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# TRANSFORMING HEALTHCARE

## Precision Medicine and Pharmaceutical Innovations

**Editors:**
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**Dr. D. Brighty**
**Dr. Pralhad Rege**
**Dr. E. Thenpandiyan**

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# Transforming Healthcare:

# Precision Medicine and Pharmaceutical Innovations

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## *PREFACE*

Healthcare is undergoing a transformation driven by advances in biomedical science, precision medicine, digital technologies, and pharmaceutical innovation. Transforming Healthcare: Precision Medicine and Pharmaceutical Innovations presents perspectives on approaches reshaping disease prevention, diagnosis, treatment, and patient care. The volume highlights movement from generalized healthcare models toward personalized, evidence-based, patient-centred approaches.

Precision medicine integrates genetic, molecular, clinical, and lifestyle information to support disease characterization and therapeutic decisions. Advances in genomics, biomarkers, molecular diagnostics, artificial intelligence, and data analytics are creating opportunities for early detection, risk assessment, targeted therapies, and improved treatment outcomes. Alongside these developments, pharmaceutical research is evolving through drug-discovery strategies, biotechnology, drug-delivery systems, personalized therapeutics, and approaches to clinical development.

The chapters in this book explore the intersection of discovery and healthcare application, emphasizing how collaboration can accelerate innovation. Contributions address trends in pharmacology, biotechnology, medical sciences, diagnostics, therapeutic development, healthcare technologies, and related fields. Attention is given to challenges associated with translating scientific discoveries into safe, effective, accessible, and sustainable healthcare solutions. The volume also recognizes that progress must be accompanied by ethical considerations, regulatory oversight, data security, affordability, and equitable access. Responsible innovation is essential to ensure that benefits of precision healthcare and pharmaceutical advances reach diverse populations and contribute to public health.

This book is intended to serve as a reference for students, researchers, academicians, healthcare professionals, pharmaceutical scientists, and technology practitioners. It aims to encourage interdisciplinary dialogue, stimulate further research, and promote innovative thinking in modern healthcare.

We sincerely acknowledge the contributions of all authors, reviewers, and collaborators whose expertise and efforts have made this volume possible. We hope the book inspires scientific inquiry and advances toward a more precise, innovative, and patient-centred healthcare future.

**- Editors**

**TABLE OF CONTENT**

# TRADITIONAL MEDICINAL PLANTS USED IN THE TREATMENT OF HEPATITIS

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**Abstract**
Hepatitis, the inflammation of the liver, is a disease caused by RNA viruses. The hepatitis diagnoses most prevalent in India are of types A, B, C and E, with acute cases leading to severe complications. While there are several effective anti-viral treatments of hepatitis, they are often not accessible to patients in India. This, combined with India's rich history of traditional medicine and plant-based approaches makes surveying the existing plant-based treatments prudent. Thus, the text highlights the critical role of both modern antiviral therapies and traditional medicine in the management of hepatitis. Specifically, it elucidates the use and medicinal properties of commonly occurring plants in India such as *Achyranthes aspera* L., *Adhatoda vasica Nees.*, *Aloe barbadensis Mill*., *Allium sativum L.*, *Artemisia iwayomogi L.*, *Silybum marianum*, *Camellia sinensis* and *Curcuma longa*. It also stresses the importance of regulatory oversight and the need for comprehensive patient education regarding traditional remedies.

**Keywords:** Hepatitis, Hepatitis Treatment, Traditional Medicine, Ayurvedic Treatment.

## 1. Introduction

Hepatitis is the inflammation of the liver. It can be caused by several factors, such as alcohol consumption, autoimmune diseases, medications, toxins, and viral infections. In India, the most prevalent types are A, B, C, and E, with hepatitis E as the leading cause of sporadic cases. Hepatitis can be acute (< six months) or chronic (> six months), with acute cases sometimes resolving spontaneously or leading to severe liver failure, while chronic hepatitis can result in significant secondary effects such as liver fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) (Abraham., 2012; Castenada *et al.,* 2021; Mehta *et al.,* 2024).

The viral hepatitis is attributed to RNA viruses, including Hepatitis A (HAV), Hepatitis C (HCV), Hepatitis D (HDV), Hepatitis E (HEV), and Hepatitis G (HPgV-1) (Abraham., 2012; Castenada *et al.,* 2021; Mehta *et al.,* 2024).

- Hepatitis A (HAV): Transmission via the fecal-oral route, primarily due to the consumption of contaminated food and water. Vaccination is effective.
- Hepatitis B (HBV): Spread through parenteral routes, sexual contact, and vertical transmission, posing a high risk for chronic infection and severe liver complications.
- Hepatitis C (HCV): Primarily transmitted through contaminated needles; chronic infections are common, complicating vaccine development.

- Hepatitis D (HDV): Requires HBV for replication; shares similar transmission routes.
- Hepatitis E (HEV): Transmission via the fecal-oral route, primarily due to the consumption of contaminated water.
- Hepatitis G (HPgV-1): Spread through infected blood and sexual contact, with unclear pathogenic significance.

**2. Antiviral Therapy**

Antiviral therapy is advised for most hepatitis C virus (HCV) patients, except those with decompensated cirrhosis and a high MELD score awaiting transplant, pregnant women, and individuals with less than 12 months of life expectancy (Castaneda *et al.,* 2021). The primary objective of therapy is to attain a sustained virologic response (SVR), defined as the absence of detectable viral RNA 12 weeks after completion of treatment. Achieving SVR is associated with a significant reduction in the risk of complications such as cirrhosis, portal hypertension, hepatocellular carcinoma (HCC), and overall mortality (Castaneda *et al.,* 2021). Treatment options include interferon-alpha, ribavirin, and direct-acting antivirals (DAAs). Interferon, particularly in its PEGylated form, improves SVR rates, while ribavirin enhances response when combined with interferon but poses risks such as hemolytic anemia and teratogenicity, especially in those with renal impairment (Castaneda *et al.,* 2021). DAAs target different phases of HCV replication such as NS3/4A inhibitors which includes Boceprevir, telaprevir, simeprevir, asunaprevir, grazoprevir, and paritaprevir, while ombitasvir, ledipasvir, daclatasvir, elbasvir, and velpatasvir function as NS5A inhibitors, and sofosbuvir and dasabuvir are NS5B inhibitors, with current HCV treatment relying on combinations of two or more direct-acting antivirals (DAAs) (Geddawy *et al.,* 2017; Castaneda *et al.,* 2021). Common combinations, such as sofosbuvir/ledipasvir and glecaprevir/pibrentasvir, are selected based on genotype and renal function. Notably, sofosbuvir/velpatasvir and glecaprevir/pibrentasvir are pangenotypic, providing broad coverage. Caution is required for protease inhibitors in advanced liver disease, and sofosbuvir should not be used with an estimated glomerular filtration rate below 30 mL/min per 1.73 $m^2$ (Castaneda *et al.,* 2021).

**3. Traditional Medicine Approaches to Treat Hepatitis**

India is known for its indigenous medicinal traditions, the relevance of which is acknowledged by the WHO. This tradition persists, with individuals often turning to traditional medicine for relief, particularly in managing conditions like hepatitis, chikungunya, and dengue fever, which frequently occur as epidemic outbreaks in the region (Chandrakumar *et al.,* 2016). Even today, a majority of Indians depend on the traditional systems of medicine as it is economically feasible and has low concern of potential side effects. The Indian Council of Medical Research (ICMR) has carried out controlled trials on herbal medicines and has demonstrated the efficacy of several herbs in the treatment of viral hepatitis, including *Picrorhiza kurroa*, *Arogyawardhini*, *Tinospora cordifolia*, *Phyllanthus amarus, Silybum marianum, and Glycyrrhiza glabra* (Chandrakumar *et al.,* 2016; Thyagarajan *et al.,* 2002).

### 3.1 Ayurveda

According to Ayurvedic principles, an imbalance in pitta from among the three factors (*doshas*) *vata*, *pitha*, and *kabha*, leads to hepatic problems in the form of jaundice. Ayurvedic preparations aim to reduce *pitha* in the patients (Chandrakumar *et al.,* 2016). Some frequently used remedies include the powdered whole dried plant of *Phyllanthus niruri* Linn. (Bhumi Amalaki Churna), the powdered whole dried plant of *Tephrosia purpurea* Linn. (Sharpunkha Churna), the powdered dried rhizome of *Picrorhiza kurroa* (Katuki Churna), and Triphala—a traditional formulation comprising *Terminalia chebula*, *Emblica officinalis*, and *Terminalia bellirica* (Chandrakumar *et al.,* 2016; Varsakiya *et al.,* 2022). Practices include decoctions (Kashaya), powders mixed with milk or juice, and dietary restrictions (e.g., avoiding meat and fried foods) (Chandrakumar *et al.,* 2016). Common homeopathic remedies for liver diseases include *Chelidonium majus*, *Thuja occidentalis*, *Kali muriaticum*, *Ferrum phosphoricum*, milk thistle (*Carduus marianus*), *Bryonia*, and *Podophyllum*. These are often selected based on specific symptoms and traditional principles (Sarter *et al.,* 2012; Chandrakumar *et al.,* 2016).

