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Dr. Poonam Mandowara

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Contents

S. No.	Chapters	Page No.
1.	Formulation and Characterization of Budesonide Pellets for Targeted Delivery in Inflammatory Bowel Disease (IBD) *Deepak Kashyap, Princy Kashyap and Dr. Manoj Kumar**	01-23
2.	Formulation, Characterization and Therapeutic Evaluation of Camptothecin-Loaded Mesoporous Silica Nanoparticles for Skin Cancer *Deepak Kashyap, Princy Kashyap and Dr. Manoj Kumar**	24-49
3.	Chapter Title: Ethical AI in Medical Practice and Patient Safety *Tripti Sharma*	50-71
4.	Artificial Intelligence in Biological Sciences: Transforming Drug Discovery and Development *Umadevi A*, Defin DV, VK Mohammed Naazil and Veeraraghavan K*	72-94
5.	Dressing the Self: Fashion Education, Religious Identity and Agency among Young Muslim Women in India *Dr. Rohit Pawar and Dr. Khushboo Shimali*	95-111
6.	Human-in-the-Loop Generative AI for Financial and Managerial Decision-Making: Automation Bias, Explainability and Risk Governance *Shubham Dogra, Sahil Dogra, Himan* and Deeksha Kumari*	112-121
7.	From Consent to Capability: Digital Financial Literacy, Open Finance and Consumer Protection in India's Account Aggregator Ecosystem *Himan*, Sahil Dogra and Deeksha Kumari*	122-138
8.	From Loom to Legacy: Rejuvenation of Traditional Textiles in India *Dr. Sunita Dixit*	139-151
9.	Slow Fashion: An Ecological Footprint for the Attainment of Sustainable Goals *Dr. Sunita Dixit*	152-166

10. Precision Reproductive Medicine in Dairy Cattle: Integrative Endometrial, Inflammatory and Metabolic Biomarkers for Early Prediction of Postpartum Uterine Disease and Reproductive Failure 167-194
Dr. Akshay Bind, Dr. Prajyot Sahebrao Dakhane, Dr. Akash Ashok Suryawanshi, Dr. Saurabh Sharma and Dr. Rushikesh A. Kantale

11. Decoding Embryo–Maternal Signalling: Molecular Mechanisms, AI-Enabled Prediction and Robotic Precision in Pregnancy Establishment in Cattle and Buffaloes 195-213
Dr. Akshay Bind, Dr. Prajyot Sahebrao Dakhane, Dr. Akash Ashok Suryawanshi, Dr. Saurabh Sharma and Dr. Rushikesh A. Kantale

12. From Classroom to Kerbside: Impact of Education on Waste Management 214-228
Samrin Mirza and Dr. Bhavna Parwani

CHAPTER 1

Formulation and Characterization of Budesonide Pellets for Targeted Delivery in Inflammatory Bowel Disease (IBD)

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Abstract

Crohn's disease and ulcerative colitis, both forms of inflammatory bowel disease (IBD), are prevalent conditions. Budesonide, a medication widely recommended as a first-line treatment for Crohn's disease, necessitates the development of formulations capable of delivering the drug to the intestinal region. Inulin, a highly effective polysaccharide and prebiotic in treating IBD, possesses inadequate film-forming abilities. However, harnessing the beneficial effects of inulin in IBD treatment, researchers designed a single-layer coating using a central composite design (CCD) that combined inulin and eudragit RS. This coating aimed to sustain the release of budesonide pellets, providing targeted therapy for Crohn's disease. The study focused on two independent variables: the percentage of inulin and the coating level. The responses evaluated were the release of the drug during a 2-h period at pH 1.2, as well as within three and 10 h at pH 6.8. The release profiles of the coated pellets were examined in media with varying pH levels to determine the optimal coating formulation. Subsequently, the optimized coated pellets underwent continuous mode dissolution testing to assess their release profile. Finally, the impact of the optimized formulation on reducing inflammation was evaluated in rats with experimentally induced colitis. The coating formulation composed of 9%

inulin and 91% eudragit RS at a coating level of 20% displayed a complete and pH-independent release profile. Approximately 85% of the drug was gradually released throughout the small and large intestines. The presence of inulin in the coating formulation rendered it susceptible to microbial degradation, resulting in an increased drug release rate in the colonic medium containing rat cecal content. *In vivo* results demonstrated the favorable therapeutic effects of this delivery system in treating inflammation in rats. The controlled release of the drug throughout the gastrointestinal tract (GIT) contributed to its efficacy in mitigating inflammation.

Keywords: Ulcerative colitis; Crohn's disease; Budesonide; Inflammation; Histopathological study.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition linked to the inflammation of the intestinal lining. The two main types of IBD include ulcerative colitis and Crohn's disease [1]. In Crohn's disease, the inflammation can affect the entire gastrointestinal tract, from the mouth to the anus [2], while in ulcerative colitis, the inflammation is primarily limited to the colon and rectum. The primary therapeutic goal in patients with IBD is to achieve mucosal healing [3], which can significantly improve their quality of life. Although the medications currently used for IBD treatment have been effective [4], their potential side effects have prompted the search for new delivery systems that offer higher safety profiles [5]. Due to its topical anti-inflammatory properties and minimal systemic absorption, budesonide has gained attention as an oral controlledrelease formulation for the treatment of IBD [6]. It is currently recommended as a first-choice therapy for Crohn's disease. While numerous budesonide dosage forms have been developed for IBD treatment [7] multi-particulate dosage forms, particularly pellets, are gaining prominence due to their potential advantages. These advantages include predictable movement within the gastrointestinal (GI) tract and reduced variations in gastric emptying compared to tablets [8]. Thus, budesonide pellet formulations have been extensively developed to target IBD treatment. Recent studies have revealed that natural products for instance prebiotics, probiotics and synbiotics can be considered as alternative therapeutic options for IBD [9]. These natural products have shown potential in alleviating gastrointestinal symptoms by lowering the pH of the intestine and increasing the population of beneficial bacteria [10]. They can be used either as a standalone treatment or in combination with standard therapy for IBD. Several studies have confirmed that the concurrent administration of probiotics with standard medications is more effective than using chemical

therapy alone [25, 26]. These findings highlight the potential benefits of incorporating natural products, particularly probiotics, into the treatment regimen for IBD. It is important to note that the use of probiotics is contraindicated in patients with compromised immune systems [11] due to the potential risks associated with the development of sepsis and bacteremia. On the other hand, the use of commercial prebiotics is generally considered safe. Additionally, the production processes and storage requirements for prebiotics are typically easier compared to probiotics [12] making them more appealing for pharmaceutical industries. Due to their safety profile and ease of production, prebiotics hold a significant interest in the pharmaceutical industry. Inulin is a widely used prebiotic in IBD patients [13]. Oral administration of inulin can reduce inflammation in both IBD patients and colitis rat models. The modulation of the intestinal flora is the primary mechanism involved in the management of IBD with inulin [14]. Additionally, the metabolites produced through inulin fermentation can help regulate constipation by affecting intestinal peristalsis [15]. One notable characteristic of inulin is its ability to withstand the acidic environment of the stomach and only be hydrolyzed by specific intestinal microflora, not digestive enzymes [16]. Therefore, in addition to its pharmacological benefits, inulin holds promise as a vehicle for targeted drug delivery to the intestinal region. Some studies have explored the encapsulation of budesonide into inulin nanoparticles for targeted delivery to the colon [17]. Furthermore, loading budesonide into inulin microparticles has shown significant effectiveness in targeting the colon. Studies have shown that loading polysaccharides within pellet matrices can lead to a decrease in drug release due to gel formation upon contact with a dissolution medium [18]. Considering that budesonide is practically insoluble in water incorporating inulin into the pellet structure and promoting gel formation may result in inadequate drug release at the desired site of action. Alternatively, using inulin as a coating material may lead to premature drug release in the upper parts of the gastrointestinal (GI) tract due to its high water solubility and poor film-forming ability [19]. To address these challenges, the combination of inulin with time-dependent polymethacrylates has been extensively studied as a system for targeted delivery to the intestinal region. Current evidence suggests that blending inulin with water-insoluble polymers can enhance film-forming ability and prevent early drug release in the upper segments of the GI tract [20]. This approach aims to optimize drug release profiles and ensure that the drug is delivered effectively to the intended site of action in the intestinal region. In this study, the objective was to develop a sustained-release formulation of budesonide pellets for the treatment of

Crohn's disease, while also leveraging the therapeutic advantages of inulin. To achieve this, an optimized single-layer coat was designed for the budesonideloaded pellets using a blend of inulin and time-dependent polymers, specifically eudragit RS [21]. The drug release profiles in these combined systems can be controlled by adjusting the concentration of inulin in the coating composition as well as the coating thickness. To optimize this system, a full factorial design was utilized, allowing for the investigation of the impact of varying inulin concentration and coating thickness on the drug release characteristics of the pellets. By systematically evaluating these factors, the researchers aimed to determine the optimal formulation parameters for achieving the desired sustained-release properties of budesonide [22].

2. Materials and methods

Budesonide was purchased from Cipla Ltd, Mumbai, Maharashtra, India. Avicel PH 102, lactose monohydrate, triethyl citrate, talc and polyvinylpyrrolidone was purchased from Loba Chemie Pvt Ltd Mumbai, Maharashtra, India. Inulin (high molecular weight) Sigma-Aldrich, Mumbai, Maharashtra, India. Isopropyl alcohol, sodium lauryl sulfate (SLS) and eudragit RS 30D was purchased from Loba Chemie Pvt Ltd Mumbai, Maharashtra, India. Other reagents and solvents utilized in this study were of analytical grade.

Preparation of budesonide pellets

Budesonide-loaded pellets were produced using the extrusionspheronization technique, following a formulation developed previously for conventional budesonide pellets. The selection of excipients and their concentrations aimed at achieving a high dissolution rate for the budesonide pellets. In summary, a blend containing budesonide (1.5% w/w), PVP K30 (5% w/w) serving as a binder, lactose monohydrate (25% w/w) as a soluble filler and Avicel PH 102 (68.5% w/w) as a pelletization aid was prepared through mixing for a duration of 20 min. Distilled water was then slowly added to the powder blend until a wet mass with the desirable plasticity was achieved. This wet mass was subsequently extruded through an axial screw extruder with a 1 mm screen. The spheronization of the extrudates was carried out for 5 min at 1200 RPM. The resulting pellets were subjected to drying in an oven (40 ◦C) for 24 h, then were sieved and the fraction of 850–1180 μm was used for further studies [23].

Design of coating formulation

The response surface method with a 32 full factorial design was implemented to mode and optimize the coating formulation, by design expert software. The independent variables which were examined at three levels were the amount of inulin in the coating composition (X1) as well as the coating level (X2). The higher and lower levels of each independent variable were designated as +1 and - 1, while the average value was denoted as 0. Considering the extension of inflammation in Crohn's disease from the stomach all the way down to the anus therefore the coating formulations should be able to offer a sustained release of the drug from the stomach to the small and large intestines. So the percentage of drug release during 2 h at pH 1.2 (Y1), the percentage of drug release within 3 h at pH 6.8 (Y2) (to avoid excessive release in the small intestine), as well as the percentage of drug release during 10 h at pH 6.8 (Y3), were chosen as response variables [24].

Coating of pellets

Preparation of coating formulations

First of all, a solution of inulin was prepared in hot distilled water (10% w/v) and then added to an appropriate amount of eudragit RS 30D under continuous stirring. As a plasticizer, triethyl citrate (TEC) was incorporated at a weight ratio of 10% to the eudragit RS and stirred for 1 h. Following this, the resulting mixture was diluted with deionized water so that the final concentration of eudragit RS in the coating suspension was 10% (w/v). Finally, to prevent the adhesion of pellets during the coating procedure, talc was added to this dispersion at a weight ratio of 10% relative to the eudragit RS. The resulting suspension was stirred for 20 min prior to use.

Coating procedures and conditions

The obtained budesonide pellets (100 g) were placed in a fluidized bed coater (Wurster insert, Werner Glatt, Germany) and then different coating formulations were sprayed employing a peristaltic pump under specific coating conditions. Once the coating percentage reached the designed levels specified in Table 2, samples of coated pellets were collected and dried for 24 h at a temperature of 45 ◦C in an oven.

Animal treatment

Acetic acid-induced colitis is a commonly utilized experimental model in IBD studies. To assess the anti-inflammatory effect of the optimized coated pellets in comparison to uncoated budesonide pellets, an acetic acid-

induced rat model of UC was utilized. Male Wistar rats, aged 10–12 weeks and weighing 250–300 g, were used in the research following ethical approval by the institutional ethics committee of Mashhad University of Medical Sciences. All the rats were housed in clean rooms at a temperature of 22 ± 3 ◦C, humidity of 40 ± 5% and a 12-h light–dark cycle. The rats were kept fasted with free access to water for 24 h before colitis induction. To induce colitis, the rats were first anesthetized with an intraperitoneal injection of ketamine (50 mg/kg) and xylazine (5 mg/kg) solution [25]. Then, 2 mL of acetic acid (3% v/v in normal saline) was rectally administered using a 2 mm diameter cannula. In the healthy control group instead of acetic acid solution, 2 mL of normal saline was administered intrarectally. To prevent draining back of the fluid from the rectal region, rats were positioned upside-down for 1 min before being returned to their cages. To allow for the development of colitis, they were left without treatment for the next 3 days [26]. The rats with induced colitis were grouped into 3 different sub-groups, each consisting of 6 rats. One group of rats was kept untreated and received 1 mL of normal saline (using an insulin syringe) during the treatment period, while two others were orally administrated an equivalent dose of budesonide (0.15 mg/day) from uncoated budesonide pellets as well as optimized coated pellets (using an insulin syringe with a cut-off head), for 7 consecutive days. During the study period, all animals were daily investigated for their weight and stool consistency. 24 h after the last dose consumption, the rats were sacrificed using CO2 asphyxiation and their entire colon was removed for additional investigations [27].

Assessment of colitis treatment

Colitis activity index

The severity of inflammation at different time points throughout the study was evaluated by calculating the colitis activity index (CAI). The CAI was determined based on several parameters, including weight loss, stool consistency and anal bleeding. The CAI scoring system provided a comprehensive assessment ranging from 0 (indicating a state of normal health) to 4 (representing the highest level of inflammation). Weight loss was evaluated and assigned scores to reflect the degree of loss. Specifically, no weight loss was given a score of 0 points, while weight loss ranging from 1% to 5% was assigned a score of 1 point. For weight loss between 5% and 10%, a score of 2 points was given and for weight loss ranging from 10% to 20% received a score of 3 points. Weight loss exceeding 20% was assigned the highest score of 4 points. Stool consistency was assessed and scored accordingly. Well-formed pellets were assigned a score of 0 points,

indicating normal consistency. On the other hand, pasty and semi-formed stools that did not stick to the anus received a score of 2 points, reflecting altered stool consistency. Lastly, liquid stools that stuck to the anus were given a score of 4 points, indicating the most severe alteration in stool consistency. The presence and extent of anal bleeding were also considered in the CAI assessment. No evidence of blood in the stool resulted in a score of 0 points. Positive findings of blood in the stool received a score of 2 points, indicating the presence of bleeding. Visible anal bleeding, representing the most severe form of bleeding, was assigned a score of 4 points. The overall CAI score, which provided a comprehensive measure of inflammation severity, was quantified by calculating the mean of the individual scores for weight loss, stool consistency and anal bleeding [28].

Colon weight ratio

Prior to sacrifice, the rats were weighed to record their body mass. Following sacrifice, the entire colon tissues, extending from the colorectal junction to the anal verge, were carefully excised. The excised colon tissues were then horizontally cut and rinsed with a cooled phosphate buffer solution at a pH of 7.4. To assess the extent of inflammation, the ratio of the mass of the colon sample to the body mass of each rat was determined. This ratio served as an inflammation index, providing a quantitative measure of the inflammation present in the colon [29].

Weight/length ratio of colon

The weight-to-length ratio of the washed colon tissue samples was used as a measure of the inflammation severity [30].

Glutathione content of the colon tissue

To determine the reduced glutathione (GSH) content in the tissue samples, a UV spectrophotometer was utilized by measuring the generation of a yellow color upon the addition of DTNB (5,5′-dithiobis-(2- nitrobenzoic acid), which reacts with sulfhydryl groups [31]. Here is a summary of the procedure: Tissue samples were homogenized (10% w/v) in phosphate-buffered saline (0.1 M, pH 7.4). To the 0.5 mL of the homogenized tissue, 0.5 mL of trichloroacetic acid (TCA) (10% w/v) was added. The mixture was then subjected to centrifugation at 25,000 g for 10 min at 4 ◦C. From the resulting supernatants, 0.5 mL was taken and combined with reaction mixtures containing 2.5 mL of phosphate buffer (pH 8) and 0.5 mL of DTNB. After 10 min, the absorbance of the resulting yellow solution was measured at 412 nm using a spectrophotometer. The GSH content (nmol/g tissue) was quantified based on a standard curve constructed using the blank

medium. By measuring the yellow color developed through the reaction between DTNB and sulfhydryl groups, the GSH content in the tissue samples was determined using UV spectrophotometry [32].

Malondialdehyde content of the colon tissue

The concentration of malondialdehyde (MDA) in the colon tissue sample can serve as an indicator of the severity of inflammation. The procedure for determining MDA is outlined as follows: Homogenized tissue samples (10% w/v) in 1.15% w/v KCl were prepared. To the homogenized samples, 3 mL of phosphoric acid (1% w/v) and 1 mL of thiobarbituric acid (TBA) (0.6% w/v) were included. The mixtures were then subjected to heating in a boiling water bath for 45 min. After the heating process, the mixtures were cooled and 4 mL of nbutanol was added. The resulting mixture was vortex-mixed for 1 min and then centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Jenway 6105 UV/Vis, UK). The concentration of MDA (nmol/g tissue) was quantified by quantifying the absorbance based on a standard curve constructed using a blank medium. By following this procedure, the concentration of MDA in the colon tissue sample, which serves as a biomarker of oxidative stress and inflammation, can be measured using spectrophotometry [33].

Histological assessment of inflammation severity

Colon tissue segments were first fixed in an aqueous solution of formalin (10% v/v) and then embedded in paraffin. The segments were stained with hematoxylin and eosin (H&E) following sectioning by microtome. The severity of inflammation in stained samples was graded on a scale ranging from 0 to 4 using an optical microscope. A score of 0 indicated no or mild inflammation, while a score of 1 was assigned when focal infiltration of inflammatory cells and a small affected area were observed. A score of 2 was given when inflammation involved a significant portion of the colon tissue with evidence of smooth muscle thickening. A score of 3 was assigned in cases where ulceration and infiltration of inflammatory cells were observed in the tissue sections. The highest score of 4 was given to cases showing extensive tissue damage characterized by necrosis and gangrene, indicating the most severe colitis [34, 35].

Statistical analysis

Statistical analysis of the collected data was done using Graph Pad Prism software (Graph Pad Prism, version 7, San Diego, CA). To compare the means and determine significant differences, ANOVA test was

performed followed by Tukey–Kramer test (P values < 0.05 indicates statistically significant).

3. Results and discussion

Fig. 1 displays the release profiles of budesonide from all pellets coated under the experimental design conditions. As it could be seen, at various pH conditions the drug release rate from these formulations was almost the same. Considering the fact that the solubility of budesonide and all other materials that were applied in these formulations is not dependent on the pH of the dissolution medium [36], this pH-independency of release was predictable and such delivery systems could be considered as a combination of time-dependent and bacterial degradation systems. In all formulations, higher coating levels were accompanied by reduced drug release levels. So that formulations with a 20% coating level led to a slower release rate than the same formulation with a 10% coating level. Since diffusion has a significant role in drug release from time-dependent systems [37], such behaviors could be related to the longer pass for diffusion. The formulation containing 20% inulin and 80% eudragit RS (ERS) with a 20% coating level released almost 15% of the drug after 2 h and more than 60% during the first 5 h of the release study (before reaching the terminal ileum), while the formulation containing 40% inulin and 60% ERS with the same amount of coating level released approximately 30% of the drug within 2 h and more than 70% of the drug during the first 5 h of the study and those pellets with no inulin in their coating formulation, released less than 10% of their drug content within 2 h and less than 50% during 5 h. These results demonstrated that increasing the amount of inulin in the coating composition could increase the drug release rate. This can be ascribed to the solubility of inulin in the dissolution medium [38] and the development of pores within the coating layer. Although it seems that formulations with no inulin in their coating compositions could be able to deliver a desired amount of drug to the colonic region, an incomplete drug release (almost 90% of drug content was released during 10 h), could be a drawback of such formulations. High molecular weight inulin was used in this study due to the slower dissolution rate and hydrolysis in the colonic environment compared to low molecular weight inulin [39].

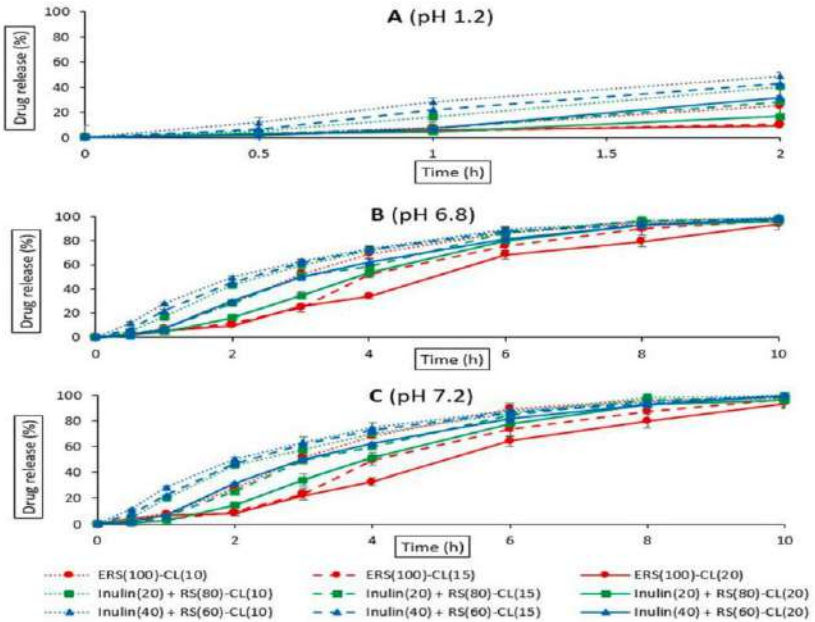


Fig. 1: The drug release profile from the pellets coated under the experimental design conditions at various pH conditions. CL: coating level.

Determination of optimum coating composition and level

Sequential p-values were computed using the design expert software and a linear model was established to investigate the three responses. Upon conducting an analysis of variance (ANOVA) on the experimental data, it was observed that the model p-value for all responses was less than 0.0001. The model F-values corresponding to responses Y1, Y2 and Y3 were 205.68, 38.31 and 28.29 respectively. These values confirm the significance of the model for all three responses. Additionally, the Rsquared (R2) values for Y1, Y2 and Y3 were determined as 0.9763, 0.8846 and 0.8500 respectively, indicating that the model offers a robust fit to the data [40]. Equations (1)–(3) represent the predicted mathematical relationship between independent variables (Xs) and responses (Ys) in terms of coded factors: The positive coefficient of X1 in all equations indicated a synergistic effect of this factor on all responses, while the negative value of X2 demonstrated an opposing effect on them. It could be concluded that an increase in the amount of inulin in the coating composition as well as a decrease in the coating level would result in more drug release. Response surface plots (Fig. 2) were also presented in order to better illustrate the impact of independent variables on each response. The optimization of the coating formulation and level was

adjusted based on the residence time of the formulation in various regions of the GIT. Since this designed delivery system was based on the time-dependent and bacterially degradable systems, to provide a suitable sustained release of the drug throughout the GIT, the optimal formulation should be able to stop excessive drug release in an acidic pH environment of the stomach within the first 2 h (Y1) and release the drug through the small and large intestines (Y3) in a sustained manner [41]. As an attempt to prevent a high amount of drug release in the initial parts of the small intestine, the drug release at pH 6.8 within 3 h was also considered as one of the responses (Y2). So, desirable responses were considered drug release of less than 20% within 2 h at pH 1.2, less than 30% within 3 h at pH 6.8, while the release of 80–100% for the rest of 10 h at pH 6.8. Based on the obtained dissolution data, a coating composition consisting of 9% inulin and 91% ERS, at a coating level of 20% was predicted as the optimized coating formulation by the model. The optimized formulation was prepared and subjected to morphological analysis and dissolution tests [42].

Dissolution studies of budesonide pellets coated with optimized coating formulation

Although based on the low standard deviation and predicted residual error sums of square values (PRESS) as well as high coefficient of determinations (R2) and enough high F-value, it seems that the chosen predicting model (Linear) could represent high predictability [69], the measured response values were also compared with the anticipated values generated by the software to assess the validity of the optimization process. According to the results presented in Table 3, close agreement between these two values showed the validity of the optimization method. The release profile of optimized coated pellets was studied in a continuous mode of dissolution test at different simulated GIT conditions. As mentioned earlier, budesonide is a practically-insoluble drug [43] and therefore incomplete drug release might be expected from its diffusion-based sustained release formulations. It could be seen in Fig. 3 that, the optimized pellets released all of their drug content during the test period. So, it could be concluded that the problem of incomplete drug release, which is one of the drawbacks of some marketed brands of budesonide and most of the time-dependent based colon drug delivery systems [44], could be addressed with this formulation. It has been shown that blending inulin with time-dependent polymers could improve the permeability of films and lead to more drug release. Optimized pellets released only 15% of their cargo during the first 2 h in the simulated gastric conditions (pH 1.2) and the remaining 85% of the drug was slowly

released through other parts of GIT. The limited drug release during 2 h in a simulated gastric environment could be attributed to the very low amount of inulin in the coating that could be dissolved in acidic media as well as the time required for ERS films to become permeable to the dissolution medium [45]. In the next 3 h, following the dissolution of a higher amount of inulin, the polymeric network of the coating system became more permeable to the drug so that almost 50% of the loaded drug was released prior to reaching the medium-simulating ileum and the remaining 35% of the loaded drug reached to the terminal ileum and colon. Therefore, it seems that optimized coated pellets could provide a favorable sustained release profile that enables effective drug delivery throughout the entire length of the small and large intestines. The optimized formulation presented a slightly faster release of budesonide at pH 6.8 when rat cecal content (SCF) is present. This is attributed to the decomposition of inulin by bacterial enzymes. Owing to this, pore formation within the coating matrix could be facilitated and accelerate drug release [46].

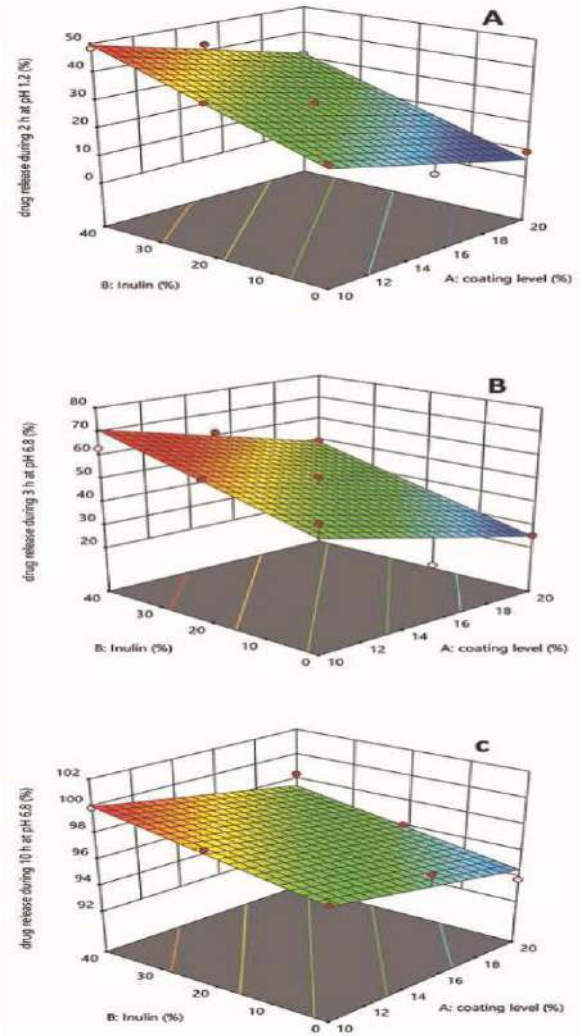


Fig. 2: The response surface plots for the responses predicted at different independent variables.

Although due to the low concentration of cecum content (4% W/V) in the dissolution medium, the difference between drug release in the medium with and without cecal content was not huge, this result could indicate enzymatic sensitivity of optimized formulation and it is expected that the higher concentration of degrading enzymes in the human digestive tract could result to the marked difference in release between formulations without inulin and formulations containing inulin [47].

Morphological characteristics

SEM images of the optimized coated pellets illustrated the formation of a smooth and uniform coat with a thickness of about 70 μm around the core of the pellets (Fig. 4A & B).

***In-vivo* therapeutic efficacy in rats**

The therapeutic efficacy of the optimized coated pellets was assessed and compared to uncoated pellets. To evaluate the severity of inflammation in the colon tissue, various parameters were examined, including the colitis activity index colon weight, weight/length ratio of the colon, the level of malondialdehyde (MDA), the amount of reduced glutathione (GSH) in colon tissue as well as histological studies. Three days after rectal administration of acetic acid, every animal exhibited signs of weight loss and suffered from diarrhea. This observation as well as increasing the level of CAI (Fig. 5), demonstrated the development of experimental colitis in all animals [48]. During the treatment period, the level of CAI in treatment groups decreased consistently, so all rats that received treatment exhibited a significant reduction in CAI in comparison with untreated colitis rats on the day of sacrification ($p < 0.05$). Although these results demonstrated the reduction of inflammation in colonic tissue following the administration of the drug it seems that optimized coated pellets are significantly more effective than uncoated pellets ($p < 0.05$) (see Fig. 6). As it can be seen in Fig. 6A & B, in both treated groups, there was a remarkable decrease ($p<0.05$) in both the colon/body weight ratio and the weight/length ratio in comparison to the untreated control group. This result could be related to the reduction of inflammation in colonic tissue [49]. Similarly to the CAI results, more therapeutic effects were seen by the administration of optimized coated pellets compared to uncoated pellets ($p<0.05$). Fig. 6C and D illustrated that in treated rats the amount of GSH profoundly enhanced ($p < 0.05$), while the level of MDA exhibited a significant decrease ($p < 0.05$) in comparison to the untreated control rats. Since MDA is a marker that increases tissue injuries and also GSH shows the anti-oxidative activity of cells following treatment these results could demonstrate the reduction of inflammation upon the treatment. The macroscopic observation of colon specimens (Fig. 6E) showed a decrease in colon length in untreated colitis rats when compared to the healthy controls (almost 8 cm). Furthermore, the colon tissue of colitis rats exhibited visible signs of thickened bowel wall and ulceration. Although the signs of inflammation appeared to be reduced in both treated groups, the rats treated with optimized coated pellets exhibited the greatest increase in colon length (almost 4 cm). Fig. 6F represents the

colitis damage score and Fig. 7 shows the histological sections of the colon tissues under a microscope. In Fig. 7A, normal colon histology is depicted, showing no signs of tissue abnormality or disruption [50]. This image received a colitis score of zero. Conversely, in Fig. 7B, the colon tissue of untreated colitis rats exhibited tissue damage, goblet cell depletion and necrotic mucosal structure, warranting the highest score of four. Fig. 7C displayed some signs of smooth muscle thickening in the colon tissue of rats treated with uncoated pellets, resulting in a colitis score of two. Microscopic observation of the colon tissue from rats that received optimized coated pellets showed trace regions of focal inflammatory cells infiltration, as shown in Fig. 7D, which earned a colitis score of one. Although both treatment groups exhibited some improvement in the microscopic characterization of the colonic tissues compared to the untreated group, the administration of optimized coated pellets demonstrated a condition that was much closer to the normal colon histology. In summary, the findings from the *in vivo* studies indicated that the use of optimized coated pellets for the treatment of colitis, exhibited superior effectiveness compared to uncoated pellets. This higher efficacy demonstrated that designed coating systems successfully inhibited the rapid release of the drug in the stomach and delivered the drug in a sustained manner throughout the small intestine and colon and exhibited enzymatic sensitivity in the presence of cecal content in *in vitro* studies indicating that the combined effects of budesonide and inulin might contribute to the effectiveness of this formulation in the treatment of colitis inflammation [51].

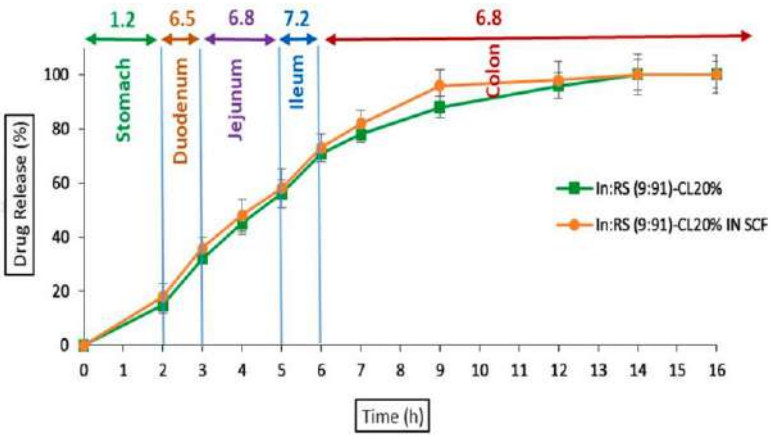


Fig. 3: The figure illustrates the release profile of budesonide from the optimized coated pellets in a continuous dissolution test, both with and without cecal content.

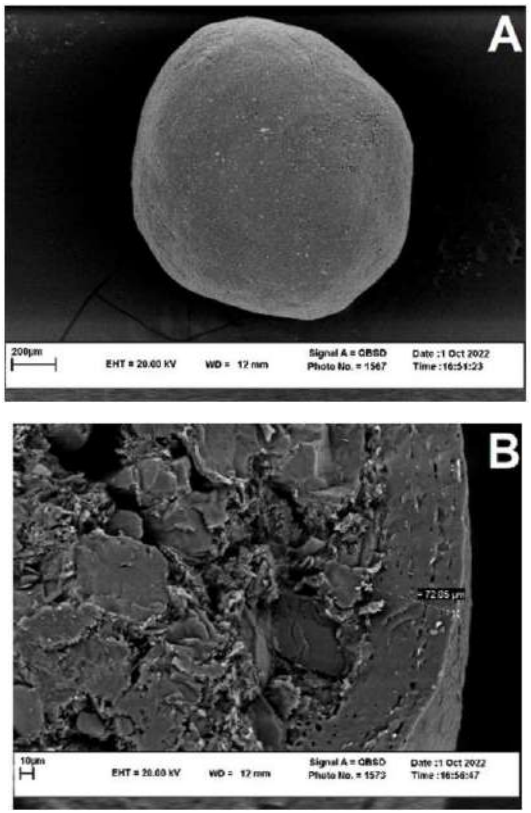


Fig. 4: Scanning electron microscopy of (A) the optimized coated pellets and (B) the cross-sectional image of optimized coated pellets.

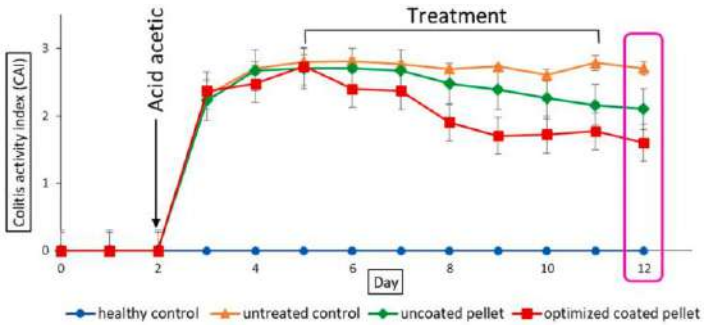


Fig. 5: Colitis activity index of the different rat groups. Data are presented as mean ± SD (n = 6 animals/group).

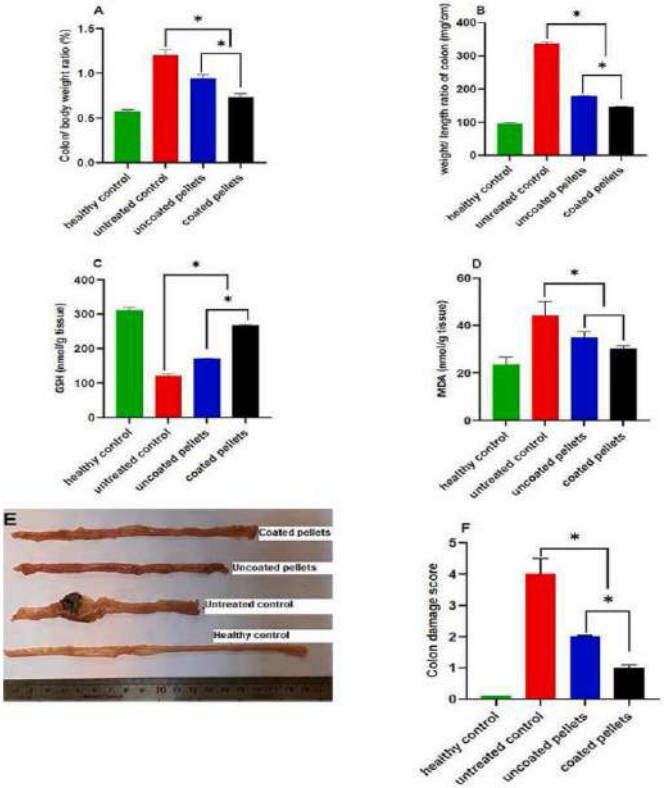


Fig. 6: The figure shows (A) Colon/body weight ratio, (B) Weight/length ratio of colon, (C) Amount of GSH, (D) Level of MDA, (E) Photographs for macroscopic examination and (F) Colon damage score of the colon tissues. The data are presented as mean ± SD, with each group consisting of 6 animals. Significant differences between marked groups are denoted by an asterisk (*) with a significance level of $p < 0.05$.

4. Conclusion

In this particular study, the researchers aimed to develop an optimized single-layer coating system for budesonide pellets by combining inulin and eudragit RS. The primary objective was to utilize a central composite design (CCD) to sustain the delivery of budesonide pellets while harnessing the therapeutic benefits of inulin in the treatment of inflammatory bowel disease (IBD). The optimized coating formulation comprised 9% inulin and 91% eudragit RS, with a coating level of 20% (w/w). This formulation effectively prevented premature drug release within the acidic environment of the stomach and facilitated complete and controlled drug release throughout the entire length of the intestine. By carefully tailoring the coating parameters,

the researchers achieved the desired sustained release characteristics for budesonide. The efficacy of the optimized coated pellets was evaluated in comparison to uncoated pellets using a rat model of colitis. The results demonstrated that the optimized coated pellets exhibited superior therapeutic effects in treating colitis when compared to the uncoated pellets. This finding suggests that the optimized coating system effectively protected the drug from premature release, allowing for targeted drug delivery and improved treatment outcomes in the context of colitis. Overall, this study highlights the successful design and optimization of a single-layer coating system combining inulin and eudragit RS for budesonide pellets. The optimized formulation demonstrated controlled drug release properties, which were advantageous for the treatment of IBD, particularly in the context of colitis.

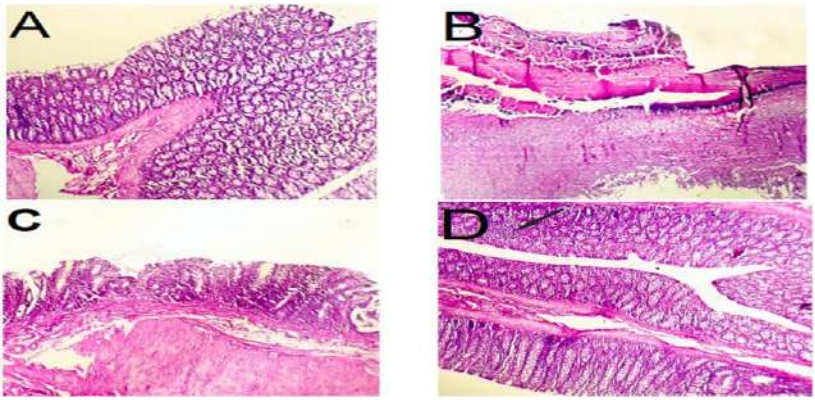


Fig. 7: The figure displays the histopathological characteristics of the colon tissues in rats subjected to different treatments. The subfigures show: (A) a rat with normal colonic tissues, (B) an untreated rat, (C) a rat treated with uncoated pellets and (D) a rat treated with coated pellets.

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CHAPTER 2

Formulation, Characterization and Therapeutic Evaluation of Camptothecin-Loaded Mesoporous Silica Nanoparticles for Skin Cancer

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Abstract

The therapeutic efficacy of camptothecin (CPT), a potent antitumor alkaloid, is hindered by its hydrophobic nature and instability, limiting its clinical use in treating cutaneous squamous cell carcinoma (SCC). This study introduces a novel nano drug delivery system (NDDS) utilizing functionalized mesoporous silica nanoparticles (FMSNs) for efficient CPT delivery. The FMSNs were loaded with CPT and subsequently coated with chitosan (CS) for enhanced stability and bioadhesion. Importantly, CpG oligodeoxynucleotide (CpG ODN) was attached onto the CS-coated FMSNs to leverage the immunostimulatory properties of CpG ODN, augmenting the chemotherapy's efficacy. The final formulation FMSN-CPT-CS-CpG displayed an average size of 241 nm and PDI of 0.316 with an encapsulation efficiency of 95 %. Comprehensive *in-vitro* and *in-vivo* analyses, including B16F10 cells and DMBA/TPA-induced SCC murine model, demonstrated that the FMSN-CPT-CS-CpG formulation significantly enhanced cytotoxicity against B16F10 cells and induced complete regression in 40 % of the *in-vivo* subjects, surpassing the efficacy of standard CPT and FMSN-

CPT treatments. This study highlights the potential of combining chemotherapeutic and immunotherapeutic agents in an NDDS for targeted, efficient skin cancer treatment.

Keywords: Camptothecin; Silica nanoparticles; Drug loading; Formulation; Cutaneous squamous cell carcinoma.

1. Introduction

Epidermal keratinocyte-derived carcinomas, particularly cutaneous squamous cell carcinoma (SCC), are the most prevalent malignancies worldwide. Several factors, including UVinduced damage from the sun or artificial sources, chronic inflammation and immunosuppression, contribute to the development and progression of SCC. Surgical resection is the first-line therapeutic approach for localized SCC, whereas systemic treatment is reserved for metastatic SCC [1]. However, these approaches may be limited by risks of recurrence, resistance and undesirable aesthetic outcomes. Chemotherapy results in serious side effects due to frequent dosing, lack of specificity and selectivity and poor availability of active components at the tumor site. Topical treatments such as 5-fluorouracil and imiquimod are only affective for superficial SCCs [2]. Epidermal growth factor receptor (EGFR) inhibitors offer a new nonsurgical option for advanced SCC but often result in skin side effects, for instance, papulopustular eruptions, pruritus, paronychia and xerosis. Furthermore, the overall costs of the management and treatment of SCC represent a major economic burden on individuals and national healthcare systems emphasizing the need for the exploration and implementation of more efficient alternatives to alleviate this healthcare challenge. Camptothecin (CPT) is an indole alkaloid that acts as a DNA topoisomerase I inhibitor with broad activity as an anticancer drug in preclinical models [3]. CPT functionality is hampered by its hydrophobicity, poor biostability, rapid physiological inactivation, high risk of systemic toxicity and multidrug resistance. The development of nano-drug delivery systems (NDDSs) has shown the potential to improve the safety and efficacy of treatment by controlling the rate and timing of drug release at specific locations. Nanocarrier systems, including liposomes, amphiphilic copolymers, dendrimers, hydrogels, micelles, metal nanoparticles (NPs) and mesoporous silica nanoparticles (MSNs) have been developed to deliver several hydrophobic drugs [4]. Among these, MSNs are actively investigated for developing next-generation inorganic materials for biomedical applications particularly in the field of drug delivery systems, owing to their

unique mesostructured properties such as large surface area, large pore volume, modifiable particle size, easy surface functionalization, outstanding biocompatibility and unique surface-decorating properties with targeting moieties due to the presence of abundant silanol groups (Si-OH). Water molecules can degrade mesoporous silica by hydrolyzing and dissolving the silica framework. However, the degradation of MSN can be reduced using a higher pH, lower temperature and coating of the silica framework [5]. Tailoring the pore characteristics and surface properties of MSNs with other molecules, including organic and inorganic entities, drug molecules and biological membranes, offers a promising approach for the development of formulations, capable of efficient drug loading and release of hydrophobic drugs into tumors. Among various polymeric materials, chitosan (CS) is a natural polysaccharide derived from chitin found in the exoskeletons of crustaceans and the cell walls of fungi. CS has been widely used as a protective shell around the core owing to its biocompatibility, biodegradability, nontoxicity, solubility, bioadhesiveness/ mucoadhessiveness and pH-sensitive properties [6]. Its application in DDS has been extensively explored for its ability to modulate drug release profiles, enhance cellular uptake and protect encapsulated drugs from degradation. It forms electrostatic interactions with negatively charged molecules on biological surfaces through its carboxyl and amino groups. Various strategies have been proposed to prolong the drug residence time by enhancing contact with the cell surface, thus improving the therapeutic efficacy of local drug delivery. Combination approaches in NDDS are promising for the treatment of cancer that can simultaneously transport and deliver different types of therapeutic molecules, such as drugs, immunomodulatory agents, or genetic material. Furthermore, various ligands, including single- and double-stranded nucleic acid aptamers, have been employed to improve the accumulation of drug-loaded NPs at tumor sites and to reduce off-target side effects [7]. These oligonucleotide strands, characterized by their specific 3D folding, engage with distinct cell surface markers, facilitating the selective transportation of tagged NPs to tumor tissues. CpG oligonucleotides (CpG ODN) are synthetic, single-stranded DNA molecules comprising unmethylated cytosine-phosphate guanine motifs, with specific sequences resembling bacterial DNA. The immune system recognizes these sequences through toll-like receptor 9 (TLR9), expressed in certain immune cells and activates antigen-presenting cells (APCs), such as dendritic cells (DCs), B cells and macrophages [8].

Preclinical and clinical studies have confirmed the potential of CpG ODNs as immune adjuvants in cancer immunotherapy. The use of MSN as carriers with or without surface modification has been reported to successfully deliver various anticancer drugs, including methotrexate doxorubicin paclitaxel gemcitabine curcumin and CPT. The acidic pH of the tumor microenvironment has become the main focus of various studies because of its difference from the normal extracellular physiological pH, which leads to the development of pHresponsive NDDS. Numerous molecules and polymers have been used to modify MSN to formulate pH-sensitive and bioadhesive NPs, including folic acid poly acrylic acid, polyethylenimine (PEI) transferrin, polylactic acid-hyperbranched polyglycerol (PLA-HPG) and alginate [9]. In this study, we developed a nanoplatform for CPT delivery by encapsulating CPT within MSN and then coating it with CS to enhance biocompatibility, control and prevent drug release to off-target sites due to difference in the pH of healthy and diseased cells. Finally, the system was functionalized with CpG ODNs to enable targeted delivery and immunostimulation, to maximize the therapeutic efficacy. This strategy was evaluated *in-vitro* using B16F10 melanoma cells and *in vivo* in a murine skin carcinoma model using local drug delivery via intratumoral injection. The formulated NDDS demonstrated good biocompatibility and high anti-tumor cytotoxic effects both *in vitro* and *in vivo*, particularly when delivered synergistically as a ligand-targeting strategy [10].

2. Materials and Methods

For the NPs formulation, tetraethyl orthosilicate (TEOS, 98 %), (3-aminopropyl) triethoxysilane (APTES, ≥98 %), cetyltrimethylammonium bromide (CTAB, 25 wt%), ammonium hydroxide, glutaraldehyde (GA) solution (25 wt%) and, (S)-(+)-Camptothecin (CPT ≥ 90 %), ethidium bromide (EB), phosphate buffered saline (PBS) were purchased from Sigma-Aldrich, Mumbai, India. Ethanol (EtOH, HPLC grade), dimethyl sulfoxide (DMSO), hydrochloric acid (HCl, ACS reagent, fuming, 37 %), chitosan (CS) and glacial acetic acid (<99 %) were purchased from Loba Chemie Pvt Ltd, Mumbai, India. All reagents and solvents used were of analytical grade. Deionized (DI) water was used in all experiments.

Synthesis of functionalized MSNs

MSNs were formulated using a modified version of the previously reported Stober ¨ method, a type of sol-gel process. Briefly, 250 mg of CTAB was dissolved in 120 mL of DI water, followed by the addition of 1

mL of 2 M NaOH. This solution was then heated to 80 ◦C. Upon reaching this temperature, 1.08 mL TEOS and 110 µL of APTES were added in a molar ratio of 10:1. This mixture was sonicated, stirred and maintained at 80 ◦C overnight to form a gel. Subsequently, the particles were washed repeatedly at 6000 rpm for 25 mins, 3 times with ethanol and 3 times with DI water to remove the surfactant, until the supernatant appeared clear and free of foam. The NPs were redispersed in DI water and dried under a vacuum. The functionalized MSNs are referred to as FMSN, while the non-functionalized ones, as MSNs [11].

Drug loading

MSNs and FMSNs, each weighing 45 mg, were dispersed in 1 mL of a CPT solution (2.5 mg/mL) in DMSO. To disperse the mixture, it was first sonicated on ice for 5 mins and then stirred at a cold temperature while protecting it from light. The resulting suspension was collected at three different time points (6 h, 12 h and 24 h), centrifuged at 10,000 rpm for 20 mins at 4 ◦C and this process was repeated 4 times with DI water. The NPs were then resuspended in a specific volume of DI water and lyophilized. The drug-incorporated NPs are referred to as MSN-CPT and FMSN-CPT [12].

Chitosan coating of NPs and crosslinking by glutaraldehyde (GA)

FMSNs or FMSN-CPT were dispersed in DMSO. 1 % (v/v) GA was added to the reaction mixture, dropwise and allowed to react for 1 h followed by the addition of 1 % (w/v) CS (4 mL) in 1 % (v/v) acetic acid. After sonication, the mixture was stirred for 8–10 h. The blank NPs were coated with CS using the same method. The resulting suspensions were then washed by centrifugation at 10,000 rpm at 4 ◦C for 20 mins, four times with DI water, resuspended in a certain amount of DI water and lyophilized. The NPs were denoted as FMSN-GA-CS and FMSN-CPT-GACS [13].

Characterization of NPs

The size distribution and surface morphology of the NPs were investigated using a Scanning Electron Microscope (SEM) equipped with an energy-dispersive X-ray microanalysis system (EDX). The hydrodynamic size, polydispersity index (PDI) and zeta potential of the NPs were determined using Dynamic Light Scattering (DLS) and electrophoretic light scattering (ELS)**.** Brunauer-Emmett-Teller (BET) analysis (Gemini VII 2390 V1.03) was used for surface characterization, including pore characteristics. To evaluate the thermal behavior (Δw %) and extent of surface modification

of MSNs, thermogravimetric analysis (TGA) (TGA/SDTA851e, was carried out at a heating rate of 10 ◦C. min- 1 up to 700 ◦C. Further, the chemical interactions between CS, CPT and carrier were studied using Fourier-Transform Infrared Spectroscopy (FTIR) by analyzing a sample in the range of 4000 cm- 1 to 450 cm- 1 with an average of 16 scans per sample**.** The CPT loading within the NPs was quantified using ultraviolet–visible spectroscopy analysis [14].

***In-vitro* release assay**

To evaluate the rate of drug release from all groups of NPs, a known volume of CPT (100 µL) with known concentration was loaded into a Slide-A-Lyzer Mini dialysis units 10 KDa MWCO (ThermoFisher) in triplicate. These units were then incubated at 37 ◦C. The drug was allowed to be released in 1 mL of 1X PBS at pH 7.4 and pH 6.4, with gentle shaking to facilitate the process. The released medium was collected and replaced with fresh 1x PBS at specific intervals. The CPT concentration in the dialyzed fluid was quantified using a Multiskan Sky microplate reader [15].

In-vitro cytotoxicity assay

The cytotoxicity of NDDS was assessed using the MTT assay as previously described. The murine melanoma cell line B16F10 was cultured in 96-well plates (5,000 cells per well) in DMEM medium containing 1 % (v/v) antibiotic (penicillin–streptomycin) and 5 % heat-inactivated fetal bovine serum at 37 ◦C, in a 5 % CO2-humidified atmosphere. The cells were washed with PBS after 24 h and treated with various concentrations of FMSN, free CPT, FMSN-CPT, FMSN-CPT-GACS and FMSN-CPT-GA-CS-CpG for 24 h, 48 h and 72 h and analyzed with MTT reagent (2 mg/mL) solution by spectrophotometry (570 nm) [16].

Anticancer potential of NDDS against the carcinogenic model

A preclinical cutaneous chemical carcinogenesis model was established, based on a previously reported protocol with slight modifications made for experimental refinement. This protocol was approved by the Research Ethics Committee of the National University of Sciences and Technology (NUST) (IRB No. 04–2021-02/36), in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male BALB/c mice, 4 to 6 weeks old and averaging 20 g in weight, were acclimatized for 1 week [17]. The dorsal skin of these mice was shaved (approximately 3 × 3 cm) using electric clippers. Before starting the experiment, a preliminary study

was conducted to determine the dose and frequency of carcinogens and drugs required to induce and treat skin cancers. A single dose of DMBA (200 nmol) was applied to the shaved dorsal skin of mice to initiate tumorigenesis. Starting one week after initiation, TPA (8 nmol) was applied twice a week (up to 10 weeks) to promote tumor progression. The mice were monitored daily and weighed weekly**.** Only tumors with a diameter of more than 2 mm that remained for not less than one week were classified as papillomas and were recorded. The volume of tumors was calculated using the formula: 1/2 × (length × width2). Following the establishment of papillomas, animals were randomized into five distinct groups (n = 7): PBS, free CPT, FMSN-CPT, FMSNCPT-GA-CS and FMSN-CPT-GA-CS-CpG. The PBS group served as a positive control. The free CPT group received intratumoral injections twice a month, with a 15-day interval between the administrations. The remaining groups followed the same treatment schedule. The experimental plan is illustrated in Fig. 6A. The animals were euthanized at the end of the study and skin samples were excised and collected for histological evaluation [18].

Statistical analysis

All data were acquired in triplicate and expressed as mean ± standard error of the mean (SEM). The results were statistically analyzed using Origin pro2023b and GraphPad Prism 8 with a *t*-test or one-way

ANOVA as applicable. Post Hoc, Tukey's Multiple Comparison Analysis was employed for multiple comparisons, with the significance level set at $p < 0.05$ [19].

3. Results and Discussion

Synthesis, modification and characterization of MSNs

A schematic representation of the NPs formulation process is shown in Fig. 1. MSNs were synthesized according to the Stober method (sol-gel), with some modifications. Hydrolysis and condensation reactions were performed using TEOS as the silica precursor and CTAB as the template. The modification of hydroxylated MSNs (MSN-OH) with APTES created a siloxane network with positively charged NH2 head groups that can electrostatically bind to negatively charged molecules, such as DNA, RNA, proteins, drugs, anionic dyes and polysaccharides. This allowed the successful incorporation of the negatively charged CPT through hydrophobic interactions. CS was then coated onto the drugincorporated FMSNs using

GA as a crosslinker. GA is a commonly used crosslinking agent that reacts with primary amines to form an imine (N = C) bond between the primary amines and aldehyde carbon to form a stable coating. When the CS coating was carried out without GA, only a minor change was observed in the weight of the end product after washing and drying the NPs. This might be due to the lightly bound CS layer, whereas the use of GA resulted in a more stable and firmly bound layer on the surface of the FMSNs, which was also supported by previous studies. The yield of MSNs obtained was 454 mg ± 23 mg, but it varies typically with reaction conditions and particularly the method of drying. The percentage of CS coated on the FMSNs was evaluated by recording the weight of the FMSNs before and after coating (initial and final weights) to determine the weight gain of the end product ($\Delta W\% = (Wf - Wi/Wi) \times 100$). The weight gain, $\Delta W = 45$ % was calculated after coating FMSNs with 1 % CS. The last step in the fabrication process was the binding of CpG ODN to FMSN-CPT-CS to stimulate and enhance the therapeutic action. The successful fabrication of NPs was characterized and confirmed at each step, starting from the synthesis of bare MSNs to the binding of CpG ODN, ensuring the accessibility and controllable release of the encapsulated drug [20].

Dynamic light scattering (DLS) and electrophoretic light scattering (ELS)

The hydrodynamic size and PDI for the particle size distribution and zeta potential were measured using DLS and ELS, respectively. MSN-OH particles showed an estimated average hydrodynamic size of 195 nm with a zeta potential of - 23.7 mV (Fig. 2E, 2F) because of the presence of siloxane groups (- Si-O-), derived from the deprotonation of silanol groups (Si-OH). Modification of MSN-OH to FMSN slightly increased the size but shifted the zeta potential to + 35.5 mV (Fig. 2F) because of the protonation of the NH2 group. Loading of the drug into the FMSN increased the overall size of the NPs to approximately 244 nm and decreased the zeta potential to + 21 mV because of the negatively charged CPT (Fig. 2F). The coating of FMSN NPs with a positively charged polymer, CS, further modified their size to 269 nm and increased their charge to + 43.6 mV. Binding CpG to CS-coated FMSN caused a further increase in size to 291 nm and reduced the charge to + 29.8 mV (Fig. 2F). CpG ODN typically carries a negative charge due to its phosphate backbone, while CS is a cationic polymer due to the presence of NH2 groups. The electrostatic interaction between the negatively charged CpG and positively charged CS results in a reduction in the overall charge.

The PDI of all types of NPs was retained from 0.3 to 0.66. Fig. 2B shows the uniformity of the hydrodynamic size of the prepared NPs. A single sharp and prominent peak signifies the synthesis of uniformly distributed NPs. An alternate shift in the zeta potential after each step illustrates the conversion of MSNs to the end product FMSN-CPT-CS-CpG [21, 22].

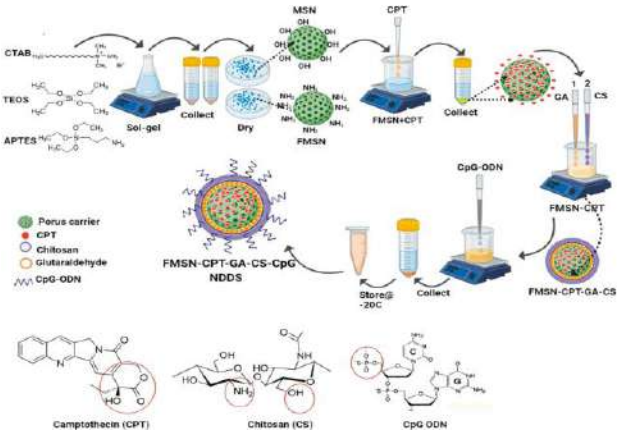


Fig. 1: Schematic representation of NDDS formulation, beginning with the synthesis of MSN to FMSN-CPT-GA-CS-CpG. The chemical structures of CPT, CS and CpG ODN with encircled active groups, involved in the interaction.

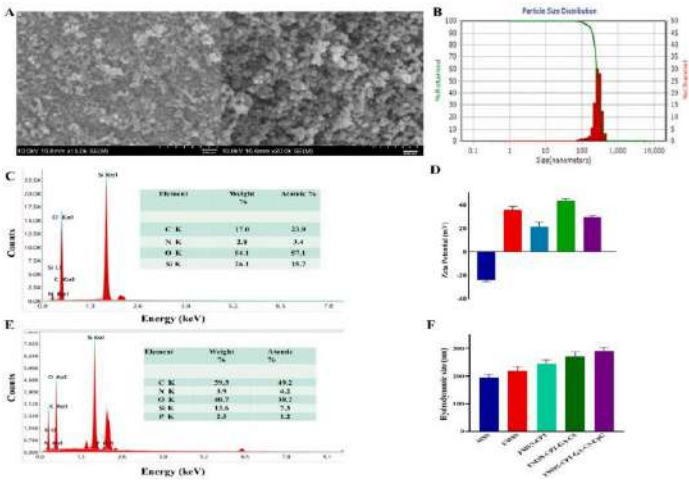


Fig. 2: Physical characterization of NPs (A) Scanning Electron Microscopy (SEM) images (B) Hydrodynamic size distribution (C) Energy-dispersive X-ray spectroscopy (EDX) of FMSN-CPT (D) EDX with CpG ODN (E) Zeta potential and (F) Hydrodynamic size.

Scanning Electron microscopy (SEM)

SEM characterization was performed to assess the dry size of NPs. Approximately 50 NPs were chosen and evaluated using the ImageJ software. The bare MSNs presented a uniform, slightly rod-shaped morphology, which can be classified as MCM-41, as shown in Fig. 2A. The observed sizes of the FMSNs and CPT-loaded FMSNs were 180 nm and 205 nm, respectively. In contrast, coating NPs resulted in a slightly rough morphology with well-segregated particles and a slightly larger size (235 nm) compared to the uncoated counterparts, owing to the additional CS layer. A comparison of the DLS and SEM analyses is presented in Table 1. However, the deviation of the microscopy results from the DLS study results could be attributed to swollen NPs. Since the DLS method was applied in the liquid phase, it showed a larger mean hydrodynamic particle size compared with SEM, which is the diameter of the dried NPs. The EDX spectrum revealed the presence of Si, C, N and O (Fig. 2C) in the samples from all groups. The addition of CpG resulted in the appearance of phosphate signals (Fig. 2D), confirming the presence of ODN on the surface of the NPs [23, 24].

Brunauer-Emmett-Teller (BET) surface area analysis

Brunauer-Emmett-Teller (BET) surface area analysis was conducted to evaluate the surface areas and pores. The recorded surface area of bare MSNs was 735.58 m2/g, indicating that MSNs have a large available surface area for the efficient adsorption and loading of guest molecules. The nitrogen adsorption curve of MSN was identified as the typical type IV isotherms according to the IUPAC classification, which is characteristic of mesoporous materials. Higher quantity of adsorption typically indicates a larger surface area available for gas adsorption [25]. The highest curve of blank MSNs among the three materials (Fig. 3A), suggests that they have the most space available for gas molecules to adhere to their surface, compared to FMSN-CPT and FMSN-CPT-GA-CS-CpG. The pore size measured using the BET method was 7–13 nm and the pore volume measured using the Density Functional Theory (DFT) method was 0.91 cm3/g (Fig. 3A). This reduction could be attributed to the successful incorporation of the drug and the subsequent CS coating of the FMSNs. The drug and CS layers occupy the surface, leading to a reduction in the total available surface area [26].

Thermogravimetric analysis (TGA)

To evaluate the thermal behavior (Δw %) and extent of surface modification of MSNs, TGA was conducted at a heating rate of 10 ◦C min- 1 to 700 ◦C, as shown in Fig. 3B. All the curves typically exhibit a two-step weight loss. The first weight loss below 150 ◦C can be ascribed to adsorbed water molecules, condensation of surface silanol groups (SiOH) and evaporation of the remaining solvent (Dau *et al*., 2021). Weight loss above 150 ◦C could be attributed to surface modifications, including APTES functionalization, CPT loading, GA linking, CS coating and CpG binding, indicating the detachment of particular groups at their respective degradation temperatures [27]. Between 150–700 ◦C, FMSN lost almost 12.72 % of its weight, which indicates the total decomposition of the NH2 groups attached to its surface. FMSN-CPT lost 12.28 % within 150 ◦C and 700 ◦C, of which 3.24 % (190 ◦C - 310 ◦C) could be attributed to weight % of CPT. The remaining weight percentage can be considered as the total weight of the MSNs. The differences in Δw % among all the groups are shown in Fig. 3B. The weight loss increased to 27 % after CS coating, indicating that the weight percentage of CS and the weight further decreased to 31 % and 35.5 % for the CS coating with GA crosslinking and after CpG binding, respectively. The curves with GA were observed more stable below 200 ◦C (Fig. 3B) owing to the crosslinking ability of GA, which can enhance the stability and mechanical strength of CS coating [28].

Fourier-Transform Infrared spectroscopy (FTIR)

The fabrication of MSN and the changes in chemical bonding after each step were investigated using FTIR spectroscopy (Fig. 3C and 3D.). The characteristic absorption peaks of MSN appeared at 945 cm- 1,1045 cm- 1 and a wide band at 3000 cm- 1–3700 cm- 1. These peaks can be assigned to the asymmetric vibration of Si-OH, the stretching vibrations of Si-O-Si and hydrogen-bonded water molecules. The presence of the NH2 group after APTES modification was confirmed by the N–H stretching bands detected at 3200–3500 cm- 1, NH2 bending at around 1652 cm- 1 and C-N stretching vibrations at 1200–1300 cm- 1 [29]. The APTES modification could also be verified by the appearance of peaks at 2997 cm- 1 and 2933 cm- 1, related to the stretching vibration of C–H bond in the propyl chain of APTES (Fig. 3C). The loading of CPT to FMSN displayed changes in the intensity and shapes of certain peaks, including peaks that are typical of esters (organic compounds that have a carbonyl group (C = O) and –OH). The bands related

to the Si-OH stretching vibrations at approximately 3755 cm- 1 exhibited a change in intensity. The peak at approximately 1640 cm- 1 is likely due to the stretching of the C = O bond, whereas the peak at approximately 3200–3400 cm- 1 is likely due to the stretching of the O–H bond. The peaks at approximately 1600–1500 cm- 1 were attributed to the vibrations of the carbon–carbon double bonds (C = C) in the rings. The presence of NH2 groups on the surface of the FMSNs allowed potential interactions with CPT (Fig. 3C) [30].

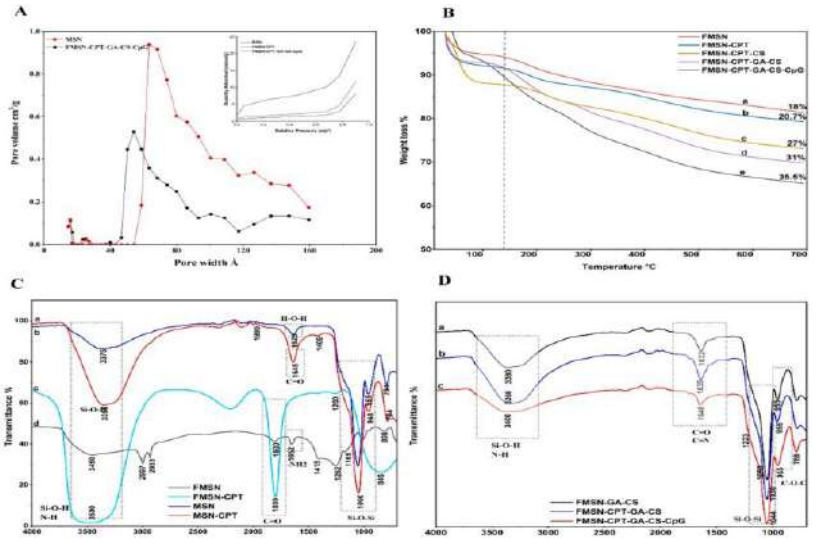


Fig. 3: (A) Brunauer-Emmett-Teller (BET) surface characterization (B) Thermogravimetric Analysis TGA thermal behavior of NPs (C, D) Fourier-Transform Infrared Spectroscopy (FTIR), Chemical characterization.

Bands typically related to CS can be observed after CS coating. The peaks at 3300–3500 cm- 1 correspond to the O–H stretching vibrations due to the interaction between the OH groups in CS and FMSN (Fig. 3D), suggesting the presence of H bonding between the two materials. The peaks at 1640 cm- 1 and 946–960 cm- 1 are associated with the stretching vibrations of amide bonds and the vibration of the C-O-C bonds. The bands in the region 1600–1700 cm- 1 specify the presence of imine bonds (C = N) due to the interaction of GA with the NH2 groups in (Fig. 3D). This suggests a successful cross-linking reaction between GA and CS. The presence of CpG can modify CS-specific bands in the FTIR spectrum. CpG contains phosphate groups, which typically show peaks at 1200 cm- 1 –1300 cm- 1

and 1096 cm- 1 that are overlapped by sharp peaks of characteristic MSN peaks at 980 cm- 1 to 1280 cm- 1 (Tao *et al*., 2015; Xu *et al*., 2015) (Fig. 3C,D). The FTIR spectra confirmed the successful fabrication of MSNs, functionalization, drug loading and CS coating [31].

Drug loading

The hydrophobic anticancer drug, CPT, was loaded into the nanocarrier. DMSO was chosen as the solvent to maximize drug loading, owing to the hydrophobicity of CPT. The pores of MSN can accommodate both hydrophobic and hydrophilic molecules, making them highly versatile for biomedical and pharmaceutical applications. The drug incorporation efficiency of CPT into MSN, FMSN and CS-coated FMSN was evaluated using UV spectrophotometry. The drug loading capacity (DLC%) of MSN-OH was 3.24 % out of 5 % and the drug entrapment efficiency (EE%) was 64.8 %, whereas FMSN showed a DLC% of 4.2 % and an EE% of 84 %. The obtained results confirm that both MSN-OH and FMSN are capable of effectively loading CPT [32]. However, FMSN showed a higher drug loading capacity and better entrapment efficiency, suggesting that the presence of NH2 groups on the MSN surface enhances the drug loading process because of electrostatic interactions between the positively charged NH2 groups and the reactive sites on CPT. These findings are consistent with those of previous studies on the loading of CPT into MSN and FMSN. The CS coating on the surface, with or without the incorporation of GA, further improved the drug loading. DLC % and EE% calculated for CS-coated FMSN were 4.5 % and 90 %, respectively, while those for crosslinking were 4.75 % and 95 %, respectively [33]. The UV spectrum (Fig. 4A) shows that the incorporation of GA crosslinking in CS-coated MSNs possibly caused a slight improvement in drug loading, probably because of the increased stability of the CS coating and reduced drug leakage during the loading and washing process, which has also been reported in other studies. The average yield of NPs was 80 mg/mL, with a maximum drug loading of 4.75 % for FMSNCPT-GA-CS. This means 5 mg of NPs could retain almost 0.23 mg of CPT. CpG ODN binding to the nanocarrier was detected using a UV spectrophotometer (Fig. 4B). FMSNs were used as blank to avoid background signals. Free CpG ODN exhibited a characteristic ODN band with the highest intensity at approximately 260 nm (Fig. 4B) and CpG ODN attached to NPs showed a peak a little ahead due to interaction with CS, as shown in Fig.4B. 81 % CpG ODN were detected and the rest were either washed away or could not bind and thus could not be detected. CpG ODNs,

owing to the phosphate groups in their backbone, carry a negative charge and interact with the positively charged (NH2) of the CS layer on FMSN via electrostatic interactions. Hydrogen bonding between CS and CpG also facilitated binding, thereby improving the stability of the complex [34].

***In-vitro* drug release**

An *in vitro* drug release study was performed to evaluate the applicability of FMSNs as carriers for the delivery of hydrophobic drugs. The release pattern of drugs from NDDS is influenced by several factors, such as the chemical and molecular properties of the NPs surface, the physicochemical properties of the encapsulated drug and the pH of the release media. Drug release assays were carried out in PBS at pH 7.4 and pH 6.4 at 37 ◦C, mimicking physiological and tumor microenvironment. The release profile displayed an initial burst release during the first 6 h at both pH values from all groups (Fig. 4C, D, E), likely due to drug adsorption on the NPs surface. All CPT molecules from the free CPT group were released into the release medium within 24 h. MSN-OH exhibited an initial burst release (50 %) within the first 8 h, followed by a sustained release phase throughout 48 h and cumulative drug release reached 85 % within 72 hrs. 65 % of the drug was released from FMSN-CPT within 120 h. CPT release from MSNCPT and FMSN-CPT was not affected, particularly by changes in pH (Fig. 4C,4D), whereas a significant difference was noted in the drug release patterns of the CS-coated group over 120 h. FMSN-CPT-GA-CS released approximately 51 % and 20 % of its loaded CPT at pH 6.4 and 7.4, respectively. This slow and sustained release profile can be explained by the fact that CS-coated FMSN were significantly affected by pH, which shows that CS is a pH-sensitive polymer that is dependent on the nature of the environment (acidic or neutral) to release the drugs [35]. At pH 6.4, the NH2 groups of FMSN and OH of CPT were protonated, resulting in repulsive interactions between the positive charges, followed by swelling and pore opening, leading to higher drug release. However, after moving away from the acidic pH, it contracted, forming a film that shielded the pores and revealed a significant reduction in CPT release at higher pH. Thus, CS, a pH-responsive polymer, provided a sustained CPT release over time [36]. The release of CpG ODN was also found to be pHdependent and was faster at pH 6.4, probably due to the increased hydrophilicity of CpG ODNs under acidic conditions (Fig. 4E). This release was faster than that of CPT, possibly due to its hydrophilicity and attachment to the outer surface. The rapid initial release was also observed for CpG ODN during the first 6 h, followed by the

controlled release that reached 60 % in 60 h at pH 6.4. Other studies have also found almost the same release curves for CpG ODN bound to cationic polymers. Thus, the rate of release is inversely proportional to the pH of the external medium, which means that it increases with a decrease in pH. A small amount of CPT and CpG released under normal physiological conditions (pH 7.4) indicates that NDDS is well protected from drug release to normal tissues, which is essential for limiting the high toxicity of these drugs to healthy cells. This also suggests the possibility of prolonging the therapeutic effects of the drug and reducing its administration frequency [37].

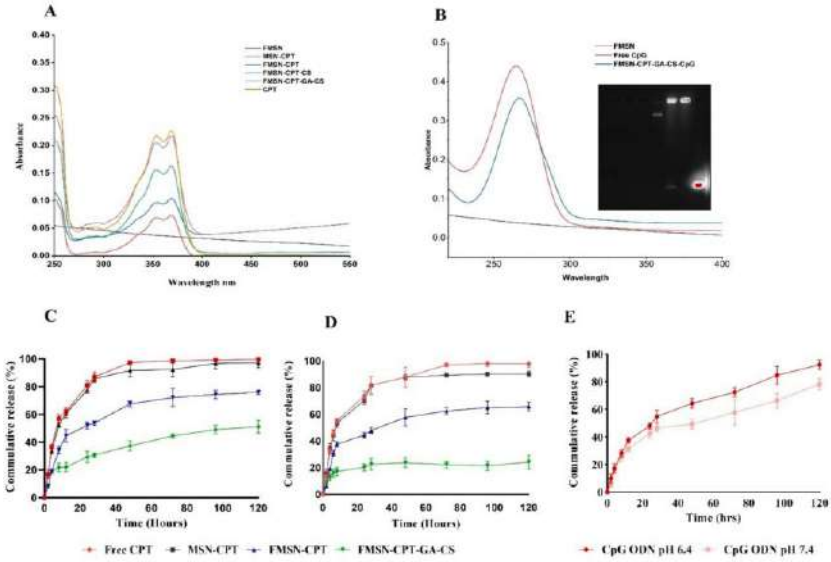


Fig. 4: (A) UV spectrum of CPT, blank FMSN, MSN-CPT, FMSN-CPT, FMSN-CPT-CS, FMSN-CPT-GA-CS (B) UV spectrum of CpG ODN and gel electrophoresis detection of CpG ODN (C, D) Cumulative release of CPT, MSN-CPT, FMSN-CPT and FMSN-CPT-GA-CS at pH 6.4 and pH 7.4, respectively, (E) Cumulative CpG ODN release at pH 6.4 and pH 7.4.

In-vitro cytotoxicity

An MTT assay was conducted to evaluate the cytotoxicity of the NPs formulations and to evaluate the synergism of CPT and CpG ODN when delivered together as a targeted NDDS against B16F10 cells. The cytocompatibility of bare MSN, FMSN and CS-coated FMSN is well established against many other cancer cell lines, including MCF7, CHO, C26, 3 T3, HepG2, Glioblastoma and U87 MG cells. Only one study was

performed on B16F10 cells, where MSN-CPT was coated with poly(ethylene glycol)-poly(b-aminoester urethane) (PAEU) and delivered in the form of a hydrogel, either alone or in combination with doxorubicin. Herein, we demonstrated the cytocompatibility of blank MSNs, FMSNs and CS-coated FMSNs and the cytotoxicity of CPT-loaded FMSN against B16F10 cells. When treated with blank FMSN and FMSN-CS, 84 % (FMSN-CS) and 77 % (FMSN) of cells were viable at 800 µg/mL even after 48 h of incubation (Fig. 5B), which confirmed that the blank NPs exhibited good biocompatibility and that the CS coating further enhanced biocompatibility due to the shielding effect of CS [38]. Next, we evaluated the concentration-dependent responses of B16F10 cells to various formulations, including free CPT, FMSN-CPT, FMSN-CPT-GA-CS and FMSN-CPT-GA-CS-CpG, incubated at 37 ◦C for 48 h. The results revealed that free CPT and FMSN-CPT exhibited comparable cell-killing potency, with no statistically significant difference, as shown in Fig. 5C. Free CPT displayed slightly enhanced cytotoxicity owing to its efficient release compared to FMSN-CPT. However, the addition of CS to FMSN-CPT resulted in a reduction in cell viability compared to free CPT and FMSN-CPT (Fig. 5C) because of an additional positively charged CS layer on the surface that could interact with the negatively charged cell membrane, accumulating inside or around the cell, influencing the drug release kinetics (pH-dependent) and releasing the drug within the cancerous cell (slightly acidic) [39]. The binding of CpG ODN to the surface of CPT-loaded FMSN vividly exhibited exceptionally high cytotoxicity, with only 10 % viable cells remaining after 48 h at 7 mM concentration ($p < 0.00001$). Time dependent analysis was conducted to evaluate the drug release from the nanocarrier over time. As shown in Fig. 5D, a long incubation time was required for drug release from FMSN-CPT and FMSN-CPT-GA-CS to display its cytotoxic effect (Fig. 5D); however, free CPT was more toxic because of its quick release, while the distribution of drug-loaded nanocarriers increased cytotoxicity over time [40]. After 24 h, FMSN-CPT-GA-CS significantly reduced cell viability compared to free CPT ($p = 0.0001$). After 72 h of incubation, FMSN-CPT and FMSN-CPT-GA-CS showed reduced cell viability compared with free CPT, which could be attributed to the slow and sustained release of CPT from the pores of the nanocarrier. FMSN-CPTGA-CS-CpG showed significantly lower ($p = 0.0003$) viable cells (15 %) than the other groups after 72 h (Fig. 5D). This significantly heightened cytotoxicity upon co-delivery of CPT with CpG ODN, emphasizing the synergistic potential of this multifunctional NDDS.

CpG ODN stimulates the immune response by activating Toll-like receptor 9 (TLR9), resulting in improved recognition, cellular uptake and localized/targeted release of CpG ODN and CPT into cancer cells, leading to more potent and sustained therapeutic efficacy as compared to their free counterparts [41].

Cutaneous chemical carcinogenesis and in vivo anticancer potential

To investigate the antitumor efficacy of the formulated NPs, a tumor model was developed using DMBA/TPA in BALB/c mice. Tumors with a minimum size of 3 mm that did not regress for two weeks were considered for treatment. Established tumors were treated locally via intratumoral injections of PBS, free CPT, FMSN-CPT, or FMSN.CPT-GACSand FMSN-CPT-GA-CS-CpG. Systemic toxicity in each group was assessed by monitoring the body weight and survival rates following the respective formulations. Distinct trends were observed among the treatment groups. A decline in body weight was observed in the animals treated with free CPT. One mouse died within 24 h and 48 h following injection, after each dose of free CPT, which can be ascribed to the late systemic toxicity of free CPT, as reported previously. Moreover, free CPT initially demonstrated a reduction in papillomas following two to four days post-injection; however, the papillomas aggressively regrown until the second dose. This could be attributed to the instability of the free drug in the plasma, leading to conversion of the active lactone ring of CPT into the carboxylate form (inactive) [42]. Animals treated with FMSN-CPT displayed a similar regression trend but a slight delay in tumor regrowth. Notably, FMSN-CPT-GA-CS treatment resulted in significantly fewer tumors than the free CPT and FMSN-CPT treated groups, suggesting a potential impact of the CS coating in inhibiting tumors by adhering to the negatively charged dermal cells via strong electrostatic interactions. Furthermore, encapsulation and coating can prevent deactivation of the drug, enhance cellular uptake and subsequently improve its effectiveness. Numerous studies have confirmed enhanced dermal penetration and improved efficacy of drugs due to the presence of a CS coating. Most strikingly, the FMSN-CPT-CS-CpG group displayed an exceptional decrease in papillomas for two weeks and papillomas did not show aggressive regrowth, even in the absence of injection [43]. This effect persisted for 20 weeks until the end of the experiment (Fig. 6B, C, D). This *in vivo* synergism of CPT with CpG ODN in the form of FMSN-CPT-GA-CS-CpG was evident compared to control groups, validating the advantages of combination treatment in the murine

SCC model as the reduction in the cumulative number of tumors; posttreatment was much more pronounced with 100 % survival (Fig. 6D). Furthermore, administration of drugs via intratumoral injection offers the major advantage of reducing systemic exposure by maintaining the maximum therapeutic concentration within the tumor microenvironment, showing immediate tumor responses and facilitating controlled modulation of local immune cascades [44]. This treatment combination has also been reported in PDVC57 tumors when treated with either non-adhesive or bioadhesive NPs of CPT alone or in combination with CpG ODN, which improved the percent survival relative to controls and single therapy, revealing the potential of local tumor synergistic immunotherapy. We also found that codelivery of an adjuvant immunosensitizing agent, CpG ODN, may augment the efficacy of FMSN-CPT-CS in eliminating tumors and prolonging animal survival (100 %) by stimulating a local immune response to treatment, which is also supported by previous reports where CpG as a TLR9 ligand-activated immune response when used with chemotherapy and radiotherapy against cutaneous malignancies [45].

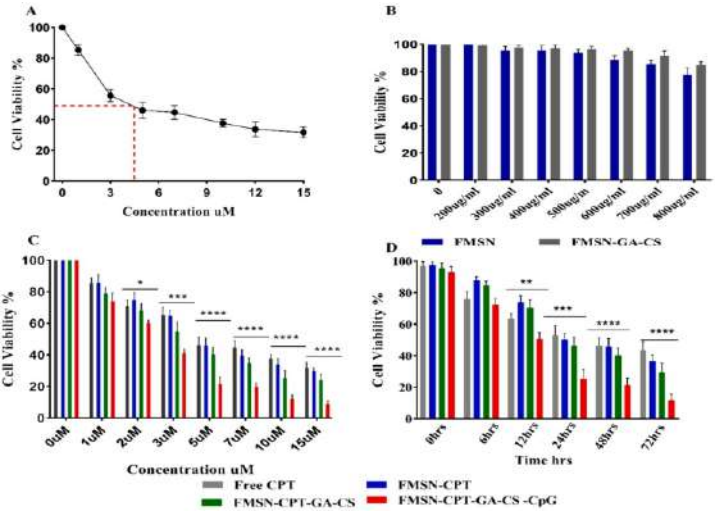


Fig. 5: *In vitro* cytotoxicity of formulated NDDS against B16F10 cells, the drug was used as 5 % of the carrier in all groups. *p* - value > 0.05 represents not significant (ns), *p* - value ≤ 0.05 represents significance (*), *p* - value ≤ 0.01 (**), *p* - value ≤ 0.001 (***) and *p* - value ≤ 0.0001 (****) (A) Cell viability % against free CPT (B) Cytocompatibility of blank FMSNs in increasing concentrations (C) Concentration dependent cytotoxicity for 48 h (D) Time dependent cytotoxicity at a concentration of 5 µM.

Histopathological evaluation

Histological (H&E staining) examination of the mouse skin from all groups was conducted to assess the internal morphology of the tumor tissues. The negative control exhibited a well-defined epidermal morphology with underlying dermis and subcutaneous tissue (Fig. 7B, a). DMBA/TPA resulted in ulceration with well-developed hyperchromatia, thickened corrugated epidermis and hyperkeratosis as key features. Upon synergistic treatment with local chemotherapy and adjuvant immunotherapy, the tumor burden substantially decreased. The skin had an intact epidermis and an average deep dermis with mild inflammation (Fig. 7A, a,b and Fig. 7B,c).