### 3.2 Siddha Medicine

Siddha medicine includes polyherbal formulations derived from plants such as Manjal (*Curcuma longa*), Keezhkaai Nelliver (*Phyllanthus amarus*), Keezhanelli (*Phyllanthus amarus*), Sirunerinjil Vaer (*Tribulus terrestris*), Seeragam (*Cuminum cyminum*), Vilvamver (*Aegle marmelos*), Sirukeerai Ver (*Amaranthus tricolor*), Karisalai Karpam (*Eclipta alba*), Karisalankanni (*Eclipta prostrata*), Sombu (*Foeniculum vulgare*), Milagu (*Piper nigrum*), Valmilagu (*Piper nigrum*), Chukku (*Zingiber officinale*), Nilavembu (*Andrographis paniculata*), Vilamichai ver (*Plectranthus vettiveroides*), Vetiver (*Vetiveria zizanioides*), Santanam (*Santalum album*), Korai kizhangu (*Cyperus rotundus*), Parpatakam (*Mollugo cerviana*), and Pei Pudal (*Trichosanthes dioica*) (Chandrakumar *et al.,* 2016; Ramaswamy *et al.,* 2024).

### 3.3 Unani System

Qurse-e-istisqua (Q-e-I) is a polyherbal formulation from the Unani system of medicine, specifically designed to address hepatitic diseases. This intricate blend combines a diverse array of herbal ingredients, including *Nardostachys jatamansi*, *Cucumis sativus*, and *Portulacea oleracea*, alongside *Cuscuta reflexa*, *Apium graveolens*, and *Pistacia lentiscus*. Additionally, it features potent botanicals such as *Berberis aristata*, *Rheum emodi*, and various species of chicory seeds (*Cichorium intybus*), as well as *Solanum nigrum*, known for their therapeutic properties. Other notable components include decoctions of traditional herbs like *Fumaria officinalis* and *Swertia chiraita*, alongside East Indian globe thistle, red sandalwood, and henna. Capsules with combinations of herbs like Piper cubeba and Chenopodium album are also used. The above formulation exemplifies the holistic approach of Unani medicine, utilizing the therapeutic power of natural resources to promote liver health and enhance overall wellness (Rehan *et al.,* 2015; Siddiqui & Ansari, 2016; Chandrakumar *et al.,* 2016).

## 4. Medicinal Plants Used in the Treatment of Hepatitis

Many medicinal plants are used for the treatment of hepatitis. Details of a few important plants are given below.

### 4.1 *Achyranthes aspera* L.

*Achyranthes aspera* L. (Amaranthaceae) is recognized as a widespread weed throughout India (Figure 1), tropical Asia, and various regions worldwide. This plant is commonly utilized in traditional medicine within tropical Asian and African countries as a folk remedy. It is reported to possess a wide range of medicinal properties, including diuretic, cardiac stimulant, anti-inflammatory, antihypertensive, and antioxidant effects, along with antimicrobial, larvicidal, hypoglycemic, antifertility, anti-anasarca, immunostimulant, and hypolipidemic activities. Additionally, it demonstrates analgesic, antipyretic, antinociceptive, prothyroid, antispasmodic, and hepatoprotective potential. Phytochemical analyses have identified the presence of various compounds, including sterols, alkaloids, saponins, sapogenins, cardiac glycosides, and ecdysterone in different parts of the plant. Notably, the ethanolic extract of *A. aspera* L. seeds has been shown to inhibit the increase in serum levels of total bilirubin, total protein, serum alanine transaminase, aspartate transaminase, and alkaline phosphatase, indicating a hepatoprotective effect in cases of jaundice (Krishnaswamy *et al.,* 2012). Furthermore, the seeds exhibit antihyperlipidemic and antioxidant properties, potentially leading to improvements in serum lipid profiles and blood antioxidant status, as well as displaying hyper triglyceridemic and hypocholesterolemic effects without detectable adverse impacts on liver and cardiac functions (Khan *et al.,* 2015).

**Figure 1:** ***Achyranthes aspera*** **L.**

**Figure 2:** ***Adhatoda vasica*** **Nees**

### 4.2 *Adhatoda vasica* Nees.

*Adhatoda vasica* Nees., a well-known Ayurvedic medicinal plant from the Acanthaceae family, is commonly found across India, Tibet, and the Yunnan province of China. In India (Figure 2), it is referred to as “Vasaka,” while in Tibet it is known as “BaXiaga.” In Tibetan medicine, one-fifth of the prescriptions for hepatitis include *A. vasica*. Historical records in Tibetan Annals of Traditional Chinese Medicine indicate that a decoction of *A. vasica* is recommended for treating hepatitis, with a suggested dosage of 10–30 grams. Recent studies have shown that the leaf extracts of *A. vasica* exhibit significant protective effects against liver damage induced by D-galactosamine (D-GaIN), aflatoxin B1 (AFB1), and carbon tetrachloride (CCl4) in vivo (Lu *et*

*al.,* 2020). *A. vasica* is abundant in vitamin C and contains various chemical constituents, including flavonoids, alkaloids, steroids, tannic acid, saponins, and phytosterols (Lu *et al.,* 2020). The aqueous extract of *A. vasica* also shows promise as a vaccine adjuvant, although it requires effective preservatives for long-term clinical use. Overall, finding an adjuvant that enhances the immune response with minimal toxicity while accommodating the aqueous extract of *A. vasica* could improve the formulation of vaccines aimed at preventing and controlling viral diseases (Gupta & Chaphalkar, 2016).

### 4.3 *Aloe barbadensis Mill.*

*Aloe barbadensis Mill* (*Aloe vera*) belongs to the Aloaceae family and is native to the arid regions of Africa, Asia, and Southern Europe, particularly the Mediterranean areas. This succulent and xerophytic plant is well-adapted to environments with limited water supply (Figure 3). Aloe vera stores significant amounts of water in its leaves, which are the part of the plant utilized for their healing properties. Traditionally, Aloe vera has been recognized as a folk remedy for various ailments. It contains the polysaccharide compound "Acemannan" along with other antioxidant agents. Previous studies in animal models have indicated its potential hepatoprotective effects in cases of alcoholic hepatitis and chemical-induced liver damage, such as those caused by carbon tetrachloride, lindane, and azoxymethane (Werawatganon *et al.,* 2014). Aloe vera demonstrates protective effects on the liver through detoxification processes mediated by glutathione. Glutathione (γ-glutamyl cysteinyl glycine, GSH) is a sulfur-containing antioxidant, antitoxin, and cofactor for enzymes, primarily located in the cell cytosol and other fluid compartments in living organisms (Abinaya *et al.,* 2025). The leaves of Aloe vera are rich in various biologically active compounds, with the most extensively researched being acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones, and a variety of lectins. Acemannan and other polysaccharides in *Aloe vera* enhance the levels of reduced glutathione, thereby helping to mitigate oxidative damage. The aqueous extract of *Aloe barbadensis* demonstrates a significant capacity to restore the integrity of hepatocytes, as evidenced by improvements in physiological parameters, enhanced excretory function (BSP retention) of hepatocytes, and increased stimulation of bile flow secretion (Chandan *et al.,* 2007).