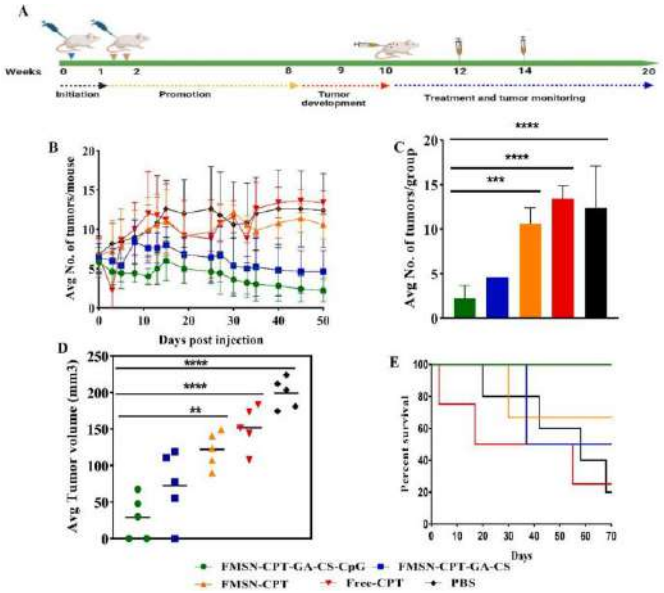


Fig. 6: *In vivo* tumor killing in murine model, p - value > 0.05 represents not significant (ns), p - value ≤ 0.05 represents significance (*), p - value ≤ 0.01 (**), p - value ≤ 0.001 (***) and p - value ≤ 0.0001 (****) (A) treatment schedule, initiation by DMBA, promotion by TPA followed by NPs injection at week 10, CPT dose of 0.25 mg/mouse, distributed into tumors via intratumoral injections depending on tumor size (B) Average number of tumors per mouse post treatment (C) Average number of tumors per group at the end of study showing the comparison of FMSN-CPT-GA-CS-CpG group with FMSN-CPT-GA-CS (p = ns), FMSN-CPT ($p \leq 0.001$), free CPT ($p \leq 0.0001$) and DMBA/TPA ($p \leq 0.0001$), (D) Average tumor volume showing significant difference between FMSN-CPT-GA-CS-CpG and FMSN-CPT, free CPT ($p \leq 0.0001$) and DMBA/TPA group ($p \leq 0.0001$), (E) Kaplan-Meier curve showing percent survival of all treatment groups.

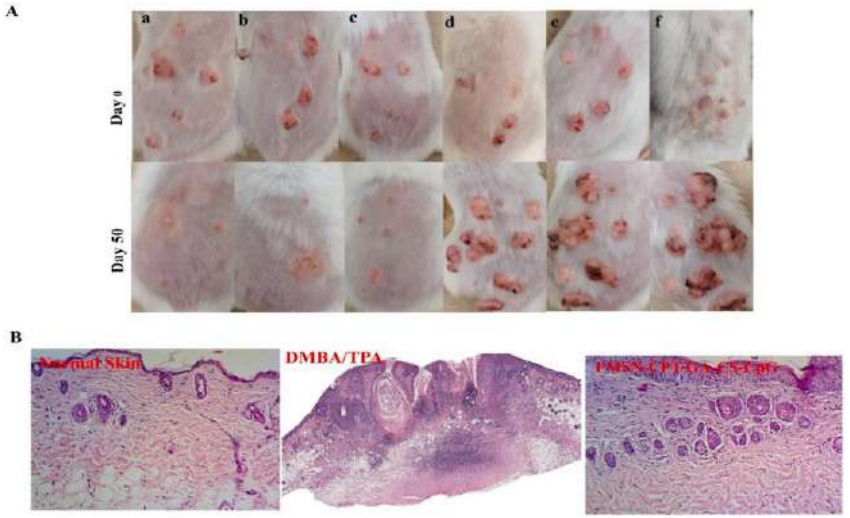


Fig. 7: (A) Mice papillomas at day 0 of intratumoral injections and at day 50 (a) FMSN-CPT-GA-CS-CpG (b) FMSN-CPT-GA-CS-CpG (c) FMSN-CPT-GA-CS (d) FMSNCPT (e) Free CPT (f) PBS. (B) H & E staining of normal mouse skin, skin treated with FMSN-CPT-GA-CS-CpG and only DMBA/TPA treated skin after 20 weeks.

4. Conclusion

Our study demonstrates the successful development of a multifaceted NDDS that effectively delivered the hydrophobic, anticancer drug, CPT to skin cancer cells, exhibiting remarkable efficacy both *in vitro* and *in vivo* preclinical studies. The CS coating contributed to the sustained and pH-dependent release of CPT because of the pH-responsiveness of CS, as the release was faster at an acidic pH (tumor microenvironment) than at a neutral pH (normal physiological pH). The incorporation of CpG ODN into the NDDS serves to immunostimulate the local chemotherapy, amplifying the therapeutic impact significantly, leading to a remarkable synergistic effect both *in vitro* in B16F10 cells and the pronounced tumor regression *in vivo* in DMBA/TPA-induced SCC in a murine model. The formulation's success is particularly notable given the poor stability of free CpG ODN, which limits its clinical applications. However, binding it to the surface of aminated NPs can substantially improve its stability. The findings propose that the combined localized anticancer strategy without resorting to surgical approaches, holds great potential for enhanced skin cancer therapy, paving the way for future advancements in NDDS based synergistic therapies for

cutaneous malignancies. The efficacy of local (intratumoral) injections in delivering NDDS to the tumor site, with minimal distribution to nontarget areas underscores the need for additional studies to validate this delivery method fully. Furthermore, comprehensive research with a large sample size is required to completely explore the immune cascade involved in combination therapy and the underlying molecular mechanisms. Determining whether local treatment provides efficacy in the clinical setting and whether response rates would approach those of intravenous or orally delivered agents also requires additional clinical studies.

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3
CHAPTER

Chapter Title: Ethical AI in Medical Practice and Patient Safety

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Abstract

Artificial Intelligence (AI) is rapidly transforming medical practice by supporting diagnosis, clinical decision-making, medical imaging, drug discovery, patient monitoring, personalized treatment and healthcare administration. AI-based systems can process large volumes of medical data and identify patterns that may be difficult to recognize through conventional methods. However, the increasing use of AI in healthcare also creates significant ethical, legal and patient-safety challenges. Errors in AI systems can directly affect human health, while inappropriate use of patient data may compromise privacy and confidentiality. Other concerns include algorithmic bias, lack of transparency, inadequate human oversight, cybersecurity threats, accountability and unequal access to AI-enabled healthcare.

Ethical AI in medical practice requires more than technical accuracy. AI systems should be designed and deployed according to principles such as patient autonomy, beneficence, non-maleficence, justice, transparency, accountability, privacy, security and human oversight. Healthcare professionals must understand the capabilities and limitations of AI and should not treat AI-generated recommendations as unquestionable clinical decisions. This chapter examines the role of AI in medical practice, major ethical concerns, patient-safety risks, responsible AI principles, governance requirements and strategies for developing trustworthy AI-based healthcare systems. It also discusses the responsibilities of healthcare professionals,

developers, healthcare institutions, policymakers and patients in ensuring that AI contributes to safer and more equitable healthcare.

Keywords: Artificial Intelligence, Ethical AI, Healthcare, Patient Safety, Medical AI, Algorithmic Bias, Data Privacy, Explainable AI, Clinical Decision Support, Responsible AI.

1. Introduction

Artificial Intelligence has become one of the most important emerging technologies in modern healthcare. The rapid growth of computational power, machine learning, deep learning, medical imaging technologies, electronic health records, wearable devices and health-related datasets has created new opportunities for applying AI to medical problems.

AI systems can assist healthcare professionals in detecting diseases, analyzing medical images, predicting risks, monitoring patients, recommending treatments and managing healthcare resources. For example, machine-learning models can analyze radiological images to identify abnormalities, while predictive systems can help identify patients who may be at increased risk of certain complications.

The potential benefits of AI in healthcare are considerable. AI may help reduce diagnostic delays, improve workflow efficiency, support personalized medicine and assist clinicians in handling large amounts of information. However, healthcare is a high-risk domain. A technically sophisticated AI system can still cause harm if its training data are inadequate, its recommendations are misunderstood, or its limitations are not communicated clearly.

Therefore, the question is not simply whether AI can perform a medical task accurately. A more important question is whether the AI system can be used **safely, fairly, transparently and responsibly in real-world medical practice**.

Ethical AI refers to the development and use of AI systems in accordance with accepted ethical principles, human rights, professional responsibilities and applicable laws and regulations. In medical practice, ethical AI must place patient welfare and safety at the center of technological development.

2. AI in Medical Practice

AI can be used at different stages of healthcare delivery. Its applications can broadly be categorized into the following areas.

2.1. Medical Diagnosis

AI-based diagnostic systems can analyze clinical information, laboratory results, medical images and patient histories.

Examples include:

- Detection of abnormalities in X-rays and CT scans
- Analysis of MRI images
- Identification of diabetic retinopathy
- Detection of certain types of cancer
- Risk prediction for cardiovascular diseases
- Clinical decision-support systems

AI can function as an additional support mechanism for healthcare professionals rather than necessarily replacing them.

2.2. Medical Imaging

Medical imaging is one of the important areas of AI application. Deep-learning models can analyze images and identify patterns associated with diseases.

AI can support:

- Image classification
- Tumor detection
- Segmentation of organs
- Detection of abnormalities
- Image enhancement
- Quantitative analysis

However, an AI model trained on images from one population or healthcare environment may not perform equally well in another environment.

2.3. Personalized Medicine

Patients may respond differently to the same treatment. AI can combine information such as:

- Medical history

- Genetic information
- Laboratory results
- Lifestyle information
- Previous treatment responses

to support personalized treatment decisions.

2.4. Patient Monitoring

AI can be integrated with wearable devices and monitoring systems to identify changes in patient conditions.

For example, AI may assist in monitoring:

- Heart rate
- Blood oxygen levels
- Blood pressure
- Glucose levels
- Sleep patterns
- Physical activity

Such systems can potentially provide early warnings, but incorrect alerts or missed alerts can also create patient-safety concerns.

2.5. Drug Discovery

AI can support pharmaceutical research by analyzing biological and chemical data and predicting potential drug candidates.

It may help researchers:

1. Identify potential molecular targets.
2. Predict molecular interactions.
3. Screen candidate compounds.
4. Estimate possible drug properties.
5. Reduce the time required for certain stages of drug development.

2.6 Administrative Applications

AI is also used for non-clinical activities such as:

- Appointment scheduling
- Medical documentation
- Billing support
- Hospital resource management
- Patient communication
- Workflow optimization

Although administrative AI may appear less risky than clinical AI, errors can still affect patient access, communication, or treatment.

3. Ethical Principles of AI in Healthcare

Ethical medical AI should be based on established ethical principles.

3.1. Respect for Patient Autonomy

Patient autonomy means respecting an individual's right to make informed decisions about their healthcare.

Patients should have appropriate information about the use of AI when it materially affects their care.

For example, if an AI system contributes significantly to a medical recommendation, patients may reasonably need to understand:

- That AI is being used
- The purpose of its use
- The role of the healthcare professional
- Important limitations of the system

AI should support patient decision-making rather than remove the patient's ability to participate in decisions.

3.2. Beneficence

Beneficence refers to the responsibility to promote the well-being of patients.

AI should be implemented because it provides a meaningful healthcare benefit, such as:

- Improving diagnostic support
- Reducing preventable errors
- Improving patient monitoring
- Supporting healthcare professionals
- Increasing access to appropriate care

The adoption of AI merely because it is technologically advanced does not automatically make its use ethically justified.

3.3. Non-Maleficence

Non-maleficence means avoiding unnecessary harm.

In AI-based healthcare, harm may result from:

- Incorrect diagnosis
- Incorrect treatment recommendations
- False-positive results
- False-negative results
- Poor-quality training data
- Software failures
- Cybersecurity incidents
- Misinterpretation of AI outputs

A healthcare institution should therefore assess potential risks before deploying an AI system.

3.4. Justice and Fairness

AI should not systematically disadvantage particular groups.

Bias may occur because of differences in:

- Age
- Sex
- Geography
- Socioeconomic conditions
- Ethnicity or population characteristics

- Language
- Availability of healthcare data

If a model is trained primarily on data from one population, its performance may differ when applied to another population.

Therefore, fairness testing and representative datasets are important components of responsible medical AI.

3.5 Transparency

Patients and healthcare professionals should have meaningful information about how an AI system is being used.

Transparency may involve:

- Explaining the purpose of the AI system
- Describing its intended use
- Communicating important limitations
- Providing information about performance
- Maintaining appropriate documentation

Transparency does not necessarily mean revealing every technical detail of a complex algorithm to every user. Rather, users should receive information sufficient to understand the system's role and limitations.

4. Major Ethical Challenges

4.1 Algorithmic Bias

Algorithmic bias is one of the major concerns associated with medical AI.

An AI system learns patterns from data. If the training data contain systematic biases or insufficient representation of certain populations, the resulting model may reproduce or amplify those problems.

For example, if a diagnostic model is trained mainly using data from one demographic population, its performance may not generalize equally to other groups.

Causes of Bias

Common causes include:

- Unrepresentative datasets

- Historical inequalities
- Missing data
- Measurement differences
- Sampling bias
- Incorrect labeling
- Data imbalance

Methods for Reducing Bias

Possible strategies include:

- Using diverse datasets
- Testing models across different populations
- Monitoring performance after deployment
- Conducting subgroup analysis
- Reviewing training and validation procedures
- Involving multidisciplinary experts

5. Patient Data Privacy

Healthcare generates highly sensitive information.

Examples include:

- Patient names
- Medical histories
- Diagnostic reports
- Laboratory results
- Genetic information
- Images
- Medication records
- Biometric information

Unauthorized access or misuse of such information can have serious consequences.

AI systems may require large datasets for training and operation. Therefore, privacy protection should be integrated into the entire AI lifecycle.

Privacy Protection Measures

Important measures include:

1. Data minimization
2. Access control
3. Encryption
4. Secure data storage
5. De-identification where appropriate
6. Authentication
7. Audit trails
8. Secure data transmission
9. Appropriate retention policies

Healthcare organizations should also establish clear policies regarding who can access AI-related patient data and for what purpose.

6. Explainability and the Black-Box Problem

Some advanced AI systems, particularly deep-learning models, can be difficult to interpret.

A model may produce a prediction such as:

"High risk of disease"

but the healthcare professional may not easily understand why the model reached that conclusion.

This is commonly referred to as the black-box problem.

Lack of explainability can create difficulties because healthcare professionals may need to evaluate whether an AI recommendation is clinically reasonable.

Importance of Explainable AI

Explainable AI (XAI) attempts to make AI outputs more understandable.

Explanation techniques may help identify:

- Important input features

- Relevant image regions
- Contributing variables
- Confidence levels
- Factors influencing predictions

However, an explanation should not automatically be treated as proof that the AI's decision is correct. Explanations themselves need careful evaluation.

7. Patient Safety and AI

Patient safety should be a central consideration throughout the AI lifecycle.

A medical AI system can create several categories of risk.

Risk	Example
False positive	AI incorrectly identifies a disease
False negative	AI fails to identify a disease
Data error	Incorrect patient information
Model error	Incorrect prediction
Automation bias	Clinician trusts AI without adequate review
Cybersecurity risk	Unauthorized access to medical data
System failure	AI becomes unavailable
Poor generalization	Model performs poorly in a new population

Patient safety therefore requires more than achieving high accuracy during model development.

8. Automation Bias and Human Oversight

Automation bias occurs when people place excessive trust in automated recommendations.

In healthcare, a clinician may accept an AI-generated recommendation without independently evaluating it.

This can be dangerous because AI systems are not infallible.

A safer approach is human-in-the-loop decision-making, in which qualified healthcare professionals remain involved in important clinical decisions.

Human Oversight Should Include:

- Reviewing AI recommendations
- Considering patient-specific information
- Identifying unusual cases
- Overriding inappropriate recommendations
- Monitoring AI performance
- Reporting system errors

AI should generally function as a tool that supports professional judgment rather than an unquestionable authority.

9. Accountability and Responsibility

A major ethical question is:

Who is responsible when an AI-supported medical decision causes harm?

Possible stakeholders include:

- AI developers
- Software vendors
- Healthcare institutions
- Doctors and other healthcare professionals
- Data providers
- System administrators
- Regulators

Responsibility should not become unclear merely because AI is involved.

Healthcare institutions should establish clear procedures for:

- System approval
- Clinical validation
- User training
- Error reporting
- Incident investigation

- Performance monitoring
- System updating

Clear accountability is essential for patient safety.

10. Cybersecurity Threats

Healthcare organizations are increasingly dependent on digital technologies. AI systems can therefore become targets for cyberattacks.

Potential threats include:

- Unauthorized access
- Data theft
- Malware
- Ransomware
- Model manipulation
- Data poisoning
- Adversarial attacks
- Denial-of-service attacks

Security Measures

Organizations should consider:

1. Strong authentication
2. Role-based access control
3. Encryption
4. Network security
5. Regular security testing
6. Backup and recovery procedures
7. Incident-response plans
8. Software updates
9. Employee cybersecurity training

Security should be treated as an ongoing process rather than a one-time activity.

11. Generative AI in Medical Practice

Generative AI systems can produce text, summaries, explanations and other content. They may assist healthcare professionals with documentation, communication, education and information retrieval.

However, generative AI introduces additional risks.

One important problem is hallucination, in which an AI system generates information that appears plausible but is incorrect.

For medical applications, hallucinated information can be particularly harmful.

Therefore, generated medical content should be appropriately reviewed by qualified professionals before being used for clinical decision-making.

Potential Uses

Generative AI may assist with:

- Drafting clinical documentation
- Summarizing patient information
- Preparing educational material
- Supporting patient communication
- Research assistance
- Administrative documentation

Important Limitations

Users should consider:

- Factual errors
- Outdated information
- Missing context
- Privacy risks
- Bias
- Hallucinations

- Inappropriate confidence

12. Data Quality and Reliability

The quality of an AI system depends heavily on the quality of its data.

The commonly used principle "garbage in, garbage out" is particularly relevant to AI.

If training data are:

- Incomplete
- Incorrect
- Outdated
- Biased
- Poorly labeled

The resulting model may produce unreliable outputs.

Therefore, data governance should include:

- Data quality assessment
- Data validation
- Appropriate labeling
- Documentation
- Version control
- Monitoring for data drift

13. Model Validation and Continuous Monitoring

An AI model that performs well during development may not perform equally well after deployment.

Changes may occur in:

- Patient populations
- Medical practices
- Disease patterns
- Equipment
- Data sources

- Clinical workflows

This phenomenon can contribute to **model drift**.

Therefore, medical AI should be continuously monitored.

Monitoring Activities

Organizations can monitor:

- Accuracy
- Sensitivity
- Specificity
- False-positive rates
- False-negative rates
- Subgroup performance
- Error patterns
- User feedback
- Patient-safety incidents

If performance deteriorates, the system may need recalibration, retraining, modification, or withdrawal.

14. Ethical AI Lifecycle

Ethical considerations should be incorporated throughout the AI lifecycle.

Stage 1: Problem Identification

The healthcare problem should be clearly defined.

Stage 2: Data Collection

Data should be collected responsibly and securely.

Stage 3: Data Preparation

Data quality, representativeness, privacy and bias should be assessed.

Stage 4: Model Development

Developers should select appropriate algorithms and evaluation procedures.

Stage 5: Validation

The system should be tested using appropriate datasets and relevant performance measures.

Stage 6: Clinical Evaluation

Qualified healthcare professionals should assess whether the system is useful and safe in real clinical environments.

Stage 7: Deployment

Deployment should occur with appropriate training, documentation and safeguards.

Stage 8: Monitoring

Performance and safety should be continuously evaluated.

Stage 9: Updating or Retirement

The AI system should be updated or discontinued when it becomes unreliable, unsafe, outdated, or unsuitable.

15. Role of Healthcare Professionals

Healthcare professionals play a central role in responsible AI adoption.

They should:

- Understand the purpose of AI tools
- Know their limitations
- Verify important recommendations
- Consider patient-specific factors
- Communicate appropriately with patients
- Report AI-related errors
- Maintain professional responsibility
- Participate in AI training

AI literacy should increasingly become part of healthcare education and professional development.

16. Role of AI Developers

Developers have important responsibilities in creating safe medical AI.

They should:

1. Use appropriate and representative datasets.
2. Document model development.
3. Test for bias.
4. Evaluate performance across relevant groups.
5. Implement security controls.
6. Clearly communicate limitations.
7. Maintain appropriate documentation.
8. Support monitoring after deployment.
9. Provide mechanisms for error reporting.
10. Follow relevant regulatory and ethical requirements.

Technical performance should not be the only development objective.

17. Role of Healthcare Institutions

Hospitals and healthcare organizations should create governance structures for AI adoption.

An AI governance framework may include:

- AI approval committees
- Ethical review
- Clinical validation
- Cybersecurity assessment
- Data governance
- User training
- Incident reporting
- Performance monitoring

Before deployment, organizations should ask:

Is the AI system technically accurate?

But they should also ask:

Is it safe, fair, secure, explainable, clinically appropriate and suitable for our patient population?

18. Patient Participation

Patients should not be treated merely as sources of medical data.

Where appropriate, patients should be informed about how their data are used and how AI contributes to healthcare decisions.

Patient participation can improve:

- Trust
- Transparency
- Acceptance
- Shared decision-making

Healthcare professionals should communicate AI-related information in language that patients can understand.

19. Regulatory and Governance Considerations

Medical AI operates within a complex environment involving healthcare regulation, medical-device regulation, privacy requirements, professional standards and institutional policies.

Regulatory approaches vary across countries and depend on the intended use and risk of the AI system.

Organizations should therefore identify applicable requirements before deploying AI.

Internationally, organizations such as the World Health Organization (WHO) have emphasized principles including human autonomy, safety, transparency, accountability, inclusiveness and sustainability in the responsible development and use of AI for health.

20. AI and Health Equity

AI has the potential to improve healthcare access, but it may also increase existing inequalities.

For example, advanced AI systems may initially be available mainly in well-funded hospitals or urban healthcare environments.

Potential barriers include:

- High implementation costs
- Limited digital infrastructure

- Lack of trained professionals
- Poor internet connectivity
- Language barriers
- Limited access to digital devices

Responsible AI should therefore consider not only technological performance but also accessibility and equity.

21. Benefits and Risks of Ethical AI in Healthcare

Benefits	Potential Risks
Faster analysis	Incorrect predictions
Diagnostic support	Algorithmic bias
Personalized healthcare	Privacy concerns
Patient monitoring	False alerts
Workflow automation	Automation bias
Medical research support	Data misuse
Resource optimization	Unequal access
Decision support	Lack of explainability
Early risk detection	Cybersecurity threats

The goal is not to eliminate every possible risk, which may be unrealistic, but to identify, reduce, monitor and manage risks appropriately.

22. Framework for Responsible Medical AI

A practical responsible-AI framework can be organized around eight principles:

1. Safety

The system should be designed and evaluated to minimize preventable harm.

2. Privacy

Patient information should be protected throughout the data lifecycle.

3. Fairness

AI performance should be evaluated across relevant populations.

4. Transparency

Users should understand the intended role and limitations of the system.

5. Explainability

Important decisions should be supported by understandable information where appropriate.

6. Accountability

Responsibilities should be clearly assigned.

7. Human Oversight

Qualified professionals should remain involved in appropriate clinical decisions.

8. Continuous Monitoring

AI systems should be monitored after deployment and updated when necessary.

23. Future Directions

The future of medical AI is likely to involve increasingly sophisticated systems capable of integrating multiple forms of information.

Emerging directions include:

- Multimodal AI
- AI-assisted precision medicine
- AI-enabled remote monitoring
- Wearable health technologies
- Federated learning
- Privacy-preserving machine learning
- Explainable AI
- Human-AI collaboration
- Generative AI for healthcare
- AI-supported medical research

However, technological advancement should be accompanied by stronger governance, professional education, cybersecurity and ethical oversight.

Future AI systems should not be evaluated only by their computational performance. Their real-world impact on patients, healthcare professionals and society should also be considered.

24. Conclusion

Artificial Intelligence has significant potential to transform medical practice. It can support diagnosis, patient monitoring, personalized medicine, medical research, clinical decision-making and healthcare administration. However, medical AI is fundamentally different from many ordinary technology applications because errors can directly affect human health and safety.

Ethical AI therefore requires a patient-centered approach based on safety, autonomy, beneficence, non-maleficence, fairness, transparency, privacy, accountability and human oversight. Algorithmic bias, data privacy, explainability, cybersecurity, automation bias and unclear responsibility are among the major challenges that must be addressed.

AI should be viewed as a tool for supporting healthcare professionals rather than as an independent replacement for human judgment in situations requiring clinical expertise and responsibility. Developers, healthcare professionals, institutions, policymakers and patients all have roles in creating a trustworthy AI ecosystem.

Ultimately, the success of AI in medicine should not be measured solely by how accurately an algorithm performs a technical task. It should also be evaluated by whether the technology contributes to safe, equitable, transparent, responsible and patient-centered healthcare.

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CHAPTER 4

Artificial Intelligence in Biological Sciences: Transforming Drug Discovery and Development

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Abstract

Artificial intelligence is transforming biological sciences by enabling faster, more accurate and data-driven approaches to drug discovery and development. Advances in multi-omics technologies, high throughput screening, electronic health records and biomedical databases are leading to the production of enormous amounts of large-volume, complex data, which cannot be handled by traditional methods of analysis. AI technologies, such as machine learning, deep learning, graph neural network, transformer models and generative AI, have become important tools for deriving biological insights and for accelerating the pharmaceutical research. This chapter gives a detailed description of the application of Artificial Intelligence in biological fields such as Genomics, Proteomics, Transcriptomics, Metabolomics, Systems Biology and Precision medicine. It also includes details on the applications of AI across the entire drug discovery process, including target identification, hit discovery, virtual screening, molecular docking, lead

optimization, de novo drug design and drug repurposing. In addition, the chapter explores how AI technologies are transforming preclinical research, clinical trial planning, patient screening, biomarker identification and pharmacovigilance, which have the potential to boost efficiency, cut costs and inform better decision making. In addition, it addresses current challenges such as data quality, model interpretability, regulatory acceptance, ethical concerns and workforce readiness. In summary, this chapter offers a detailed view of the impact that AI is having on biological sciences and how this is driving the search for safe and effective drugs at a faster rate.

Keywords: Artificial intelligence, Drug discovery, Drug development, Precision medicine and Pharmacovigilance.

1. Introduction

Artificial Intelligence (AI) is one of the most significant technologies of today's biomedical research. The increase in computing capabilities, availability of large biological datasets and improved algorithms for artificial intelligence have enabled the usage of AI to facilitate almost all aspects of drug discovery and drug development. The traditional drug development process from target identification to regulatory approval is expensive, complex and a long process. It is typically a process spanning 10 to 15 years, the expense of a successful drug from approval is greater than US $2.6 billion and less than 10% of all drug candidates enter the marketplace. The identified challenges underscore the need for more efficient and rapid solutions, which is where AI can assist in enhancing the drug discovery pipeline [1].

Modern biological research produces a huge amount of data that is difficult to analyses manually. For instance, next generation sequencing has the ability to generate vast amounts of genomic information, mass spectrometry can yield millions of information points in one experiment and electronic health records (EHRs) can contain a wealth of clinical information for millions of patients over a period of time. These complex datasets need advanced computational tools to analyse and interpret. Use of artificial intelligence (AI) systems can detect underlying relationships in these patterns, analyse various variables at once and process vast-scale information, giving researchers valuable biological insights and improving their ability to make informed decisions in drug discovery [2].

The impact of artificial intelligence (AI) is already evident throughout the drug discovery process. For instance, AlphaFold2 can accurately predict the

three-dimensional structure of proteins, which are near to laboratory experiments and which are beneficial in drug designing, structure-based drug designing. Compared to the traditional drug molecule design process, which takes years, Generative AI can come up with new drug molecules in a short span of days or weeks with the required biological properties. AI-powered knowledge graph technologies can facilitate the identification of novel therapeutic indications in approved drugs, allowing drug repurposing to be faster and more cost-effective. Besides, deep learning models can analyse clinical trial data to locate the right patient population and make better predictions regarding treatment outcomes, thereby improving potential clinical trials and minimizing failures in the drug development process's late stages [3].

This book chapter provides a comprehensive overview of how artificial intelligence (AI) is applied in biological sciences and throughout the drug discovery and development process. It starts with an overview of the fundamentals of AI and their application to biology. The chapter further focuses on the contributions of AI to multi-omics research systems biology and precision medicine. It also outlines the use of AI in various phases of drug discovery, such as target identification, virtual screening, lead optimization and drug repurposing. This chapter also covers AI applications in pre-clinical, clinical studies and pharmacovigilance. The chapter ends with a summary of the key benefits and existing challenges associated with AI in healthcare and a look forward to the continued evolution of AI in biological research and drug development.

2. Fundamentals of Artificial Intelligence

AI Technique	Description	Application	Reference
Artificial Intelligence (AI)	Computational systems designed to perform tasks that normally require human intelligence.	Drug discovery, diagnosis, precision medicine	[4]
Machine Learning (ML)	A branch of AI in which algorithms learn from data rather than explicit programming.	Bioactivity and toxicity prediction	[5]
Deep Learning (DL)	Machine learning approach based on multi-layer artificial	Medical imaging and genomics	[6]

	neural networks that automatically learn complex features from raw data.		
Convolutional Neural Networks (CNNs)	Deep learning models specialized for image and spatial data analysis.	Medical imaging	[6]
Recurrent Neural Networks (RNNs) and Long Short-Term Memory (LSTM)	Neural networks designed for sequential data processing.	DNA and molecular sequence analysis	[6]
Transformer Models	Self-attention-based deep learning models capable of understanding long-range dependencies in data.	Protein and clinical text analysis	[6]
Graph Neural Networks (GNNs)	Neural networks that represent molecules and biological systems as graphs.	Drug-target interaction prediction	[6]
Generative AI	Creates novel molecules.	*De novo* drug design	[7]
Transfer Learning	Adapts pre-trained models.	Specialized biomedical research	[7]
Federated Learning	Trains models without sharing patient data.	Privacy-preserving healthcare AI	[7]

3. AI in Biological Sciences

3.1. AI in Omics Sciences

Omics technologies, such as genomics, transcriptomics, proteomics and metabolomics contribute to researchers in understanding the molecular make-up and processes of living organisms. The big data produced by these technologies is often very complex and cannot be processed with conventional methods. To deal with these datasets, to extract useful biological information and its hidden patterns, Artificial Intelligence (AI) is becoming an inevitable tool nowadays [8].

In genomics, AI has greatly improved the analysis of genetic data. With next generation sequencing (NGS) data, Deep Learning Models can be used

to reliably identify genetic variations and polymorphisms such as single nucleotide polymorphisms (SNPs) and insertions/deletions (indels). Transformers-based AI models like Enformer and Nucleotide Transformer can predict gene regulatory events like chromatin accessibility, transcription factor binding and histone modification from DNA sequence alone. Machine learning models also improve the prediction of psychiatric diseases, CAD, type 2 diabetes and other complex diseases in an attempt to get early diagnosis and personalization [9].

In proteomics, AI has revolutionized protein structure prediction. DeepMind's AlphaFold2 helped to solve the gaps in structural biology and structure-based drug design by allowing the prediction of three-dimensional protein structures with an extraordinary level of accuracy. Researchers now have access to more than 200 million predicted protein structures in what's called the AlphaFold Protein Structure Database. AI is also used to enhance peptide identification in mass spectrometry data, to foresee post-translational modification sites and to analyze protein–protein interaction networks through the use of Graph Neural Networks (GNNs) [10].

In transcriptomics, AI can guide the analysis of gene expression data at the single-cell level, leveraging single-cell RNA sequencing (scRNA-seq) data. Deep learning models like scVI and SCVI-tools lower the complexity of the data, correct batch effects, discover and separate cell types and predict cell development pathways. Advanced foundation models such as Geneformer and scGPT enhance the analysis of gene networks, prediction of cellular responses to genetic changes and aid in the identification of therapeutic targets [11].

In metabolomics, AI improves the identification of metabolites by analyzing mass spectrometry data with greater accuracy than conventional methods. GNNs can be used to predict previously unknown metabolites from their mass spectra, increasing the number of metabolites that may be identified. Blood, urine and tissue metabolomics are also used as an input to machine learning models, which are trained for the discovery of disease biomarkers for diagnosis, treatment response and patient stratification. Moreover, AI can combine data from different sources, such as genomics, transcriptomics, proteomics and metabolomics, to generate a holistic picture of the complex nature of diseases and facilitate precision medicine and drug development [12].

3.2. AI in Systems Biology

Systems biology focuses on understanding the interactions of the various components of the biological system (genes, proteins, cells, etc.) as a system, not as components. These biological networks are very complex in nature and many times, the traditional approaches to calculation are not effective to analyse them. Artificial Intelligence (AI) supports the efficient development, analysis and simulation of these complex networks, thereby contributing to understanding of mechanism of disease and biological functions [13].

One of the key tools in AI for systems biology is the knowledge graph, which describes genes, proteins, drugs, diseases and biological pathways as nodes that are all connected. AI algorithms, such as TransE and RotatE, are used to discover new relationships in these networks, for example new gene–disease connections or drug–target relationships. Biomedical knowledge graphs, such as DRKG, Hetionet and OpenBioLink, integrate information from more than one biological database and assist scientific researchers in deriving novel scientific hypotheses for drug discovery and disease research [14].

Finally, AI is a key part of network medicine, which helps uncover clusters of proteins that interact and are involved in certain diseases. These disease related networks aid in understanding the disease mechanism, predicting drug actions and finding the possibility for drug repurposing. Graph Neural Networks (GNNs) better predict essential genes and protein interactions along with effective drug combinations by taking into account the relationships between biological molecules. Moreover, AI-powered mathematical modeling allows for the simulation of cellular response to various treatment options, aiding in the development of more effective combination therapies and in the optimization of drug development [15].

3.3. AI in Precision Medicine

Precision medicine would be the effective chance of delivering right treatment to the right patient at the right time by taking into account an individual's genetic profile, clinical data, physiologic traits and lifestyle. Artificial Intelligence (AI) in precision medicine involves data analysis to aid in individual treatment recommendation, especially in analysing large data sets of patients [16].

Multi-omics data from a large number of patients can be used to train ML models to recognize various molecular subtypes of diseases, especially cancer.

These models generate future predictions of disease progression and identify those patients who are likely to benefit from targeted therapies or immunotherapies. Additionally, AI algorithms can be used to analyse the genetic markers found in the tumour environment, including tumour mutational burden (TMB), microsatellite instability (MSI) and gene expression profiles, contributing to making more informed clinical decisions and selecting the right treatments. Moreover, deep learning models when analyzing digital pathology images, can assess tumour architecture, immune infiltration and cellular morphology and in so doing, offer more accurate prediction of response to therapy or patient survival, compared to traditional pathological approaches [17].

AI is also enhancing healthcare by deciphering Electronic health records (EHRs), consisting of structured medical information and unstructured clinical notes. When it comes to suitable patient identification for precision therapies and clinical trials, AI is able to do this using natural language processing (NLP). Machine learning algorithms are used to create polygenetic risk scores, which calculate likelihood of the disease happening during a particular person's life based on responses from several genes, in addition to influences from the environment. In addition, AI technologies are used to analyze data from wearable devices and other digital health technologies, providing a constant stream of data on disease progression and treatment effectiveness. These real-time insights can enhance personalized care for patients and boost the efficiency of clinical trials [18].

4. AI in Drug Discovery

4.1. Traditional Drug Discovery Process

The traditional method of drug discovery involves a step-to-step procedure: identification of a biological target connected with a disease to regulatory approval of a new drug. A typical sequence of events involved in this process is target identification and its validation, hit identification via high throughput screening (HTS), lead optimization and preclinical studies prior to clinical trials. This is a long, complex and expensive process and can take 10–15 years to develop a new medicine [19].

Pharmaceutical research has improved considerably in the last few decades, but the success rate in the development of drugs is still very small. Approximately 30% of drugs make it to Phase II trials with less than 9.6% successfully making the transition to regulatory approval after entering Phase

I clinical trials. The main reasons for clinically failing are poor drug efficacy, safety/toxicity concerns and sub-optimal pharmacokinetic characteristics: poor absorption, distribution, metabolism and excretion. Such challenges are frequently attributed to a lack of knowledge of disease biology, the lack of preclinical models and the lack of patient selection. Artificial intelligence (AI) can overcome many of these limitations by providing better target selection, predicting drug safety and efficacy and aiding patient stratification [20].

Historically, the method for finding out whether a chemical could be a drug candidate is to test 500,000 to 2 million compounds in high throughput screening (HTS) against a biological target. HTS is an effective technique, but its cost and time requirements are burdensome and it covers very small fraction of the vast chemical space of potential drug molecules. Once scientists identify the possibilities for the hits, lead optimization is then carried out, which consists of successive cycles of synthesis of new compounds, biological tests and structure–activity relationship (SAR) analysis to improve the drug candidate. This alone is likely to take 3-5 years and involves the synthesis of hundreds of chemical analogues. Today, AI can revolutionize all the steps of this old-fashioned process, accelerating and streamlining drug discovery and reducing costs [21].

4.2. Target Identification and Validation

Target identification involves identifying proteins, genes or biological pathways that can be targeted for the treatment of a disease. Traditionally, scientists have identified those targets using laboratory experiments, observations and phenotypic screening that is, observing the effects of compounds on cells or organisms in a way that did not involve knowledge of the specific target. With the dawn of artificial intelligence (AI), this process has become quick and accurate by analysing extensive amounts of biological data. This enabled AI to integrate knowledge from multiple fields including genomics, proteomics and transcriptomics, as well as protein interaction networks and genetic studies and to use these to discover the most promising therapeutic targets with greater degree of confidence [22].

One important application of AI is in analysing data from genome-wide association studies (GWAS). GWAS help discover genetic differences that are linked to various diseases. AI combines GWAS results with other biological information to pinpoint those most likely to be involved in disease, including analysis of expression quantitative trait loci (eQTL), chromatin interactions, protein–protein interaction networks and phenome-wide association studies.

Human genetic data is better for success in clinical development of targets than conventional methods. Also, by employing natural language processing (NLP) AI can analyse scientific literature, clinical trial databases and patent publications to identify further evidence of the involvement of particular genes or proteins in disease processes [23]

Once a target has been identified, AI can be used to validate the target by evaluating large genomic datasets, Mendelian randomisation data sets and studies involving functional better-than-expected, CRISPR-based genomic libraries. AI also provides druggability assessments for validated targets based on protein structure, binding sites, sequence conservation and other molecular features. These methodologies help optimize target identification, minimize spend and time in research and boost the likelihood of creating effective small molecule drugs or biological therapies [24]

4.3. Hit Identification and Lead Optimisation

Hit identification is the identification of a chemical compound that has the potential to interact with a biological target and elicit the desired therapeutic effect. It was a traditional, mainly laboratory screening process using huge numbers of compounds. Hit identification has changed with the use of AI, using computation in conjunction with experimental testing. Deep learning models can be used to score how tightly virtual compounds bind the target proteins by analysing large databases of bioactivities, e.g., ChEMBL, PubChem and BindingDB. This enables researchers to appraise a much bigger chemical space and to prioritize the screening time of promising compounds as compared to the traditional laboratory screening [25].

AI can also enhance hit identification by leveraging the power of advanced machine learning algorithms including graph neural networks (GNNs) and message-passing neural networks (MPNNs) to predict the biological activity of compounds from their molecular structure. Active learning strategies are used to choose the most informative set of compounds to test for a set of experiments and increase the likelihood of finding effective drug candidates while decreasing the number of compounds that are unnecessarily tested. Further, AI has the potential to assess data from DNA-encoded chemical libraries (DELs) that allow the screening of billions of compounds, to uncover critical structure–activity relationships that can guide drug discovery [26].

Once promising hits are identified, they undergo lead optimisation to

improve their potency, selectivity, safety, pharmacokinetic properties (ADMET) and ease of synthesis. In addition to saving time and speed, AI can optimise several molecular properties at once, as compared to the traditional method. Additionally, it helps predict the impact of changes to the structure, enhances the precision of biological activity prediction via deep-learning methods and assists in the creation of feasible synthetic pathway ideas with the tools of retrosynthetic analysis and AI. These capabilities are used to speed drug molecule development in both a safe and effective way [27].

4.4. Virtual Screening and Molecular Docking

Virtual screening (VS) is a computational method which searches through the databases with a vast pool of chemical structures to pick out the most promising structures to bind to a biological target. AI can foresee chemical compounds with the greatest prospective biological activity, avoiding the necessity of laboratory testing of millions of compounds. Structure Based and Ligand Based are two types of the virtual screening (VS) and there is the addition of AI [28].

Structure-based virtual screening is an in-silico method that designs also predict the binding mode of the compound to the protein target and more important, the strength of the interaction. These predictions are more accurate using AI-based scoring techniques than traditional docking methods. The improved generation of more reliable binding poses with advanced AI models like DiffDock, combined with the analysis of three-dimensional molecular geometry for more accurate prediction of binding affinity with equivariant graph neural networks (GNNs), offer significant advantages in the field of drug discovery [29].

With integration of effective compound scoring along with high performance computing, screening of billions of compounds at speed has also become possible through AI. Ligand based virtual screening can be performed to discover new active compounds, which may involve compounds with non-descendants scaffold that are not identified by similarity-based virtual screening and can be identified by deep learning-based analyses of molecular fingerprints, molecular graph or three-dimensional structure of the compounds. Fast AI-based screening, coupled with conventional physics-based screening further enhances the efficiency and precision of virtual screening, enabling the researchers to find potential drug candidates faster [30].

4.5. De-Novo Drug Design

De novo drug design involves the design of new drug molecules rather than the selection from available chemical libraries. AI has emerged as a valuable instrument to this end by scanning the extensive chemical space to produce new molecules that exhibit certain biological and chemical properties of interest. This empowers researchers to find out about potential drug candidates that may not show up in existing collections of compounds [31].

AI uses different generative models to design new molecules. For instance, recurrent neural networks (RNNs) are trained in the structure of existing drug molecules and used to generate new molecules of similar characteristics. The other ones like variational autoencoders (VAEs), optimize for molecular properties including potency, selectivity, ADMET (absorption, distribution, metabolism, excretion and toxicity) and synthetic accessibility. The diversity of newly generated drug candidates is further extended with other methods such as generative adversarial networks and normalising flow models [32].

Successful binding is more likely using the three-dimensional structure of a protein targeted to develop molecules. In contrast to previous approaches, these models have the advantage of utilizing target structure information in the molecule generating process. The success of INS018_055, developed by Insilico Medicine, provides a clear example of the capabilities of AI-suggested molecules to promote drug discovery and development. acestle a molecule went from target identification to a pre-clinical candidate in approximately 18 months [33].

4.6. Drug Repurposing

Drug repurposing is a search for new applications of an approved, investigational or previously unsuccessful drug. The fact that these drugs already exist means they are known to be safe and have a formula and manufacturing process which can help to save a lot of time, money and risk for drug development. AI has emerged as a valuable resource for drug repurposing because of its capacity to process vast amounts of biomedical data from varied sources and uncover previously unnoticed drug–disease connections [34].

AI-based drug repurposing uses knowledge graphs, protein interaction networks and natural language processing (NLP) to identify potential new uses for existing drugs. In this regard, knowledge graphs analyze the

interactions between drugs, genes, proteins and diseases, while network-based approaches discover the drugs that target proteins that are close to disease pathways. Additionally, NLP examines existing scientific studies, clinical trial databases, pharmacovigilance (PV) reports and case studies to identify any promising findings for unexpected therapeutic applications and/or repurposing opportunities [35].

AI can also be used to analyze gene expression data from platforms like the Connectivity Map (CMap) and LINCS L1000 potential drugs that may be able to reverse what is observed with disease related gene expression. For example, several new therapeutic applications have been found through this approach such as repurposing baricitinib in COVID-19, antiparasitic drugs to fight cancer and cardiovascular drugs for fibrotic diseases. AI-led drug repurposing can speed up the process to detect promising drug candidates, potentially shorting drug development from 10-15 years to 3-5 years [36].

5. AI in Drug Development

5.1. Preclinical Studies

Preclinical development includes the testing of the safety, pharmacokinetics and initial effectiveness of a drug candidate prior to its testing in humans. Artificial intelligence (AI) is changing all that by advancing the precision of prediction, detecting potential trouble upstream and decreasing the need for animal testing via new in silico (computer-based) and in vitro (laboratory-based) techniques [37].

One of the most important applications of AI in preclinical development is the prediction of absorption, distribution, metabolism, excretion and toxicity (ADMET) properties. AI models, especially deep learning algorithms, can be trained with large experimental datasets and predict important drug properties from the chemical structure of the molecule, including aqueous solubility, lipophilicity, intestinal permeability, plasma protein binding, blood-brain barrier penetration, cytochrome P450 enzyme interactions and hepatic metabolic clearance. Multi-task learning models based on the same model structure that predicts multiple ADMET properties simultaneously tend to yield better results than models that are designed to predict a single ADMET level, since the multi-task learning models have the ability to learn common molecular features. AI also enhances physiologically based pharmacokinetic (PBPK) models and accurately estimates tissue distribution and a drug's clearance, facilitating its more accurate prediction for the first drug dose to be

given into human beings [38].

Toxicity prediction is another important application of AI because unwanted toxic effects are responsible for about 30% of clinical trial failures and are the leading cause of drug withdrawal after marketing. CNN models can be trained with data from Tox21, ToxCast and drug-induced liver injury (DILI) databases to predict various types of toxicity, such as liver toxicity, heart toxicity (hERG inhibition), genetic toxicity, reproductive toxicity and skin sensitisation. The graph neural network (GNN) models added information about the structure and connectivity of atoms in a molecule, which helped to more accurately predict toxicity than traditional models. Furthermore, toxicogenomic, which is an integration of machine learning and gene expression analysis, elucidates why a compound is toxic and enables researchers to develop a safer version by altering the chemical structure of the compound [39].

The use of high-content imaging, when coupled with deep learning, enables the automatic and detailed analysis of the impact of drugs on cell shape, organelle structure and cellular activities. This method, called "morphological profiling," or Cell Painting, allows the comprehensive microstructural assessment of metabolic profiles of the drugs, hence revealed the mechanism of action, specificity and off-target activity and predict in vivo toxicity from in vitro studies. Organ-on-chip and three-dimensional (3D) organoid models, in combination with AI-assisted image analysis, also offer more human-like preclinical testing systems than 2D cell culture models. Consequently, they enhance the precision of preclinical research and boost the likelihood that what is done in the lab will work in the clinic [40].

5.2. Clinical Trials

Clinical trials can be the costliest, time consuming and the most failure intensive ($hitpital) phase of drug development. AI has the potential to address numerous challenges in this stage, including optimized trial design, patient recruitment, biomarker identification and data analysis, as well as regulatory determinations [41].

In clinical trials, AI can enhance the design of trials by simulating scenarios and forecasting the likelihood of successful outcomes to optimize protocols. Bayesian Adaptive Trial Designs (ATDs) can also be enhanced using AI and are designs that either change treatment allocation, sample size or stopping rules during trial execution based on trial results. AI models can

help forecast if the trial will achieve success based on a set of parameters gathered during the interim analyses. Data from past clinical trials can also be used to train machine learning models which help to estimate the probability of success depending on the drug target, type of disease, mechanism of action and trial design. This assists pharmaceutical companies to prioritize promising drug candidates and to make investment decisions. A new solution being developed is the use of digital twin technology, which involves creating an AI simulation of a patient, which can act as a digital placebo for trials, decreasing the amount of patients needed in the control group without compromising the correctness of the trial results [42].

Patient recruitment is one of the largest problems in clinical trials and more than 80% of clinical trials have trouble recruiting the right number of patients within the required timeframe. The AI-based patient identification feature leverages natural language processing (NLP) to accurately screen and identify eligible patients from structured and unstructured electronic health records (EHRs) as compared to manual screening. AI can identify which patients are at risk of disengaging from a study, violating protocol or suffering adverse events, enabling researchers to take steps to prevent this and increase the likelihood of trials being completed. Furthermore, federated learning can allow several hospitals and/or research centres to identify eligible patients without having to disclose the sensitive data of the patients, preserving patient privacy while increasing the number of patients that are available for the learning process [43].

A significant use case for AI is in the field of precision medicine, where biomarkers are identified. AI can analyse multi-omics, imaging and genomic information to aid in determining which biomarkers more accurately predict treatment response than current methods can. It also uses tumour sequencing information to detect genomic markers that are linked to tumour drug responsiveness. Moreover, AI-powered processing of liquid biopsy information, such as circulating tumour DNA and tumour cells, allow for non-invasive tracking of the patient's situation and the early identification of treatment resistance. Such progressions assist in the development of personalised treatment methods and the identification of biomarkers by AI tools is gaining recognition in the eyes of regulatory agencies, including the FDA and EMA, for companion diagnostics [44].

5.3. Pharmacovigilance

Pharmacovigilance, which is the science of collecting, interpreting,

understanding and preventing undesired effects of drugs, helps safeguard public health by maintaining the safe surveillance of marketed drugs. The traditional approach to pharmacovigilance is largely dependent on adverse event reporting which suffers from a variety of under reporting biases, inconsistencies in terminology as well as significant backlogs of adverse event reviews. By leveraging AI, pharmacovigilance enhances the detection of signals in real time, automatically and at scale, across a multitude of sources [45].

NLP algorithms applied to spontaneous reports in the FDA Adverse Event Reporting System (FAERS) and WHO VigiBase automatically extract drug–adverse event pairs, normalise event terminology to MedDRA codes, classify causality and perform disproportionality analysis to detect emerging safety signals significantly faster and with greater consistency than manual case processing. Named entity recognition and relation extraction models trained on pharmacovigilance corpora identify adverse event mentions and their drug associations from scientific literature, social media, patient forums and electronic health records in near real time, providing surveillance coverage beyond formal reporting systems [46].

Using of social media data such as patient forums, Twitter and health applications with NLP and deep learning has proven useful for identifying signals of safety issues weeks to months before they appear in spontaneous reporting systems, helping to complement ascertainment bias and notoriety bias inherent in spontaneous systems. The ability of deep learning models trained on EHRs to predict incidence rate of adverse events for a specific drug in real-world patient populations also introduces another layer of early warning. Predictive pharmacovigilance, the ability of ML to predict adverse events of likely drugs based on structural, pharmacological and genomic properties, gives the potential to prevent adverse event occurrences in advance and creates an opportunity for designing studies with target post-marketing data [47].

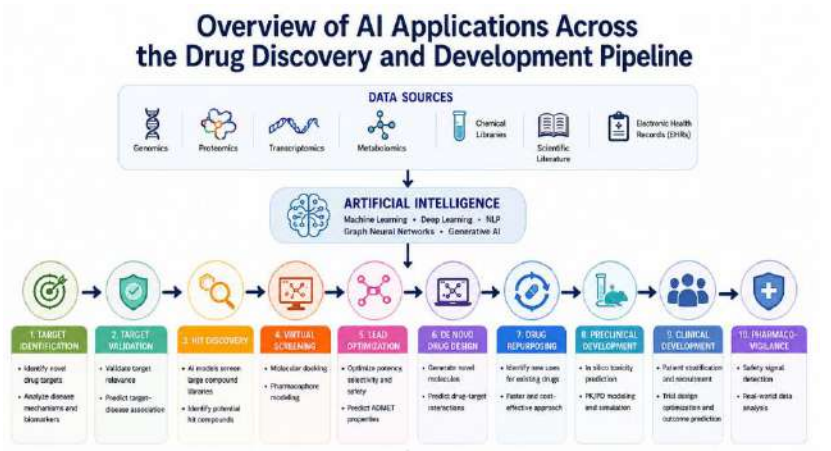


Fig. 1: Overview of Artificial Intelligence Applications Across the Drug Discovery and Development Pipeline

6. Advantages Of AI in Drug Discovery And Development

i. **Speeds up drug discovery**: AI can do complex computational analysis in hours or days rather than years, speeding up the drug discovery process. In 18 months, AI-driven platforms have discovered new drug candidates that took over five years using traditional methods. In addition, AI has the ability to virtually screen billions of compounds in a matter of days, something that simply couldn't be done with conventional high throughput screening [48].

ii. **Decreases costs of development**: AI decreases the costs of development by screening out the best possible set of drug candidates before they are synthesized and tested in the lab. By predicting the ADMET properties early, experiments and studies on animals are minimized as unsuitable compounds are eliminated early in the process. The use of AI in drug repurposing also reduces the development costs by 50–60% as existing safety data will be at hand [49].

iii. **Chemical and biological space expansion**: AI helps to explore a vast chemical space of around 10^{60} drug-like molecules and to discover new compounds that may not be identified through conventional screening. It also tackles biological networks to discover new disease–gene associations and therapeutic targets [50].

iv. **Optimises multiple drug properties simultaneously**: AI can improve several molecular properties, including potency, selectivity, solubility, pharmacokinetics and toxicity, at the same time. ADMET properties will be optimized in this multi-parameter optimization, saving the optimisation cycles and reducing the risk of drug failure at the last moments due to its poor ADMET properties [51].

v. **Enhances clinical trial success**: AI can help precision medicine efforts by selecting the best people for a particular treatment based upon genomic, clinical and biomarker information. Scientifically efficient patient selection for better treatment efficacy and clinical trial success rates in Phase II and Phase III studies [51].

7. Challenges And Limitation

i. **Data limitations and poor data quality**: AI models need large, high-quality data sets for accurate predictions. Bioactivity databases, however, may have contradictory information, varying experimental procedures and publication bias for the ones that have been successful. Limited reporting of inactive compounds, which contributes to low reliability and generalizability of AI models [52].

ii. **Limited interpretability of the models**: AI models, especially deep learning models, can be "black boxes," which means it may be hard to understand how predictions are made. This non-transparency affects the use of them in pharmaceutical research and regulation acceptance. While techniques such as Explainable AI (XAI) help in interpreting the model to a greater extent, they are still not enough in order to develop complete regulatory confidence [53].

iii. **Limited applicability to new drug candidates**: AI models might be highly effective at identifying hits in historical data but perform poorly when predicting "totally new" molecules and novel drug targets. Subjecting the model to prospective validation studies is necessary because of the differences between the training data and the real-world scenarios that can cause a reduction in model performance [54].

iv. **Regulatory and ethical issues**: Regulation of AI in drug development is still in a developmental stage. There are few clear guidelines for validation of AI-generated evidence. AI systems should also consider aspects of patient privacy, data security and

bias; not representative datasets can result in unequal performance in various patient groups [55].

v. **Organizational and workforce challenges**: Collaboration is needed between computer scientists, medicinal chemists, biologists, clinicians and regulators for successful implementation of AI. Numerous pharmaceutical enterprises are lacking on correct computational foundation, data managing systems and AI professionals too. There is also evidence of resistance to the use of AI in decision-making, which hinders the widespread use of AI in routine drug development [56].

8. Conclusion

Artificial intelligence (AI) has become a transformative technology in biological sciences and pharmaceutical research. It is transforming every stage of the drug discovery and development process, including understanding disease mechanisms, predicting protein structures, designing novel drug molecules and optimising clinical trial design. By leveraging AI, researchers can more efficiently analyse large and complex biological data, discover potential drug candidates faster and make informed decisions during the drug development process with greater accuracy. AI has significantly improved the speed, cost-effectiveness and overall efficiency of pharmaceutical research compared with traditional approaches.

The future of drug discovery lies in the integration of AI across the entire drug development pipeline. AI systems can continue to learn from fresh experimental and clinical findings to enhance future predictions and drug designs. New technologies like foundation models, generated by extensive biological datasets and self-driving laboratories, powered by AI for lab automation, promise to push the pace of drug discovery even further alongside cutting down on extensive experimental tests. These advancements will help scientists make safer and more effective medicines more rapidly.

However, there are a number of challenges that need to be overcome for AI to be fully integrated into routine pharmaceutical research. Issues around the availability of high-quality data, interpretability of the AI model, the necessity for prospective validation and patient privacy, fairness and approval from regulators are important concerns. These challenges can be addressed through the development of transparent AI models, standardized data-sharing frameworks and close collaboration among researchers, pharmaceutical

companies, healthcare professionals and regulatory authorities. Furthermore, the use of AI in conjunction with other recent technologies, like organ-on-chip systems, cryo-electron microscopy, single-cell multi-omics and spatial transcriptomics will further enhance the accuracy and clinical relevance of drug discovery via AI.

Over the next few years, AI is projected to transform from a decision-support system into an intelligent research assistant that can formulate scientific hypotheses, design experiments, analyse experimental data and inform strategies for drug discovery. The responsible use, robust ethical and regulatory frameworks and ongoing interdisciplinary cooperation, however, will be crucial for the successful integration of AI. These advancements present an opportunity for AI to revolutionize drug discovery and development, making the process faster, more efficient, cost-effective and patient-centric, while also contributing to the creation of safer and more effective drugs and better healthcare outcomes worldwide.

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5
CHAPTER

Dressing the Self: Fashion Education, Religious Identity and Agency among Young Muslim Women in India

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Abstract

The relationship between fashion and religious identity is often viewed through a simple binary, where contemporary fashion is associated with modernity, while religious clothing, particularly the hijab, is seen as a symbol of tradition or restriction. This perspective becomes limiting when we consider young Muslim women who actively pursue fashion education while at the same time navigating their religious values and expectations around modesty. This qualitative study explores why young Muslim women in India pursue fashion design and how they negotiate fashion, hijab, religious identity, family expectations and future career aspirations. The study followed an interpretivist approach to understand the experiences and perspectives of Muslim women pursuing fashion design. Data were gathered through focus group discussions and individual interviews with students from three colleges in Maharashtra. To provide a broader perspective and strengthen the findings, six semi-structured interviews were also conducted with religious scholars, academics and professionals from the fashion and textile industry. These conversations

helped triangulate and contextualize the experiences shared by the students. Four higher-order themes: passion, identity and self-expression; modesty, hijab and fashion compatibility; social and institutional ecology; and future orientations towards careers and entrepreneurship were observed in Thematic Analysis of the collected data. The findings suggest that pursuing fashion education does not necessarily mean moving away from religious values. For many participants, fashion was seen as a way to express creativity, build confidence and develop their sense of identity. Rather than viewing modesty as a restriction, several participants approached it as a creative element that could be incorporated into their design choices. Family support, a good educational environment and evolving social attitudes emerged as key factors that encouraged these young women to pursue fashion education. Many participants also envisioned careers in modest fashion, boutique ownership and entrepreneurship, seeing these paths as opportunities to combine creativity, financial independence and religious values. Drawing on ideas of situated fashion, agency, cultural hybridity and religious identity, the study suggests that these women are not choosing between faith and fashion. Instead, they are continuously shaping their identities, using clothing and fashion as a way to negotiate and express who they are within their social and religious worlds.

Keywords: Muslim women, fashion education, hijab, modest fashion, identity, self-expression, India, qualitative research

1. Introduction

Clothing is much more than something we wear. The way people dress can communicate their sense of belonging, values, aspirations, social position and even how they see themselves. This suggests that fashion simply does not mean an aesthetic or creative practice; it also works as a form of social communication through which individuals express who they are and negotiate their place within society. Kaiser (2012) highlights the strong connection between fashion and cultural identity, while Tarlo (2010) shows how dress can become an important space where religion, politics and everyday identity come together.

This relationship becomes especially interesting when looking at young Muslim women who choose to pursue fashion education despite the tradition of Hijab which is associated with modesty, faith and religious identity. Fashion is often associated with changing trends, experimentation with appearance and Western ideas of beauty and dress. At first glance, these may

appear to represent two very different worlds. This raises an important question: why would a young Muslim woman who observes hijab choose to study fashion design?

However, the question itself assumes that fashion and religious modesty are necessarily in conflict. This assumption does not fully reflect the experiences described in existing research. Lewis (2015), for example, explains modest dress as a situated practice through which Muslim women actively negotiate their identities within contemporary fashion and style cultures. Similarly, Tarlo (2010) demonstrates how visibly Muslim women use dress to navigate the relationship between their religious beliefs and everyday social life. Sandıkcı and Ger (2010) further show that, in certain contexts, veiling has moved beyond being viewed only as a stigmatized practice and has become connected with fashion, consumption and personal expression.

Building on these perspectives, the present study shifts attention to the educational experiences of young Muslim women. Rather than focusing primarily on established designers, fashion consumers or social media influencers, it looks at women who are still learning, experimenting with design and beginning to imagine their professional futures. This stage is particularly significant because choosing to study fashion is itself a statement of aspiration. By entering a fashion programme, these students engage with a field whose practices and conventions may sometimes appear difficult to reconcile with religious expectations. Yet they have consciously chosen to enter this space.

The study therefore explores four interconnected questions: Why do young Muslim women choose to pursue fashion design? How do they experience and negotiate hijab and modesty within fashion education? What influence do family, community and educational institutions have on their decisions? And how do they envision their future careers within the fashion industry?

The central argument of the study is that pursuing fashion education does not necessarily represent a move away from religious identity. For these young women, fashion education can instead become a space where creativity, personal aspirations and religious values come together. Their experiences suggest that identity is not always formed by choosing between faith and fashion; rather, it can be continuously shaped through the ways they express, negotiate and ‘dress’ the self within both worlds.

2. Fashion, Hijab and the Negotiation of Identity

Research on Muslim women's dress has increasingly moved beyond the traditional debate of whether the hijab represents oppression or empowerment. Earlier discussions often placed veiling within a narrow framework, interpreting it either as an expression of patriarchal control or as a symbol of religious devotion. More recent scholarship, however, recognises that the hijab can carry different meanings depending on a woman's personal experiences, social environment, culture and circumstances. Bilge (2010), for instance, approaches the agency of veiled Muslim women from an intersectional perspective, while Tarlo (2010) highlights how dress can allow Muslim women to negotiate multiple aspects of their identity at the same time.

Ahmed L. (2011) similarly situates the renewed visibility of the veil within changing social and political contexts. The meaning of the hijab, therefore, cannot be assumed to be the same for every woman. For some, it may reflect religious commitment or modesty; for others, it may express belonging, cultural identity, personal choice or several of these meanings simultaneously. Research also suggests that understandings of Islamic dress differ considerably across cultural and community contexts (El Guindi, 1999; El Guindi & Zahur, 2009).

This complexity becomes particularly interesting when considered in relation to fashion. If clothing is one of the ways through which people communicate identity, then the hijab cannot simply be placed outside the world of fashion. The growing visibility of modest fashion illustrates how religiously informed dress can engage with contemporary aesthetics, markets and changing ideas of style. Gökarıksel and Secor (2010) explore the connections between Islamic dress, consumption and fashion, demonstrating that modest clothing participates in contemporary consumer culture rather than existing separately from it. Lewis (2015) similarly presents Muslim fashion as an evolving and diverse style culture rather than as a fixed or uniform category.

The work of Sandıkcı and Ger (2010) is particularly relevant to this discussion. Their research shows how veiling can take on new meanings as it becomes part of contemporary fashion practices. This does not necessarily suggest a rejection of religious values. Instead, fashion can provide new ways for women to express and make visible their faith while engaging with contemporary forms of style and self-presentation.

The question of agency is equally important in understanding these choices. Mahmood (2005) challenges the idea that agency must always be expressed through resistance to established traditions. Agency can also be found in the ways individuals consciously adopt, interpret and negotiate practices that are meaningful to them. This perspective offers a useful way of understanding young Muslim women studying fashion. A student who chooses to design modest clothing, for example, may not see herself as challenging religion. Rather, she may be using her creativity and professional aspirations within a religious framework that she personally values.

The concept of cultural hybridity adds another dimension to this understanding. Bhabha (1994) suggests that cultural identities can develop within an 'in-between' space, rather than fitting neatly into fixed categories. This is particularly relevant to young Muslim women navigating contemporary fashion. Their ideas about dress may bring together elements of local culture, global fashion trends, youth aesthetics and religious values. Their choices, therefore, do not necessarily belong exclusively to either 'tradition' or 'modernity'; instead, they may reflect an ongoing process of combining and negotiating different influences.

Building on these perspectives, the present study shifts the focus from Muslim women primarily as wearers or consumers of fashion to their experiences as students, aspiring designers, creators and potential professionals within the fashion industry. It seeks to understand what happens when religious identity and fashion aspirations meet within the educational space and how young Muslim women navigate this intersection while imagining their own futures.

The Indian Context and the Research Gap

The Indian context brings additional dimensions to this discussion. Head covering is not practiced exclusively by Muslim women; different forms of covering can be found across religious and regional communities in India. Even within Muslim communities, ideas about modesty and appropriate dress vary considerably. The literature reviewed for this study suggests that these practices are influenced not only by religious beliefs but also by family expectations, community norms, cultural backgrounds and individual circumstances.

The educational and professional experiences of Muslim women also need to be understood within the broader socio-economic conditions in which

they live. Earlier studies have highlighted the various constraints women may experience in areas such as mobility, employment, dress and family expectations (Jeffery & Jeffery, 2006). The Sachar Committee Report (Government of India, 2006) similarly drew attention to the educational and socio-economic challenges experienced by sections of India's Muslim population.

At the same time, these circumstances are changing. The literature points towards increasing participation of Muslim women in education and employment, along with evolving aspirations among younger generations. The growth of digital platforms and the emergence of modest fashion have further expanded the ways in which Muslim women can engage with the fashion industry. They are increasingly visible not only as consumers but also as entrepreneurs, designers, content creators and influencers. Lewis (2015), Jones (2019) and Moors (2020) highlight the growing connections between modest fashion, digital culture and economic participation.

An important gap identified in the existing literature is that much of the research has focused on Muslim women as consumers, established designers, entrepreneurs or participants in Western fashion contexts. Comparatively less attention has been given to the experiences and motivations of young Muslim women who are entering fashion education, particularly within the Indian context. The present study addresses this gap by focusing on women at an early and primary stage of their journey towards becoming fashion professionals.

This distinction is important because choosing fashion is not the same as simply consuming it. There is a significant difference between asking what Muslim women choose to wear or purchase and asking why a young Muslim woman chooses to **learn how to create fashion herself.** The shift from consumer to creator opens up questions of aspiration, creativity, identity and agency and provides a valuable lens through which to understand how young Muslim women envision their place within the fashion industry.

4. Methodology

The study used an exploratory qualitative design grounded in an interpretivist approach. This approach was chosen because the research aimed to understand how young Muslim women themselves make sense of fashion, faith, modesty and their decisions to pursue a career in fashion. Rather than

reducing these experiences to numerical measures, the study focused on the meanings and interpretations participants attached to their own experiences.

The research involved young Muslim women studying fashion across three colleges in Maharashtra. Data were collected through focus group discussions, followed by individual interviews, to gain a deeper understanding of their motivations, experiences and perceptions. Approximately eight to ten focus group discussions were conducted. In addition, six semi-structured interviews were held with experts from fields such as fashion design, Islamic studies and related areas. Their perspectives provided an additional lens through which the experiences shared by the students could be understood.

The focus group discussions were deliberately kept open-ended, allowing participants to express their views in their own words and respond to the experiences and opinions of others. This format also created space for issues to emerge naturally that might not have been captured through a more structured questionnaire. The expert interviews complemented these discussions and provided another perspective for interpreting and contextualising the student narratives.

The interviews and group discussions were transcribed and analysed using thematic analysis. The researchers engaged closely with the transcripts, reading them repeatedly, identifying meaningful patterns and developing both descriptive and interpretive codes. These codes were gradually brought together into broader categories and themes. In total, 35 initial codes were identified, which were first organised into 14 provisional themes and subsequently refined into four overarching themes.

The analysis was guided by the six-phase thematic analysis framework proposed by Braun and Clarke. Throughout the process, attention was also given to researcher positionality, recognising that researchers bring their own experiences and perspectives to the interpretation of qualitative data. This helped ensure that the analysis remained closely connected to participants' accounts rather than being shaped primarily by preconceived assumptions.

Ethical considerations were given particular importance throughout the study. Participants were informed about the nature of the research and their involvement, while confidentiality and anonymity were maintained. Since discussions around religion, family expectations, hijab and personal choices can be sensitive, care was taken to create a respectful and comfortable environment in which participants could share their experiences openly.

5. Findings and Discussion

5.1 Fashion as Passion, Identity and Self-Expression

One of the strongest findings was that participants rarely viewed fashion simply as an interest in following trends. For many, their connection with fashion had developed much earlier, often through everyday creative activities such as drawing, embroidery, stitching, dressing dolls or experimenting with clothes. Pursuing fashion education therefore appeared less like a sudden career decision and more like a natural continuation of an interest and creativity that had been part of their lives from an early age.

Participants also spoke about fashion as an important part of their personality and self-expression. They described it as a "way to express your personality" and, for some, even as a "lifestyle." These perspectives suggest that their interest in fashion went beyond clothing as a product. Fashion was closely connected to how they understood themselves and how they wished to present themselves to others.

A sense of confidence also emerged repeatedly in the participants' narratives. Clothing and design were described as ways of feeling attractive, comfortable, presentable and more confident. This is reflected in the recurring codes identified during the analysis, including "Fashion as Self-Expression," "Identity Through Fashion," and "Confidence Through Fashion."

These findings are consistent with Kaiser's (2012) understanding of fashion as a cultural practice through which identity is both expressed and shaped. They also correspond with Bernier's (2023) observation that Muslim women engaged in fashion can reinterpret and reshape clothing creatively rather than being restricted to conventional or stereotypical representations of Muslim dress.

Importantly, the participants did not describe their interest in fashion as a way of distancing themselves from their religious identity. Instead, fashion education appeared to provide them with a space where creativity, personal expression, confidence and religious identity could exist alongside one another. In this sense, fashion became not an alternative to their faith, but another way of expressing and understanding different aspects of who they are.

5.2 Modesty as a Design Resource Rather Than a Barrier

One of the most prominent themes emerging from the study was the perceived compatibility between hijab, modesty and fashion. The thematic analysis identified 47 excerpts relating to "modesty, hijab and fashion compatibility," making it the most frequently discussed theme. This was followed by social and institutional ecology with 29 excerpts, passion, identity and self-expression with 28 and future orientations with 8.

The significance of this finding goes beyond the number of references. What stood out was the way participants themselves understood the relationship between modesty and fashion. Rather than seeing the hijab as something that prevented them from studying or engaging with fashion, they described practical ways of adapting fashion to their understanding of modest dress.

Participants spoke about using opaque fabrics, layering, fuller silhouettes, longer hemlines and greater garment volume to create designs that remained fashionable while meeting their expectations of modesty. They discussed combinations such as loose trousers with longer tops, scarves and jilbabs, while also exploring different colours, fabrics and styling possibilities.

This represents an important shift in how modesty can be understood within fashion. For these participants, modesty was not necessarily a rule that restricted creativity; instead, it could become a **design consideration or parameter**. Working within certain boundaries sometimes encouraged them to think differently and search for creative solutions. Their interest in designing innovative abayas, hijabs and other modest garments illustrates how religious considerations can become part of the creative design process.

At the same time, participants did not suggest that every fashion opportunity should take priority over their religious values. When they felt that a particular design requirement or professional expectation crossed a boundary that was important to them, religious considerations generally took precedence. Some also described modifying their clothing choices depending on the situation, taking into account factors such as privacy, safety, comfort and social context.

This finding can be understood through Mahmood's (2005) concept of agency. Agency does not always have to take the form of challenging or rejecting established religious practices. It can also involve the ability to interpret those practices, make personal choices within them and creatively incorporate them into everyday life. In this context, choosing to work with

modesty rather than against it can itself be understood as an expression of agency.

The expert interviews further supported this interpretation. Religious scholars generally viewed hijab and contemporary fashion as compatible as long as the underlying principles of modesty were respected. Academic and industry experts also acknowledged the growing space for modest fashion, while some raised concerns about highly decorative or attention-oriented designs that could, in their view, conflict with the ethical intentions associated with modesty.

The findings therefore do not suggest that the relationship between religion and fashion is completely free of tension. Instead, they reveal something more nuanced: the space between religious values and fashion can itself become a space for negotiation, experimentation and creativity. For these young women, the challenge was not necessarily to choose between faith and fashion, but to find ways of making the two work together.

5.3 Family, Community and the Institutional Environment

Individual choices did not take place in isolation. Family emerged as one of the strongest influences on participants' decisions about education and career. Interestingly, family influence was not experienced in the same way by everyone. While some participants encountered expectations and restrictions, others spoke about considerable encouragement from their parents, particularly from fathers who recognised their daughters' abilities and supported their interest in fashion. One participant recalled, "My father said I have it in me and got me admission."

At the same time, support from immediate family did not always extend to the wider family or community. Some participants spoke about relatives questioning their decision to study fashion or encouraging them to choose more conventional career paths. This is reflected in the recurring codes **"Family Support," "Family Expectations Conflict,"** and **"Community Resistance."** Taken together, these accounts suggest that family cannot simply be described as either supportive or restrictive. The same social environment may provide encouragement while also creating expectations that young women have to negotiate.

This complexity also challenges the familiar idea of a straightforward conflict between a "traditional family" and a "modern daughter." Participants' experiences were more layered than this binary suggests. Family could

simultaneously act as a source of emotional and practical support and as a space where expectations about appropriate education, career, dress and behaviour were negotiated.

Public attitudes also shaped participants' everyday experiences. Some recalled being questioned or criticised because of their hijab or modest clothing, particularly in public or social settings. Others appeared more confident in responding to such reactions and emphasised their right to make their own choices. One participant captured this sense of personal agency by stating, "You are your own leader; you can do whatever you want."

Educational institutions emerged as an important mediating space between individual aspirations and these wider social influences. Most participants described their colleges as relatively supportive environments where wearing the hijab did not prevent them from participating in academic or creative activities. Academic experts similarly pointed to the importance of inclusive institutions in creating an environment where young women could pursue their interests without feeling that their religious identity was a barrier.

This finding is particularly significant in the context of fashion education. A supportive educational environment can influence how students perceive their possibilities. When students feel accepted within the classroom and studio environment, fashion can become a realistic and legitimate career pathway rather than a field that appears incompatible with their religious identity.

The expert interviews also highlighted the diversity within these experiences. Differences related to urban and rural settings, family values, community expectations and individual interpretations of religious practice were repeatedly recognised. These differences caution against treating Muslim women as a single, homogeneous group. The participants' experiences demonstrate that educational aspirations are shaped by multiple and intersecting social contexts and that fashion is one of many possible pathways through which young Muslim women may choose to build their futures.

Overall, the findings suggest that agency is shaped not only by individual choice but also by the social environments in which those choices are made. Family support, community attitudes and institutional acceptance can either create space for young women to pursue their aspirations or make that process more difficult. The participants' narratives therefore show that choosing

fashion is not simply an individual decision; it is a process negotiated within relationships, institutions and wider social expectations.

5.4 From Student to Designer: Imagining a Future in Modest Fashion

The fourth theme relates to how participants envisioned their lives and careers after completing their fashion education. Many expressed an interest in starting their own boutiques, developing home-based businesses or pursuing entrepreneurship. These options were appealing not only because they offered opportunities to earn an income, but also because they could provide greater independence, flexibility and control over the way they worked while remaining compatible with their personal, family and religious expectations.

A particularly strong aspiration was to work within modest fashion. Participants perceived a growing need for clothing that could be contemporary and fashionable while still reflecting their understanding of modesty. They spoke about the possibility of designing abayas, hijabs, long dresses and fusion garments that could appeal to younger consumers. One participant expressed a desire for "more innovation in modest fashion," while another suggested creating hijabs and abayas "in different styles."

For these participants, entrepreneurship therefore meant more than simply starting a business. It represented an opportunity to have greater control over their professional lives and to create working environments that suited their circumstances. A home-based studio or boutique, for example, could offer flexibility in managing professional responsibilities alongside family commitments. Entrepreneurship thus emerged as both an economic aspiration and a way of negotiating personal and social priorities.

The expert interviews broadly supported these aspirations. Industry professionals viewed modest fashion as an increasingly relevant area of the fashion sector rather than a marginal niche. Religious scholars also generally considered fashion design compatible with religious values when the work remained within the boundaries of modesty and ethical practice.

These findings also connect with Al-Dajani and Marlow's (2013) discussion of entrepreneurship as a potential pathway towards empowerment. Economic participation can create greater opportunities for women to make independent choices, while entrepreneurship can also allow them to build professional identities that reflect their own priorities and values.

Taken together, the findings point towards an important shift from wearer to creator. The young women in this study are not only looking for modest clothing that fits their needs; some are imagining themselves as the designers, entrepreneurs and decision-makers who can shape what modest fashion looks like in the future. In this sense, fashion education becomes more than preparation for employment—it becomes a pathway through which they can turn their creative interests, religious values and professional aspirations into their own forms of expression.

6. Rethinking Agency: Beyond the Faith-versus-Fashion Binary

Taken together, the findings suggest that describing the relationship as "fashion versus hijab" does not adequately reflect the experiences of the young women in this study. Their narratives point towards a more nuanced understanding. Rather than asking whether they must choose between faith and fashion, a more meaningful question is how fashion can be practised, created and experienced in ways that remain consistent with their understanding of faith.

This distinction has important theoretical implications. Mahmood's (2005) concept of agency helps explain why following religious practices should not automatically be understood as a lack of independence or choice. Agency can also be expressed through the ways individuals interpret and consciously live within practices that hold meaning for them. Similarly, Lewis (2015) demonstrates that modest fashion can be an active and creative way of negotiating identity, while Bhabha's (1994) concept of hybridity provides a useful lens for understanding identities that develop between and across cultural influences rather than fitting neatly into fixed categories.

The experiences of the participants reflect this kind of negotiated space. They engage with mainstream principles of fashion education, experiment with contemporary silhouettes and materials, remain aware of global trends and use digital platforms, while at the same time maintaining personal boundaries around modesty. Their experiences therefore cannot be easily placed into categories such as "traditional" or "Westernised." Instead, their identities and choices are continuously negotiated.

The idea of "dressing the self" captures this process particularly well. Dress becomes more than an outward appearance; it becomes one of the ways in which these women work through questions of identity, faith, self-expression and aspiration. What they wear reflects not only how they

understand themselves but also how they wish to be perceived and the kind of future they hope to create.

The findings also point to the important role of the educational environment in this process. The fashion classroom can become a space where assumptions about what fashion should look like are questioned and reconsidered. The presence of hijab-wearing students demonstrates that there is no single way of participating in fashion and that creativity can take many different forms.

Seen in this light, these young women are not simply entering an established fashion system. Through their choices, design interests and aspirations, they may also be expanding the boundaries of what fashion can include. Their presence introduces new perspectives on modesty, identity, creativity and representation, potentially contributing to a more diverse understanding of contemporary fashion.

7. Conclusion

This study sought to understand why young Muslim women choose to pursue fashion design despite the commonly held perception that the hijab and religious modesty may conflict with contemporary fashion. The findings suggest that this perceived contradiction is not as straightforward as it may initially appear.

For the participants, the decision to study fashion was closely linked to creativity, interests that had often developed from an early age, self-expression, confidence and the desire to build a professional identity. Fashion was not understood simply in terms of appearance or following trends. Instead, it offered a way for participants to express their personalities, explore their creativity and imagine themselves within a professional field.

At the same time, religious identity remained an important part of their experiences. Participants generally did not feel that they needed to give up modesty in order to participate in fashion. Instead, they described adapting silhouettes, fabrics, layering and styling practices to fit their understanding of modest dress. When they perceived a clear conflict between a fashion requirement and an important religious principle, faith generally took priority. The prominence of the theme relating to hijab, modesty and fashion compatibility reinforces how central this negotiation was to their experiences.

Family, community and educational institutions also shaped this process. While some participants encountered questions or resistance from relatives

and community members, others received considerable encouragement from their parents, particularly their fathers. Colleges, meanwhile, often provided supportive environments in which students could pursue fashion education without their religious dress becoming a significant barrier. These experiences highlight that the choices of young Muslim women are shaped not only by individual preferences but also by the social environments surrounding them.

The participants' aspirations for the future further demonstrate the broader significance of fashion education. Many expressed interest in boutique ownership, home-based businesses and entrepreneurship, with modest fashion emerging as a particularly meaningful area of interest. These aspirations indicate that the participants do not see themselves only as consumers or wearers of fashion. Some also imagine themselves as designers, entrepreneurs and creators who can influence what modest fashion looks like in the future.

The central contribution of this study is therefore to move beyond the familiar "tradition versus modernity" framework. The experiences of these young Muslim women suggest that they are not necessarily required to choose one identity over another. Instead, they negotiate multiple aspects of identity simultaneously. Religious commitment, creativity, professional ambition and engagement with contemporary fashion can coexist, although bringing these different dimensions together may require ongoing negotiation.

The title "Dressing the Self" captures this process. Dressing is not simply an outward act of choosing clothes; it can also become a way of expressing identity, creativity, values and agency. For the women in this study, fashion education appears to provide a space not to move away from their faith, but to explore what it means to be creative, professionally ambitious and religiously grounded at the same time.

The study also points towards several areas for future research. Following these women beyond their educational years and into employment or entrepreneurship could provide valuable insight into whether the aspirations they expressed as students translate into actual opportunities within the fashion industry. Further research could also explore how fashion curricula can engage more meaningfully with modest fashion and how educational institutions can accommodate diverse religious and cultural expressions without reducing them to stereotypes. Such research could contribute to broader conversations around fashion, gender, religion, education, identity and women's agency.

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6
CHAPTER

Human-in-the-Loop Generative AI for Financial and Managerial Decision-Making: Automation Bias, Explainability and Risk Governance

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Abstract

The applications of GenAI in financial analysis, strategic planning and managerial decision making aren't limited to the quality of the output—they are also about the balance between models and humans. Based on the available evidence, the two AI functions of information synthesis and option generation/scenario exploration are more effective at GenAI, whereas the choice phase is more prone to calibration errors and over-reliance. Confidentiality, third-party concentration, market-correlation and auditability risks are added to these vulnerabilities in the financial world. The chapter suggests a seven-stage approach to both the separation of generation from independent verifier and the explicit retention of human decision powers. It also covers the following practical controls: Provenance, communicating uncertainty, multi-model or multi-prompt checking, role-based approval and outcome monitoring. The main message is that GenAI is not a decision-

making system, but it is a decision-making that supports system. The framework connects productivity in the workplace and quicker analysis with transparent verification responsibilities, escalation processes and audit trails to ensure that productivity increases do not come at the cost of human accountability.

Keywords: generative AI; human-AI collaboration; managerial decision-making; automation bias; risk governance; finance

1. Introduction

Large language models have transformed the economics of decision support by being able to read, summarise, compare and generate text-based analyses at very low marginal cost. In organizations, this means that AI is not just about prediction anymore, but also about problem framing, scenario construction, due-diligence support and strategic evaluation. Recent reviews do not focus on a single forecasting task but rather map GenAI to multiple decision stages and Schulte and Kanbach (2026) list a variety of generative tasks, including attention, intelligence, design, choice, implementation and feedback. The potential is therefore great: managers can interrogate large sets of documents, develop alternatives and drafts for decision making much faster than with traditional workflows.

The same capabilities pose a quality-control issue. GenAI can confidently state inaccurate or poorly supported statements as if they were accurate. Experimental findings on automation bias indicate that users can accept incorrect AI support despite their own reasoning leading to a superior solution (Wingerter *et al*., 2025). Similarly, Doshi *et al*. (2025) discover that individual model evaluations can be inconsistent or biased, but aggregation across models, prompts or roles can help align with expert judgement. The message is not that GenAI is not useful for important decisions, but that the architecture of use is important.

This question is particularly relevant in the fields of finance and risk management. The literature on GenAI in finance has focused on its use in decision-making, sentiment analysis, ESG analytics, fraud and explainable AI and the importance of interpretability and governance gaps (Ali *et al*., 2025). The Financial Stability Board (FSB, 2024) and the Bank for International Settlements (Crisanto *et al*., 2024) also highlight model risk, data challenges and third-party reliance and other risks linked to broader use of AI in financial services. This chapter thus poses the question: What are the areas where GenAI can be most useful in organizational decision-making, where human

judgment is most essential and what are the safeguards that can enhance the reliability of the hybrid system?

2. Methodology and Analytical Scope

The chapter employs a narrative review of peer-reviewed studies and institutional guidance, structured around a decision-workflow narrative. The search terms included "generative AI", "large language models", "decision support", "organizational decision-making", "finance", "automation bias", "explainability", "human oversight" and "AI risk management". Recent systematic reviews, empirical research on human-AI decision making and authoritative risk-governance documents such as NIST, FSB and BIS were prioritized. Given the fast pace of change in GenAI tools and model capabilities, the analysis focuses on mechanisms that are likely to endure, such as task allocation, verification, provenance, calibration and accountability.

Table 1: Selected evidence informing the chapter

Source	Evidence base	Main finding	Use in this chapter
Schulte & Kanbach (2026)	Systematic review; 68 publications	GenAI performs different roles across six recurring decision components.	Supports stage-specific task allocation.
Doshi *et al*. (2025)	Two studies of strategic business-model evaluation	Single AI evaluations can be inconsistent; aggregation improves alignment with experts.	Supports independent and plural checking.
Wingerter *et al*. (2025)	Controlled experiment on faulty AI assistance	Users can over-rely on plausible AI output; warning nudges reduce the effect.	Supports calibrated reliance and interface controls.
Ali *et al*. (2025)	Review of 84 finance studies	Finance applications are expanding, alongside bias, transparency and governance concerns.	Connects decision support to finance-specific risks.
FSB (2024); Crisanto *et al*. (2024)	Financial-sector risk and regulatory analyses	AI may amplify model, data, cyber, third-party and concentration vulnerabilities.	Extends controls from individual decisions to institutions.