**Figure 3:** ***Aloe barbadensis Mill.***

**Figure 4:** ***Allium sativum*** **L.**

### 4.4 *Allium sativum* L.

*Allium sativum*, commonly known as garlic, has been utilized traditionally for its diverse medicinal properties, particularly its potential hepatoprotective effects and its role in the management of diabetes. This perennial herb, which belongs to the Amaryllidaceae family, is regarded as one of the most versatile medicinal plants (Figure 4), employed in traditional herbal medicine to prevent and treat a wide array of diseases, including diabetes, hypertension, thrombosis, hyperlipidemia, atherosclerosis, and cardiovascular disorders (O *et al.,* 2023b). Research indicates that garlic can provide protection against acetaminophen-induced hepatic damage. The consumption of garlic powder has been shown to enhance antioxidant status, mitigate oxidative stress, and protect rodents from gentamicin-induced liver injury (Rouf *et al.,* 2020). Furthermore, garlic has been observed to alleviate the hepatotoxic effects of nitrate in rat models. Garlic extract may bolster the antioxidant defense mechanisms and reduce lipid peroxidation (Rouf *et al.,* 2020). Garlic contains organosulfur compounds (OSCs) believed to be the principal bioactive constituents contributing to its distinctive odor. Garlic contains over thirty sulfur-containing compounds, which are primarily classified into two main chemical groups: l-cysteine sulfoxides and γ-glutamyl-l-cysteine peptides. Among these, alliin (S-allyl-l-cysteine sulfoxide) is the most abundant sulfur compound, present in both fresh and dried garlic at concentrations of approximately 10–30 mg/g. The effectiveness of garlic in regulating inflammation and oxidative stress in non-alcoholic fatty liver disease (NAFLD) has been assessed across nine animal studies (Rouf *et al.,* 2020). The administration of garlic has been shown to suppress the expression of genes associated with inflammation, including nuclear factor kappa-light-chain enhancer of activated B cells (NF-kB) and protein kinase B (PKB)/Akt, in NAFLD. Additionally, garlic significantly reduces serum levels of interleukins IL-6 and IL-1β, tumor necrosis factor alpha (TNF-α), and the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) (Pourreza *et al.,* 2022).

### 4.5 *Artemisia iwayomogi* L.

*Artemisia* L., a diverse genus within the Anthemideae tribe with over 500 species mainly found in Asia, Europe, and North America, includes the perennial herb *Artemisia iwayomogi*, known as haninjin or dowijigi in Korea, which has been traditionally used in food and beverages (Figure 5), such as tea and rice cake, while also serving medicinal purposes to treat conditions like hepatitis, inflammation, and immune-related diseases (Lee, Y. K. *et al.,* 2017; Nigam *et al.,* 2019). *Artemisia iwayomogi* L. (AI) has been shown to counteract high-fat diet (HFD)-induced reductions in plasma adiponectin levels and hepatic AMPK phosphorylation in mice by activating AMPK, which leads to the inhibition of signaling pathways related to lipogenesis and promotes fatty acid oxidation (Lee, J. *et al.,* 2017). Additionally, AI reduces triglyceride (TG) synthesis by downregulating key enzymes (GPAT, DGAT, GPDH, PAP) and upregulating hydrolysis enzymes (HTGL, ACS) in the liver. Consequently, AI improves hypertriglyceridemia, body weight, hepatic lipid accumulation, and insulin resistance in HFD-fed mice. The effects of AI on hypertriglyceridemia occur through enhanced adiponectin-AMPK signaling and the

regulation of molecules involved in TG assembly (Lee, J. *et al.,* 2017). *Artemisia iwayomogi Kitamura* treatment reduced liver injury markers and restored glutathione levels in bile duct ligation (BDL). It also decreased cholestatic liver damage and collagen deposition while lowering expression of fibrogenic factors like alpha-smooth muscle actin and transforming growth factor β (Han *et al.,* 2012).

**Figure 5: *Artemisia iwayomogi* L.**

***Figure 6: Silybum marianum* (L.) Gaertn**

**4.6 *Silybum marianum*** (L.) Gaertn.

*Silybum marianum* (Milk thistle), originating from the Mediterranean and now grown in Asia and Europe, is a plant whose seeds yield an extract called silymarin (Figure 6). This extract, primarily composed of silybin, is known for its potential benefits in treating various liver injuries, such as alcoholic liver disease and drug-induced hepatotoxicity. Research has shown that silymarin derived from *Silybum marianum* L. Gaertn. can significantly improve liver enzyme levels, HCV-RNA titer, hepatic fibrosis, and overall quality of life for patients (Polyak *et al.,* 2012; Abenavoli *et al.,* 2018: Kalantari *et al.,* 2011) Silymarin protects liver cells from injury through various mechanisms, including free radical scavenging, stabilizing cell membranes, stimulating protein synthesis, modulating immune response, and exhibiting antiviral effects against hepatitis C virus by inhibiting its entry and replication (Kalantari *et al.,* 2011).

**4.7 *Camellia sinensis* (L.) Kuntze**

Green tea's beneficial effects are largely due to catechins, particularly (-) - epigallocatechin gallate (EGCG) and other isomers. Derived from the *Camellia sinensis* plant (Figure 7), it has been consumed for thousands of years and is the second most popular beverage after water. Regular intake of green tea is linked to various health benefits, including protection against diseases such as diabetes, inflammation, and several types of cancer. Research has demonstrated that green tea can reduce liver injury from various toxins and prevent chemically induced liver cancer (Shivashankara *et al.,* 2019; Mahmood *et al.,* 2016).

**4.8 *Curcuma Longa* L.**

*Curcuma longa* (*C. longa*), a member of the Zingiberaceae family and the *Curcuma* genus, produces underground rhizomes (Figure 8). Traditionally, it has been utilized as a spice, natural dye, and herbal remedy due to its bioactive compounds. In Asian medicinal practices, it has been employed to manage gastrointestinal disorders, arthritic inflammation, urinary tract infections,

and liver-related conditions (Wei *et al.,* 2017; Tung *et al.,* 2019). Recent research has investigated the effects of *C. longa* on liver and bile duct diseases, including fatty liver, hepatitis, cirrhosis, and hepatic tumors (Wei *et al.,* 2017).

Several studies have highlighted the therapeutic potential of *C. longa* in animal models exposed to toxins, alcohol, heavy metals, and other harmful agents (Humbal *et al.,* 2024). In rats with liver injury induced by the carcinogen diethylnitrosamine (DEN), *C. longa* rhizomes were found to decrease mortality, reduce the hepatosomatic index, and lower serum levels of AST, ALT, and ALP. The methanolic extract of *C. longa* demonstrated greater cell viability compared to curcuminoids, indicating its potential to mitigate alcohol-induced toxicity. Additionally, the ethanol extract of *C. longa* rhizomes enhanced the expression of hepatic microsomal proteins that are essential for detoxification (An *et al.,* 2020). Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], derived from the rhizome of *Curcuma longa* L. (family Zingiberaceae), exhibits antibacterial, antiviral, antifungal, antiparasitic, anti-inflammatory, and antioxidant activities (Wei *et al.,* 2017, Tung *et al.,* 2019; Kumari and Kumar, 2024). Curcumin exerts inhibitory effects on hepatitis B virus (HBV) by downregulating the gluconeogenesis coactivator PGC-1α or by enhancing transcriptional activity and stabilizing p53. It limits intracellular HBV replication and decreases the expression of hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg), while strongly suppressing HBV covalently closed circular DNA (cccDNA). Additionally, curcumin lowers the levels of both chromosomal and cccDNA-associated histones H3 and H4, leading to marked inhibition of HBV mRNA transcription, protein synthesis, and DNA replication (Wei *et al.,* 2017).

Additional constituents of *C. longa*, including β-elemene, germacrone, ar-turmerone, and bisacurone, have demonstrated beneficial effects on liver and bile duct disorders. *C. longa* is recognized for its antioxidant, hepatoprotective, lipid-lowering, anti-inflammatory, antifibrotic, antitumor, and cholagogic properties, which are mediated through the regulation of apoptosis, CYP2E1, Nrf pathways, lipid metabolism-related factors, TGF-β, NF-κB, CYP7A1, and other molecular targets. These effects are linked to its influence on cell death, certain enzymes, and factors involved in metabolism and inflammation. Among these compounds, β-elemene stands out for its strong liver-protective, anti-inflammatory, antifibrotic, and antitumor actions (An *et al.,* 2020).

**Figure 7: *Camellia sinensis* (L.) Kuntze**

**Figure 8: *Curcuma Longa* L.**

**Conclusion**

The application of medicinal plants is extensively practiced in traditional healthcare systems in many tropical, sub-tropical and equatorial regions such as East Asia, Southeast Asia, Africa, South America with abundant medicinal flora. As discussed above, different plants contain specific bioactive compounds that are strongly anti-inflammatory, antimicrobial, and antineoplastic, making them a potential alternative to modern medicine. It is essential to determine safe, non-toxic dosages and to integrate traditional knowledge with modern healthcare to mitigate the adverse effects of chemotherapeutic agents.