3. Where Generative AI Adds Decision Value

3.1. Intelligence and design: compressing search and expanding alternatives

The best argument for GenAI is often upstream of the final decision. In the intelligence phase, models can summarise long reports, identify recurring themes, compare policy or business documents, translate technical jargon and help decision makers identify questions that warrant further investigation. They can create scenarios, alternative strategies, counterarguments and

structured checklists during the design phase. Large language models expand the role of traditional decision-support systems, as the same interface can be used for information collection, interpretation and option generation (Handler *et al*., 2024). Schulte and Kanbach (2026) also conclude that the use of GenAI is spread throughout the decision-making process, particularly for attention, intelligence and design tasks.

These capabilities are useful because managerial decisions are limited by time and bounded attention. However, productivity is not synonymous with validity. A short synthesis may not mention an inconvenient source; a seemingly exhaustive list of alternatives may be framed; a convincing explanation may be unsupported. The unit of analysis is thus not "AI output" but a controlled workflow that results in an intermediate artefact that can be challenged.

3.2. Choice: the point at which fluency becomes most dangerous

The choice phase is a higher evidentiary standard because a recommendation can directly impact capital allocation, pricing, hiring, investment, credit or strategic commitment. Doshi *et al*. (2025) demonstrate the need for caution when using single-model judgement, as individual judgements can differ between prompts or roles, while aggregation can help stabilize judgements. This indicates a pragmatic difference between the use of GenAI to provide reasons and the use of GenAI to make the decision. The former can expand the set of options, the latter can mask sensitivity to the wording of the prompt, lack of context or stochastic variation. In high impact environments, the human role is not just to "approve" an AI answer. It is to set goals, determine what evidence is relevant, to test hypotheses, to resolve value conflicts and to take responsibility for the action.

4. A Human-in-the-Loop Decision Architecture

1. Frame the decision	**2. Ground the evidence**	**3. Generate / analyse**	**4. Verify independentl y**	**5. Human judgeme nt**	**6. Implement**	**7. Learn & audit**
→ Scope, objectiv e, decision rights	→ Approve d data, retrieval, constraint s	→ Summarie s, scenarios, alternative s	→ Cross-check sources, numbers, logic	→ Accept, revise or reject output	→ Record action and accountabilit y	Measure outcomes; update prompts/contro ls

Fig 1: Seven-stage human-in-the-loop architecture for GenAI-supported financial and managerial decisions. Source: Authors' conceptualisation based on NIST (2024), FSB (2024) and recent human-AI decision research.

Figure 1 shows the separation between the generation and the verification. Stage 1 refers to the decision, goal, constraints and decision rights prior to calling the model. Stage 2 anchors the task in recognized evidence, preferably as a structured retrieval task or a data environment created for the task and not as a free invention of the task. Stage 3 employs GenAI to synthesize, analyze scenarios or generate other options. Stage 4 intentionally includes friction: numerical statements, citations, causal statements and key assumptions are examined individually and compared with the source information or a second approach. Stage 5 holds back evaluation in terms of substance and approval until the human decision-maker is responsible. Implementation is then recorded and Stage 7 compares the outcomes against what was expected to make sure that prompts, controls and data will be improved.

This structure works well to overcome a major limitation of conversational AI: persuasive language can make a system seem more reliable than the supporting evidence warrants. Wingerter *et al*. (2025) present experimental data showing that faulty AI assistance can impair user performance; warning nudges about possible over-reliance can partially mitigate this effect. Therefore, a good workflow should include visible uncertainty, checkpoints for verification and the authority to reject the model. “Human-in-the-loop” does not imply mechanical approval by a person, but rather review by someone who has the information, authority and time to disagree.

4.1. Explainability, provenance and calibrated reliance

Explainability for generative systems should be understood operationally. A fluent rationale does not always provide a faithful explanation of how the model arrived at its answer. Useful controls are source provenance, retrieval traces, versioned prompts, explicit assumptions, uncertainty flags and reproducible calculations outside the language model. NIST's Generative AI Profile (Autio *et al*., 2024) emphasizes the need for monitoring and documentation, aligning with the lifecycle approach to GenAI risk management. In practice, calibrated reliance is the idea of increasing human involvement when evidence is weak, stakes are high and the task is one that requires value judgement and decreasing human involvement when the task is reversible, well-bounded and easily verified.

5. Financial and Managerial Risk Governance

Table 2: Risk-control matrix for GenAI-supported decisions

Risk	How it enters the decision	Possible consequence	Control
Hallucination / factual error	Unsupported claim, invented citation or incorrect calculation	Wrong forecast, memo, valuation input or recommendation	Grounded retrieval; source checking; external calculation.
Automation bias	User accepts fluent output without sufficient challenge	Poor choice despite available contradictory evidence	Independent first judgement; warning nudge; mandatory challenge step.
Data confidentiality	Sensitive data is exposed to an unsuitable model or provider	Privacy, contractual or competitive harm	Approved environments; data minimisation; access controls.
Model / prompt instability	Output changes materially across wording, model or run	Inconsistent strategic or financial judgement	Sensitivity testing; multi-prompt or multi-model aggregation.
Third-party concentration	Critical workflow depends on a small set of external AI providers	Operational dependency and correlated failure	Fallback process; vendor due diligence; model inventory.
Weak auditability	No record of evidence, prompt, model version or approval	Difficult review, accountability and learning	Decision log; provenance record; named human approver.

5.1. Verification patterns for real workflows

There are three levels of verification design. At the evidence level, the reviewer verifies if the cited document, number or event supports the generated claim. At the reasoning level, the reviewer checks if the conclusion is supported by the evidence and if the reviewer has taken into account plausible counterarguments. The organization reviews at the system level to determine if there are consistent error trends due to model selection, prompt design, retrieval settings or vendor dependence. These levels should not be reduced to a single "fact-check" step. A model can cite a correct number but make an incorrect inference from it or one run can be correct, but the workflow can be fragile because a slight change in the prompt can change the recommendation.

This separation is especially helpful for financial analysis. Deterministic software can be used to reproduce arithmetic, ratios and time-series calculations, judgemental recommendations can be challenged by a second human or model-based challenge and statements can be tied to document identifiers. The objective is not to make every decision slow. Lightweight

checks can be used for routine, low impact decisions and heavier checks and approvals can be used for irreversible or high value decisions. This risk-tiered approach ensures that oversight is commensurate with the impact of failure, not every GenAI interaction.

Finance introduces two complications to the ordinary managerial use. Leverage, speed or correlated behaviour can amplify an error. According to the FSB (2024), broader AI use can interface with third-party dependencies, market correlations, cyber risk and model risk. BIS analysis also highlights that generative AI introduces well-known model and data-governance challenges and introduces specific risks of hallucination and anthropomorphic trust (Crisanto *et al*., 2024). Second, financial decisions require a repeatable process from data to conclusion. A language model is thus best used in conjunction with deterministic tools for arithmetic, databases for evidence and an audit trail to differentiate between machine-generated analysis and approved human judgement.

Four propositions for future research are derived from the combined literature. P1: GenAI will enhance decision performance more consistently in intelligence and design tasks than in final choice tasks. P2: Independent verification will be most useful when output fluency is high and evidence quality is not easily discernible by users. P3: Multi-model, multi-prompt or multi-role aggregation will decrease the idiosyncratic evaluation variance but will not remove the shared data or model-family bias. P4: Human oversight will only enhance outcomes if the reviewer has real decision making power, domain expertise and enough time to question the system.

5.2. Decision criticality and control intensity

A practical extension of the risk-tiered approach is to match control intensity to the reversibility, financial exposure and accountability requirements of the decision. Routine uses such as summarising an internal document, drafting a checklist or comparing already-approved information can often be handled with lightweight verification because the output is easy to check and the consequences of error are limited. A middle tier includes tasks such as scenario development, preliminary forecast commentary, vendor comparison or management reporting, where the model may influence interpretation even if it does not make the final choice. These uses benefit from source checks, explicit assumptions and a second review before the output enters a formal decision process. A high-impact tier covers decisions involving capital allocation, credit, pricing, hiring, regulatory reporting or irreversible

strategic commitments. Here, independent evidence review, reproducible calculations, named human approval and a retained decision log should be treated as part of the workflow rather than as optional quality assurance. This is consistent with the lifecycle logic in NIST guidance and with the financial-sector concern that model, data and third-party risks can become more consequential when AI is embedded in critical processes (Autio *et al*., 2024; FSB, 2024).

The same logic also helps define decision rights. A person who approves a GenAI-supported recommendation should be able to see the relevant evidence, understand the important assumptions and request a different analysis. If the reviewer lacks access to the source material, has no time to challenge the result or is expected to approve the model by default, the presence of a human reviewer adds little protection. Role-based approval is therefore most useful when responsibility is assigned before the model is used. For example, an analyst may prepare the evidence set, GenAI may generate alternatives, a separate reviewer may test calculations and citations and a manager may retain the final decision right.

This distinction minimizes the possibility of confusion between fluency and validation and simplifies subsequent audit. It also resolves the automation-bias issue that Wingerter *et al*. (2025) note, since the workflow includes a challenge step rather than the users having to spot a plausible error when engaging in normal dialogue with the model.

Plural checking is not a mechanical process but can be added selectively. Doshi *et al*. (2025) demonstrate that prompt, role or model aggregation can enhance consistency with expert judgement in cases where individual judgements are volatile. Practically, this may involve requesting a second prompt to question the initial analysis, comparing the results of more than one model or a human reviewer to test the best counterargument. Nevertheless, consensus among various outputs cannot be regarded as evidence of accuracy. The same framing can be reproduced by models sharing training data, assumptions or failure modes and repeated prompts. Plural checking is thus aimed at revealing sensitivity and disagreement, rather than establishing a false sense of certainty. To be used financially and managerially, the best control is a traceable route between source evidence and calculation, judgement and final approval.

6. Implications and Research Agenda

The most helpful organizational question is not whether GenAI will "replace" or "assist" managers in general. Repetitive synthesis, document comparison and option generation are natural areas where there is potential for greater AI support. Human responsibilities are confused goals, ethical decisions, irreversible commitments and final responsibility. Hence, the focus of research should be on the quality of the decision at the workflow level and not just on the accuracy of the model. There is also a financial issue whether the same set of foundation models are used across institutions and analysts and thus generate correlated judgement errors.

The key takeaway for the future of work is that productivity gains should be co-designed with accountability, not as distinct goals. The time spent on document review and initial analysis and option generation can be reduced with GenAI, potentially freeing up time for managers to focus on judgement, negotiation and implementation. The benefit is not so obvious when faster production merely leads to more uncontrolled output. Digital leadership thus involves determining what tasks can be delegated to a model, what evidence should be kept in view of the employees and when a decision should be escalated to a more experienced reviewer. The human-in-the-loop architecture offers a means to make these choices explicit. It also proposes that organizations should not only measure the accuracy of the model or the time saved, but also. Useful outcomes are the frequency of corrections, the number of unsupported claims that are detected before use, the stability of recommendations across prompts, the time needed to verify and whether or not accountable decision makers actually use their authority to revise or reject model output. These measures relate to the use of AI in the workplace and its impact on productivity, rather than the automation itself being the goal. They also establish a more transparent research agenda for studying the conditions under which human-AI collaboration enhances decision quality and when it simply speeds up the shortcomings of organizational processes.

7. Conclusion

Generative AI can enhance the speed and breadth of financial and managerial analysis; however, it is not a foolproof solution for sound decision-making. The human-in-the-loop approach is therefore not a cosmetic safety measure, but a mechanism for making a probabilistic text generator into a more accountable and responsible decision-support system. The ultimate goal is to keep the scale and agility of GenAI while making evidence, uncertainty,

authority and responsibility transparent in the decision-making process.

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7
CHAPTER

From Consent to Capability: Digital Financial Literacy, Open Finance and Consumer Protection in India's Account Aggregator Ecosystem

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Abstract

India has introduced a paradigm shift for the flow of financial information among regulated institutions with the Account Aggregator (AA) framework. The network has now reached a significant scale. This growth, however, raises the question that transaction numbers cannot answer—the quality of customer consent and how well customers understand what they approve. This chapter reviews the AA ecosystem from the perspective of digital financial literacy, open finance and consumer protection. It is based on secondary evidence from the directions issued by the Reserve Bank of India, statistical data of various companies in the Sahamati ecosystem, World Bank materials and recent research on open banking, financial data portability and informed consent. A meaningful consent should also involve an understanding from the customer of the data that is sought, the purpose for which it is sought, who the recipient is, how long or how often it will be accessed and how it can be revoked or modified. In fact, behavioural science research confirms that interface clarity is important because users often opt for digital permissions without thoroughly reading or comprehending them and explanations can help users better

understand them. Therefore, the chapter promotes ‘consent literacy' as a practical implementation of digital financial literacy. The chapter argues that the effectiveness of open finance should be assessed through more than connected entities and granted consents; it should also include understanding, control, equitable use of data and financial outcomes arising from data sharing.

Keywords: Account Aggregator, Consent Literacy, Digital Financial Literacy, Open Finance, Consumer Protection

Introduction

The digital-finance narrative in India is typically one of access – bank accounts, UPI, mobile apps and quicker onboarding. The Account Aggregator framework adds a different layer. It addresses the issue of what occurs once an individual has become a part of the formal financial system and wishes one regulated financial institution to access information that is held by another. In that context, the question is no longer just about whether to use a digital service, but whether to permit a specific set of financial information to flow for a specific purpose.

The regulatory design is deliberately consent-led. Under the RBI’s Account Aggregator directions, financial information cannot be retrieved, shared or transferred without the customer’s explicit consent. The consent artefact must identify the information requested, its purpose, the intended recipient and the creation and expiry conditions. Customers must be told these attributes and must be able to revoke consent, including consent relating to part of the information (RBI, 2024). The AA itself is not meant to become a storehouse of customer data and cannot ask for passwords, PINs or similar authentication credentials.

This architecture matters because transaction data can improve financial decisions. A lender may use verified cash-flow information to assess repayment capacity. A personal-finance application may bring several accounts into one view. An insurer or investment service may use verified information to reduce documentation and improve suitability. Open-banking research shows that customer-controlled data access can encourage FinTech entry and help some consumers obtain advice and credit (Babina *et al*., 2025). In the Indian context, interoperable payment information has also been shown to add predictive value to conventional credit-bureau data in small-business lending (Rishabh, 2026).

The network is no longer small. Sahamati’s dashboard records 390.92

million cumulative fulfilled consents in January 2026 and 493.50 million by June 2026. A wider benchmark shows the scale more clearly: Sahamati reported 248+ million fulfilled consents in June 2025, so the June 2026 figure is about 99 per cent higher than a year earlier (Sahamati, 2025; Sahamati, 2026a). The dashboard defines a fulfilled consent as a consent request against which data were successfully delivered at least once. It is therefore an operational outcome, not a direct measure of how well the customer understood the request.

That gap is the starting point for this chapter. A customer may complete a consent journey correctly and still have a weak grasp of what the approval permits. Research outside finance has repeatedly found that digital users accept privacy statements or permissions without reading them carefully, often because the task is repetitive, lengthy or treated as a necessary step to reach the service (Zhu & Zhang, 2025). The problem is behavioural as much as legal. Information must be available, but it must also be presented at a moment and in a form that a user can reasonably process.

The chapter uses the term consent literacy to describe that practical capability. It does not propose a new legal category. It simply brings an existing part of digital financial life into the financial-literacy discussion: knowing what data are being requested, why they are required, who will receive them, how long the permission lasts and how it can be withdrawn. Seen this way, AA is not only a piece of open-finance infrastructure. It is also a useful test of whether digital inclusion is producing genuine user control.

Objectives

- To examine the current scale and structure of India's Account Aggregator ecosystem using published secondary data.
- To assess the RBI consent architecture from the perspective of digital financial literacy and consumer capability.
- To review recent evidence on open banking, interoperable financial data and access to credit or financial advice.
- To identify behavioural frictions that can separate formal consent from informed consent in digital financial journeys.
- To propose measurable indicators for moving from consent at scale to consumer capability and financial empowerment at scale.

Review of Literature and Research Gap

Open banking and open finance allow customers to permit the movement

of financial information instead of leaving those data locked within one institution. The expected gains are lower information frictions, easier switching and more room for new providers (World Bank, 2024a). The study by He *et al*. (2023) shows that borrower-controlled data sharing can change screening and competition between incumbents and FinTech lenders. Their model also shows why the welfare effect need not be identical for every customer: better information can improve competition while also enabling more precise pricing and segmentation.

Empirical evidence broadly supports these gains but does not justify a simple 'open banking is always beneficial' conclusion. Babina *et al*. (2025) find greater FinTech entry after open-banking adoption and evidence that consumers gain access to advice and credit. Ryan and Mukherjee (2026) find higher digital-payment participation, especially among less-educated adults, but no significant increase in mobile or internet banking transactions per 1,000 adults. Participation and intensity, in other words, do not move in exactly the same way.

Indian evidence adds a credit-information perspective. Rishabh (2026) studies payment histories linked to non-bank small-business loans in India and finds that interoperable payment data improve screening and monitoring beyond traditional credit-bureau information. Payment histories appear to capture current repayment ability, while bureau data capture slower-moving repayment behaviour. The study also identifies distributional trade-offs: data-rich borrowers may benefit more than borrowers who are thin on both bureau and payment records.

A second stream of research concerns willingness to share data. Grassi (2024), studying open-finance data sharing in insurance, shows that customers do not evaluate data requests only in terms of financial benefit; privacy and the perceived value of the proposed service matter as well. Blanchard *et al*. (2026) frame financial-data exchange as a fairness problem involving consumers, incumbent institutions, third-party firms and technical intermediaries. Trust can weaken when customers have little visibility into what is exchanged or how the value created from their data is distributed.

Digital financial literacy provides the third strand. Choung *et al*. (2023) link digital financial literacy to financial well-being and emphasise the ability to protect oneself from digital risks. Chopra *et al*. (2026) place digital public infrastructure within India’s wider financial-inclusion story and argue that infrastructure works best when institutional support and user capability

develop alongside it. These studies support a broader view of literacy that goes beyond operating an application.

The remaining gap is quite specific. Open-finance studies generally focus on competition, credit access or data portability. Financial-literacy studies focus on knowledge, capability and safe use of digital services. The decision to authorise financial data sharing sites between these two literatures but is rarely treated as a capability in its own right. This chapter addresses that gap by separating infrastructure access, consent comprehension and the financial outcome that follows.

Methodology

The study uses a descriptive and analytical secondary-data design. The purpose is not to estimate how many AA users understand consent; no dataset presently supports a defensible population-level estimate of that kind. Instead, the chapter brings together three forms of evidence that can be examined without overstating what the data show: current ecosystem statistics, the formal consent rules and research on open banking and digital-consent behaviour. A primary survey was therefore not used because the present objective is conceptual and evaluative rather than prevalence estimation. Claims about AA-specific comprehension are deliberately avoided.

The monthly ecosystem analysis covers January to June 2026 because Sahamati reports comparable dashboard measures for these months. A one-year benchmark is provided by a public snapshot from June 2025. The regulatory provisions are sourced from the RBI Master Direction as updated in September 2024, while the behavioural and open-finance studies are from the period 2020–2026. The dates are maintained on a source-by-source basis and not as a single panel.

The analysis proceeds in four steps. First, the Sahamati monthly series is used to describe the growth of fulfilled consents. Second, the institutional breadth of the ecosystem is examined through the number of AAs, FIPs, FIUs and live regulated entities. Third, RBI requirements are mapped to the questions a financially capable user should be able to answer before approving a request. Fourth, evidence from open banking, financial inclusion and digital-consent research is used to interpret what the infrastructure can and cannot establish about consumer capability. The result is a structured synthesis rather than a causal test.

Table 1: Secondary Sources and Their Analytical Use

Source	Period / version	Information used	Role in analysis
Sahamati AA Dashboard	Jun 2025 benchmark; Jan-Jun 2026 monthly series	Historical benchmark plus monthly/cumulative consents, accounts and data shares	Measures one-year scale-up and current monthly activity.
Sahamati June 2026 update	June 2026	Operational AAs, FIPs, FIUs and live entities	Describes institutional breadth.
RBI Master Direction - NBFC-AA	Updated Sep 2024	Consent artefact, revocation, data-use and grievance requirements	Defines the formal consent architecture.
World Bank Digital Finance Inclusion (2024b)	2024 policy guidance	Consent, data privacy, consumer understanding and provider responsibility	Frames consumer-protection risks.
Peer-reviewed open-finance literature	2020–2026	Competition, credit, inclusion, transparency and consent behaviour	Interprets likely benefits and behavioural constraints.
UK Open Banking / Australia CDR	Current public frameworks	Consent choice, cancellation, accreditation and oversight	Provides a limited comparative benchmark for consumer control.

Source: *Authors' compilation from the sources cited in the chapter.*

Results / Findings

Growth of the Account Aggregator Ecosystem

Between January and June 2026, cumulative fulfilled consents increased from 390.92 million to 493.50 million, a rise of about 26.2 per cent. Monthly new fulfilled consents remained between 17.85 million and 23.50 million. The pace therefore varied from month to month even while the cumulative series rose steadily. Figure 1 presents both measures so that ecosystem scale is not confused with the current monthly flow.

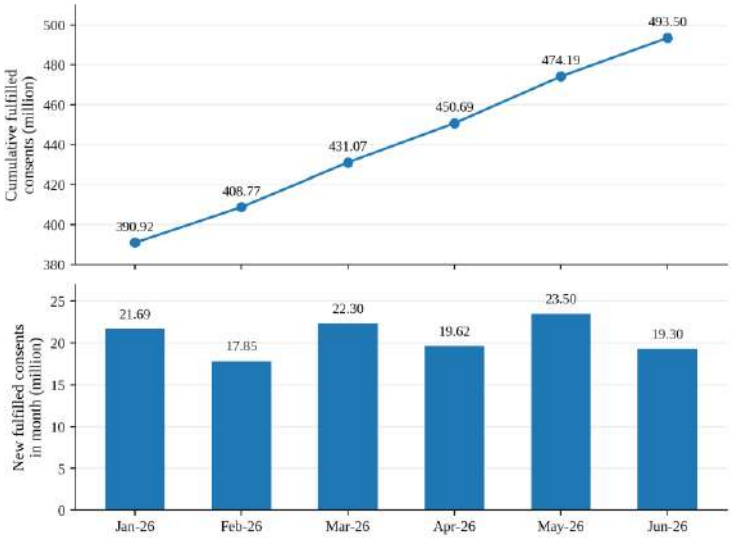


Fig 1: Cumulative and Monthly New AA Consent Requests Fulfilled, January-June 2026

Source: Sahamati Account Aggregator Ecosystem Adoption Metrics (2026a). The monthly series counts new consent requests against which data were successfully delivered at least once.

Institutional participation is also uneven in scale. In June 2026 the ecosystem reported 17 operational AAs, 176 FIPs, 1,076 FIUs and 1,117 live regulated entities. These categories overlap because the same regulated institution may perform more than one role, so they cannot be added to obtain a unique participant count. Figure 2 uses a logarithmic scale only to make the very different role counts readable on one axis.

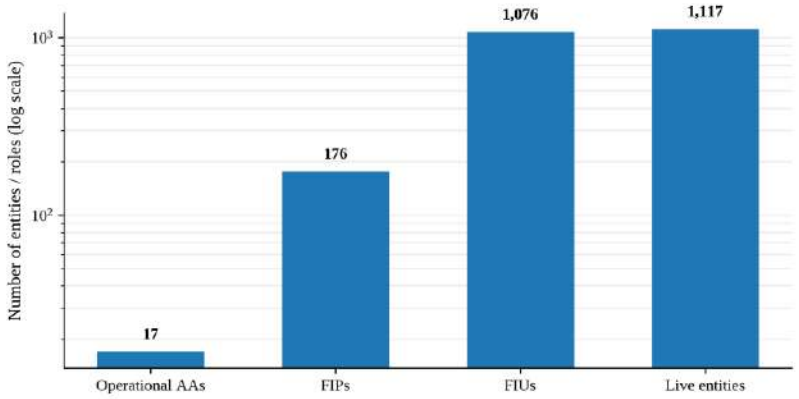


Fig. 2: Institutional Breadth of the Account Aggregator Ecosystem, June 2026

Source: *Sahamati (2026b). Log scale is used because the role counts differ sharply. Categories overlap and are not additive.*

What the Consent Architecture Requires

The AA model is unusually explicit about what a valid consent event should contain. The RBI requires a standardised consent artefact that identifies the customer, the nature of the financial information requested, the purpose, the recipient and the creation and expiry conditions. The customer must be informed of these attributes before consent is obtained. Electronic consent must be capable of being logged, audited and verified and the customer must have a way to revoke future access (RBI, 2024).

The same directions place limits on the AA itself. Customer financial information accessed from an FIP should not reside with the AA and the information cannot be used outside the terms of the consent artefact. AAs cannot ask for or store customer authentication credentials such as passwords and PINs. Customers are also entitled to access a record of the consents they have given and the FIUs with which their information has been shared. These provisions reduce several risks that existed in older, less structured forms of data aggregation.

Table 2: From Regulatory Consent to Consent Literacy

Consent element	Formal requirement in the AA framework	Question a capable user should answer	Likely friction point
Data scope	Nature of financial information requested	Exactly which account or data are being requested?	Broad or technical labels may make the scope hard to judge.
Purpose	Purpose of collecting the information	Why does this service need these data?	Purpose wording can be read quickly without being evaluated.
Recipient	Identity of the information recipient	Which regulated entity will receive the information?	Brand, FIU and service-provider identities may be confused.
Time boundary	Creation, expiry and usage conditions	For how long and how often, can the consent be used?	Recurring access and expiry conditions may receive less attention.
Revocation	Functionality to revoke consent, including part of the information	Can I stop future access if I change my mind?	Revocation may be understood in principle but hard to locate in practice.
Audit and redress	Consent must be loggable/auditable; grievance route must be available	Can I review what I approved and where do I complain?	Users may not know where consent history or grievance routes sit.

Source: Authors' analytical mapping based on RBI (2024).

Behavioral Frictions in Digital Consent

The rules specify the information that should be included in a valid consent request. Behavioural research can contribute to a different question: Do people process such information when a digital decision becomes routine? Zhu and Zhang (2025) demonstrate how users can comply with privacy policies without fully comprehending their content. Betzing *et al*. (2020) find that clearer explanations can improve understanding of data-processing permissions on mobile devices without significantly reducing permission acceptance. These studies examine mechanisms relevant to repeated digital consent but do not directly examine the AA consent journey.

The analogy should not be over-extended. Financial data sharing may receive greater attention than ordinary app permissions because the stakes are more immediate. It may also become commonplace when users repeatedly make the same type of request. Therefore, the privacy studies cited here are used to inspire AA-specific questions; it is not suggested here that the Indian

AA users are careless. Direct evidence would require testing real AA consent journeys with users.

From a consumer-protection perspective, the World Bank (2024b) raises a related concern. Digital-finance users likely do not fully grasp how their data are gathered, shared or used and there is a need for purpose limitation, secure processing, rights of access and correction, revocation and provider responsibility (World Bank, 2024b). These protections are significant in the AA context, as a proper consent artefact represents permission, but meaningful consent also requires that the customer understand the request.

Comparative Consumer Control Perspective

The framework for AAs in India can be contrasted with other open data frameworks to highlight aspects that need to be measured. UK Open Banking operates on an opt-in basis and allows users to decide what information a provider can access and for how long, with the ability to cancel access (Open Banking Limited, n.d.). The Consumer Data Right in Australia is also consent-based, adds accreditation of data recipients and requires government regulation over privacy, security and compliance (Australian Competition and Consumer Commission [ACCC], n.d.). Neither model needs to be replicated directly in India; the comparison instead highlights withdrawal, access history, data quality and redress as dimensions of consumer control that should be considered alongside transaction volume.

From Data Portability to Financial Inclusion

The economic case for AA is strongest where useful financial information remains fragmented across institutions. A borrower may have regular cash flows but a weak conventional credit profile, while a personal-finance service may require a consolidated view rather than multiple statements. Permissioned portability can allow access to such information in a structured format, without requesting customer credentials or the need to upload the same documents repeatedly.

Evidence from Indian small-business lending makes this argument more concrete. Rishabh (2026) finds that interoperable payment histories can improve loan screening and monitoring beyond conventional credit-bureau data. Payment histories provide a snapshot of repayment capacity, while bureau information provides a longer-term record of willingness to repay. The two information sources are complementary rather than substitutes. This is the kind of information gap that open finance can address.

There are also distributional questions. While better data can improve underwriting, it can also make segmentation more accurate. He *et al.* (2023) show theoretically that customer-controlled data sharing alters competitive screening and may not benefit all borrowers equally. Grassi (2024) further finds that willingness to share data varies with the perceived value of the service and privacy concerns. The AA ecosystem thus needs to be more than just technically portable. It must also be a fair exchange from the customer's perspective.

Digital financial literacy therefore becomes part of infrastructure quality rather than an add-on to it. When the scope, purpose or duration of the consent flow is not understood well, it may result in weak user control even when technically correct. At the same time, simply adding more disclosure pages could make the journey impractical. The intention in practice is more limited: to ensure that the information required for a decision can be made visible, comprehensible and easily recalled.

Discussion / Analysis: From Consent Volume to Consumer Capability

The evidence can be understood in three stages. The first is infrastructure access, where the relevant FIP and FIU are connected and a valid request is made. The second is consent capability, where the customer understands what they are consenting to and can exercise control over it. The third concerns economic outcomes, where data sharing may improve access to credit, advice, insurance, investment services or personal-finance management. These three stages should not be combined into a single measure. The vast majority of the public AA indicators are at the infrastructure stage. They mainly report associated accounts, fulfilled consents and completed data shares. These are helpful measures of adoption, but they do not show whether a customer recognised the FIU, understood the purpose or expiry condition or could later revoke access. These are different outcomes and should be measured separately.

The second stage can be measured without rating every consent request. Periodic customer research could check whether users can identify the recipient, purpose, scope and duration after completing a consent journey. It can also measure whether users can locate their consent history, revoke access and complete a grievance journey. These comprehension measures should remain separate from operational measures because a smooth journey does not necessarily mean an informed one.

Table 3: Proposed Indicators for Measuring Consent Capability

Indicator	How to measure	Primary data source	What it would show
Recipient recognition rate	Ask users to identify the FIU immediately after a consent journey.	Periodic user-comprehension sample	Whether users know who receives the data.
Purpose recognition rate	Ask users to state the purpose in their own words.	Periodic user-comprehension sample	Whether the reason for sharing was understood.
Scope recognition rate	Ask which account/data categories were approved.	Periodic user-comprehension sample	Whether users understood what they shared.
Expiry / frequency recognition	Ask when consent expires or how often data may be fetched.	Periodic user-comprehension sample	Understanding of the time boundary.
Revocation task success	Measure the percentage able to locate and revoke an active consent without assistance.	AA/FIU usability logs or supervised task	Practical control after approval.
Consent-history retrieval success	Measure whether users can locate previous and active consents.	AA interface / usability study	Auditability from the customer side.
Grievance resolution time	Track time from complaint filing to closure for consent/data-sharing complaints.	AA/FIU grievance records.	Whether formal rights are operationally usable.

Source: Authors' proposed measurement framework. These indicators are not reported as current AA ecosystem statistics.

The third stage asks whether data portability improves the customer's financial position. In lending, relevant outcomes include turnaround time, approval quality, pricing, repeat borrowing and performance among thin-file customers. In personal finance, outcomes may include better aggregation and budgeting without unnecessary data sharing. Ecosystem activity, consent capability and financial well-being should therefore be reported as separate but related outcomes. There is also a question about the incentive structure. FIUs may benefit from greater data availability through improved screening and personalisation or cross-selling, potentially at the expense of customer privacy and with the potential of more refined segmentation. He *et al*. (2023) demonstrate the impacts of borrower-controlled data on screening and pricing and Blanchard *et al*. (2026) suggest that the evaluation of financial data

exchange depends in part on the value or benefit consumers receive and the degree of transparency and control they experience. It is therefore appropriate that the policy goal be one of proportionate data use, rather than maximum data extraction.

Findings and Discussion

- The AA ecosystem has passed the first pilot stage. Published Sahamati data show that cumulative fulfilled consents increased from 390.92 million in January 2026 to 493.50 million by June 2026, with more than one thousand live regulated entities in the ecosystem.
- The RBI framework contains strong formal safeguards, including explicit consent, purpose specification, recipient identification, expiry conditions, revocation, auditability, restrictions on AA data storage and a grievance route.
- Behavioural evidence shows why these safeguards need to be supported by clear presentation: users may accept digital permissions without fully understanding them, while greater transparency can improve comprehension.
- In the area of open banking, evidence suggests that it brings benefits in terms of competition, advice, credit and digital participation, although these benefits are not consistent across the board. Equally, better data can help to fine-tune segmentation, making fairness and proportionality relevant.
- Therefore, consent literacy ought to be part of digital financial literacy. However, using an app does not mean that users understand the extent and purpose of financial-data sharing or how to withdraw permission.
- Future assessment of AA should combine scale indicators with measures of comprehension, control, grievance handling and financial outcomes.

Limitations and Future Research

The extent of weak consent comprehension among AA users cannot be estimated in this chapter because no public dataset currently links consent events with user understanding, revocation behaviour and subsequent financial outcomes. Privacy and mobile-permission studies therefore provide evidence about relevant behavioural mechanisms rather than AA-specific

evidence. A helpful next step would be a field study using actual AA screens in different Indian languages, followed by tests of recipient, purpose, scope and expiry knowledge and a practical revocation task. The work might also investigate whether comprehension varies according to age, education, digital experience and financial risk of the service requested.

Policy Recommendations

Add a plain-language consent summary. For important or recurring requests, the first screen should show the data requested, purpose, recipient, frequency, duration and revocation option in a compact form before the customer approves.

Measure the quality of consent in addition to volume. Ecosystem reporting should differentiate between requests raised, fulfilled, declined, expired and revoked and separately report evidence of usability and comprehension, where technically feasible. These measures should not be aggregated into a single score as they address different questions.

Explain in multiple languages and with assistance. A consent-based infrastructure that serves many Indians should not presume that familiarity with a smartphone means familiarity with formal financial or legal jargon.

Implement data minimisation at the use-case level. FIUs should seek only information reasonably related to the purpose of the request; sector-level templates can help minimise unnecessary variations in consent requests.

Make consent history easy to use. Customers should be able to identify active, expired and revoked consents, view the FIU and stated purpose and be able to stop future access without having to search through a multi-step process. Periodic task-based testing can show whether that control is effective in practice.

Link inclusion claims to outcomes. Lending use cases should include whether AA-based underwriting results in greater access, better pricing or faster turnaround for thin-file customers without increasing distress. Similar outcome metrics should be created for insurance, investment and personal-finance applications.

Keep responsibility shared. While financial literacy can help customers make better decisions, it cannot supplant provider responsibilities related to security, purpose limitation, fair use and clear communication and grievance redressal.

Conclusion

India's Account Aggregator framework has created a regulated route for customer-authorised financial-data sharing at substantial scale. Its consent architecture is unusually explicit about purpose, recipient, expiry, revocation and auditability. That is an important institutional achievement, but it answers only the first question: whether data can move under a valid authorisation.

The second question is whether customers can exercise that control with reasonable understanding. Current public statistics cannot answer it. Fulfilled consents are operational events indicating successful data delivery, while the behavioural studies used in this chapter come from adjacent digital settings rather than AA itself. That boundary should remain visible rather than being filled with assumptions.

The policy test is therefore threefold: can the data move securely, can the customer understand and control that movement and does the resulting use of data improve the customer's financial position? Consent literacy links the second question to the other two. If future AA reporting measures comprehension and practical control alongside scale and financial outcomes, India can move from consent at scale to a stronger form of data-enabled financial inclusion.

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8
CHAPTER

From Loom to Legacy: Rejuvenation of Traditional Textiles in India

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Abstract

The traditional handloom weaving is a part of the country's cultural ethos and it forms a precious part of its own generational legacy. Indian textiles are known for their intricate designs, vibrant colors and unique craftsmanship and efforts are being made to revive and promote these art forms. India has a rich heritage of traditional textiles and the government has undertaken various initiatives to rejuvenate and promote these textiles. The handloom industry plays a key role in the Indian economy — from providing employment to rural populations to being a carrier of India's rich cultural heritage to other countries. According to the handloom census 2019-20, the handloom sector creates around 35 lakhs of direct employment. However, handlooms do not form a major portion of Indian textile exports. According to the Handloom Export Promotion Council (HEPC), the handloom exports from India fell from ₹ 2,353.33 crore in 2015-16, to ₹ 2,245.33 crore in 2019-20.The government has treated this sector as a rural enterprise and have been offering different schemes for its survival. The initiatives like Make in India aims to ignite the dying aspiration of many weavers and help them to get their products recognized for a better world standing in terms of its visibility and viability.

Introduction

The rejuvenation of traditional textiles in India is an important aspect of preserving the country's rich cultural heritage and supporting local artisans and weavers. Traditional Indian textiles are known for their intricate designs,

vibrant colors and unique craftsmanship and efforts are being made to revive and promote these art forms.

India has a rich heritage of traditional textiles and the government has undertaken various initiatives to rejuvenate and promote these textiles. Here are some notable government initiatives in this regard:

Government Support: The Indian government has launched various schemes and programs to promote traditional textiles. One such initiative is the Ministry of Textiles' "Handloom Mark" certification, which ensures the authenticity and quality of handloom products. Additionally, the "India Handloom" brand has been established to promote handloom products both domestically and internationally.

Government Initiatives:

Handloom Reservation Act: The Handloom Reservation Act ensures the reservation of certain textiles exclusively for handloom weavers. This initiative aims to protect the livelihoods of traditional weavers and promote handloom textiles.

Cluster Development Program (CDP): The Cluster Development Program by the Ministry of Textiles focuses on promoting the growth and development of handloom clusters. It provides support for infrastructure development, skill upgradation, design innovation and market linkages.

With development and changes in consumers' behaviours, traditional handicraft markets have given way to the high end segment of the domestic market and export niches. Artisans, unaware of the macro economic changes, continued to supply their local market this either directly or through traders, resulting in sometimes drastically falling returns. The issue was addressed by a UNIDO development programme in a block printed fabrics cluster in Jaipur, Rajasthan, from 1997 to 2001. The intervention was shaped by a demand-led perspective and multilateral trust building, joint action and balancing of activities were among the implementation tools of the chosen strategy. By the mid 1990s, the cluster had reached a stage where it required a serious rethinking for further and sustainable growth. The major issues identified for intervention were: -

- Market intelligence and development
- Working capital support
- Infrastructure support

- Institutional support
- The creation and strengthening of implementation vehicles (Beddig, n.d.)**.**

In the face of growing competitiveness in the textile industry both in the national and international markets and the free trade opportunities emerging in the post MFA environment, a growing need has been felt for adopting a focused yet flexible and holistic approach in the sector to facilitate handloom weavers to meet the challenges of a globalized environment. A need has also been felt to empower weavers to chart out a sustainable path for growth and diversification in line with the emerging market trends. The Integrated Handlooms Cluster Development Programme is an attempt to facilitate the sustainable development of handloom weavers located in identified clusters into a cohesive, self managing and competitive socio-economic unit. Government of India Ministry of Textiles Office of the Development Commissioner for Handlooms has started handloom cluster development programme *(*Directorate of Textiles, Handlooms, Spinning Mills, Silk Weaving & Handloom Based Handicrafts Division, n.d.)**.**

National Handloom Development Program (NHDP): The NHDP aims to provide comprehensive support to handloom weavers by promoting their welfare, skill development and infrastructure development. It includes various components like yarn supply, design innovation, marketing support and financial assistance.

Geographical Indication (GI) handloom products are traditional authentic products with irreplaceable uniqueness and quality. Indian handlooms play a prominent role in the nation's socio-economic development. The Fourth Handloom Census pinpointed that millions of weavers are employed and are contributing to India's GDP growth. Nationwide pandemic COVID–19 affected this local community. Still, the Government's Make in India Scheme moving towards the Atmanirbhar Bharat Abhiyan Scheme implemented many sub-schemes and government programmes for GI tagged products and weavers. One among the noticeable centrally launched schemes is National Handloom Development Programme (NHDP) **(Udayan *et al*., 2021).**

India is a nation that is quite famous for its handloom products. However, despite having such credibility in handloom production, this industry is not as grown as it should be. Therefore, the Government of India emphasized enhancing and growing the handloom industry of India. To maintain this

handloom industry, several schemes and programs were launched by the Government of India, which included the National Handloom Development Programme (NHDP). As a fact, the Indian handloom system brought a great amount of revenue as well as employment opportunities to the country. Therefore, these schemes are revised under the guidance of the Government of India to improve the opportunity for employment for these handloom weavers, enhance and integrate the growth of these Indian handloom industries as well as focus on the welfare of the workers working in these industries. With the improvement of technology, it is very much essential to bring these new technologies into the market of handloom products (**'**What Is National Handloom Development Programme?**'**, 2022**).**

Handloom Weavers' Comprehensive Welfare Scheme: This scheme provides financial assistance and social security measures for handloom weavers. It includes components like health insurance, life insurance, educational assistance for children and housing support.

India Handloom Brand: The India Handloom Brand was launched to promote high-quality handloom products and create a niche market for them. It certifies handloom products that meet specific quality standards and helps in branding and marketing these products globally.

Handloom Mark: The Handloom Mark is a certification mark introduced by the Ministry of Textiles to differentiate handloom products from powerloom or mill-made products. It helps consumers identify authentic handloom products and supports the livelihoods of handloom weavers.

Silk Mark: The Silk Mark is a quality assurance label for silk products. It ensures the purity and quality of silk and promotes consumer confidence in silk textiles. The initiative aims to protect the interests of silk producers and weavers.

Design and Technology Upgradation Scheme (DTUS): The DTUS focuses on upgrading the design and technological capabilities of handloom weavers. It provides support for design development, product diversification and adoption of new technologies. These initiatives by the Indian government aim to preserve and promote traditional textiles, improve the livelihoods of weavers, enhance product quality and create market opportunities for traditional textiles both domestically and internationally.

Case studies reveal that traditional industries have suffered in recent years due to change in demand, preferences and designs and the failure of these

industries in changing themselves to the changed requirement. Case study on textile industry reveals that if an institution is meant for technical upgradation of both large and small enterprises, the main beneficiaries are only the large enterprises and often the conditions are so framed that keeps the small, micro and unorganised enterprises out of the purview of *benefits (*Design and Technology Upgradation Research Paper - Google Search, n.d.)**.**

Governments, anxious to assist the SMEs in their countries to survive and grow, face not only policy choices about how to assist with technology, financial resources and business direction, but also the complex issues of how to design organizations that can deliver these policies effectively (Kolodny *et al*., 2001)**.**

Weavers' Cooperatives: Weavers' cooperatives play a crucial role in the rejuvenation of traditional textiles. These cooperatives provide support and resources to weavers, such as training, raw materials, design inputs and marketing assistance. They help weavers improve their skills, preserve traditional techniques and access wider markets for their products.

Design Intervention: Design intervention involves blending traditional textiles with contemporary designs to make them more appealing to modern consumers. Designers collaborate with weavers to create innovative designs that retain the essence of traditional textiles while making them relevant to current trends. This approach helps attract younger generations and expand the market for traditional textiles.

Skill Development and Training: Skill development programs are being implemented to train the next generation of weavers and artisans. These programs focus on teaching traditional weaving techniques, dyeing processes and textile design. By passing on these skills to younger artisans, the continuity of traditional textiles is ensured.

Skill Development and Training

By focusing on skill development and training, the rejuvenation of traditional textiles in India ensures the preservation of cultural heritage, promotes economic empowerment of artisans and contributes to the sustainable growth of the textile sector. Skill development and training are vital components of the rejuvenation of traditional textiles in India. They play a crucial role in preserving indigenous knowledge, enhancing craftsmanship and ensuring the continuity of traditional textile techniques. Here are some key aspects of skill development and training in the rejuvenation process:

1. **Artisan Training Programs:** Skill development programs are designed to provide training to artisans in traditional textile techniques. These programs focus on both practical skills and theoretical knowledge, covering areas such as weaving, dyeing, printing, embroidery and fabric manipulation. Training is conducted by experienced master artisans who possess deep knowledge of traditional techniques.
2. **Apprenticeships and Guru-Shishya Parampara:** The traditional guru-shishya (master-apprentice) system is often utilized to transfer skills and knowledge from experienced artisans to the younger generation. Apprenticeships allow aspiring artisans to learn directly from masters, working alongside them to acquire technical skills, understand design concepts and grasp the nuances of traditional textile production.

   In Indian tradition, knowledge has always been transferred through the Guru-Shishya way. This training programme aims to tap that channel. Under it, master craftsmen train artisans on craft skills, on which they have some knowledge or that are entirely new to them. This enables transfer of traditional knowledge from master craftsmen to the new generation, ensuring the sustenance of the craft.
3. **Design and Innovation Workshops:** Workshops and training sessions that focus on design innovation within the context of traditional textiles are organized. These workshops encourage artisans to experiment with new design elements, motifs, color combinations and product diversification while staying true to the essence of traditional techniques. Design experts and mentors provide guidance and support during these workshops.
4. **Entrepreneurship and Business Skills:** Skill development programs also include components that help artisans develop entrepreneurship and business skills. This includes training in market research, product development, pricing, marketing, branding and financial management. By empowering artisans with business skills, they can effectively market and sell their products and establish sustainable livelihoods.

   The handloom sector is yet to produce an ample size of entrepreneurship despite its potential in the globalization era. However, even after continuous intervention by the Government and other entities, the sector has miserably failed in creating an entrepreneurial climate for the community. A number of skilled and semi-skilled weavers express to join

as an entrepreneur if their issues and development factors like better credit system, a research center for trend forecasting at the local level, technological upgradation, regular quality training, feeling of ownership of stakeholders and incubation center, etc., could be taken care of by the Government and other private-entity companies and NGOs. Besides, most of the master weavers claim that lack of getting entrepreneurial status is debarred from the government benefits although they are already into handloom enterprises. Although the Government has many schemes for their upliftment, not a single weaver from the communities has registered himself as a business entity under the companies' act or even operate as a sole proprietor. So, there is an opportunity for entrepreneurship in the cluster if impending issues are considered and implemented **(Tarai, 2020).**

5. **Technology and Sustainable Practices:** Skill development programs also focus on incorporating technology and sustainable practices into traditional textile production. Artisans are trained to use modern tools and equipment while ensuring the preservation of traditional techniques. Training on sustainable dyeing methods, waste reduction, water and energy conservation and eco-friendly materials is also provided.
6. **Collaboration with Designers and Institutions:** Collaboration with designers, fashion institutes and textile research institutions plays a vital role in skill development and training. Designers bring fresh perspectives and contemporary design sensibilities to traditional textiles, while institutions provide access to technical expertise, resources and exposure to the broader design community.
7. **Continued Learning and Knowledge Exchange:** Skill development is an ongoing process. Artisans are encouraged to participate in knowledge exchange programs, workshops, seminars and exhibitions where they can interact with fellow artisans, designers and industry experts. This continuous learning helps artisans stay updated with new techniques, trends and market demands.
8. **Recognition and Certification:** Recognizing the skills acquired through training programs, certification and accreditation schemes are implemented. This adds value to the artisans' work and enhances their marketability. Certifications also help consumers identify and appreciate authentic traditional textiles produced by skilled artisans.

Reviving Indigenous Techniques:

Various efforts are underway to revive ancient weaving techniques and dyeing processes that were on the verge of being lost. Documentation of traditional practices, research on historical textiles and collaborations between weavers and researchers are helping in the revival and preservation of these techniques. Reviving indigenous techniques is a crucial aspect of rejuvenating traditional textiles in India. These techniques hold cultural and historical significance and contribute to the uniqueness of Indian textiles. Here are some ways in which indigenous techniques are being revived and integrated into the rejuvenation process:

1. **Documentation and Research:** Reviving indigenous textile techniques begins with extensive documentation and research. Scholars, artisans and organizations collaborate to study and understand traditional textile techniques, including weaving, dyeing, printing and embroidery. This research helps in preserving and reviving ancient knowledge and skills.
2. **Artisanal Training and Skill Development:** Training programs are conducted to impart the knowledge of indigenous techniques to the younger generation and interested artisans. Skilled craftsmen and weavers pass down their expertise to apprentices, ensuring the continuity of these techniques. Skill development initiatives also focus on improving the quality of craftsmanship and encouraging innovation within traditional frameworks.
3. **Preservation of Tools and Equipment:** Traditional textile techniques often involve specific tools and equipment. Efforts are made to preserve and maintain these tools, which may include looms, printing blocks, dyeing vats and embroidery needles. By preserving these tools, artisans can continue to use them to produce authentic traditional textiles.
4. **Collaboration with Artisans and Communities:** Revival initiatives actively involve artisans and communities in the rejuvenation process. Collaboration helps in understanding the cultural significance of the techniques and ensures that the knowledge and skills are transferred from one generation to the next. It also provides an opportunity for artisans to share their insights and suggestions for the preservation and promotion of traditional techniques.

5. **Market Access and Promotion:** Rejuvenation efforts focus on creating market opportunities for textiles produced using indigenous techniques. This involves organizing exhibitions, fashion shows and craft fairs that showcase the unique beauty and craftsmanship of these textiles. Online platforms and social media are also utilized to reach a wider audience and promote the products to both domestic and international markets.
6. **Design Innovation and Contemporary Adaptation:** To make traditional textiles relevant to modern consumers, design innovation and contemporary adaptation play a crucial role. Designers collaborate with artisans to create contemporary designs that incorporate traditional techniques. This fusion of traditional and contemporary aesthetics helps attract a broader customer base while maintaining the authenticity of the textiles.
7. **Policy Support and Recognition:** Government policies and support are essential for the successful revival of indigenous techniques. Recognizing the cultural and economic value of traditional textiles, governments can provide financial assistance, infrastructure support and incentives to artisans and organizations working in this field. This recognition helps create a conducive environment for the preservation and promotion of indigenous techniques.

Sustainable Practices:

Many initiatives are emphasizing the use of sustainable practices in textile production. This includes promoting natural dyes, organic fibers and environmentally friendly production processes. The focus on sustainability helps preserve traditional textiles while aligning with contemporary concerns for the environment. India has a rich heritage of traditional textiles and there is a growing interest in rejuvenating and preserving these art forms using sustainable practices. Here are some sustainable practices that are being employed in the rejuvenation of traditional textiles in India:

1. **Natural and Organic Materials:** Traditional textiles are often made using natural fibers like cotton, silk, wool or jute. Rejuvenation efforts focus on using organic and locally sourced materials to promote sustainability and reduce the environmental impact. Natural dyes derived from plants, roots and flowers are also preferred over synthetic dyes.

2. **Handloom Weaving:** Handloom weaving is an integral part of many traditional textile techniques in India. Rejuvenation initiatives support and promote handloom weavers who use traditional techniques. Handloom weaving is labor-intensive but has a minimal carbon footprint compared to mechanized processes. It also helps in preserving traditional weaving skills.
3. **Upcycling and Recycling:** Rejuvenation efforts often involve upcycling or recycling old textiles to create new products. This can be done by repurposing old garments or textile scraps to make quilts, rugs or accessories. By giving new life to existing textiles, it reduces waste and conserves resources.

Upcycling and recycling are innovative approaches to sustainable fashion that have gained popularity worldwide, including in India. India has a rich heritage of traditional textiles and the rejuvenation of these textiles through upcycling and recycling practices has become a significant trend in recent years. Upcycling involves taking existing textiles or materials and transforming them into new products with a higher value. This process not only extends the lifespan of the original textile but also reduces waste and promotes creative reuse. In the context of traditional textiles in India, upcycling can involve repurposing old saris, dupattas or other garments into new clothing items or accessories.

Recycling, on the other hand, focuses on breaking down textile waste into raw materials and using them to create new textiles or products. This process often involves shredding, melting or chemically treating the textiles to obtain fibers that can be spun into yarns or used for other applications. In the case of traditional textiles in India, recycling can involve collecting fabric scraps from textile mills or garment factories and transforming them into new fabrics or materials.

The rejuvenation of traditional textiles through upcycling and recycling offers several benefits. Firstly, it helps preserve the cultural heritage embedded in these textiles, as their unique patterns, motifs and techniques are showcased in new and contemporary designs. It also promotes sustainable practices by reducing the demand for new materials and minimizing the environmental impact associated with textile production.

In India, numerous initiatives and organizations are working towards upcycling and recycling traditional textiles. Artisans and designers collaborate

to transform old saris into stylish dresses, skirts or jackets, combining traditional aesthetics with modern designs. Some projects focus on converting textile waste into yarns or fabric, which can be used for weaving or knitting new products. Additionally, community-based enterprises provide training and employment opportunities to artisans, empowering them with skills in upcycling and recycling techniques.

The upcycling and recycling rejuvenation of traditional textiles in India not only contributes to sustainable fashion but also supports local artisans, promotes cultural heritage and fosters creative innovation. By embracing these practices, India can continue to be at the forefront of sustainable textile production while celebrating its rich textile heritage.

4. **Community Empowerment:** Sustainable rejuvenation initiatives focus on empowering local communities and artisans. By providing fair wages, skill development programs and access to markets, these initiatives support the livelihoods of traditional artisans. This helps in preserving the indigenous knowledge and skills associated with traditional textiles.
5. **Water and Energy Conservation:** Sustainable practices in textile rejuvenation also involve minimizing water and energy consumption. Efforts are made to implement water-efficient dyeing and washing techniques, such as using natural dyes that require less water. Energy-efficient technologies and renewable energy sources are also utilized to reduce the carbon footprint of textile production.
6. **Transparent Supply Chains:** Sustainable rejuvenation efforts emphasize transparency in the supply chain. This includes tracing the origin of materials, ensuring fair trade practices and promoting ethical production processes. Consumers are increasingly interested in knowing the story behind the textiles they purchase, including the social and environmental impact of their production.
7. **Education and Awareness:** Rejuvenation initiatives often include educational programs and awareness campaigns to highlight the value of traditional textiles and sustainable practices. By educating consumers and the public about the significance of these textiles and the importance of supporting sustainable initiatives, there is increased appreciation and demand for these products.

Conclusion

The handloom industry plays a key role in the Indian economy — from providing employment to rural population to being a carrier of India's rich cultural heritage to other countries. According to the handloom census 2019-20, the handloom sector creates around 35 lakhs of direct employment. These efforts collectively contribute to the rejuvenation of traditional textiles in India. By creating awareness, providing support to artisans, preserving traditional techniques and adapting to evolving market demands, traditional textiles can continue to thrive and be appreciated both within India and globally.By adopting these sustainable practices, the rejuvenation of traditional textiles in India can help preserve cultural heritage, support local communities and contribute to a more environmentally conscious fashion industry.

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CHAPTER 9

Slow Fashion: An Ecological Footprint for the Attainment of Sustainable Goals

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Abstract

The fashion industry is one of the largest global contributors to environmental degradation, resource depletion and unethical labor practices. As a counter-response to the rapid production and consumption cycle of fast fashion, the slow fashion movement promotes sustainability, ethical production and mindful consumption. This paper explores how slow fashion reduces the ecological footprint of the apparel industry and contributes to the achievement of global sustainable development goals (SDGs), especially SDG 12 (Responsible Consumption and Production) and SDG 13 (Climate Action). Through an analysis of ecological impacts, sustainable practices and consumer behavior, this research highlights how adopting slow fashion principles can lead to a more resilient and responsible fashion ecosystem.

Keywords: Slow fashion, Sustainable fashion, Sustainable Development Goals (SDGs); Responsible consumption, Circular economy, Carbon emissions; Conscious consumerism; Green supply chains; Handloom and crafts; Zero-waste fashion.

1. Introduction

The global fashion industry is valued at over $2.5 trillion, employing millions worldwide. However, beneath its glamorous exterior lies an alarming environmental and social cost. Fast fashion—characterized by mass production, low-quality materials and short-lived trends—has created a culture of disposability. It is estimated that the fashion industry contributes to

about 10% of global carbon emissions and consumes 79 trillion liters of water annually (Ellen MacArthur Foundation, 2020). The fashion industry is one of the major industries contributing to environmental degradation, due to fast fashion practices and cheaply manufactured clothing. For instance, nearly three-quarter of fast fashion apparel ends up in landfills. To uplift sustainable consumption patterns, slow fashion and thrift shopping arise as alternatives to fast fashion systems. There is an imperative need for companies to engage in sustainable production processes as consumers are getting increasingly aware of environmental issues. Accordingly, consumers obtain moral satisfaction by adapting their purchasing behaviors, from reducing waste and extending the clothing lifecycle. Thus, companies with the ability to adapt to consumers' sustainability demands are more likely to succeed in the long term (Castagna *et al*., 2022).

As climate change, pollution and waste management emerge as global concerns, the concept of slow fashion has gained importance. Coined by Kate Fletcher (2007), slow fashion advocates for quality over quantity, ethical labor, environmental awareness and **l**ongevity in design. The movement aligns closely with the United Nations Sustainable Development Goals (SDGs), particularly focusing on sustainability in production, consumption and environmental conservation.

This paper aims to examine how the slow fashion movement creates an ecological footprint that supports sustainable development goals. It explores its philosophy, environmental benefits and socio-economic implications, emphasizing its role in reshaping the fashion industry towards sustainability.

The challenge is to change the way consumers think, rooted in society, in order to achieve sustainable fashion, reinventing consumption and production habits and abandoning fast fashion methods in favour of alternatives such as slow fashion, which proposes a change of vision. It is important to emphasise that this change will only happen with the awareness of everyone. Conscious consumption considers collective well-being, the preservation of resources, good working conditions and fair pay.

The relation between fashion and sustainable development is complex, as it involves reconsidering the practice of consumption. Thus, a new paradigm is needed to guide society and companies towards satisfying the needs of consumers, who are increasingly aware of environmental issues, but tend to be consumerists in an increasingly digital age (Vassalo *et al*., 2024).

Slow fashion is a progressive movement within the fashion industry that promotes ecological integrity, ethical labor practices and mindful consumption. It stands as a direct response to the rapid, high-volume and environmentally damaging operations of the fast-fashion sector. As growing concerns about climate change, resource depletion and textile waste intensify, slow fashion has emerged as a more sustainable pathway that aligns with global environmental and social priorities. The movement emphasizes buying less, choosing high-quality garments, caring for clothes longer and valuing transparency in the entire fashion supply chain. It seeks harmony between fashion, the environment and society, making it a key strategy for achieving long-term sustainability goals (Fletcher, 2010; Gwilt, 2020).

The term "slow fashion" was introduced by Kate Fletcher (Centre for Sustainable Fashion, London College of Fashion) in 2007. She conceptualized it as a fashion system that prioritizes quality over quantity, local production, eco-friendly materials and ethical labor conditions (Fletcher, 2007). Slow fashion encourages consumers to make deliberate and conscious clothing choices, including repairing, reusing, recycling and investing in timeless styles.

The concept draws inspiration from the "Slow Food Movement," advocating for a pace of production that respects natural cycles, human rights and environmental limitations. Key principles of slow fashion include:

- Durability and longevity of products
- Transparency in sourcing and manufacturing
- Fair wages and ethical treatment of workers Environmental responsibility through reduced carbon emissions, less water consumption and minimal chemical usage
- Circularity, such as recycling, upcycling and closed-loop systems (Clark, 2008)

Thus, slow fashion is both a philosophy and a practical framework aimed at reducing the negative ecological footprint of the fashion industry while improving social welfare.

2. Evolution of the Slow Fashion Movement

The slow fashion movement evolved gradually as a counter-movement to the harmful impacts caused by the industrialized fast-fashion model. Its development can be understood through several key phases:

(i) Early Awareness (1990s–2000s)

Environmental activists and ethical fashion researchers began highlighting the consequences of mass production, global supply chain exploitation and the rise of disposable clothing. Reports on textile pollution, sweatshop labor scandals and overconsumption initiated public discourse on sustainable alternatives.

(ii) Invention of the Term (2007)

Kate Fletcher formally coined "slow fashion" in 2007, drawing direct comparison to the slow food philosophy. Her work emphasized the need for regional production, artisan craftsmanship and long-lasting garments.

(iii) Mainstream Recognition (2010–2015)

Growing environmental awareness, along with social media activism, increased interest in ethical fashion. Influential documentaries such as *The True Cost* (2015) exposed the severe human and ecological costs of fast fashion, pushing consumers and brands toward slow-fashion principles.

(iv) Integration into Global Sustainability Agendas (2015–present)

With the introduction of the UN Sustainable Development Goals (SDGs), slow fashion became linked to broader frameworks like responsible consumption (SDG 12), decent work (SDG 8) and climate action (SDG 13). Brands began adopting circular models, using sustainable fibres and improving supply chain transparency (Niinimäki & Hassi, 2011).

Today, slow fashion continues to expand as consumers demand ethical practices, traceability and environmentally conscious products.

3. Slow Fashion and Sustainable Development Goals (SDGs)

Slow fashion aligns with several UN Sustainable Development Goals. The following are key linkages:

SDG 12: Responsible Consumption and Production

Slow fashion encourages conscious consumerism. It promotes reusing, repairing, upcycling and reducing waste. Brands adopting circular economy models—like Patagonia, Eileen Fisher and Reformation—design products for longevity, thereby minimizing resource extraction and waste.

SDG 13: Climate Action

By reducing carbon emissions through localized production, renewable energy use and natural fabrics, slow fashion mitigates climate change. Organic cotton, bamboo and hemp require fewer chemicals and energy, contributing to lower emissions.

SDG 8: Decent Work and Economic Growth

Ethical production ensures fair wages, gender equality and safe working conditions. Slow fashion supports artisans and craftspeople, sustaining traditional skills and providing stable livelihoods.

SDG 6 & 15: Clean Water and Life on Land

Sustainable textile practices—like natural dyeing and organic farming—help conserve water and protect ecosystems. Slow fashion reduces landfills and pollution, directly benefiting terrestrial and aquatic biodiversity.

4. The Rise of Sustainable Fashion

In light of these severe environmental impacts, there has been rising public pressure on the fashion industry to adopt more sustainable practices. The idea of "sustainable fashion" encompasses materials and manufacturing processes that minimize harm to the environment and communities involved. This includes initiatives like using organic and recycled fabrics, non-toxic dyes, closed-loop recycling programs, monitoring of working conditions and carbon offsetting. Major brands like H&M, Zara, Nike and Levi's have launched sustainability programs and clothing lines, while smaller eco-fashion labels have emerged. However, sustainable fashion currently makes up only a small percentage of the overall Rs.1.5 trillion global clothing market. Motivating widespread consumer adoption remains a key challenge. Consumer Demand for Sustainable Fashion Despite the growing range of sustainable apparel options, there remains a distinct "attitude-behavior" gap, where consumers who express environmental concern often fail to translate these values into purchasing sustainable fashion. Previous studies have identified several potential barriers including higher prices, limited availability, skepticism around brand greenwashing claims and lack of style options. However, the determinants behind ethical fashion consumption are still not fully understood (Guria, 2024).

5. Case Studies Illustrating Slow Fashion Practices

Case studies of global and Indian brands demonstrate how slow fashion principles can be integrated into real-world business models. These brands and

platforms highlight sustainable materials, ethical labor, transparency, recycling initiatives and cultural preservation. The following examples show how slow fashion is feasible across diverse contexts—luxury markets, outdoor wear, traditional crafts and circular economy systems.

(i) Patagonia: Pioneers of Environmental Responsibility

Patagonia is widely regarded as a global leader in sustainable and ethical fashion. Founded in 1973, the company embeds environmental responsibility into every stage of its business model. Patagonia emphasizes durability, repairability, recycling and transparent supply chains.

Key Slow-Fashion Practices:

- **Worn Wear Program:** Encourages repairing, reusing and reselling products instead of buying new ones.
- **Recycled Materials:** Uses recycled polyester, nylon, wool and down to reduce waste and carbon emissions. Supply Chain Transparency: Publicly discloses factories and environmental impacts.
- **Environmental Activism:** Donates 1% of its revenue to grassroots environmental organizations.

Patagonia's business model illustrates how slow fashion can coexist with commercial success while reducing ecological impact.

(ii) Stella McCartney: Luxury Fashion with Sustainability at Its Core

British designer Stella McCartney is one of the first luxury fashion leaders to integrate sustainability into high-end fashion. Since launching her brand in 2001, she has eliminated harmful practices commonly associated with the luxury sector.

Key Slow-Fashion Practices:

- **No Animal Products:** No leather, fur or exotic skins—reducing reliance on unethical supply chains.
- **Innovative Eco-Materials:** Uses recycled polyester, regenerative cotton and plant-based alternatives like *Mylo* (mushroom leather).
- **Circularity in Luxury:** Collaborates with Ellen MacArthur Foundation to promote circular fashion.

- Ethical Production: Implements strict labor standards across global suppliers.

McCartney's brand shows that luxury fashion can prioritize sustainability without compromising design or quality.

(iii) FabIndia and Khadi India: Blending Slow Fashion with Indian Craft Heritage

FabIndia and Khadi India represent India's own slow-fashion ecosystem, rooted in handloom weaving, local production and artisanal empowerment. FabIndia works with over 55,000 rural artisans, promoting handcrafted textile traditions while ensuring fair wages and employment stability. FabIndia represents a large-scale model of culturally sustainable fashion built on traditional craftsmanship.

Slow-Fashion Elements:

- Craft Revival: Supports handloom weaving, block printing, natural dyeing and embroidery.
- Sustainable Materials: Focuses on cotton, linen, silk and natural fibre-based fabrics.
- Community Development: Operates through producer-owned companies, ensuring inclusive growth.

(iv) Khadi India

Khadi, promoted by the Khadi and Village Industries Commission (KVIC), symbolizes hand-spun, hand-woven, eco-friendly and decentralized production. Khadi India is a national model demonstrating how traditional, low-energy craftsmanship aligns with modern sustainability goals.

Slow-Fashion Principles:

- **Zero-Carbon Production:** Since khadi is hand-spun and handwoven, its carbon footprint is significantly lower than mill-made fabrics.
- **Local Manufacturing:** Empowers rural artisans and supports village economies.
- **Cultural Preservation:** Keeps traditional Indian craft skills alive.
- **Minimal Environmental Impact:** Uses natural fibres such as cotton, silk and wool.

6. Ecological Footprint of Slow Fashion

Slow fashion significantly reduces the ecological burden created by the global textile and apparel industry. Unlike fast fashion—which accelerates production cycles, increases resource consumption and contributes heavily to pollution—slow fashion emphasizes environmentally responsible processes, long-lasting materials and circularity. The movement seeks to minimize environmental impacts associated with water consumption, energy use, carbon emissions, chemical discharge and textile waste across the entire product life cycle. The following components illustrate how slow fashion achieves a lower ecological footprint and supports global sustainability goals.

(i) Reduction in Water Usage

The fashion industry is one of the world's largest consumers of freshwater, using approximately 93 billion cubic meters of water annually (UNEP, 2020). Slow fashion mitigates this impact through sustainable fiber choices, water-efficient dyeing technologies and reduced production volumes.

How Slow Fashion Reduces Water Use

- Organic cotton uses up to 91% less water compared to conventional cotton because it relies on rain-fed systems instead of irrigation (Textile Exchange, 2021).
- Natural dyes derived from plants and minerals require significantly less water and reduce harmful wastewater discharge.
- Degrowing production levels—producing fewer garments—directly reduces the volume of water-intensive washing, dyeing and finishing.
- Handloom fabrics such as khadi require minimal water since no large-scale industrial wet processing is involved.
- Waterless dyeing technologies (e.g., CO_2 dyeing) adopted by sustainable brands drastically reduce freshwater use.

By prioritizing eco-friendly fibers and low-water processes, slow fashion addresses water scarcity and aligns with SDG 6: Clean Water and Sanitation.

(ii) Lower Carbon Emissions

Global fashion emits an estimated 1.2 billion tonnes of CO_2 annually, more than international flights and maritime shipping combined (Ellen

MacArthur Foundation, 2017). Slow fashion reduces these emissions by lowering production, using renewable energy and adopting low-impact fibers.

Key Mechanisms:

- Low-volume, high-quality production decreases energy use in manufacturing.
- Energy-efficient artisan production (e.g., handloom weaving, hand-spinning) uses 4–6 times less energy than mechanized mills (Nayak & Padhye, 2015).
- Local sourcing reduces transportation emissions. Recycled fibers (recycled polyester, wool and cotton) release up to 80% fewer CO_2 emissions compared to virgin fibers.
- Lifecycle extension—wearing garments longer—minimizes the need for new clothing production. Slow fashion encourages decarbonization by shifting away from fossil fuel–based synthetics and labor-intensive mass production.

(iii) Recyclability and Circular Economy

Circularity is a fundamental pillar of slow fashion, emphasizing an extended product life cycle and minimizing waste. A circular fashion system eliminates the traditional *take–make–dispose* model and replaces it with reuse, repair, upcycling, recycling and rental systems.

Circular Strategies in Slow Fashion

- Closed-loop recycling: Fibers such as cotton, wool and polyester are recycled into new textiles, reducing raw material extraction.
- Extended garment lifespan: High-quality, durable clothing allows more wears and reduces waste generation.
- Repair and mending programs (e.g., Patagonia's Worn Wear) divert textile waste from landfills.
- Rental and resale platforms reduce the environmental burden of single-use garments.
- Design for disassembly ensures garments can be more effectively recycled at end of life.

Circularity can reduce material waste by up to 50%, depending on design and system efficiency (EMF, 2017).

(iv) Eco-Friendly Materials Used in Slow Fashion

A significant component of slow fashion's ecological footprint reduction comes from responsible material selection. The industry focuses on fibers that are biodegradable, low-energy, low-water and non-toxic.

Organic Cotton

- Free from synthetic pesticides and fertilizers.
- Uses 91% less water and 62% less energy than conventional cotton.
- Improves soil health through regenerative agricultural practices.

Hemp

- Requires *50% less water* than cotton.
- Grows without pesticides; enriches soil through phytoremediation.
- Produces one of the strongest natural fibers, extending garment **lifespan.**
- Absorbs more CO_2 per hectare than most crops.

Bamboo

- Regenerates rapidly without replanting.
- High CO_2 absorption capacity.
- Low water requirements.
- When mechanically processed (not chemically), bamboo linen is biodegradable and eco-friendly.

Linen (Flax Fiber)

- Among the most eco-friendly fibers with low pesticide and water use.
- Fully biodegradable and durable.
- Requires 6–7 times less water than cotton during cultivation.

(v) Zero-Waste Production Methods

Zero-waste fashion aims to eliminate textile waste at the design stage, addressing the issue that approximately 92 million tonnes of textile waste are generated annually (Global Fashion Agenda, 2020).

Zero-Waste Strategies in Slow Fashion:

- **Zero-waste pattern cutting:** Uses patterns that utilize nearly 100% of fabric (Timo Rissanen's techniques).
- **3D knitting:** Produces garments directly to shape, minimizing offcuts.
- **Upcycling:** Transforms post-consumer or industrial waste into new garments.
- Made-to-order models reduce overproduction—one of the biggest contributors to waste.
- Modular and multi-purpose garments maximize usability and minimize disposal.
- Fabric scrap recovery and patchwork (e.g., Indian *kantha* or *boro* techniques).

Zero-waste methods not only reduce landfill accumulation but also conserve resources by minimizing the need for virgin materials.

7. Economic and Social Dimensions of Slow Fashion

Slow fashion is not only an environmental movement—it also represents a transformative socio-economic model rooted in fairness, cultural preservation and equitable growth. While the fast-fashion industry is often associated with exploitative labor, low wages, production outsourcing and unsafe working conditions, slow fashion promotes ethical labor practices, supports local artisans, preserves traditional craftsmanship and encourages resilient small-scale enterprises. Together, these dimensions contribute to a more inclusive and just fashion ecosystem.

Fair Wages and Safe Working Conditions

A defining characteristic of slow fashion is the prioritization of human rights within the production process. The fast-fashion industry often relies on workers in the Global South who earn below-living wages and operate in hazardous conditions—a reality highlighted by the 2013 Rana Plaza collapse, which killed over 1,100 garment workers in Bangladesh.

How Slow Fashion Ensures Ethical Labor:

- **Living wages:** Brands adopting slow fashion commit to paying fair compensation aligned with local economic needs, not minimum legal wages.

- **Safe work environments:** Complying with International Labour Organization (ILO) standards and ensuring adequate ventilation, safety equipment and regulated working hours.
- **Transparency:** Many slow fashion brands publish factory lists, audit reports and workforce welfare data.
- **Ethical certifications:** Fair Trade, SA8000, WFTO and GOTS provide third-party assurance of ethical labor practices.

This approach creates more stable livelihoods and improves socio-economic mobility for garment workers.

Local Artisan Empowerment

Slow fashion supports skilled artisans who preserve localized textile practices. These artisans often come from rural or marginalized communities and the slow-fashion movement strengthens their economic independence and social status.

Key Contributions to Artisan Empowerment:

- **Decentralized production:** Instead of mass industrialized manufacturing, slow fashion supports small artisan clusters, allowing work within home-based or community settings.
- **Fair compensation:** Artisans receive equitable payment reflecting skill, time and craftsmanship.
- **Skill development programs:** Brands like FabIndia and Dastkar train artisans in design adaptation, quality standards and market access.
- **Market access:** Slow-fashion platforms allow artisans to reach global consumers through direct-to-market models.
- **Social equity:** Empowering women, who constitute a large share of the artisan workforce, improves household income and community development.

By integrating artisans into sustainable value chains, slow fashion boosts rural employment and preserves intangible cultural heritage.

Revival of Traditional Crafts and Handloom

Traditional crafts and handloom weaving—which represent centuries-old practices—have declined due to industrialization and rapid fast-fashion manufacturing. Slow fashion helps revive these traditions by acknowledging their value in sustainability, creativity and cultural identity.

How Slow Fashion Supports Craft Revival:

- **Low-energy production:** Handloom textiles consume minimal electricity and rely heavily on human skill, making them inherently eco-friendly.
- **Use of natural dyes and traditional techniques:** Ajrakh, kalamkari, chikankari, ikat, banarasi silk and khadi represent ancient craft forms revived through slow-fashion initiatives.
- **Heritage branding:** Craft-based clothing appeals to consumers seeking authenticity, prompting fashion designers and NGOs to collaborate with artisans.
- **Intergenerational knowledge transfer:** Sustainable markets ensure artisans continue teaching craft traditions to younger generations.

Traditional handloom and craft clusters (e.g., Kanchipuram, Varanasi, Kutch, Kota Doria) benefit economically while preserving cultural identity.

Small-Scale Sustainable Business Models

Slow fashion encourages small-scale, community-based and environmentally conscious enterprises. Unlike fast fashion's centralized, mass-production model, slow fashion supports entrepreneurial ecosystems that promote sustainability and local development.

Types of Sustainable Business Models:

a. Artisanal and Cooperative Enterprises

Cooperatives allow artisans to collectively manage production, pricing and profits—enhancing bargaining power and financial stability.

b. Made-to-Order and Limited Production Brands

These brands reduce overproduction, minimize textile waste and ensure product exclusivity.

c. Social Enterprises and NGOs

Organizations such as SEWA, Dastkar and Barefoot College provide training, microfinance and market linkages.

d. Thrift, Rental and Repair Businesses

Circular business models extend garment life and reduce consumption intensity.

e. Farm-to-Fashion Models

Brands that grow their own cotton (e.g., organic cotton collectives) promote transparency, traceability and fair pricing.

Economic Benefits:

- Increased local employment
- Reduced capital requirement compared to large-scale factories
- Higher product value due to craftsmanship and quality Enhanced economic resilience due to diversified skills and markets

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10
CHAPTER

Precision Reproductive Medicine in Dairy Cattle: Integrative Endometrial, Inflammatory and Metabolic Biomarkers for Early Prediction of Postpartum Uterine Disease and Reproductive Failure

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Abstract

Postpartum uterine disease and reproductive inefficiency remain major constraints on the biological and economic sustainability of dairy production worldwide. The transition from pregnancy to lactation involves extensive endocrine, metabolic, immunological and structural changes that influence uterine involution, endometrial repair, ovarian resumption and the establishment of subsequent pregnancy. Failure to coordinate these processes may result in clinical or subclinical uterine inflammation, delayed conception, embryonic mortality and prolonged calving-to-conception intervals. Conventional diagnostic approaches, although indispensable, frequently identify reproductive disorders after clinically relevant pathological processes have already developed. Precision reproductive medicine offers an opportunity to move beyond symptom-based diagnosis towards individualised, risk-based reproductive management.

This chapter critically examines the biological rationale, current evidence and translational potential of integrating endometrial, inflammatory, metabolic, endocrine and microbiome-associated biomarkers to predict postpartum uterine disease and subsequent reproductive performance in dairy cattle. Particular emphasis is placed on the interactions among uterine immune regulation, microbial communities, negative energy balance, oxidative stress, systemic inflammation and ovarian–uterine signalling. Established diagnostic approaches, including clinical examination, vaginal discharge scoring, uterine cytology and reproductive ultrasonography, are considered alongside emerging molecular technologies, such as targeted metabolomics, transcriptomics, proteomics and microbiome profiling.

The chapter further evaluates approaches for developing and validating multimodal predictive models, including longitudinal sampling, multivariable regression, survival analysis, machine learning, calibration and external validation. Methodological challenges associated with confounding, temporal variability, sample contamination, population heterogeneity, overfitting and limited transportability are discussed. Finally, a translational framework is proposed for integrating biomarker-based risk stratification into herd-level reproductive management, with specific consideration of Indian dairy production systems and their relevance to international research. A central argument is that clinically useful precision reproduction will depend not on the discovery of isolated biomarkers, but on reproducible, biologically interpretable and externally validated combinations of measurements that improve decisions beyond conventional veterinary examination.

1. Introduction

Reproductive efficiency is a major determinant of dairy herd productivity, replacement requirements, lifetime milk production and the economic sustainability of cattle enterprises. Successful reproduction depends on the coordinated recovery of the reproductive tract after parturition, restoration of ovarian activity, establishment of a receptive endometrium, successful fertilisation, conceptus development and maintenance of pregnancy. Disruption at any stage may reduce the probability of conception or increase the time required to achieve pregnancy.

The postpartum period is particularly important because the uterus must recover from pregnancy-associated enlargement, placental separation, tissue injury, bacterial exposure and extensive immunological remodelling. Simultaneously, the cow enters a period of rapidly increasing milk production and frequently experiences negative energy balance. These concurrent

demands create a physiological environment in which inflammatory regulation, immune competence, metabolic adaptation and reproductive function are closely interconnected.

Postpartum uterine disorders include puerperal metritis, clinical endometritis and subclinical endometritis, although their diagnostic definitions, timing and clinical significance differ. These disorders should not be treated as interchangeable entities. Puerperal metritis is generally associated with an enlarged uterus, fetid watery uterine discharge and systemic illness during the early postpartum period. Clinical endometritis is commonly identified later through abnormal uterine discharge, whereas subclinical endometritis involves uterine inflammation without the characteristic purulent discharge of clinical disease. Cytological criteria and diagnostic thresholds depend on postpartum stage and the protocol used.

The consequences extend beyond the uterus itself. Persistent inflammation may interfere with endometrial function, ovarian activity and the uterine environment required for early embryonic development. Conversely, cows with metabolic or immune dysfunction may be more susceptible to postpartum disease. Thus, uterine pathology and metabolic maladaptation may represent interconnected components of a broader transition-period disorder rather than independent clinical events.

Traditional reproductive management relies on clinical examination, rectal palpation, ultrasonography, vaginal examination, reproductive history and observation of oestrus. These tools remain fundamental because they are practical and directly relevant to veterinary decision-making. However, they may not fully identify subclinical inflammatory changes or distinguish animals that will recover uneventfully from those likely to experience persistent reproductive impairment.

Precision reproductive medicine seeks to address this limitation by combining information about the animal's physiological state, inflammatory activity, metabolic status, reproductive tract condition and subsequent reproductive outcomes. The objective is not simply to detect disease earlier, but to estimate an individual animal's probability of developing a clinically meaningful outcome and to identify the management decisions most likely to improve that outcome.

An integrative biomarker approach could therefore support three complementary functions:

1. **Early risk prediction:** identifying cows likely to develop postpartum uterine disease before conventional clinical diagnosis.

2. **Prognostic stratification:** estimating the probability of subsequent conception, pregnancy maintenance or timely pregnancy.
3. **Decision support:** identifying animals that may benefit from intensified monitoring, targeted investigation or evidence-based intervention.

Nevertheless, the development of such systems requires caution. A biomarker associated with disease is not necessarily a reliable predictor and a highly accurate predictive model is not necessarily clinically useful. A valid precision-reproduction strategy must demonstrate reproducibility, incremental predictive value, calibration in the intended population and an ability to improve decisions without causing unnecessary treatment or expenditure.

The central objective of this chapter is to establish a biologically grounded and methodologically rigorous framework for integrating endometrial, inflammatory and metabolic information into predictive reproductive medicine for dairy cattle.

2. Biological Foundations of Postpartum Reproductive Recovery

2.1. Uterine involution and endometrial restoration

Following parturition, the bovine uterus undergoes substantial anatomical and functional remodelling. Myometrial contractions, tissue repair, removal of lochial material, restoration of the endometrial epithelium and changes in local immune activity contribute to the recovery process.

Bacterial exposure of the postpartum uterus is common. The presence of bacteria alone, however, does not establish clinically significant uterine disease. Disease development depends on interactions among microbial characteristics, tissue injury, host immune responses, endocrine conditions, nutritional status and the effectiveness of local defence mechanisms.

Neutrophils and other immune cells participate in the removal of microorganisms and damaged tissue. Inflammation is therefore a necessary component of postpartum recovery. Problems arise when the inflammatory response is insufficient to control pathogenic organisms or remains excessive or unresolved beyond the period compatible with normal tissue restoration.

Persistent endometrial inflammation may alter the uterine environment and interfere with subsequent reproductive events. The degree of impairment is likely to depend on the severity, duration and anatomical distribution of inflammation, as well as the stage of the reproductive cycle and the animal's overall physiological condition.

For biomarker research, this distinction is important. A measurement taken shortly after calving may reflect normal physiological inflammation, whereas a similar measurement several weeks later may indicate delayed recovery. Consequently, postpartum sampling time must be incorporated into both the study design and the interpretation of diagnostic thresholds.

2.2. Innate immunity and inflammatory regulation

The postpartum uterus depends on innate immune mechanisms for early defence. Pattern-recognition receptors, including Toll-like receptors, recognise microbial-associated molecular patterns and initiate signalling pathways that regulate cytokine production, immune-cell recruitment and antimicrobial responses.

Inflammatory mediators such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α) and chemokines may participate in these responses. Their biological effects depend on concentration, location, duration and the broader regulatory environment. Increased concentrations may indicate inflammatory activation, but they cannot be interpreted as disease-specific in isolation.

The acute-phase response provides another potential source of information. Haptoglobin and serum amyloid A are among the proteins used to investigate systemic inflammation in cattle. Their concentrations may be influenced by uterine disease, mastitis, lameness, tissue injury and other concurrent conditions. A biomarker panel must therefore distinguish reproductive disease-associated inflammation from inflammation arising elsewhere in the animal.

The relationship between local and systemic inflammation is particularly relevant. A cow may exhibit substantial local endometrial inflammation without a proportionate systemic acute-phase response. Conversely, a systemic inflammatory marker may rise because of a non-reproductive disorder. Combining local and systemic measurements may improve interpretation, but this proposition must be tested empirically.

An additional consideration is inflammatory resolution. A single measurement provides limited information about whether the inflammatory response is increasing, stable or declining. Repeated measurements may therefore offer a more meaningful representation of the transition from inflammation to tissue recovery.

2.3. Endometrial receptivity and reproductive competence

The endometrium is not simply a passive surface on which the embryo

develops. It participates actively in the establishment and maintenance of pregnancy through hormonal responsiveness, cellular differentiation, nutrient transport, immune regulation and signalling with the developing conceptus.

Progesterone-dependent changes in endometrial function contribute to the preparation of the uterus for pregnancy. In cattle, conceptus-derived interferon tau plays a central role in maternal recognition of pregnancy by preventing the luteolytic process that would otherwise compromise corpus luteum function.

Endometrial dysfunction may arise from persistent inflammation, altered endocrine signalling, tissue damage or metabolic disturbance. However, uterine cytology, gene expression and circulating inflammatory measurements reflect different biological dimensions. They should not be assumed to be interchangeable indicators of endometrial receptivity.

An important research objective is to determine whether a defined combination of inflammatory, metabolic and endometrial measurements can identify cows whose reproductive tract appears clinically recovered but remains associated with a lower probability of successful pregnancy.

This question requires prospective follow-up. Associations with inflammation alone cannot establish that a proposed panel predicts conception or embryonic survival.

3. Postpartum Uterine Disease: Clinical and Subclinical Phenotypes

3.1. Clinical metritis

Puerperal metritis is generally diagnosed during the early postpartum period using a combination of uterine enlargement, abnormal fetid discharge and systemic signs such as fever or reduced general condition. The precise case definition should be specified in any research protocol.

Metritis is often associated with substantial inflammatory activation and can coexist with metabolic disturbances, reduced feed intake and other transition-period diseases. These concurrent conditions complicate biomarker interpretation because an observed association may reflect disease severity, systemic inflammation or the broader metabolic state rather than a uniquely uterine mechanism.