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# BUCCAL DRUG DELIVERY SYSTEM

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**Abstract**
Buccal drug delivery is an effective transmucosal drug delivery system that delivers drugs through the buccal mucosa for local or systemic therapeutic effects. This route bypasses hepatic first-pass metabolism and gastrointestinal degradation, thereby improving the bioavailability of many drugs. Buccal dosage forms such as tablets, films, patches, and gels are formulated using mucoadhesive polymers to prolong residence time and provide controlled drug release. This chapter discusses the anatomy and physiology of the buccal mucosa, mechanisms of drug absorption, theories of mucoadhesion, formulation components, dosage forms, design approaches, manufacturing methods, evaluation parameters, advantages, limitations, applications, and recent advances in buccal drug delivery systems.

**Keywords**: Buccal Drug Delivery System, Buccal Mucosa, Mucoadhesion, Mucoadhesive Polymers, Buccal Tablets, Buccal Films, Buccal Patches, Buccal Gels, Transmucosal Drug Delivery, Controlled Drug Release.

## 1. Introduction

Buccal drug delivery system is a transmucosal drug delivery approach in which the drug is administered through the buccal mucosa (inner lining of the cheek) for local or systemic therapeutic action. The buccal mucosa is highly vascularized, allowing rapid drug absorption directly into the systemic circulation.

This route avoids hepatic first-pass metabolism and gastrointestinal degradation, thereby improving the bioavailability of drugs with poor oral absorption. It is a non-invasive, painless, and patient-friendly method of drug administration.[1]

Buccal drug delivery systems, such as tablets, films, patches, and gels, are commonly formulated using mucoadhesive polymers to prolong residence time and provide controlled or sustained drug release. Due to these advantages, buccal drug delivery has become a promising alternative to conventional oral and parenteral drug delivery systems.[2]

## 2. Anatomy and Physiology of Buccal Mucosa

The buccal mucosa is the inner lining of the cheeks and forms part of the oral mucosa. It provides a suitable site for drug absorption because of its rich blood supply, good accessibility, and relatively low enzymatic activity. The average thickness of the buccal mucosa is

approximately 500–800 µm, making it more permeable than the skin but less permeable than the sublingual mucosa.[3]

The buccal mucosa consists of three main layers:

- Epithelium: The outermost layer composed of non-keratinized stratified squamous epithelial cells. It acts as the primary barrier for drug permeation.
- Basement Membrane: A thin membrane that connects the epithelium with the connective tissue and provides structural support.
- Connective Tissue (Lamina Propria): Contains collagen fibers, blood vessels, nerves, lymphatics, and salivary glands, which help in nutrient supply and drug absorption.

The buccal mucosa is continuously moistened by saliva, which maintains tissue hydration, facilitates drug dissolution, and improves patient comfort. Due to its high vascularization, drugs absorbed through the buccal mucosa enter the systemic circulation directly, thereby bypassing hepatic first-pass metabolism.[4]

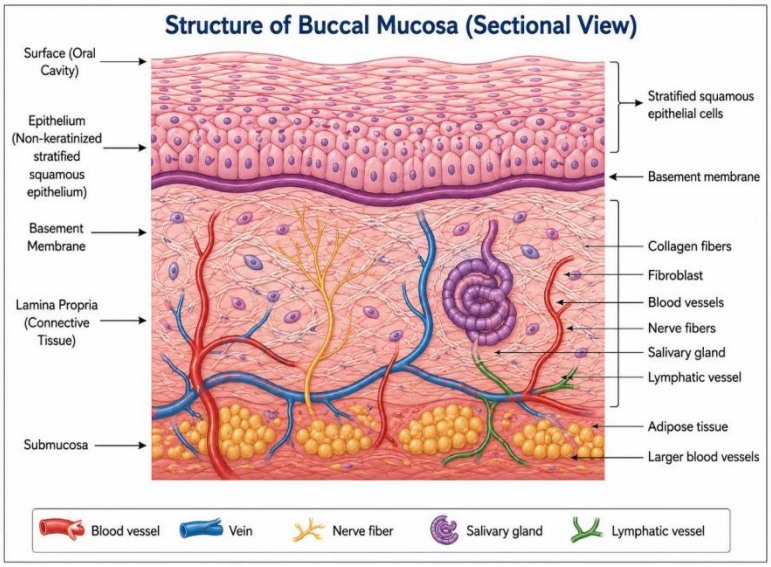


**Figure 1: Structure of buccal mucosa**

## 3. Mechanism of Drug Absorption Through Buccal Mucosa

Drug absorption through the buccal mucosa mainly occurs by passive diffusion, where the drug moves from a region of higher concentration to a region of lower concentration without requiring energy. The buccal epithelium acts as the primary barrier, and only drugs with suitable physicochemical properties can effectively permeate the mucosal membrane.[5]

Two major pathways are involved in buccal drug absorption:

### 3.1 Transcellular Pathway

In the transcellular pathway, drug molecules pass through the epithelial cells. This route is mainly preferred by lipophilic (lipid-soluble) drugs because they can easily diffuse across the cell membranes. It is considered the major pathway for the absorption of many drugs through the buccal mucosa.

### 3.2 Paracellular Pathway

In the paracellular pathway, drug molecules pass between adjacent epithelial cells through the intercellular spaces. This pathway is generally used by hydrophilic (water-soluble) drugs. However, drug absorption through this route is limited due to the presence of tight intercellular junctions.

Overall, the extent of buccal drug absorption depends on factors such as drug molecular weight, lipophilicity, degree of ionization, saliva secretion, contact time, and the permeability of the buccal mucosa.[6]

## 4. Theories of Mucoadhesion

Mucoadhesion is the process by which a drug delivery system adheres to the mucosal surface for an extended period. This prolonged contact improves drug residence time, enhances drug absorption, and increases therapeutic efficacy. Several theories have been proposed to explain the mechanism of mucoadhesion.[7]

### 4.1 Electronic Theory

The electronic theory states that mucoadhesion occurs due to electron transfer between the mucoadhesive polymer and the mucus layer. This transfer creates an electrical double layer, producing attractive electrostatic forces that help the dosage form adhere to the mucosal surface.

### 4.2 Adsorption Theory

According to the adsorption theory, mucoadhesion results from secondary chemical interactions between the polymer and the mucus. These interactions include hydrogen bonding, van der Waals forces, hydrophobic interactions, and electrostatic attractions, which strengthen adhesion.

### 4.3 Diffusion Theory

The diffusion theory suggests that polymer chains and mucin glycoprotein chains interpenetrate and entangle with each other. The extent of interpenetration depends on the flexibility of polymer chains, contact time, and diffusion coefficient, resulting in stable mucoadhesion.

### 4.4 Wetting Theory

The wetting theory explains that mucoadhesion depends on the ability of the adhesive material to spread uniformly over the mucosal surface. A lower contact angle indicates better wetting, leading to stronger adhesion between the dosage form and the mucosa.[8]

## 5. Mucoadhesive Polymers

Mucoadhesive polymers are materials that adhere to the buccal mucosa and retain the dosage form at the site of application for an extended period. These polymers improve drug residence time, enhance drug absorption, and provide controlled or sustained drug release.[9]

Classification of Mucoadhesive Polymers

1. Natural Polymers

- Chitosan
- Sodium alginate
- Pectin

- Gelatin
- Guar gum

2. Semi-Synthetic Polymers

- Hydroxypropyl methylcellulose (HPMC)
- Sodium carboxymethyl cellulose (NaCMC)
- Hydroxypropyl cellulose (HPC)
- Methyl cellulose (MC)

3. Synthetic Polymers

- Carbopol (Carbomer)
- Polyvinyl alcohol (PVA)
- Polyvinylpyrrolidone (PVP)
- Polyethylene oxide (PEO)

Ideal Properties of Mucoadhesive Polymers

- Good mucoadhesive strength
- Non-toxic and non-irritant
- Biocompatible and biodegradable
- Good swelling property
- Compatible with the drug
- Stable over a wide pH range
- Provides controlled drug release
- Easy to formulate and manufacture.[10]

**6. Components of Buccal Drug Delivery System**

A buccal drug delivery system consists of several components that work together to ensure effective drug delivery, prolonged mucoadhesion, controlled drug release, and improved patient compliance. The selection of appropriate components depends on the physicochemical properties of the drug and the desired therapeutic effect.

**Components of Buccal Drug Delivery System**

**1. Active Pharmaceutical Ingredient (API)**

The active pharmaceutical ingredient (API) is the therapeutic agent responsible for producing the desired pharmacological effect. The drug should possess good potency, suitable molecular weight, and adequate permeability through the buccal mucosa.