Studies evaluating predictive biomarkers should distinguish the prediction of incident metritis from the diagnosis of disease already present. Samples collected after clinical signs emerge may be useful for diagnostic research but provide weaker evidence for genuinely early prediction.

3.2. Clinical endometritis

Clinical endometritis is usually characterised by abnormal uterine discharge beyond the earliest postpartum period, in the absence of the severe systemic illness typical of puerperal metritis. Diagnostic criteria vary among studies and clinical systems.

The practical advantage of discharge-based assessment is its relative simplicity. Nevertheless, the relationship between discharge, endometrial inflammation and subsequent fertility is not uniform across animals. Differences in timing, disease severity, reproductive management and case definitions can produce substantial variation in reported associations.

Research should therefore document the examination method, postpartum day, scoring system and criteria used to classify disease. Standardisation is necessary for comparing biomarker performance across herds and countries.

3.3. Subclinical endometritis

Subclinical endometritis refers to endometrial inflammation identified in the absence of the characteristic clinical discharge used to diagnose clinical endometritis. Endometrial cytology is frequently used to identify an increased proportion of polymorphonuclear neutrophils (PMNs).

The threshold used to define a positive cytological result is not universal. Appropriate cut-offs depend on postpartum timing, sampling technique, study population and the diagnostic objective. A single threshold applied indiscriminately across all postpartum stages may misclassify cows.

Subclinical disease presents a particularly important opportunity for precision medicine because routine observation may not reveal the underlying inflammatory condition. However, cytology itself is not a perfect reference standard. Sampling variability, cellular recovery, observer interpretation and the biological dynamics of inflammation may influence classification.

A rigorous biomarker study should therefore report cytological methods in detail and, where feasible, evaluate the reproducibility of the classification procedure.

3.4. Why phenotype definition matters

A central methodological problem in reproductive biomarker research is the use of broad or inconsistent disease categories. Combining metritis, clinical endometritis and subclinical endometritis into a single outcome can obscure meaningful differences in pathophysiology and predictive performance.

A better strategy is to define separate outcomes for:

- Early postpartum metritis.
- Later clinical endometritis.
- Cytologically defined subclinical endometritis.
- Time to first insemination.
- Conception following insemination.
- Pregnancy loss following confirmed pregnancy.
- Time to pregnancy and calving-to-conception interval.

These outcomes should be analysed separately when biologically and statistically justified. A composite outcome may be useful for selected applications, but its components must remain transparent.

4. Endometrial Biomarkers

4.1. Endometrial cytology

Endometrial cytology provides a direct indication of local inflammatory-cell activity. Samples may be obtained using cytobrush techniques or other validated collection methods. The proportion of PMNs is commonly assessed relative to other recovered cells.

Cytology has several advantages: it is comparatively accessible, can be performed in field-oriented studies and measures a biological process directly relevant to endometrial inflammation. It is therefore an important comparator for emerging biomarker panels.

Its limitations include sampling variability, dependence on operator technique, cell-preservation quality, observer variation and the time-dependent nature of postpartum inflammation. Cytological classification also does not fully characterise microbial activity, inflammatory resolution, tissue architecture or endometrial molecular function.

In a precision-reproduction framework, cytology can serve as a phenotyping tool, a candidate predictor or a component of a reference definition. These roles should be clearly distinguished. If cytology is used to define the outcome, its value should not be overstated as an independent predictor of that same outcome.

4.2. Endometrial gene expression

Gene-expression analysis can provide insight into local immune activity, epithelial function, tissue repair and endocrine responsiveness. Candidate pathways may include innate immune signalling, inflammatory regulation,

cellular stress responses, epithelial integrity and progesterone-associated pathways.

Targeted quantitative PCR may be appropriate when there is a strong biological rationale and a limited number of candidate genes. RNA sequencing can provide broader discovery opportunities but generally requires greater resources, more extensive quality control and careful control of multiple testing.

A critical distinction must be maintained between discovery and validation. Genes identified through exploratory analyses should not be described as established predictive biomarkers until their performance has been evaluated in independent samples.

Endometrial gene expression is also sensitive to cell composition. An apparent increase in an inflammatory transcript may reflect a greater number of infiltrating immune cells rather than altered expression within a specific cell population. Where feasible, interpretation should account for cellular composition and sampling conditions.

4.3. Endometrial proteins and inflammatory mediators

Protein-level measurements can provide information about inflammatory signalling and local tissue responses. Depending on the study question, candidate measurements may include selected cytokines, chemokines, antimicrobial proteins and markers of tissue injury.

Protein abundance does not necessarily correspond directly to messenger RNA abundance. Post-transcriptional regulation, protein degradation, secretion and local binding can all affect observed concentrations.

Assay selection should be guided by analytical validity, sensitivity, specificity, sample-matrix compatibility, reproducibility and practical cost. A biologically interesting marker with poor analytical performance is unlikely to be suitable for a clinical prediction system.

4.4. Endometrial receptivity signatures

A more advanced approach is to investigate whether combinations of endometrial molecular features are associated with subsequent reproductive success.

Such signatures could incorporate inflammatory pathways, epithelial function, hormone responsiveness and tissue-repair processes. However, a molecular profile associated with established disease should not automatically be described as a receptivity signature. Demonstrating receptivity-related

prognostic value requires appropriately timed sampling and prospectively measured pregnancy outcomes.

A particularly useful research question is whether molecular measurements add predictive information beyond clinical examination, postpartum timing, parity, previous disease and cytological assessment.

5. Inflammatory Biomarkers

5.1. Systemic acute-phase proteins

Haptoglobin and serum amyloid A are established research candidates for evaluating systemic inflammatory responses in cattle. Their concentrations can reflect inflammatory activation, but neither is specific to postpartum uterine disease.

Their potential value lies in the context they provide. When combined with clinical examination, metabolic indicators and reproductive measurements, acute-phase proteins may help characterise the systemic response associated with disease.

Interpretation requires attention to concurrent disorders, including mastitis, lameness, respiratory disease and tissue injury. Failure to account for these conditions may lead to an overestimation of reproductive disease-specific predictive performance.

5.2. Circulating cytokines

Circulating cytokines can provide information about immune activation, but their concentrations may be low, transient and influenced by assay characteristics. Biological variability and pre-analytical handling are especially important considerations.

A cytokine should not be included in a prediction panel solely because it has a plausible inflammatory function. Its inclusion should depend on evidence of reproducible measurement, a defined relationship with the outcome and incremental value over simpler measurements.

5.3. Leukocyte profiles

Total leukocyte counts, neutrophil-to-lymphocyte ratios and other haematological measures may provide information about systemic immune status. These measurements are comparatively accessible, but their specificity is limited.

Haematological profiles may be influenced by stress, infection, physiological state and medication. They should therefore be treated as

contextual predictors rather than definitive diagnostic indicators of uterine disease.

5.4. Oxidative stress and antioxidant status

The transition period is associated with substantial changes in metabolic demand and oxidative balance. Oxidative stress may influence immune-cell function, cellular integrity and reproductive processes.

Potential research measurements include selected oxidative damage products and antioxidant indicators. However, assay standardisation and interpretation remain important challenges. A large panel of oxidative markers can increase cost and multiplicity without necessarily improving prediction.

A focused strategy should select markers based on a clear biological hypothesis and evaluate whether they provide information beyond metabolic and inflammatory measurements.

6. Metabolic Biomarkers and the Transition Period

6.1. Negative energy balance

The onset of lactation increases nutrient requirements at a time when feed intake may not fully meet energy demand. Many dairy cows experience negative energy balance during this period.

The magnitude and duration of negative energy balance vary with milk production, parity, body condition, diet, management and individual metabolic adaptation. These differences may influence immune competence, inflammatory activity and reproductive recovery.

Negative energy balance should therefore be considered a dynamic physiological state rather than a single universal disease category.

6.2. Non-esterified fatty acids and beta-hydroxybutyrate

Circulating non-esterified fatty acids (NEFA) reflect the mobilisation of body fat, while beta-hydroxybutyrate (BHB) provides information about ketone-body metabolism. Both are widely used in research on transition-cow metabolic health.

Higher concentrations during relevant postpartum windows may be associated with metabolic challenges and adverse health outcomes. Their relationship with uterine disease and subsequent fertility, however, is influenced by sampling time, dietary conditions, lactation stage and concurrent illness.

NEFA and BHB should therefore be interpreted using predefined

sampling windows and appropriate reference or decision thresholds for the intended population. The same threshold may not perform identically across management systems.

6.3. Glucose and insulin-related measures

Glucose homeostasis and insulin-related signalling are connected to nutrient partitioning, ovarian activity and reproductive function. These pathways provide a biological rationale for evaluating selected endocrine-metabolic indicators.

However, a complex endocrine panel may not be practical for routine herd-level screening. The research objective should be to determine whether these measurements provide clinically meaningful incremental value beyond readily available metabolic indicators.

6.4. Body condition and body-condition change

Body-condition scoring provides an inexpensive estimate of body energy reserves. Both excessive body condition at calving and substantial postpartum body-condition loss may be associated with management and health challenges.

Although body-condition scoring is less molecularly sophisticated than metabolomics, it can be valuable in a prediction model because it is inexpensive, repeatable and widely accessible. A precision system should not automatically favour expensive biomarkers over practical clinical measurements.

6.5. Linking metabolism to uterine recovery

Metabolic indicators may influence reproductive outcomes through several interacting mechanisms, including altered immune function, systemic inflammation, oxidative stress and changes in ovarian activity.

A useful model should distinguish three possibilities:

1. A metabolic marker predicts uterine disease independently of other risk factors.
2. A metabolic marker provides information about a pathway that also influences uterine disease.
3. A metabolic marker acts mainly as a proxy for broader physiological stress.

These explanations are not equivalent. Predictive models can identify useful associations, but causal claims require stronger study designs and

additional evidence.

7. The Uterine Microbiome

7.1. From microbial presence to disease-associated ecology

The postpartum uterus is exposed to microorganisms, but the relationship between microbial presence and disease is complex. Disease may reflect particular organisms, microbial interactions, host susceptibility, the uterine environment or combinations of these factors.

Culture-based methods remain useful for identifying viable organisms under appropriate conditions. Molecular methods can detect a broader range of microbial DNA but may not establish organism viability or biological activity.

Microbiome profiling therefore needs to be interpreted alongside clinical phenotype, cytology, sampling methodology and relevant host-response measurements.

7.2. Culture-independent approaches

16S ribosomal RNA gene sequencing is commonly used to characterise bacterial community composition. Shotgun metagenomic sequencing can provide greater taxonomic and functional resolution, although it usually requires additional resources and more complex analysis.

These approaches have important limitations in low-biomass samples. Contamination from collection equipment, extraction reagents, laboratory environments and adjacent anatomical sites can distort the apparent microbial profile.

Appropriate negative controls, extraction controls, sequencing controls and transparent reporting of contamination-management procedures are essential. A detected microbial sequence should not automatically be interpreted as evidence of an active uterine infection.

7.3. Host–microbe interactions

The most informative microbiome research may be that which evaluates relationships between microbial characteristics and the host inflammatory response.

For example, investigators could examine whether specific microbial community patterns are associated with persistent cytological inflammation, altered local immune markers or subsequent reproductive outcomes.

However, microbiome associations do not establish causality. Reverse causation is plausible because inflammation and changes in the uterine

environment may themselves alter microbial community composition.

Longitudinal sampling and carefully controlled study designs can help distinguish the temporal sequence of these processes.

7.4. Is microbiome profiling necessary in every study?

No. Microbiome profiling can add considerable complexity, cost and analytical risk. It is most justified when the research question specifically concerns host–microbe interactions or when a clear hypothesis suggests that microbial features may improve prediction.

For a first-stage clinical prediction study, standardised examination, cytology, selected inflammatory measurements and metabolic indicators may provide a more feasible starting point. Microbiome profiling can then be added in a mechanistic substudy if the study resources and biological rationale justify it.

8. Hormonal Regulation and Reproductive Outcomes

8.1. Progesterone and ovarian resumption

Progesterone is central to the establishment and maintenance of a uterine environment compatible with pregnancy. Postpartum ovarian activity, corpus luteum function and progesterone exposure are relevant to subsequent reproductive performance.

However, progesterone concentration varies with ovarian status and sampling time. A single measurement may not reliably represent the hormonal environment over an entire reproductive interval.

Serial progesterone measurements or validated measures of ovarian activity may be more informative in studies investigating relationships between uterine recovery and reproductive function.

8.2. Oestradiol and follicular dynamics

Oestradiol is associated with follicular development, oestrous behaviour and changes in the reproductive tract. Its interpretation depends on follicular stage, ovarian dynamics and the timing of sampling.

Hormonal measurements can contribute useful context to an integrated reproductive model, but they should not be assumed to provide disease-specific signals.

8.3. Hormones as predictors and explanatory variables

A major design decision is whether hormonal measures are included to improve prediction, explain a biological mechanism or adjust for differences

in reproductive status.

These objectives require different analyses. A variable that improves prediction may not establish a causal pathway and adjustment for a variable that lies on the pathway between disease and outcome can alter the interpretation of estimated associations.

The statistical plan should therefore define the role of each hormone before data analysis.

9. Embryonic Loss and Subsequent Fertility

9.1. Defining reproductive failure

Reproductive failure is not a single outcome. It may involve failure to conceive, fertilisation failure, early embryonic mortality, later pregnancy loss, failure to maintain pregnancy or prolonged time to pregnancy.

These outcomes should not be treated as interchangeable. They arise at different biological stages and may have different relationships with postpartum uterine disease.

A study should define the pregnancy-confirmation method, examination schedule and criteria for classifying pregnancy loss. If pregnancy is not detected initially, it may be impossible to distinguish fertilisation failure from embryonic loss before the first confirmation.

9.2. Early pregnancy establishment

Successful pregnancy establishment depends on coordinated embryo development, maternal recognition of pregnancy, corpus luteum function and an appropriate uterine environment.

Persistent inflammation may be associated with an unfavourable endometrial environment. Metabolic stress may also influence reproductive physiology. Nevertheless, the relative contribution of each factor is likely to vary across animals and management conditions.

Research should distinguish evidence for association from evidence for a specific mechanism. A relationship between postpartum inflammation and later pregnancy loss does not, by itself, establish that the inflammatory process directly caused embryo death.

9.3. Conception rate and pregnancy per insemination

Conception rate and pregnancy per insemination are practical outcomes for evaluating reproductive performance, but their denominators and observation periods must be defined clearly.

Analyses should account for differences in insemination timing, semen and insemination practices, parity, reproductive management and the probability that a cow receives insemination during the observation period.

A biomarker model that predicts pregnancy in a tightly managed experimental herd may perform differently in commercial herds with different breeding policies.

9.4. Time to pregnancy

Time-to-event analysis can capture the interval between calving and a defined reproductive event, such as conception or confirmed pregnancy. It is often more informative than a binary outcome measured at a single time point.

Cows that are culled, die, remain uninseminated or are lost to follow-up require explicit treatment in the analysis. The choice of censoring rules and competing-event methods should reflect the biological and management questions.

9.5. Embryonic loss as a distinct endpoint

To study pregnancy loss, the protocol must first establish pregnancy using a defined diagnostic method and subsequently repeat examination at an appropriate interval.

The exact timing of loss may remain uncertain between examinations. Consequently, analyses should account for interval censoring or acknowledge the limitations of the observed event time when relevant.

Pregnancy-loss research also requires sufficient numbers of confirmed pregnancies and loss events. A study designed primarily to predict uterine disease may not contain enough pregnancy-loss events to support a reliable secondary model.

10. Integrating Multimodal Biomarkers

10.1. Why integration may outperform isolated measurements

Each biomarker class reflects a different aspect of postpartum physiology:

- Endometrial measurements characterise local uterine inflammation or molecular function.
- Systemic inflammatory measurements characterise aspects of the host response.
- Metabolic measurements reflect nutrient mobilisation and metabolic adaptation.

- Hormonal measurements provide information about ovarian and endocrine status.
- Microbiome measurements characterise selected features of microbial communities.
- Clinical measurements provide directly observable information about health and reproductive tract condition.

Integration may improve prediction if these measurements contribute complementary information. It may also fail if markers are highly correlated, noisy, expensive or weakly related to the clinical outcome.

The objective should therefore be to demonstrate incremental predictive value rather than merely to assemble a large panel.

10.2. A tiered biomarker strategy

A feasible translational strategy is to develop the model in stages.

Tier 1: Routine clinical predictors. These include parity, calving history, postpartum timing, body condition, clinical examination, reproductive history and relevant herd-management variables.

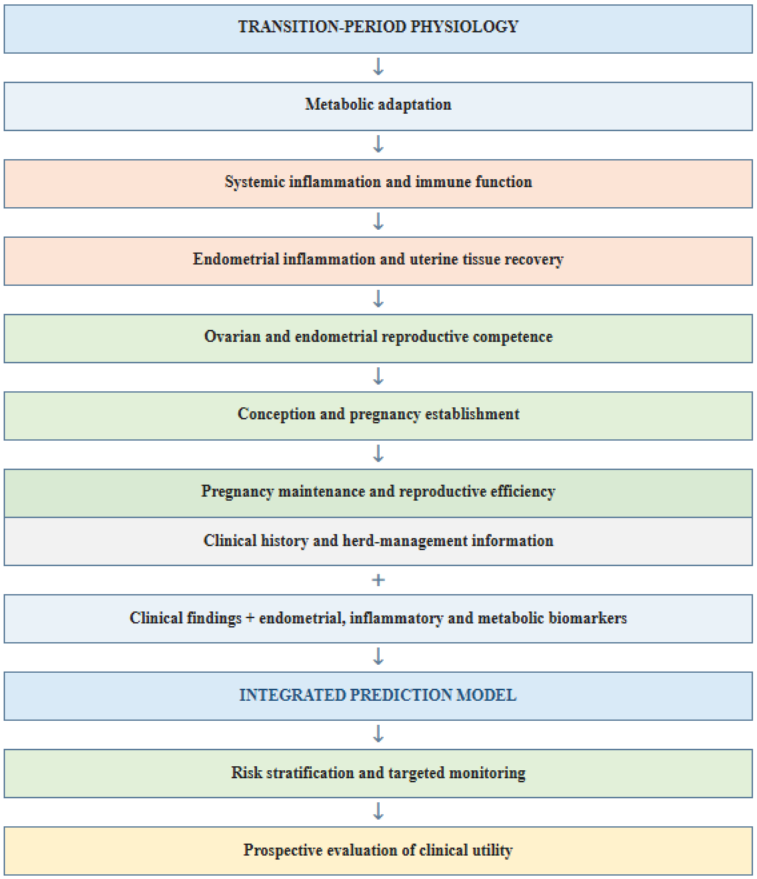
Tier 2: Accessible laboratory measurements. These may include selected metabolic indicators, acute-phase proteins, haematological measures and endometrial cytology.

Tier 3: Advanced molecular measurements. These may include targeted gene expression, selected cytokines, metabolomics, proteomics or microbiome profiling.

Each tier should be compared with the preceding tier. This approach reveals whether advanced technologies provide enough additional value to justify their cost and complexity.

10.3. A proposed conceptual framework

The following framework describes a research hypothesis rather than an already validated



PREDICTION AND CLINICAL-UTILITY PATHWAY

11. Study Design for an Internationally Competitive Research Programme

11.1. Establishing the research question

A suitable primary question is:

Can a prespecified combination of clinical, endometrial, inflammatory and metabolic measurements obtained during the early postpartum period improve prediction of subsequent uterine disease compared with routine clinical assessment alone?

Secondary questions may examine whether the same measurements improve prediction of pregnancy establishment, pregnancy loss or time to pregnancy.

The study should avoid trying to answer every reproductive question with one small cohort. The primary endpoint should determine the sample-size calculation and the main analytical strategy.

11.2. Prospective longitudinal cohort design

A prospective longitudinal cohort is appropriate because the measurements must precede the outcomes they are intended to predict.

Cows should be enrolled according to predefined eligibility criteria, with postpartum assessments scheduled at biologically justified intervals. Repeated clinical and laboratory measurements can characterise changes over time.

The protocol should document parity, breed or genetic group, body condition, calving difficulty, retained fetal membranes, relevant concurrent diseases, diet, housing, milk production and reproductive management. These variables may act as confounders, predictors or indicators of population heterogeneity.

A multicentre study may improve external validity, particularly when it includes herds with different climates and management systems. However, multicentre recruitment requires consistent case definitions, sample handling and outcome assessment.

11.3. Selecting sampling windows

Sampling times should be chosen to reflect the intended prediction task. A measurement collected before disease onset can support a claim of early prediction; a measurement obtained after clinical disease is apparent is more appropriately evaluated as a diagnostic or severity marker.

A protocol might include an early postpartum assessment, one or more intermediate assessments and a later reproductive follow-up. Exact days should be selected after reviewing the intended disease definition, expected timing of the outcome, animal-welfare considerations and the feasibility of repeated sampling.

Sampling schedules should be fixed before outcome analysis wherever possible. Flexible sampling based on emerging clinical signs can introduce bias if not handled appropriately.

11.4. Sample collection and laboratory quality assurance

Blood, endometrial cytology samples and other biological materials require standardised collection, processing, storage and transport.

The protocol should specify the collection equipment, anticoagulants

where relevant, processing intervals, storage temperatures, freeze–thaw limits and assay procedures. Laboratory staff should ideally be blinded to reproductive outcomes.

Quality assurance should include analytical controls, duplicate measurements where appropriate, prespecified rules for excluding failed assays and documentation of missing samples.

For microbiome studies, contamination controls are essential, especially when bacterial biomass is low. For molecular studies, sample integrity and appropriate normalisation procedures are equally important.

11.5. Outcome adjudication

Outcome assessment should use standardised criteria and, where feasible, assessors blinded to the biomarker results.

Clinical disease should be distinguished from cytologically defined inflammation. Pregnancy establishment should be confirmed using a prespecified method and subsequent pregnancy status should be assessed at defined intervals.

When more than one veterinarian or diagnostic team participates, interobserver agreement should be evaluated where feasible.

12. Statistical Modelling and Predictive Validation

12.1. Prediction is not the same as association

A statistically significant association does not necessarily produce a useful prediction model. A biomarker can be associated with an outcome but contribute little discrimination once established clinical predictors are included.

Similarly, a model can discriminate between animals with different outcomes yet systematically overestimate or underestimate their absolute risk.

Predictive research should therefore assess discrimination, calibration and clinical usefulness rather than relying exclusively on p-values or apparent associations.

12.2. Baseline model development

The baseline model should include established clinical and management variables selected on biological and practical grounds. Candidate biomarkers can then be added in prespecified stages.

The key question is whether the integrated model improves prediction beyond this baseline. The answer should be evaluated using appropriate

performance measures and uncertainty intervals.

A large number of candidate predictors relative to the number of outcome events increases the risk of overfitting. The sample-size strategy should be based on the expected event frequency, the intended model complexity and the desired precision.

12.3. Regression approaches

Multivariable logistic regression may be appropriate for binary outcomes such as a defined postpartum disease diagnosis or pregnancy status at a prespecified time.

Cox proportional hazards regression or other survival models may be appropriate for time-to-pregnancy outcomes when their assumptions and censoring structures are suitable.

Repeated measurements may require mixed-effects models or other longitudinal methods that account for within-cow correlation. Herd-level clustering should also be considered.

Model assumptions, missing-data procedures and variable-selection decisions should be reported transparently.

12.4. Machine learning

Machine-learning methods, including penalised regression, random forests and gradient-boosting approaches, may help model nonlinear relationships or interactions among biomarkers.

However, these methods are not inherently superior to simpler regression models. Their value depends on sample size, data quality, the complexity of the underlying relationships and the availability of independent validation data.

Feature selection and hyperparameter tuning must occur within the training process. Performing these steps on the complete dataset before validation can cause information leakage and produce overly optimistic performance estimates.

12.5. Discrimination and calibration

The area under the receiver operating characteristic curve may summarise discrimination for a binary outcome. Sensitivity, specificity, predictive values and likelihood ratios can provide additional information at clinically relevant thresholds.

Calibration should assess agreement between predicted and observed

risks. Calibration plots and suitable summary measures can reveal whether the model systematically overpredicts or underpredicts risk.

For time-to-event outcomes, performance measures must account for event timing and censoring.

12.6. Internal and external validation

Internal validation using bootstrap resampling or appropriately structured cross-validation can estimate optimism in model performance.

External validation is a separate requirement. A model should be tested in independent animals and preferably in herds not used for model development. When international transferability is a goal, validation across different management systems and climatic conditions becomes particularly important.

Randomly splitting individual cows from the same herds into training and test sets may overstate generalisability because the groups can share management practices, environmental exposures and herd-level characteristics.

12.7. Clinical utility and decision analysis

A prediction model should be assessed in relation to the decisions it is intended to support. Decision-curve analysis or other appropriate utility assessments may help evaluate whether model-guided decisions provide greater net benefit than treating all cows, treating none or relying on routine assessment.

The consequences of false-positive and false-negative classifications should be considered explicitly. Unnecessary treatment, repeated sampling and laboratory expense may outweigh the benefit of a small increase in predictive accuracy.

13. Translational Relevance to Indian Dairy Systems

India offers an important setting for veterinary reproductive research because its dairy sector includes indigenous cattle, crossbred cattle and buffaloes managed under diverse production systems.

However, the Indian dairy population is not homogeneous. Differences in breed composition, nutrition, housing, thermal exposure, disease burden, access to veterinary services and reproductive management can affect biomarker distributions and disease prevalence.

A prediction model developed in a high-input commercial dairy herd may therefore require recalibration before use in smallholder systems. Conversely,

a model developed in a specific Indian population should not automatically be assumed to perform well in temperate commercial dairy herds.

Research should explicitly define the intended population and use a sampling strategy that reflects the desired clinical application. Breed, parity, production system and climatic conditions may need to be incorporated into validation analyses.

13.1. Indigenous and crossbred cattle

Indigenous and crossbred cattle may differ in production characteristics, adaptation and management exposure. These differences provide a rationale for investigating model transportability and population-specific calibration.

Nevertheless, genetic-group comparisons require adequate sample sizes and appropriate control of management and environmental confounding. Differences in observed biomarker values should not automatically be attributed to breed genetics.

13.2. Buffaloes as a related but distinct research population

Buffalo reproduction presents additional questions relating to reproductive seasonality, heat stress, management and the timing of ovarian activity. Findings from cattle may inform buffalo research, but diagnostic thresholds and predictive relationships should be validated rather than transferred without testing.

Where buffaloes are included, the study should treat species as a prespecified population factor and evaluate whether the biological assumptions and measurement protocols remain appropriate.

13.3. Affordability and field implementation

A useful precision-reproduction system must be compatible with the economic and logistical realities of the intended setting.

The most feasible model may rely on a small number of accessible measurements combined with clinical history and standardised examination. Advanced molecular measurements could be reserved for selected animals, referral laboratories or research-oriented applications.

Cost-effectiveness analysis should consider laboratory expenses, veterinary time, repeat examinations, treatment costs, breeding outcomes and the potential economic consequences of incorrect risk classification.

14. Limitations and Major Research Gaps

Despite progress in reproductive diagnostics, several challenges limit the

routine application of integrated biomarker panels.

First, biomarker specificity is often limited. Inflammatory and metabolic measurements may reflect multiple physiological or pathological processes, making them difficult to interpret without clinical context.

Second, timing strongly influences results. Biomarker distributions change during postpartum recovery. Sampling schedules and disease definitions must therefore be standardised.

Third, study populations are heterogeneous. Differences in breed, parity, management, nutrition, environment and disease prevalence can reduce transportability.

Fourth, molecular research can be vulnerable to overfitting. High-dimensional data combined with limited outcome events may produce apparently impressive but poorly generalisable models.

Fifth, prediction does not establish causation. A biomarker may be a useful predictor without being a causal driver of disease. Mechanistic claims require additional evidence.

Sixth, advanced testing may not be economically practical. A small improvement in statistical performance may not justify expensive assays or complicated sampling.

Seventh, external validation remains essential. A model that performs well in its development cohort may fail in another herd, region or production system.

Eighth, clinical utility is frequently underexamined. The benefit of predicting disease depends on whether earlier identification leads to an effective, safe and economically justified response.

These limitations support a staged research programme in which biological discovery, analytical validation, predictive development, external validation and clinical implementation are treated as distinct phases.

15. Future Perspectives: Towards Precision Reproductive Medicine

The next generation of reproductive prediction systems will likely combine clinical information with selected laboratory measurements and longitudinal physiological data.

Multimodal approaches may help identify distinct biological profiles among cows with similar clinical presentations. For example, animals with comparable uterine cytology results may differ in metabolic status, systemic

inflammatory activity or subsequent reproductive outcome.

Longitudinal modelling could improve interpretation by distinguishing a persistently abnormal biomarker from a transient physiological change. Repeated measurements may also reveal recovery trajectories that cannot be captured by a single sample.

Advances in targeted metabolomics, proteomics and molecular diagnostics may provide additional candidate markers. Their adoption should depend on analytical robustness and demonstrated incremental value rather than technological novelty alone.

A particularly important future direction is the development of interpretable prediction models that link risk estimates to practical veterinary decisions. A useful system should communicate not only that an animal is at elevated risk, but also the degree of uncertainty, the factors contributing to that estimate and the appropriate next clinical step.

The integration of precision reproductive medicine with herd-management software may eventually support more consistent monitoring, targeted reproductive examinations and improved allocation of veterinary resources. Nevertheless, implementation should follow independent validation and prospective assessment of clinical and economic benefit.

16. Proposed Research Framework

Based on the biological and methodological considerations discussed above, a focused research programme could be organised into four stages.

Stage I: Phenotyping and candidate-marker assessment

Establish standardised clinical, cytological, inflammatory and metabolic measurements in a well-characterised postpartum cohort. Identify the temporal relationships between candidate markers and clearly defined reproductive outcomes.

Stage II: Model development

Develop a baseline model using clinical and management variables. Add candidate biomarker groups sequentially to determine whether they improve discrimination, calibration and decision-relevant performance.

Stage III: Independent validation

Evaluate the locked model in independent herds. Examine calibration, subgroup performance and the consequences of differences in disease prevalence, breed composition and management.

Stage IV: Clinical utility evaluation

Assess whether model-guided monitoring improves reproductive management compared with routine practice. Include relevant clinical outcomes, resource use, animal welfare and economic consequences.

This staged structure separates exploratory research from clinical claims and provides a clearer pathway from biological discovery to implementation.

17. Conclusions

Postpartum reproductive failure in dairy cattle emerges from the interaction of uterine recovery, host immunity, metabolic adaptation, endocrine regulation and the establishment of pregnancy. No single biomarker is likely to capture all these dimensions across every production system.

Integrating endometrial cytology or molecular measurements with selected inflammatory and metabolic indicators offers a biologically plausible strategy for improving risk stratification. However, the usefulness of such an approach must be demonstrated through prospective sampling, clearly defined outcomes, appropriate statistical methods, external validation and assessment of clinical utility.

The most important research priority is not the creation of the largest possible biomarker panel. It is the identification of a parsimonious, reproducible combination of measurements that provides meaningful predictive improvement over routine veterinary assessment.

For India and the international dairy sector, this approach could support a more evidence-based transition from reactive reproductive diagnosis to individualised monitoring. Achieving that goal will require collaboration among veterinary theriogenologists, reproductive immunologists, metabolic researchers, molecular biologists, statisticians and dairy-management specialists.

Precision reproductive medicine should ultimately be judged by its ability to improve animal health, reproductive efficiency, welfare and the sustainability of dairy production—not by the number of biomarkers measured or the complexity of the technology employed.

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CHAPTER

Decoding Embryo–Maternal Signalling: Molecular Mechanisms, AI-Enabled Prediction and Robotic Precision in Pregnancy Establishment in Cattle and Buffaloes

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Abstract

Successful pregnancy establishment in cattle and buffaloes depends on the precise temporal coordination of bidirectional communication between the developing conceptus and the maternal reproductive tract. During early pregnancy, the conceptus must develop, elongate and signal its presence while the endometrium establishes a hormonally responsive, metabolically supportive and immunologically regulated environment. Disruption of these interdependent processes can compromise pregnancy establishment and contribute to early embryonic loss, a multifactorial phenomenon that cannot be explained by a single molecular pathway or diagnostic marker.

This chapter critically examines the biological mechanisms underlying embryo–maternal communication, with particular emphasis on interferon tau (IFNT), endometrial receptivity, extracellular vesicles (EVs), non-coding RNAs, transcriptomic and proteomic signatures, maternal metabolic status,

thermal stress and the influence of in-vitro embryo production systems. Building on these mechanisms, it proposes an integrated precision-reproduction framework that combines molecular and omics-based measurements with embryo morphology, time-lapse imaging, reproductive history, environmental sensor data and longitudinal pregnancy outcomes. Artificial intelligence (AI) and machine learning are evaluated for their potential to support embryo assessment, multimodal prediction of pregnancy failure, biomarker prioritisation and evidence-based reproductive decision-making. Automation and robotics are explored in relation to laboratory processing, sample traceability, embryo handling, automated imaging, micromanipulation and precision-assisted reproductive procedures, while acknowledging current limitations in reliability, affordability, accessibility and field-level implementation.

Particular attention is given to the physiological, reproductive and management differences between cattle and buffaloes, underscoring the need for species-specific and population-level validation before clinical adoption. The chapter also addresses the challenges of reproducibility, data integration, model interpretability, external validation and the translation of promising molecular associations into clinically useful diagnostic tools. A staged translational roadmap is proposed, encompassing mechanistic discovery, analytical validation, model development, independent external validation and prospective assessment of clinical utility. Ultimately, the chapter advocates a biologically grounded, interpretable and cost-conscious approach that integrates molecular insight with emerging digital technologies to improve the prediction and management of early pregnancy loss and strengthen reproductive efficiency in cattle and buffalo production systems.

1. Introduction

Early pregnancy is a period of exceptionally high biological vulnerability in ruminants. Following fertilisation, the embryo undergoes cleavage and blastocyst formation, hatches from the zona pellucida and develops into a conceptus that elongates before the onset of later attachment and placentation. Throughout this sequence, development depends on the oviductal and uterine environments, while the maternal reproductive tract responds to signals arising from the conceptus. Successful pregnancy therefore emerges from reciprocal communication rather than from embryo quality or uterine condition considered in isolation.

In cattle, maternal recognition of pregnancy depends critically on conceptus-derived interferon tau (IFNT), which modifies endometrial signalling and prevents the luteolytic process that would otherwise lead to

corpus luteum regression. Yet IFNT is one component of a wider communication network involving hormones, cytokines, growth factors, lipids, metabolites, extracellular vesicles and RNA species. The endometrium responds to conceptus signals and the developing conceptus is itself influenced by maternal secretions. This reciprocal relationship has been described as a dialogue because each partner contributes information that can influence the other's biological state (Bazer *et al*., 1997; Spencer *et al*., 2004; Forde & Lonergan, 2017; Mazzarella *et al*., 2024).

Embryonic loss is not a single disease entity. It can arise from chromosomal or developmental abnormalities, suboptimal embryo culture conditions, inadequate maternal support, endocrine disruption, heat stress, metabolic imbalance, uterine dysfunction or interactions among these factors. The time at which pregnancy is assessed also changes the apparent loss rate: losses occurring before first pregnancy diagnosis may be missed or classified differently from losses documented after a confirmed pregnancy. Consequently, research must define its endpoint precisely and distinguish failure to establish a detectable pregnancy from loss after pregnancy confirmation.

Emerging molecular technologies make it possible to investigate biological states at greater resolution. Transcriptomics can describe gene-expression patterns, proteomics can characterise protein abundance, metabolomics can profile biochemical state and extracellular-vesicle research can explore a potential mechanism for transfer of molecular cargo between cells. At the same time, AI-enabled imaging and data integration are being explored in reproductive technologies. These advances create opportunity, but also risk: large data sets can produce apparently persuasive associations that fail in independent populations if the study is small, poorly controlled or insufficiently validated.

This chapter therefore has four aims: (i) explain the biology of embryo–maternal signalling and endometrial receptivity; (ii) critically evaluate molecular and environmental determinants of pregnancy establishment; (iii) examine where AI, automation and robotics can responsibly contribute; and (iv) propose a rigorous translational roadmap relevant to cattle and buffalo production systems, including Indian dairy contexts and international research.

2. Developmental Biology of Pregnancy Establishment

2.1. From fertilisation to the elongating conceptus

After fertilisation, early development proceeds through cleavage

divisions and formation of a blastocyst containing the inner cell mass and trophectoderm. Following hatching, the conceptus undergoes elongation in ruminants. This stage is biologically important because the conceptus must increase its capacity to communicate with the maternal endometrium before later placental development. A conceptus with limited developmental competence may produce an inadequate or mistimed signal, while an endometrium that is not appropriately responsive may fail to support further development.

Embryo morphology is useful but incomplete as a measure of competence. Morphologically similar embryos may differ in metabolism, gene expression, stress response and ability to survive cryopreservation or establish pregnancy. Conversely, molecular differences do not automatically mean that an embryo will fail. The most useful assessment strategy is therefore one that combines validated morphology or developmental criteria with molecular information only where it provides reproducible incremental value.

2.2. Maternal recognition of pregnancy and IFNT

IFNT is the key pregnancy-recognition signal in ruminants. Produced by trophectoderm cells of the developing conceptus, IFNT acts on the endometrium to alter the signalling events involved in luteolysis. This supports maintenance of the corpus luteum and progesterone secretion, helping preserve a uterine environment compatible with pregnancy. IFNT also influences endometrial gene expression and immune-related pathways, linking pregnancy recognition with broader maternal adaptation (Spencer *et al*., 2004; Forde & Lonergan, 2017).

IFNT-related measurements are scientifically valuable, but their use as stand-alone pregnancy tests requires caution. Signal abundance depends on developmental stage, conceptus size, sampling matrix, assay sensitivity and timing. The absence of a measurable signal at one time point does not necessarily indicate embryo death, while detection of a signal does not guarantee pregnancy maintenance. Research should distinguish mechanistic measurements from validated diagnostic tests.

2.3. The endometrium as an active sensor

The endometrium senses the conceptus through molecular and cellular pathways that include interferon-responsive genes, cytokine networks, growth factors, nutrient transport, lipid signalling and changes in epithelial function. Evidence indicates that the endometrial response can vary with conceptus quality, origin and developmental state. This suggests that maternal tissues do not respond uniformly to every conceptus; rather, they integrate multiple

signals within a changing endocrine and physiological context (Forde & Lonergan, 2017; Ruminant conceptus-maternal interactions, 2022).

Endometrial receptivity should not be reduced to a single gene, cytokine or transcriptomic score. It is a dynamic functional state, dependent on timing and the interaction between progesterone exposure, endometrial differentiation, inflammatory regulation and conceptus-derived signals. A candidate receptivity signature must be evaluated against prospectively measured reproductive outcomes and should be compared with simpler clinical predictors.

3. Extracellular Vesicles and Molecular Cargo

3.1. Biological rationale

Extracellular vesicles are membrane-enclosed particles released by cells and found in biological fluids. They can carry proteins, lipids and nucleic acids, including messenger RNA and microRNA. In reproductive tissues, vesicles from the oviduct, endometrium and embryo are being investigated as potential mediators of local communication. Bovine studies indicate that reproductive-tract-derived vesicles can be taken up by embryos and that vesicle exposure under selected in-vitro conditions may influence embryo development or quality. However, results vary with source, isolation procedure, dose, culture conditions and outcome measured (Almiñana *et al*., 2017; Leal *et al*., 2022; Mazzarella *et al*., 2024).

EVs are attractive because they may carry a molecular snapshot of the tissue or embryo from which they originate. This raises the possibility of developing non-invasive or minimally invasive indicators of embryo state or uterine readiness. However, a signal detected in uterine fluid, culture medium or blood may have multiple origins. Establishing tissue origin, biological activity and predictive specificity is therefore a substantial analytical challenge.

3.2. Terminology and analytical standardisation

The terms exosome and microvesicle refer to different biogenesis pathways, but routine isolation methods often do not distinguish these subpopulations with certainty. Unless biogenesis has been demonstrated, the more cautious term extracellular vesicle is preferable. Isolation and characterisation methods can influence particle yield and apparent cargo composition. Differential ultracentrifugation, size-exclusion chromatography, precipitation-based kits and other approaches have different strengths and limitations.

EV studies should report sample origin, collection timing, processing conditions, storage, isolation method, particle concentration and size distribution, marker assessment, contamination controls and the evidence supporting vesicle identity. The MISEV recommendations provide a useful general framework for EV research reporting (Théry *et al*., 2018). For reproductive applications, additional attention is needed to the reproductive-fluid matrix and potential contamination from cells, cellular debris, blood or culture supplements.

3.3. miRNA, lncRNA and other cargo

MicroRNAs can regulate gene expression post-transcriptionally, while long non-coding RNAs may participate in diverse regulatory processes. Both have been investigated in bovine pregnancy and implantation biology. Their presence in EV cargo raises the possibility that they contribute to embryo–maternal communication, but detecting an RNA molecule in a vesicle is not equivalent to demonstrating delivery, target engagement or a functional effect in recipient cells.

A strong mechanistic study should move through a chain of evidence: identify a candidate cargo; verify its reproducible presence; show uptake or transfer under suitable conditions; demonstrate a plausible effect on a recipient cell or embryo; test whether the effect depends on the candidate molecule; and determine whether the mechanism relates to an in-vivo reproductive outcome. Many candidate molecules remain at the discovery or association stage.

3.4. Potential diagnostic and therapeutic applications

EV-derived signatures could eventually contribute to embryo-quality assessment, endometrial-state classification or evaluation of culture conditions. EVs have also been discussed as possible delivery vehicles for bioactive molecules. These applications remain research directions rather than established routine treatments for cattle or buffalo infertility. Translation requires evidence of reproducible manufacture, dose consistency, safety, biological specificity and improved pregnancy outcomes.

4. Transcriptomics, Proteomics and Metabolomics

4.1. Transcriptomics

RNA sequencing can identify patterns of gene expression in endometrium, conceptus-derived material or cultured cells. It is useful for discovery because it can reveal pathways not anticipated by a narrow candidate-gene approach. However, transcriptomic studies are sensitive to tissue composition, cycle stage, pregnancy timing, sample quality, batch

effects and analytical decisions. A different proportion of immune cells in two tissue samples can produce an apparent expression difference even if expression within each cell type is unchanged.

Discovery should be followed by targeted validation using independent samples, suitable normalisation and prespecified statistical procedures. Where a transcriptomic signature is proposed as a predictor, performance must be evaluated in a population not used to select the genes. Reporting only the most statistically significant genes risks overstating biological certainty.