**2. Mucoadhesive Polymer**

Mucoadhesive polymers help the dosage form adhere firmly to the buccal mucosa, thereby increasing the residence time and enhancing drug absorption. Common examples include Carbopol, HPMC, Chitosan, PVP, and Sodium alginate.

**3. Backing Layer**

The backing layer is an impermeable layer placed on one side of the dosage form. It prevents drug loss into the oral cavity and ensures unidirectional drug release toward the buccal mucosa.

**4. Plasticizer**

Plasticizers improve the flexibility, elasticity, and mechanical strength of buccal films and patches. Common plasticizers include Glycerol, Polyethylene glycol (PEG 400), and Propylene glycol.

**5. Permeation Enhancer**

Permeation enhancers increase drug permeability across the buccal mucosa by temporarily reducing the barrier function of the epithelial membrane. Examples include Sodium deoxycholate, Oleic acid, and Bile salts.

**6. Sweetening and Flavoring Agents**

Sweeteners and flavoring agents improve the taste and acceptability of the formulation, leading to better patient compliance. Examples include Mannitol, Aspartame, Peppermint, and Menthol.[11]

**7. Types of Buccal Drug Delivery Systems**

Buccal drug delivery systems are available in different dosage forms to achieve local or systemic drug delivery. The choice of dosage form depends on the physicochemical properties of the drug, required drug release pattern, patient compliance, and therapeutic purpose.[12]

**7.1 Buccal Tablets**

Buccal tablets are solid dosage forms designed to be placed between the cheek and gum. They adhere to the buccal mucosa using mucoadhesive polymers and release the drug in a controlled manner. These tablets provide prolonged drug residence time and improve bioavailability by avoiding first-pass metabolism.

Examples: Nitroglycerin, Miconazole buccal tablet.

**7.2 Buccal Films**

Buccal films are thin, flexible polymeric films that adhere to the buccal mucosa. They are comfortable to use, rapidly hydrate upon contact with saliva, and provide either immediate or controlled drug release.

Advantages:

- Flexible and easy to administer
- Better patient compliance
- Uniform drug distribution

**7.3 Buccal Patches**

Buccal patches are multilayer dosage forms consisting of a drug-containing layer and an impermeable backing layer. They ensure unidirectional drug release toward the buccal mucosa, minimizing drug loss into the oral cavity.

Advantages:

- Prolonged adhesion
- Controlled drug release
- Improved bioavailability

### 7.4 Buccal Gels

Buccal gels are semi-solid formulations applied directly to the buccal mucosa. They spread easily over the mucosal surface and are mainly used for local treatment, although some formulations can provide systemic drug delivery.

Advantages:

- Easy application
- Good patient acceptance
- Suitable for local drug delivery.[13]

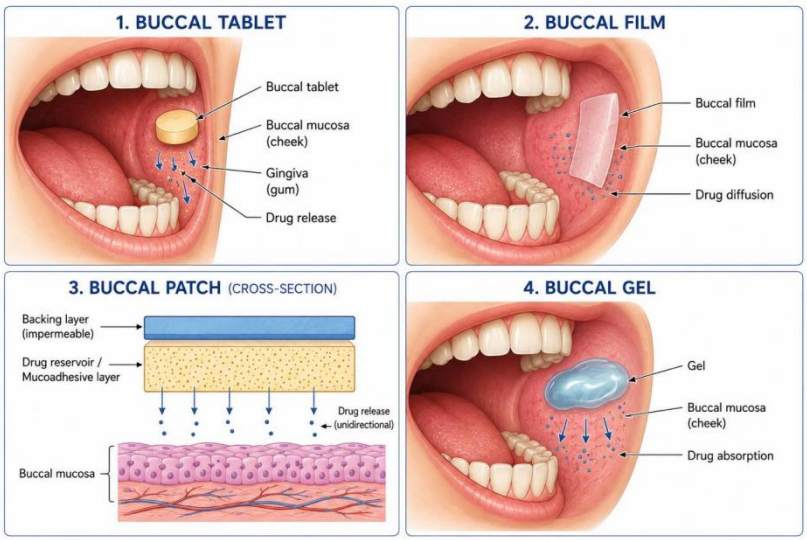


**Figure 2: Types of buccal drug delivery systems**

## 8. Design of Buccal Drug Delivery Systems

The design of a buccal drug delivery system plays a crucial role in determining drug release, mucoadhesion, and therapeutic efficacy. Based on the arrangement of the drug and polymer matrix, buccal systems are broadly classified into Matrix Systems and Reservoir Systems.

### 8.1 Matrix System

In a matrix system, the drug is uniformly dispersed throughout the mucoadhesive polymer matrix. When the dosage form comes into contact with saliva, the polymer hydrates and swells, allowing the drug to diffuse gradually from the matrix. Matrix systems are simple to manufacture and are widely used because of their good stability and sustained drug release.

Advantages:

- Simple formulation
- Uniform drug distribution
- Sustained drug release
- Cost-effective

### 8.2 Reservoir System

In a reservoir system, the drug is enclosed within a drug reservoir, which is surrounded by a rate-controlling polymer membrane. Drug release occurs in a controlled manner through the

membrane, providing a more predictable and prolonged release profile. A backing layer is often included to ensure unidirectional drug release toward the buccal mucosa.

Advantages:

- Precise control of drug release
- Prolonged therapeutic effect
- Reduced drug loss into the oral cavity.[14]

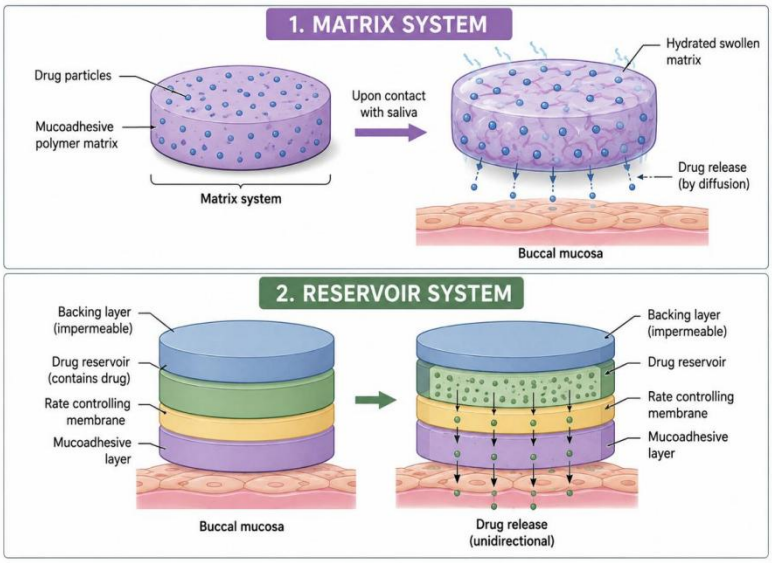


**Figure 3: Design of buccal drug delivery systems**

## 9. Manufacturing Methods of Buccal Drug Delivery Systems

The manufacturing method plays an important role in determining the quality, drug release characteristics, mechanical strength, and mucoadhesive properties of buccal drug delivery systems. The selection of the method depends on the type of dosage form and the physicochemical properties of the drug and polymers.

### 9.1 Solvent Casting Method

In this method, the drug and polymers are dissolved or dispersed in a suitable solvent to form a homogeneous solution. Plasticizers and other excipients are added, and the solution is poured into a mold or Petri dish. After solvent evaporation, a thin buccal film is obtained and cut into the required size.

Advantages:

- Simple and economical
- Uniform drug distribution
- Suitable for buccal films

### 9.2 Direct Compression Method

The drug, mucoadhesive polymer, and excipients are blended uniformly and compressed using a tablet compression machine to produce buccal tablets.

Advantages:

- Simple manufacturing process

- Low production cost
- No use of organic solvents

**9.3 Hot-Melt Extrusion**

In this method, the drug and polymer are mixed and heated until softened. The molten mixture is forced through an extruder to produce films or other dosage forms, which are cooled and cut into the desired size.

Advantages:

- Solvent-free process
- Uniform drug dispersion
- Continuous manufacturing

**9.4 Semisolid Casting Method**

A semisolid gel containing the drug and polymer is prepared and spread onto a suitable backing membrane. After drying, the film is cut into the required dimensions.

Advantages:

- Suitable for high-viscosity polymers
- Produces flexible buccal films9. Manufacturing Methods of Buccal Drug Delivery Systems.[15]

**10. Evaluation Parameters of Buccal Drug Delivery Systems**

Evaluation of buccal drug delivery systems is essential to ensure their quality, safety, stability, and therapeutic performance. Various physicochemical, mechanical, and biological tests are performed to assess the effectiveness of the formulation.[16]

**10.1 Physical Appearance**

The dosage form is visually examined for color, shape, surface smoothness, flexibility, and uniformity. It should be free from cracks, air bubbles, and other visible defects.

**10.2 Thickness**

The thickness of buccal tablets, films, or patches is measured using a digital micrometer or vernier caliper to ensure uniformity.

**10.3 Weight Variation**

The weight of individual dosage units is measured to confirm uniformity according to pharmacopeial standards.

**10.4 Surface pH**

The surface pH is determined to ensure compatibility with the buccal mucosa. An ideal formulation should have a surface pH close to the salivary pH (approximately 6.5–7.0) to minimize irritation.

**10.5 Folding Endurance**

This test is mainly performed for buccal films to evaluate flexibility. The film is repeatedly folded at the same place until it breaks, and the number of folds is recorded.

**10.6 Swelling Index**

The swelling behavior of the formulation is determined by measuring the increase in weight after exposure to saliva or a suitable buffer. This indicates the hydration capacity of the mucoadhesive polymer.

**10.7 Drug Content Uniformity**

The amount of drug present in each dosage unit is measured to ensure uniform drug distribution throughout the formulation.

**10.8 Mucoadhesive Strength**

This test measures the force required to detach the dosage form from the buccal mucosa, indicating its adhesive capability.

**10.9 In Vitro Drug Release**

Drug release studies are carried out using a USP dissolution apparatus to evaluate the drug release profile over time.

**10.10 Ex Vivo Permeation Study**

Ex vivo permeation studies are performed using animal buccal mucosa (e.g., porcine or goat buccal mucosa) to evaluate the permeation of the drug through the mucosal membrane.[17]

**11.Factors Affecting Buccal Absorption**

Buccal absorption of a drug depends on various physiological, physicochemical and formulation factors. The important factors are:

**1. Biological Factors**

- Membrane thickness: Buccal mucosa has a thin epithelial membrane which affects drug permeation.
- Blood supply: Rich blood supply increases drug absorption.
- Saliva: Salivary secretion affects drug dissolution and absorption.
- Enzymatic activity: Enzymes present in saliva and mucosa may degrade drugs.
- Mucosal integrity: Healthy mucosa enhances absorption.

**2. Physicochemical Factors**

- Molecular size: Small molecules are absorbed more easily than large molecules.
- Lipophilicity: Drugs with optimum lipid solubility show better absorption.
- pKa and ionization: Unionized form of drug is absorbed more effectively.
- Solubility: Good aqueous solubility is required for dissolution and absorption.
- Partition coefficient: Suitable partition coefficient improves membrane penetration.

**3. Formulation Factors**

- Drug concentration: Higher drug concentration increases absorption.
- Dosage form: Films, tablets, gels and patches influence absorption rate.
- Mucoadhesive polymers: Increase contact time with buccal mucosa.
- Permeation enhancers: Improve drug penetration through mucosa.
- Drug release characteristics: Controlled release enhances drug absorption.

**4. Environmental Factors**

- pH of buccal cavity: Affects drug ionization and absorption.
- Presence of food: Food particles may interfere with absorption.
- Salivary flow rate: Excess saliva may dilute the drug and reduce absorption.[18]

**Advantages of Buccal Drug Delivery System**

- Avoids first-pass metabolism: Drug directly enters systemic circulation and improves bioavailability.
- Rapid onset of action: Rich blood supply of buccal mucosa allows faster absorption.
- Improved patient compliance: Easy administration and painless route.
- Controlled drug release: Provides sustained and prolonged drug delivery.
- Protection from GI degradation: Avoids degradation in stomach and intestine.
- Easy termination of therapy: Dosage form can be removed when required.[19]

**Limitations of Buccal Drug Delivery System**

- Limited absorption area: Buccal mucosa provides a small surface area for drug absorption.
- Salivary washout: Continuous saliva flow may reduce drug retention.
- Taste problems: Bitter or unpleasant drugs reduce patient acceptance.
- Low drug loading capacity: Suitable only for low-dose drugs.
- Mucosal irritation: Some drugs may cause irritation.
- Interference with food and drinking: Eating and drinking may affect drug release.[19]

**Applications of Buccal Drug Delivery System**

i. Systemic drug delivery: Buccal route is used for delivering drugs into systemic circulation by avoiding first-pass metabolism. Example: Nitroglycerin, Nicotine.
ii. Local drug delivery: Used for treating diseases of the oral cavity such as fungal infections, ulcers and periodontal diseases.
iii. Peptide and protein delivery: Helps in delivering macromolecules that are unstable in the gastrointestinal tract.
iv. Controlled and sustained drug release: Mucoadhesive buccal systems provide prolonged drug release and maintain therapeutic concentration.
v. Emergency drug delivery: Useful for drugs requiring rapid onset of action.
vi. Hormonal drug delivery: Used for administration of certain hormones and steroids.
vii. Vaccination: Buccal route is explored for delivery of vaccines through mucosal immunity.[20,21]

**Future Perspective of Buccal Drug Delivery System**

- Development of advanced mucoadhesive polymers for improved retention and controlled drug release.
- Nanotechnology-based buccal systems to enhance drug penetration and bioavailability.
- Smart drug delivery systems that respond to physiological changes.

- Improved buccal films and patches for better patient comfort and prolonged therapy.
- Delivery of peptides, proteins and vaccines through buccal mucosa.
- Personalized drug delivery systems using advanced manufacturing technologie[22,23,24]

**Conclusion of Buccal Drug Delivery System**

- Buccal drug delivery system is a promising route for systemic and local drug administration.
- It improves bioavailability by avoiding gastrointestinal degradation and first-pass metabolism.
- It provides rapid onset of action, controlled drug release and better patient compliance.
- Advances in mucoadhesive polymers and nanotechnology may further improve the effectiveness of buccal drug delivery systems.

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# RARE-EARTH DOPED NANOMATERIALS FOR PRECISION MEDICINE AND ADVANCED HEALTHCARE APPLICATIONS

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## 1. Introduction

### 1.1 Overview of Rare-Earth Doped Nanomaterials

The lanthanide series, together with scandium and yttrium, constitutes the family of rare-earth elements (REEs) whose partially filled 4f electron shells are shielded from the surrounding chemical environment by the outer 5s and 5p orbitals. This shielding gives rare-earth ions a set of optical properties that few other classes of materials can reproduce: characteristic, narrow-line emission bands, exceptionally long excited-state lifetimes extending into the millisecond range, high resistance to photo bleaching, and emission wavelengths that can be tuned across the ultraviolet, visible, and near-infrared (NIR) regions simply by selecting the dopant ion. When trivalent lanthanide ions such as Yb3+, Er3+, Tm3+, Ho3+, Nd3+, Eu3+, Tb3+, Gd3+, and Ce3+ are incorporated as dopants into an optically inert host lattice, the resulting rare-earth doped nanomaterials (RE-NPs) combine the mechanical and colloidal stability of the host with the spectroscopic versatility of the lanthanide guest ions. Common hosts include fluorides such as NaYF4, NaGdF4, and LiLuF4, oxides such as Y2O3 and Gd2O3, phosphates such as LaPO4, and biologically derived matrices such as hydroxyapatite. Over the past decade this class of nanomaterials has evolved from a niche photo physical curiosity into a functional platform actively investigated for imaging, sensing, drug delivery, and phototherapy.

### 1.2 Importance of Precision Medicine

Precision medicine seeks to move healthcare away from generalized, population-averaged treatment protocols toward interventions that are tailored to an individual patient's genetic makeup, disease subtype, physiological state, and response to therapy. Realizing this vision depends on tools capable of detecting disease biomarkers at very low concentrations, distinguishing molecularly similar but clinically distinct conditions, and monitoring therapeutic response in real time. Conventional diagnostic modalities, including organic fluorophores and radioisotope-based imaging agents, are frequently limited by photo bleaching, short circulation times, auto fluorescence background from biological tissue, or the logistical burden of handling radioactive material. Rare-earth doped nanomaterials directly address several of these limitations, offering background-free luminescent readouts, multiplexed detection through the use of multiple dopant combinations, and integration with therapeutic payloads on a single Nano platform, thereby supporting the theranostic (therapy plus diagnostic) paradigm that underlies much of contemporary precision medicine.