4.2. Proteomics

Proteomics can characterise proteins involved in signalling, metabolism, stress responses and tissue function. It may complement transcriptomics because protein abundance is influenced by translation, secretion, degradation and post-translational modification. Nevertheless, proteomic data also depend on sample preparation, instrument platform, detection limits and bioinformatic processing. Orthogonal confirmation of selected findings is advisable before candidate proteins are promoted as biomarkers.

4.3. Metabolomics and redox biology

Metabolomics captures aspects of biochemical state and may help link maternal nutrition, energy metabolism, oxidative stress and embryo development. Heat stress and nutritional challenges may alter substrate availability, redox balance and cellular stress pathways. The challenge is that metabolites can be highly sensitive to diet, time of day, lactation stage, sample handling and environmental exposure. These factors need to be recorded and considered during analysis.

An integrated multi-omics design can generate a more complete biological picture, but it also increases cost, multiple-testing burden and risk of overfitting. A focused design with a clear biological hypothesis and a planned validation cohort may be more informative than an unrestricted survey of every available molecular layer.

5. Heat Stress, Metabolic State and Species-Specific Context

5.1. Heat stress as a reproductive modifier

Thermal stress can affect follicular development, oocyte competence, early embryo development and the maternal environment. The timing and duration of exposure matter because different developmental stages vary in sensitivity. In field studies, temperature-humidity index or other environmental measures should be linked to individual animal data rather than used as a crude seasonal label alone.

Heat stress may interact with nutritional status, milk production, housing, shade, cooling systems and breed or species. For that reason, observed differences in embryo development or pregnancy outcome cannot automatically be attributed to temperature without adequate control of these co-exposures.

5.2. Cattle and buffaloes: shared biology, distinct validation needs

Cattle and buffaloes share core ruminant reproductive mechanisms, including conceptus signalling and maternal recognition of pregnancy. Yet reproductive seasonality, management, thermal responses, reproductive tract characteristics and production systems can differ. A model developed in cattle should not be assumed to transfer directly to buffaloes or vice versa, without testing.

Indian dairy systems are heterogeneous, ranging from smallholder systems to organised commercial herds. Breed composition, feed availability, heat exposure, insemination practices, veterinary access and record quality may differ substantially. A translational programme should define its target population and validate across the settings where the tool is intended to be used.

6. Defining Early Pregnancy Loss as a Research Outcome

Pregnancy loss estimates depend strongly on when pregnancy is first confirmed and how follow-up is performed. Failure to detect pregnancy at an early examination cannot reliably distinguish failure of fertilisation, loss before detection, delayed development or diagnostic error. Studies should state the pregnancy test or ultrasonographic criteria, examination schedule and rules for classifying loss.

Repeated assessment is particularly important when the aim is to predict loss after pregnancy establishment. For example, a model intended to predict loss between two defined pregnancy examinations should be developed only among animals with a confirmed pregnancy at the first examination. If loss time is known only to lie between two examinations, the analysis should acknowledge interval uncertainty.

Potential confounders include parity, previous reproductive history, semen or embryo factors, insemination timing, ovarian status, body condition, concurrent disease, season, herd and reproductive management. The statistical plan should distinguish baseline predictors from variables that arise after the prediction time, because using later information would create information leakage.

7. AI and Machine Learning in Embryo Assessment

7.1. Image-based embryo grading

Computer vision methods can be trained to analyse embryo images, classify developmental stage or identify morphological features. In principle, these systems may improve consistency, support high-throughput assessment and reduce dependence on subjective grading. Their actual value depends on the quality and diversity of the training data, the definition of embryo-quality labels, imaging protocols and the outcome against which predictions are evaluated.

Image-based classification is vulnerable to dataset shift. Differences in microscopes, lighting, camera settings, culture systems, embryo stage, operator practices and laboratory populations can change image characteristics. A model trained on one laboratory's images may therefore perform poorly elsewhere. External validation should include data from independent laboratories and, where possible, prospective assessment.

7.2. Time-lapse imaging and developmental kinetics

Time-lapse imaging can record developmental timing and morphological transitions without repeated manual observation. AI may extract features such as cleavage timing, duration between stages and abnormal developmental patterns. Such features may add information beyond a single static image, but they require standardised definitions and careful handling of missing or ambiguous events.

Embryo-selection algorithms should not be judged only by classification accuracy. Relevant outcomes include pregnancy per transferred embryo, pregnancy maintenance, calving outcomes, reproducibility across operators and whether AI-assisted selection improves results over established embryologist assessment. Models should also be evaluated for calibration and uncertainty, not merely a single headline accuracy figure.

7.3. Multimodal AI

A future system might integrate embryo images, developmental kinetics, selected molecular measurements, donor and recipient history, environmental conditions and laboratory metadata. Multimodal learning may capture interactions that are not visible in any one data stream. However, each additional data source increases complexity and the risk of missingness, leakage and spurious correlation.

The safest development sequence is to establish a strong baseline model, add data streams one at a time and quantify the incremental value of each

addition. If a model performs well only when laboratory-specific identifiers or hidden workflow patterns are present, it may be learning the laboratory rather than embryo biology.

8. AI for Predicting Pregnancy Establishment and Loss

AI and conventional statistical models can combine reproductive history, environmental exposures, embryo features, molecular measurements and follow-up data to estimate risk. The intended prediction time must be explicit: prediction before embryo transfer, at the time of transfer, at first pregnancy diagnosis or after pregnancy confirmation are different tasks and require different permissible predictors.

Models may include penalised regression, random forests, gradient boosting or neural networks. More complex methods are not automatically better, especially in veterinary datasets where the number of pregnancy-loss events may be small. Simpler, well-calibrated models can be easier to interpret, validate and implement.

Predictive modelling should separate model development, tuning and evaluation. Feature selection and preprocessing must be performed inside the training process. Where animals are clustered by herd or laboratory, validation should be designed to test transfer across clusters rather than merely randomly splitting observations from the same setting.

The TRIPOD+AI statement provides contemporary reporting guidance for prediction models developed using regression or machine-learning methods (Collins *et al*., 2024). Its principles are relevant to transparent reporting of intended use, participants, predictors, outcome definitions, missing data, model development, performance and validation. PROBAST can also inform assessment of risk of bias and applicability when evaluating prediction-model studies.

8.1. Explainability and uncertainty

Feature-importance plots or local explanation methods can help describe which inputs influence a model's output, but they do not prove causality. An explanation should be treated as a description of model behaviour, not as proof that a biomarker causes pregnancy loss. Uncertainty estimates and clear warnings for out-of-distribution cases are important safeguards.

8.2. Fairness and population transportability

Performance should be examined across relevant subgroups, including cattle versus buffaloes, parity groups, production systems, seasons and laboratories where sample size permits. Differences in data availability can

create unequal error rates. A model should not be marketed as universally applicable if its calibration has only been assessed in a narrow population.

9. Automation in Reproductive Biotechnology

Automation can standardise repetitive laboratory steps, reduce transcription errors and improve traceability. Potential applications include sample accessioning, barcode-based chain of custody, timed incubator monitoring, image capture, culture-dish tracking, automated data entry, assay workflow control and structured quality-control alerts. These uses are generally more immediately practical than fully autonomous reproductive procedures.

Automation is valuable only when the workflow itself is well defined. If a poorly validated assay is automated, errors may simply be produced more consistently and at greater scale. Before automation, laboratories should establish standard operating procedures, instrument calibration, audit trails, error handling, data backup and human review for exceptions.

Robotic or automated systems used in embryo laboratories must protect embryo identity and prevent mix-ups between donors, recipients, culture dishes and transfer plans. A traceability system should link each embryo to its source, handling history, developmental observations, cryopreservation status, quality-control records and eventual transfer outcome, subject to privacy and data-governance requirements.

10. Robotics and Precision Reproductive Procedures

Robotics has potential applications in precision handling, microscopy, laboratory micromanipulation and repetitive movement tasks. In reproductive biotechnology, automated stages, robotic pipetting, computer-controlled microscopes and assisted micromanipulation can improve positional repeatability in suitable laboratory environments. However, their adoption depends on the specific procedure, embryo-handling constraints, contamination control, reliability and economic feasibility.

Robotics for live-animal reproductive procedures is a different and more demanding domain. Transrectal ultrasonography, insemination and embryo transfer require tactile feedback, anatomical interpretation, animal restraint, operator judgement and immediate response to unexpected conditions. A robot should not be assumed to perform these procedures safely merely because it can move a probe or instrument along a programmed path.

In the near term, the most defensible approach is decision support and operator assistance: image enhancement, procedure guidance, instrument tracking, automated record keeping and quality alerts under trained veterinary

supervision. Any autonomous or semi-autonomous system would require formal engineering validation, animal-welfare review, risk analysis, fail-safe design, operator override and prospective evaluation before clinical deployment.

10.1. Human oversight and animal welfare

Veterinary reproductive technologies operate on living animals and embryos, so technical performance must be assessed alongside welfare. Systems should minimise unnecessary restraint, repeated invasive sampling, excessive manipulation and avoidable treatment. Human oversight is especially important when a model is uncertain, the animal's condition changes or the procedure falls outside the system's validated operating range.

11. Proposed Integrated Architecture

The following conceptual architecture is a proposed research framework, not an established commercial system. It links molecular biology with data acquisition, prediction and veterinary decision-making. Its purpose is to organise testable hypotheses and identify the evidence required at each stage.

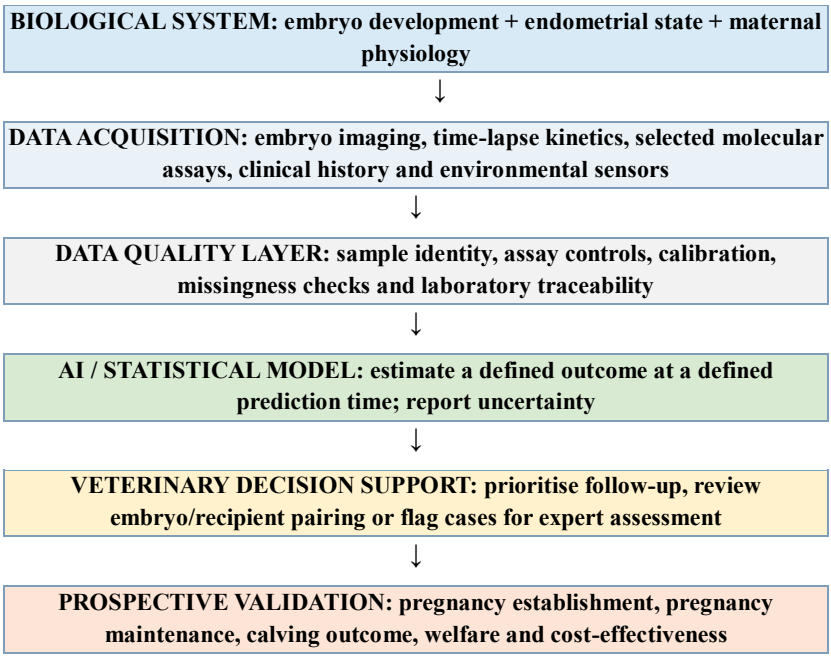


Fig. 1: Proposed integrated architecture for AI-supported embryo–maternal research and translational evaluation.

The architecture deliberately separates data collection, data quality, prediction and clinical action. This prevents a common error in technology-led research: assuming that a technically sophisticated model is useful before its inputs are reliable and its decisions have been evaluated prospectively.

12. Research Questions and Testable Hypotheses

A competitive chapter should move beyond listing technologies and articulate research questions that can be tested. The following are proposed hypotheses for future investigation; they are not established findings.

A prespecified endometrial-response signature measured at a defined stage of early pregnancy will provide incremental predictive information beyond embryo morphology and established reproductive-history variables.

Selected EV-associated cargo measurements will show reproducible associations with embryo developmental competence when sample processing, isolation and quality controls are standardised.

Environmental thermal exposure and maternal metabolic indicators will modify the relationship between embryo features and pregnancy outcome, with effect sizes that vary by population and management system.

A multimodal model combining image-derived embryo features with selected molecular and clinical measurements will outperform a baseline clinical model in independent validation, provided that the dataset contains sufficient outcome events.

Automated sample tracking and laboratory workflow monitoring will reduce preventable identification and data-entry errors compared with an otherwise equivalent manual workflow.

AI-assisted decision support will improve consistency of risk stratification without increasing unnecessary interventions, but only if the model is calibrated and evaluated prospectively.

13. Proposed Research Programme and Methods

13.1. Overall design

A feasible high-quality programme could use a staged design rather than attempting transcriptomics, proteomics, EV profiling, robotics and AI simultaneously. The discovery phase should identify a small number of biologically justified candidate features; the validation phase should test them independently; and the implementation phase should determine whether the model changes decisions beneficially.

13.2. Population and eligibility

The target population must be stated precisely: dairy cattle, buffaloes or both; in-vitro-produced embryos, in-vivo-derived embryos or both; and the relevant reproductive management system. Inclusion and exclusion criteria should be established before enrolment. If cattle and buffaloes are both included, the design should provide adequate numbers to evaluate species-specific performance rather than pooling them without examination.

13.3. Sampling and timing

Sampling windows should be selected according to the intended prediction task and biological stage. Embryo imaging and laboratory measurements should be recorded before transfer or before the outcome being predicted. Maternal samples should be collected only where justified, using welfare-conscious and standardised procedures. Repeated pregnancy assessment should use prespecified intervals and a consistent diagnostic protocol.

13.4. Sample size and events

Sample size should be calculated for the primary outcome and intended model complexity. The number of total animals is not enough; the number of pregnancy establishments, losses and other relevant events determines how much information is available. A large predictor set with few loss events is likely to produce unstable estimates. Sample-size planning should incorporate anticipated prevalence, missingness, clustering and the need for external validation.

13.5. Laboratory and imaging quality assurance

All samples and images should carry unique identifiers linked through a secure audit trail. Imaging devices and laboratory instruments should be calibrated and acquisition settings documented. Assays should include suitable controls and prespecified acceptance criteria. A subset of samples may be reanalysed to assess repeatability. Image annotation should use a written rubric and, where feasible, multiple blinded assessors.

13.6. Statistical analysis plan

The primary endpoint and prediction time should be registered in the protocol. Baseline models should be compared with models that add molecular, image-based or environmental features. Missing-data handling, preprocessing, feature selection, tuning and validation must be specified before final evaluation. Model performance should include discrimination, calibration, uncertainty and decision-relevant measures. Results should be

reported transparently, including negative findings and subgroup limitations.

13.7. External validation

External validation should involve independent herds, laboratories or time periods not used in model development. For an international application, testing should include settings that differ in management, breed composition, climate and laboratory workflow. Recalibration may be necessary, but the need for recalibration should be reported rather than hidden.

13.8. Clinical utility

The final stage should compare the proposed decision-support approach with routine practice. Relevant endpoints include pregnancy establishment, pregnancy maintenance, calving outcomes, number of transfers or examinations, time to pregnancy, adverse events, labour, cost and welfare. The model should not be considered clinically successful solely because it improves an AUC or classification accuracy.

14. Evidence Gaps and Translation Barriers

Several barriers currently separate molecular discovery from a dependable reproductive technology. First, many molecular findings are based on small cohorts, single time points or in-vitro systems. These studies can be valuable for mechanism discovery but may not reflect the complexity of commercial reproduction. Second, EV isolation and characterisation remain technically variable. Third, transcriptomic and proteomic results may not replicate across platforms or populations. Fourth, pregnancy loss is often incompletely observed because early events occur before diagnosis or between scheduled examinations.

AI systems face related challenges: biased labels, non-representative images, data leakage, small event counts, inadequate external validation and limited interpretability. Automation adds engineering risks, including equipment failure, incorrect sample identification, contamination, software updates and dependence on vendor-specific infrastructure. Robotics in live animals adds safety and welfare considerations that cannot be resolved by predictive accuracy alone.

Translation also depends on economics. A highly accurate molecular test may be unsuitable for routine herd use if sampling is invasive, turnaround is slow or costs exceed the expected benefit. A tiered system—routine clinical screening followed by targeted advanced testing for selected cases—may be more practical than universal multi-omics screening.

15. Ethics, Governance, Biosafety and Responsible AI

Research involving animals should receive the required institutional and regulatory approvals and procedures should follow applicable animal-welfare standards. Sampling should be proportionate to the scientific question and minimise pain, stress and repeated invasive procedures. Embryo manipulation and transfer should be performed by trained personnel under validated protocols.

Data governance should address ownership, access, retention, audit trails, privacy where farm or owner information is identifiable and the ability to reproduce analyses. AI development should document data provenance, model version, preprocessing and changes made after validation. A deployed model should be monitored for performance drift as equipment, populations and management practices change.

Human oversight is necessary when the model is uncertain or when decisions affect animal welfare, breeding choices or treatment. Model recommendations should not override clinical examination or established veterinary standards. Automation should include safe shutdown, manual override and procedures for handling failures.

16. A Practical Roadmap for Cattle and Buffalo Systems

Phase	Primary objective	Core outputs	Go/no-go criterion
1. Biological discovery	Identify candidate pathways or features linked to a defined outcome.	Hypotheses, candidate markers, standardised phenotypes.	Reproducible signal with plausible biology.
2. Analytical validation	Show measurements are reliable and repeatable.	Assay precision, sample protocol, quality-control rules.	Acceptable reproducibility and feasible workflow.
3. Model development	Combine predictors without overfitting.	Locked model, calibration plan, transparent code and documentation.	Improvement over a prespecified baseline.
4. External validation	Test transportability across independent settings.	Performance by herd, species, season or laboratory.	Acceptable calibration and no unacceptable subgroup failure.
5. Clinical utility	Determine whether using the tool improves decisions.	Prospective outcomes, welfare and economic evaluation.	Net benefit compared with current practice.

This roadmap encourages teams to stop or redesign a project when evidence is weak. It also makes the research more credible to international readers because each technological step is tied to an explicit validation requirement.

17. Conclusions and Future Directions

Embryo–maternal communication is a central determinant of pregnancy establishment in cattle and buffaloes. IFNT-mediated maternal recognition of pregnancy operates within a broader network of endometrial responses, extracellular vesicles, RNA and protein signals, metabolic state and environmental influences. These mechanisms offer opportunities for improved embryo assessment and risk prediction, but the field must distinguish biological plausibility from validated clinical performance.

AI may contribute to image analysis, developmental-kinetic assessment and integration of molecular and clinical data. Automation may improve traceability and consistency in laboratory workflows, while robotics may support selected repetitive or precision tasks. Yet none of these technologies removes the need for sound phenotyping, reliable assays, sufficient outcome events, external validation and veterinary oversight.

For international translation, the strongest strategy is a staged, interpretable and cost-aware framework that tests whether each added technology improves decisions and reproductive outcomes. Cattle and buffalo populations should be evaluated in their own right, with attention to the diversity of Indian dairy systems and the requirements of other global production settings. The ultimate goal is not maximal technological complexity, but safer, more reliable and biologically informed reproductive management.

Tables for Editorial Development

Table 1: Candidate data layers and their interpretive limits

Data layer	Potential contribution	Key limitation	Validation priority
Embryo morphology	Accessible baseline assessment.	Subjective grading; incomplete competence measure.	Inter-observer reliability and pregnancy outcomes.
Time-lapse imaging	Developmental timing and kinetics.	Platform and protocol dependence.	Independent-lab validation.
Transcriptomics	Pathway-level discovery.	Cell composition,	Targeted confirmation

		batch effects, multiple testing.	and independent cohort.
Proteomics/metabolomics	Functional and biochemical state.	Matrix effects, platform variation, confounding.	Analytical reproducibility and added predictive value.
Extracellular vesicles	Potential carriers of embryo–maternal signals.	Isolation heterogeneity and contamination.	Standardised characterisation and functional evidence.
Environmental sensors	Quantification of thermal exposure and context.	Sensor placement and missing data.	Calibration and animal-level linkage.
AI models	Integration and risk estimation.	Overfitting, leakage, dataset shift.	Calibration, external validation and utility.

Table 2: Key questions before a technology is adopted

Question	Why it matters
Does the measurement precede the outcome?	Prevents a diagnostic marker from being mislabelled as an early predictor.
Does it improve on routine assessment?	Demonstrates incremental value rather than technological novelty alone.
Is the result repeatable across laboratories?	Supports analytical and operational reliability.
Does the model calibrate in the intended population?	Ensures predicted risk corresponds to observed risk.
Does use of the tool improve decisions?	Connects statistical performance to clinical benefit.
Is it affordable and welfare-conscious?	Determines real-world feasibility and responsible implementation.

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12
CHAPTER

From Classroom to Kerbside: Impact of Education on Waste Management

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Abstract

Urban zero waste policies depend on citizens who segregate, reduce and reuse waste and schools are widely expected to build these habits. This chapter examines how waste management education is delivered in Ahmedabad, India and why it appears to translate only weakly into lasting behavioural change. Using a qualitative design, it draws on ten semi-structured interviews: seven school principals from the seven administrative zones of the city, an environmental engineer of the Ahmedabad Municipal Corporation (AMC), a municipal councillor and a marketing manager of a private composting company. Reflexive thematic analysis produced five themes: embedded but thin curricula; episodic and unevenly supported activities; constrained teacher capacity, time and resources; a disjuncture between school, home and locality; and a monitoring and collection system that does not reinforce what children are taught. Schools teach cleanliness and segregation, but circular economy ideas of reuse, repair and material recovery are rarely developed, no school measures learning or behavioural outcomes and municipal evaluation tracks activities rather than results. Where collected waste is re-mixed, classroom learning loses credibility. The chapter argues that education must be treated as one part of a coordinated implementation system and proposes curricular, capacity, evaluation, collection and equity measures for municipal and educational authorities.

Keywords: zero waste policy, waste management education, behavioural change, circular economy, implementation gap, Ahmedabad and municipal solid waste.

1. Introduction

1.1. Background and context

Municipal solid waste is among the most visible products of urban growth. The World Bank estimated global waste generation at about 2.01 billion tonnes in 2016 and projected a rise to about 3.40 billion tonnes by 2050, with the fastest growth expected in low- and middle-income countries.[1] Most cities still operate a linear system of collection, transport and disposal. The circular economy offers a different logic, in which materials are kept in use through reduction, reuse, repair, recycling and recovery and waste is treated as a resource rather than a residual [2, 3].

Technical infrastructure alone cannot deliver this shift. The quality of recovered material depends heavily on what households and businesses do before waste leaves their premises, above all segregation at source. India's Solid Waste Management Rules, 2016, place a duty on waste generators to segregate and hand over waste to authorised collectors [4], and the Swachh Bharat Mission has given national visibility to urban sanitation since 2014 [5]. Education is expected to support both. The 2030 Agenda, particularly the goals on quality education and responsible consumption and production, positions learning as a lever for sustainable lifestyles [6, 7] and India's National Education Policy 2020 gives weight to environmental awareness within school curricula [8].

1.2. The Ahmedabad setting

Ahmedabad is a large and rapidly growing city of 48 municipal wards grouped into seven administrative zones, according to one participant in this study. Its waste system has long been shaped by the Pirana dumping site, which, as the AMC engineer interviewed for this study noted, has operated since around 1980 and was never designed with modern environmental safeguards. Remediation through bio-mining and the diversion of recovered fractions to road construction and refuse-derived fuel, have been presented as the city's most prominent circular economy actions, while a councillor interviewed credited court intervention for forcing action [9]. Ahmedabad's Zero Waste Policy and its Road Map set a direction toward reduced landfilling through better segregation, processing and recovery. This chapter is drawn from a wider doctoral study of how that policy is being implemented and it focuses on one strand of the study: the role of schools in building the behaviours the policy requires.

1.3. Problem statement, purpose and research questions

A persistent finding in environmental psychology is that awareness does not automatically produce action [10]. Schools in Ahmedabad run rallies, cleanliness drives, projects and classroom lessons on waste, yet municipal and community actors continue to report poor segregation and littering. To the author's knowledge, few studies examine school-based waste education in an Indian city alongside the municipal, political and private-sector arrangements that surround it. The purpose of this chapter is to fill part of that gap by treating schools as implementation actors within a municipal system and asking what that system allows them to achieve. Three research questions guide the analysis.

1. ***RQ1:*** How is waste management education integrated into the curricula and school-level practices of schools across Ahmedabad's administrative zones?
2. ***RQ2:*** What institutional, pedagogical and socio-cultural barriers limit the translation of school-based waste education into sustained behavioural change at school and household level?
3. ***RQ3:*** How do municipal, political and private-sector accounts of monitoring, collection and governance help explain the education–behaviour gap and what does this imply for a circular economy-aligned zero waste policy?

The chapter first sets out the conceptual background and method, then presents findings organised by research question and finally discusses implications, limitations and conclusions.

2. Main Chapter Content

2.1. Conceptual background

Education for zero waste and the circular economy

The circular economy is commonly described as a restorative system that keeps resources in productive use for as long as possible [11]. For education this has a clear implication: learners need to understand more than where to place litter. They need to understand material flows, the waste hierarchy, composting, reuse and repair and the responsibilities of producers, municipalities and citizens. UNESCO's learning objectives for sustainable consumption and production describe such competences in cognitive, socio-emotional and behavioural terms [7]. Research on environmental education further suggests that knowledge alone rarely changes conduct; learners also need skills, positive attitudes and opportunities to act on real issues [12].

From knowledge to behaviour

Several well-established frameworks explain why the link between learning and behaviour is weak. The theory of planned behaviour holds that action depends on attitudes, perceived social norms and perceived behavioural control, that is, whether a person believes the action is feasible [13]. Stern argues that behaviour is shaped by personal values and beliefs but also by contextual forces such as infrastructure, regulation and habit [14]. Social learning theory adds that people acquire behaviour by observing models and the consequences of their actions [15]. Together these frameworks suggest that classroom teaching is only one input. The system a child sees outside school, including how waste is collected and how clean the neighbourhood is, may strengthen or erase what is taught.

The implementation gap

In this chapter the implementation gap refers to the distance between policy intent and ground practice. The wider study examines four dimensions: implementation effectiveness, stakeholder collaboration, behavioural and systemic change and circular economy integration. School education sits mainly in the third dimension but depends on the others, which is why institutional stakeholders were included in the data.

2.2. Research methodology

Design and participants

The study uses a qualitative, interpretive design based on semi-structured interviews. Participants were selected purposively. Seven principals were interviewed in October 2024, one from each administrative zone, to capture variation in location, language of instruction, school size and resources. All seven schools follow the Gujarat Secondary and Higher Secondary Education Board (GSEB) curriculum and enrol between about 300 and 2,500 students. Three institutional stakeholders were interviewed in June and July 2025: an environmental engineer in the AMC Solid Waste Management Department, an elected municipal councillor and a marketing manager of a private company working in composting and waste processing. Institutional interviews followed a ten-question schedule covering the four gaps described above; principal interviews covered curriculum, collaboration, teacher training, toolkits, field experiences, time, challenges and impact assessment. Table 1 summarises the participants, who are identified by code and zone only [9].

Table 1: Profile of participants

Code	Participant	Zone / location	Profile	Interview date
S1	School principal	East	Gujarati medium; about 700 students	17 Oct 2025
S2	School principal	North	English medium; about 1,400 students	16 Oct 2025
S3	School principal	South-West	Gujarati and English medium; about 1,700 students	11 Oct 2025
S4	School principal	Central	Gujarati and Urdu medium; about 1,600 students; run by charitable trusts	14 Oct 2025
S5	School principal	South	Gujarati and English medium; about 2,500 students	14 Oct 2025
S6	School principal	West	English medium; about 300 students	16 Oct 2025
S7	School principal	North-West	English medium; about 500 students	18 Oct 2025
A1	Environmental engineer, AMC	Central Ahmedabad	11 years of experience	1 Jul 2025
A2	Municipal councillor	Central Zone ward	20 years of experience	10 Jul 2025
A3	Marketing manager, private composting firm	City-wide	15 years of experience	28 Jun 2025

Analysis and trustworthiness

Interview records were analysed using reflexive thematic analysis following the six phases of familiarisation, coding, theme generation, theme review, definition and writing [16]. Consistent with the reflexive approach, a theme is judged by its analytical importance to the research questions rather than by how often it occurs and counting is avoided as evidence of significance [17, 18]. Where numbers appear below, they describe the sample and are not offered as proof of a theme's weight. Credibility was supported by triangulating school accounts against institutional accounts and by reporting divergences openly; reporting was guided by the COREQ checklist and by the criteria of credibility, transferability and dependability [19, 20].

Ethics and reflexivity

Participants are reported by code, role and zone and personal names are withheld. Interview material consists of participants' accounts, which are perspectives and not verified facts. Several claims, such as official segregation rates, were contested between participants and have not been independently audited; they are presented as claims. The author is a doctoral researcher in sustainability and the interpretations offered are shaped by that position.

2.3. Findings

Five themes were developed. Themes 1 and 2 address RQ1, Themes 3 and 4 address RQ2 and Theme 5 addresses RQ3. Table 2 at the end of the section summarises them.

Theme 1: Waste education is embedded in the syllabus but thin (RQ1)

Every principal described waste management as part of existing subjects, chiefly science, environmental science and social science and none described it as a stand-alone subject. In the primary years the emphasis falls on dry and wet waste, while recycling appears in higher classes (S3, S5). Teaching relies on GSEB and NCERT textbooks (S2, S4). Several principals stated that the curriculum was not designed by environmental specialists or NGOs (S1, S3, S4, S5). Updating was described variously as annual (S2, S4, S5) or only when necessary (S3). The principal of S7 said that waste management had not been included in the curriculum properly and was addressed through hands-on activities as and when needed and the principal of S6 judged the syllabus content on waste to be insufficient.

The content that is taught concentrates on cleanliness, segregation and responsible disposal. Reuse appears mainly through craft activities such as making articles from waste (S2, S7) and composting is taught only where space allows (S2). Systematic treatment of material flows, producer responsibility or repair is not evident in any account, an interpretation that should be tested further. Institutional participants described a similar picture. The AMC engineer (A1) spoke of street plays, workshops and themed events in schools and colleges but reported no curriculum-level integration or partnership with education boards. The councillor (A2) observed that Swachh Bharat had pushed schools toward general cleanliness while the deeper aspects of segregation and waste management remained inadequately addressed. The private-sector participant (A3) described the Swachh Shala programme and short films on segregation as the main school-facing tools.

Theme 2: Activities are episodic and support is uneven (RQ1)

Beyond lessons, schools run a range of activities, mostly around cleanliness and civic sense. These include rallies using a government bus and wall painting at a bus stand about four times a year (S1), daily classroom cleaning (S3), monthly school cleanliness drives (S1, S4), two-minute speeches on cleanliness after prayers three times a week (S5), a daily 'cleanest class' award (S2), Saturday 'best out of waste' activities (S7) and science projects on waste or recycling (S1, S3, S7). Government-supplied smart boards or projectors allowed teachers to show videos on waste (S1, S6, S7). Periodic government or AMC visits, such as an annual programme on compost, recycling and segregation (S6) or an annual presentation with a household questionnaire (S4), were described, but none of these was framed as part of a continuing programme.

External support was unevenly distributed. S2 reported a partnership with an NGO and a corporate partner that supplied training, compost-making, learning models and field trips and its principal reported reduced waste within the school. S1 and S7 reported occasional NGO or club activity. S3 described a 'clean Juhapura, green Juhapura' initiative with local NGOs before COVID-19 that was not followed up, after which the cleaned area became littered again. S4 and S5 reported no partnerships and no external funding and S4 noted that, with no playground and only a hall, composting and recycling activities were impossible. The councillor (A2) linked better waste management in western Ahmedabad to higher education levels and more orderly planning, while A3 relayed a second-hand account that schools in minority-dominated areas such as Sarkhej receive fewer grants and resources than schools elsewhere. S3, located on Sarkhej Road, said its school had infrastructure but no financial support. Taken together, these accounts suggest that the quality of waste education depends more on a school's location, trust and partnerships than on a city-wide standard.

One teacher-led project stands out as an example of action-oriented learning. At S4, a teacher designed a questionnaire on household waste disposal and students administered it door to door in a nearby locality. According to the principal, the analysis showed that a large majority of households (about 85 per cent) were not segregating; students then explained segregation to families and households began to segregate. The project cost little and produced both evidence and change, yet it depended on one teacher and was not institutionalised.

Theme 3: Teacher capacity, time and resources are constrained (RQ2)

No principal reported a structured, school-provided training programme in waste management for teachers. Teachers attend seminars and workshops on varied subjects that may touch on waste (S1, S5, S7) and S6 described principal-led guidance, but only S2 reported training provided by its NGO and company partners. Teacher toolkits were absent (S1, S3, S5, S7), although S1 supplied materials to students for specific activities. Time was the most consistent constraint. Principals cited a lengthy syllabus and examination pressure (S3, S4, S7), the extra workload placed on teachers by departmental guidelines (S3) and a school day of 7:30 to 12:30 (S4). A weekly cleanliness activity was attempted but found impractical (S3). Only S2 considered the time allocated sufficient. Funding was also limited: most principals reported no outside financial support (S1, S3, S4, S5, S7) and S4, which charges no fees and is run by charitable trusts, saw extracurricular activity as unaffordable.

Assessment is the clearest gap. No principal described a formal assessment of what students learn about waste or of what changes in behaviour follow. Teachers observe students informally and principals reported no impact analysis or format for one (explicitly stated by S1, S2, S3, S4, S6 and S7). This means that schools cannot know whether their activities work and reports of behavioural change, such as reduced littering after regular reminders (S5) or reduced school waste (S2), rest on impressions.

Theme 4: A disjuncture between school, home and locality (RQ2)

Principals consistently described behaviour that improved within school but weakened outside it. Regular reminders after prayers reduced littering at S5 and S3 observed fewer food wrappers and less paper wasted after visual explanations, but the principal added that the situation reverted within days. S4 and S7 reported that changed behaviour did not last. Principals frequently attributed this to family background: low or irregular incomes and parents who have little time (S4, S6), limited parental literacy (S4) and homes in areas where cleanliness is not the norm (S3, S4). The principal of S7 spoke of a generational problem in which responsibility for segregation has not been instilled. Age was also mentioned: S5 observed that primary students are more adaptable than higher secondary students.

The picture is not wholly negative. S2 and S5 reported that students carry messages home and parents appreciate what is taught and S3 saw students as catalysts for cleaner localities. Parental resistance appeared in one context: S6 does not take students on waste-related field trips because families fear

unhygienic conditions. Principals also pointed to the physical environment as part of the problem. The area outside S6 is littered and its principal repeatedly calls the municipal helpline; S3 described cleaned areas relittering when government follow-up was absent. These accounts should be read with care. Explanations that locate the problem in low-income families risk a deficit framing and the same evidence is consistent with a structural reading in which uncleaned localities and irregular follow-up deny students any chance to practise what they have learned.

Theme 5: Monitoring and collection systems do not reinforce education (RQ3)

The AMC engineer (A1) described a technologically capable monitoring system for logistics, including GPS tracking of collection vehicles and weighbridges linked to a central Command and Control Centre. She contrasted this with community engagement, which is tracked through activity indicators such as events held and participants reached, with no standard framework for evaluating behavioural impact. She also reported that AMC has no dedicated institutional structure for the Zero Waste Road Map and relies on voluntary, awareness-led approaches without penalties. She reported that about 72 per cent of domestic waste is now segregated at source and about 28 per cent remains mixed.

Other participants challenged how far such figures reflect ground practice. The councillor (A2) argued that systems for door-to-door collection and segregation exist largely on paper, that awareness campaigns once or twice a year are insufficient and that contracted collectors deploy far fewer workers than planned; his example was 50 workers deployed where 150 were required. The private-sector participant (A3) said that even when households segregate, workers often mix waste at collection and everything reaches the same site and that official data are sometimes inaccurate. His company's demonstration van, designed to show citizens how wet waste becomes compost, was used by many residents as a free disposal point. The 85 per cent non-segregation figure from the S4 locality survey sits uneasily beside the citywide 72 per cent figure. The two measure different things at different scales and cannot be reconciled with the available data, which is itself evidence of the need for independent evaluation.

These accounts point to a mechanism that matters for education. Children are taught to segregate, but if households see segregated waste re-mixed or if overstretched contract workers lack time to guide residents, the lesson loses credibility. A1 did note that frontline workers sometimes guide households on

segregation, which shows that the collection system can also reinforce learning when it is resourced to do so. Governance arrangements matter here. A3 described a procurement culture in which services are sought at little or no cost and said this had driven out a composting partner that wanted a processing fee. A1 noted that large facilities are built through private investment with AMC's role largely facilitative. Schools sit outside any of these arrangements: principals described contact with AMC only through occasional visits or helpline calls and none described a formal coordination mechanism.

Table 2: Summary of themes, evidence and implications

Theme	Core finding	Illustrative evidence	Implication
1. Embedded but thin curricula (RQ1)	Waste appears within science and social science; content stresses cleanliness and segregation over reuse and material flows	All principals; S3, S5, S7; A1, A2	Strengthen circular economy content and align with education boards
2. Episodic, uneven activities (RQ1)	Rallies, drives and projects are periodic; partnerships and resources differ by school	S1–S7; S2 partnership; S4 hall only; A2, A3	Standardise a minimum programme and equalise support
3. Capacity, time and resources (RQ2)	No structured teacher training or toolkits; syllabus pressure; no assessment of outcomes	S1, S3–S7; S4, S6, S7 on impact	Train teachers, supply toolkits, build assessment
4. School–home–locality disjuncture (RQ2)	Behaviour improves in school but fades; surroundings and family context weaken habits	S3, S4, S5, S6, S7; S2 on spillover	Engage families and clean localities; use students as change agents
5. Monitoring and collection (RQ3)	Monitoring tracks logistics and activities, not outcomes; re-mixing and understaffing undermine learning	A1, A2, A3; S4 survey	Outcome-based evaluation; align collection with segregation

3. Discussion and Analysis

3.1. Interpreting the education–behaviour gap

The findings suggest that the weak translation of education into behaviour in Ahmedabad is better understood as a system problem than as a failure of schools alone. In the terms of the theory of planned behaviour, schools work on attitudes and, to some extent, social norms through reminders and peer

activity, but perceived behavioural control remains low when collection is irregular, mixed or understaffed.[13] Stern's emphasis on contextual forces fits the reports of relittered localities and unresponsive follow-up.[14] Social learning theory adds a further layer: if the most visible models are workers who mix segregated waste or neighbours who use open points, the behaviour modelled outside school may outweigh the behaviour taught inside it.[15] The sense of repeated reminders working in school while fading at home is consistent with habit being context-bound, as the knowledge–behaviour literature would predict [10].

The absence of assessment compounds the problem. Because no school reported measuring learning or behaviour and because municipal evaluation counts events and attendance, neither level can identify which activities work. The one project that generated local evidence, the S4 household survey, shows what low-cost, action-oriented learning could add [12], but it remains an exception. The divergence between the citywide segregation estimate and the locality survey illustrates how decisions may rest on figures that no one can check.

3.2. The circular economy dimension

Education in these schools is oriented toward cleanliness and disposal, the lower end of the waste hierarchy. Reuse, repair and local composting appear sporadically, mainly through craft work and in the one school with the space and partners for compost making. The city's most visible circular actions, such as bio-mining of legacy waste, use of inert material in road construction and refuse-derived fuel for cement kilns, are centralised and technical and the AMC engineer herself noted the need for decentralised composting and community-level reuse. A3 criticised the adoption of waste-burning machines as inconsistent with circular economy principles because they yield no reusable output. If the visible circular actions are remote from daily life, students have little local practice to learn from. Embedding ward-level composting, school composting and dry-waste collection points would give circular economy education a physical setting and a feedback loop.

3.3. Equity and governance

The accounts also reveal an equity dimension. Schools serving lower-income communities, such as the charitable-trust school with no playground, have the least capacity to run activities and second-hand reports from two institutional participants suggest unequal grant distribution across areas. Because families, localities and schools face different conditions, a uniform awareness programme is unlikely to produce uniform results. The absence of

a body charged with the Road Map, noted by the AMC engineer, means no unit is positioned to coordinate education with collection, procurement and evaluation. Education therefore falls between departments.

3.4. Implications for policy and practice

The findings support the following measures, which are offered as propositions for further testing rather than as proven solutions.

1. Develop a city-level framework, in partnership with the state education board, that defines a minimum waste and circular economy learning progression for each stage and includes reuse, repair, composting and the waste hierarchy as well as segregation.
2. Provide teacher training and simple toolkits and use existing smart boards and projectors to deliver standard audiovisual modules.
3. Introduce outcome-based evaluation of school and community programmes, using baseline and follow-up surveys. The S4 student household survey is a low-cost model that could be scaled with academic or third-party support.
4. Align collection with segregation by resourcing contractors to staff routes adequately, enforcing separate handling and giving visible proof to households that segregated waste stays segregated.
5. Establish a coordinating unit or cell for the Zero Waste Road Map that includes education, solid waste, ward committees and NGOs, with regular review meetings.
6. Distribute resources and partnerships more equitably, with priority for schools that lack space, funds or external partners and sustained follow-up after campaigns.
7. Create school and ward-level composting and reuse points so that circular practice is visible and involve families through parent meetings and student-led household projects.

3.5. Limitations and future research

The study rests on ten interviews, seven of them with principals, who may present their schools favourably and who report on teaching rather than being observed. Teachers, students and parents were not interviewed and no classroom observation or document analysis was undertaken, so findings on behavioural change reflect perceptions. The principal interviews were conducted in 2024 and the institutional interviews in 2025. Several institutional claims could not be verified and participants' roles shape their

emphasis. The findings are transferable as analytical insights, not as statistical generalisations. Future work should include student and parent perspectives, pre- and post-intervention measures of segregation behaviour, observation of collection practices and comparison across cities.

4. Conclusion

This chapter examined school-based waste management education in Ahmedabad as part of the implementation of the city's Zero Waste Policy. In answer to RQ1, waste education is embedded in existing subjects and supplemented by cleanliness-oriented activities, but it is thin on circular economy content and uneven across schools. In answer to RQ2, teacher capacity, time, resources, absent assessment and a disjuncture between school, home and locality limit the translation of learning into lasting behaviour. In answer to RQ3, municipal monitoring that tracks logistics and activities rather than outcomes, together with collection practices that may re-mix segregated waste, weakens the credibility of what schools teach, while the lack of a dedicated Road Map institution leaves education uncoordinated.

The central argument is that zero waste education cannot succeed as an isolated awareness effort. It needs a curriculum that reflects circular economy principles, teachers who are trained and equipped, evaluation that measures outcomes and a collection system that visibly rewards the behaviour children are asked to adopt. Treating schools, households, workers and municipal institutions as one system and evaluating that system honestly, is a precondition for turning the Zero Waste Road Map from an aspiration into practice.

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