### 1.3 Role of Luminescent Nanomaterials in Modern Healthcare

Luminescent rare-earth nanomaterials, and up conversion nanoparticles (UCNPs) in particular, are especially well suited to biomedical use because they can be excited with NIR light in the so-called biological transparency windows (NIR-I, 700–950 nm, and NIR-II, 1000–1700 nm), where tissue absorption and scattering are minimized and light can penetrate several centimetres without causing photo damage. Because UCNPs convert lower-energy NIR photons into higher-energy visible or ultraviolet emission through anti-Stokes processes, the excitation and emission wavelengths are widely separated, virtually eliminating the auto fluorescence that plagues conventional fluorescence imaging. These characteristics have positioned rare-earth nanomaterials at the intersection of nanotechnology, photonics, and clinical medicine, where they are increasingly explored for deep-tissue imaging, ultrasensitive bio sensing, image-guided surgery, and light-activated therapy.

### 1.4 Scope and Objectives of the Chapter

This chapter provides a structured overview of rare-earth doped nanomaterials as enabling tools for precision medicine and advanced healthcare. It begins by summarizing the synthesis strategies and structural characterization techniques used to engineer these materials, then examines the photo physical mechanisms that govern their optical behaviour and biocompatibility. Building on this foundation, the chapter surveys their principal biomedical applications, spanning imaging, bio sensing, targeted drug delivery, phototherapy, oncology, and wearable diagnostics, with an emphasis on developments reported between 2023 and 2026. The chapter concludes with a critical discussion of the toxicological, regulatory, and sustainability challenges that must be resolved before these materials can transition from laboratory research to routine clinical practice, together with an outlook on emerging research directions.

## 2. Synthesis and Structural Characteristics of Rare-Earth Doped Nanomaterials

### 2.1 Conventional Synthesis Methods

The optical performance of rare-earth doped nanomaterials is dictated almost entirely by the crystallinity, phase purity, size uniformity, and surface chemistry achieved during synthesis, which makes synthetic control a central concern in this field. Thermal decomposition of lanthanide trifluoroacetate or oleate precursors in high-boiling coordinating solvents such as oleic acid and octadecene remains the benchmark route for producing monodisperse, highly crystalline hexagonal-phase NaYF4-based up conversion nanoparticles, although it requires elevated temperatures, inert atmospheres, and generates fluorinated by-products. Hydrothermal and solvothermal synthesis, carried out in sealed autoclaves at moderate temperatures, offers a more accessible alternative that yields well-defined crystal morphologies and allows convenient doping-ratio control through simple adjustment of precursor stoichiometry. Co-precipitation is the simplest and most scalable approach, producing nanoparticles rapidly at low cost, though typically with broader size distributions and lower crystallinity than solvothermal routes. Sol–gel processing and combustion synthesis are also widely used, particularly for oxide and phosphate hosts, because they allow fine compositional control and are readily adapted to thin-film or coating applications.

### 2.2 Green Synthesis Approaches

Growing concern over the environmental footprint and cytotoxic residues associated with organic solvents and surfactants used in conventional synthesis has driven interest in green synthesis routes for rare-earth nanomaterials. Plant-extract-mediated synthesis exploits phytochemicals such as polyphenols and flavonoids as reducing and capping agents, yielding nanoparticles with inherently biocompatible surface coatings. Biopolymer-templated synthesis using chitosan, alginate, or gelatine similarly produces particles with reduced cytotoxicity and improved aqueous dispensability, which is particularly advantageous for hydroxyapatite-based rare-earth nanocomposites intended for bone-related applications. Recent reviews of green synthesis strategies for hydroxyapatite nanocomposites emphasize their relevance for both biomedical and environmental applications, underscoring a broader shift in the field toward more sustainable production pipelines without sacrificing the structural quality required for clinical translation.

### 2.3 Common Host Materials and Dopants

Host selection determines the crystal-field environment experienced by the dopant ions and, consequently, the efficiency of energy transfer and the resulting emission profile. Hexagonal-phase NaYF4 and NaGdF4 remain the most extensively studied hosts for up conversion because of their low phonon energy, which minimizes non-radiative relaxation losses. LiLuF4 has gained attention for combining low phonon energy with the high atomic number of lutetium, enabling dual-mode near-infrared and computed-tomography imaging when co-doped with contrast-active ions. Calcium fluoride (CaF2) hosts co-doped with yttrium, gadolinium, and neodymium ions support multimodal near-infrared-II fluorescence, photoacoustic, and magnetic resonance imaging within a single Nano platform. Hydroxyapatite, being the principal mineral component of bone, offers an intrinsically biocompatible and osteoconductive host for rare-earth doping, with lanthanum-, cerium-, europium-, and erbium/ytterbium-doped hydroxyapatite nanoparticles under active investigation for combined bone-regenerative and theranostic functions. The most common sensitizer–activator dopant pairing is Yb3+ (sensitizer) combined with Er3+, Tm3+, or Ho3+ (activators), while Nd3+, Eu3+, Tb3+, and Ce3+ are frequently employed for near-infrared emission, red emission, green emission, and antioxidant or catalytic functionality, respectively.

### 2.4 Structural Characterization Techniques

A standard structural characterization workflow is applied across the field to confirm phase purity, morphology, and surface chemistry. X-ray diffraction (XRD) identifies crystal phase and estimates crystallite size via the Scherer equation, and is essential for distinguishing the desired hexagonal (β) phase of NaYF4-type hosts from the less luminescent cubic (α) phase. Scanning and transmission electron microscopy (SEM and TEM) provide direct visualization of particle size, shape, and, in the case of high-resolution TEM, core–shell architecture and lattice fringes that confirm epitaxial shell growth. Fourier-transform infrared spectroscopy (FTIR) verifies the presence of surface ligands, oleate or citrate capping groups, and, following bio conjugation, the successful attachment of polyethylene glycol (PEG), silica, or biomolecular targeting agents. UV–visible absorption spectroscopy is used to assess optical band gap and to confirm the loading

of co-conjugated photosensitizers or chromophores, while photoluminescence (PL) spectroscopy, often combined with luminescence lifetime measurements, characterizes emission wavelength, quantum yield, and the efficiency of sensitizer-to-activator energy transfer that ultimately determines biomedical performance.

**2.5 Recent Developments (2023–2026)**

Between 2023 and 2026 several structural innovations have advanced the field considerably. Two-dimensional rare-earth-based and rare-earth-doped nanomaterials, including layered fluorides and oxyhalides, have emerged as a distinct nanomaterial family whose super lattice assembly enables fine control over composition and optical response, expanding applications in drug delivery and magnetic resonance imaging. Multilayer, core–shell–shell architectures have been engineered to solve the long-standing problem of concentration quenching in heavily doped systems; for example, holmium-doped up conversion nanoparticles with rationally designed energy-flow pathways across multiple shells have achieved switchable emission output while suppressing cross-relaxation losses. In the hydroxyapatite subfield, pH-dependent morphological control of erbium/ytterbium co-doped hydroxyapatite has been demonstrated, along with lanthanum–strontium dual-substituted and lanthanum-doped magnesium hydroxyapatite systems that combine improved structural, magnetic, and biocompatibility profiles for bone-related theranostic use. Collectively, these developments illustrate a shift from simple single-dopant nanocrystals toward compositionally and architecturally sophisticated nanostructures engineered for specific biomedical performance targets.

**3. Optical Properties and Biomedical Functionalities**

**3.1 Photoluminescence Mechanisms**

The luminescence of rare-earth ions originates from intraconfigurational 4f–4f electronic transitions. Because these transitions are formally parity-forbidden by the Laporte selection rule, they are inherently weak when the ion is excited directly; however, the same forbiddance is responsible for the long excited-state lifetimes and narrow, atom-like emission linewidths that distinguish lanthanide luminescence from the broad, short-lived emission of organic dyes and semiconductor quantum dots. In practice, efficient luminescence is achieved either through sensitization, in which a strongly absorbing sensitizer ion or organic antenna ligand transfers absorbed energy to the emitting activator ion, or through partial relaxation of the selection rule by the host lattice's crystal field. This mechanistic basis underlies the design logic of nearly all bio medically relevant rare-earth nanomaterials, in which a high-concentration sensitizer ion such as Yb3+ absorbs excitation light and funnels the energy toward a lower-concentration activator ion responsible for the diagnostically or therapeutically useful emission.

**3.2 Energy Transfer Processes**

Energy transfer between dopant ions, and between nanoparticles and adjacent biomolecules or acceptor chromophores, underlies both the intrinsic luminescence of rare-earth nanomaterials and their use as signal transducers in bio sensing. Within the nanoparticle, sequential or cooperative energy-transfer up conversion (ETU) processes between sensitizer and activator ions generate anti-Stokes emission. At the nanoparticle–biomolecule interface, Förster resonance

energy transfer (FRET) and luminescence resonance energy transfer (LRET) are exploited analytically: when a rare-earth nanoparticle donor is brought into proximity with an acceptor fluorophore or nanoparticle through a target-specific binding event, non-radiative energy transfer quenches or activates a measurable optical signal. Because the long luminescence lifetimes of lanthanide-based donors persist well beyond the nanosecond-scale auto fluorescence of biological samples, time-gated detection can be used to record only the delayed, target-specific signal, dramatically improving the signal-to-noise ratio in complex biological matrices such as plasma or exosome suspensions.

**3.3 Up conversion and Down conversion Luminescence**

Up conversion nanoparticles absorb two or more low-energy NIR photons, typically at 980 nm or 808 nm, and emit a single higher-energy photon in the ultraviolet, visible, or shorter-NIR range through sequential energy-transfer or excited-state absorption pathways. This anti-Stokes behaviour allows excitation at wavelengths that penetrate tissue efficiently while producing emission that is easily distinguished from tissue auto fluorescence, a combination of properties that has made UCNPs the dominant rare-earth platform for deep-tissue optical imaging, sensing, and light-triggered therapy. Down conversion, or quantum-cutting, nanomaterials operate in the opposite spectral direction: absorbed higher-energy photons are converted into one or more lower-energy NIR photons, particularly within the NIR-II window (1000–1700 nm), where reduced photon scattering and negligible tissue absorption enable imaging at greater depth and with higher spatial resolution than NIR-I or visible fluorescence. Layer-by-layer surface engineering of down conversion rare-earth nanoparticles has additionally been shown to promote rapid renal clearance following systemic administration, a property directly relevant to eventual clinical use in humans.

**3.4 Biocompatibility and Surface Functionalization**

The as-synthesized surface of most rare-earth nanoparticles is coated with hydrophobic ligands such as oleic acid, which must be replaced or over coated to render the particles water-dispersible and biocompatible. Common strategies include ligand exchange with hydrophilic small molecules or polymers, encapsulation within amphiphilic polymers or lipids, and growth of a mesoporous or dense silica shell that additionally provides a chemically versatile surface for further modification. Surface PEGylating reduces non-specific protein adsorption and prolongs systemic circulation time, while conjugation of antibodies, aptamers, peptides, or folate ligands confers active targeting toward specific cell-surface receptors overexpressed in diseased tissue. Because the intrinsic toxicity of the lanthanide core is strongly influenced by the integrity and stability of its surface coating, surface engineering is now recognized as being as important to biomedical performance as the underlying crystal chemistry itself.

**3.5 Recent Advances (2023–2026)**

A prominent trend between 2023 and 2026 has been the use of rare-earth doped nanomaterials for luminescence Nano thermometry, in which temperature-dependent shifts in emission intensity ratios or lifetimes allow real-time, non-invasive monitoring of local temperature during photo thermal or photodynamic treatment, improving therapeutic precision and safety. NIR-IIb-

emitting lanthanide probes have been developed for the visualization of oxidative stress markers relevant to neurodegenerative disease, illustrating an expansion of rare-earth luminescence beyond oncology into other areas of precision diagnostics. Customized lanthanide nanobiohybrids have also been engineered for non-invasive, precise phototheranostics of localized infections, demonstrating that the sensing and therapeutic logic developed for cancer applications is transferable to infectious disease management. In parallel, up conversion hybrid Nano systems capable of spatiotemporally controlled gene regulation have been reported, in which NIR light triggers localized release or activation of gene-editing or gene-silencing payloads, pointing toward convergence between rare-earth photonics and nucleic-acid therapeutics.

## 4. Applications in Precision Medicine and Healthcare

### 4.1 Biomedical Imaging

Rare-earth doped nanomaterials are increasingly used as multimodal contrast agents that unify optical, magnetic, and radiographic imaging within a single Nano platform, allowing complementary datasets to be acquired from one administered dose. CaF2 nanoparticles co-doped with yttrium, gadolinium, and neodymium ions have been shown to support simultaneous NIR-II fluorescence, photoacoustic, and magnetic resonance imaging, combining the high spatial resolution of MRI with the real-time, high-sensitivity readout of optical and photoacoustic modalities. Neodymium-doped LiLuF4 nanoparticles similarly combine near-infrared luminescence with computed-tomography contrast, exploiting the high atomic number of lutetium for X-ray attenuation alongside lanthanide-based optical emission. Two-dimensional rare-earth nanomaterials have extended this multimodal capability further, with recent reviews highlighting their particular promise for magnetic resonance imaging applications owing to the paramagnetic character of gadolinium- and dysprosium-based layered structures. Collectively, these platforms exemplify how rare-earth doping allows a single nanomaterial to substitute for multiple separately administered contrast agents, simplifying imaging workflows in precision diagnostics.

### 4.2 Biosensors and Disease Diagnostics

Up conversion nanoparticles have become a leading transduction element in optical biosensors because their anti-Stokes emission and long luminescence lifetimes allow detection with minimal background interference, even directly in complex biological fluids. Microfluidic-chip-integrated up conversion luminescence bio sensing platforms have been developed for point-of-care virus diagnostics, combining rapid sample handling with sensitive optical read-out in a single portable device. FRET-based assays pairing up conversion nanoparticles with gold Nano rods have enabled ultrasensitive, minutes-scale detection of SARS-CoV-2 spike protein directly from clinical swabs, illustrating the clinical relevance of rare-earth bio sensing during infectious disease outbreaks. For nucleic-acid biomarkers, a pipette-tip point-of-care platform using up conversion-nanoparticle-conjugated microbeads sandwich hybridization has achieved detection limits in the low autopolar range for oncogenic microRNA, a sensitivity difficult to attain with conventional fluorescent reporters. Near-infrared, long-lifetime up conversion nanoparticles

combined with time-gated luminescence resonance energy transfer have further enabled quantification of cancer-associated microRNA directly in plasma and exosomes, successfully distinguishing cancer patients from healthy donors. Complementary work on up conversion-based lateral flow assays continues to push this technology toward low-cost, equipment-free diagnostic formats suitable for decentralized and resource-limited healthcare settings.

**4.3 Targeted Drug Delivery**

Rare-earth doped nanomaterials serve not only as diagnostic reporters but also as structural scaffolds for targeted drug delivery, in which a mesoporous silica shell, polymeric coating, or layered two-dimensional architecture provides high surface area for drug loading while the rare-earth core enables simultaneous imaging of bio distribution and release. Two-dimensional rare-earth nanomaterials, in particular, offer a large, chemically tunable surface for the covalent or electrostatic loading of therapeutic payloads, and their layered structure supports stimuli-responsive release triggered by pH, enzymatic activity, or external light. Surface conjugation with tumour-targeting ligands such as folate, RGD peptides, or monoclonal antibody fragments allows preferential accumulation at diseased sites, reducing off-target toxicity relative to free-drug administration. Because the same nanoparticle can report on its own bio distribution through luminescence or MRI contrast, rare-earth-based drug carriers inherently support the image-guided dosing strategies central to precision therapeutics, in which drug release can be verified rather than merely assumed.

**4.4 Photodynamic and Photothermal Therapy**

The capacity of up conversion nanoparticles to convert deeply penetrating NIR light into shorter-wavelength visible or ultraviolet emission has made them a natural excitation source for photodynamic therapy (PDT), in which the converted light activates a co-loaded photosensitizer to generate cytotoxic reactive oxygen species within the tumour microenvironment. NIR-II-excitable, image-guided PDT platforms based on rare-earth doped nanoparticles allow treatment to be monitored in real time using the same nanoparticle that delivers the therapeutic light-conversion function, improving spatial precision relative to conventional PDT agents that require separate imaging tracers [20]. Recent reviews describe an expanding role for rare-earth nanoparticles in precision photodynamic oncology, emphasizing their capacity for deep-tissue activation and reduced off-target photo toxicity compared with visible-light-excited photosensitizers. In photo thermal therapy, rare-earth nanoparticles are frequently combined with plasmatic or carbon-based photo thermal agents in hybrid Nano platforms, with the luminescent component simultaneously enabling real-time Nano thermometry to ensure that local tissue temperature remains within a therapeutically effective yet safe range during treatment.

**4.5 Cancer Diagnosis and Treatment**

Oncology remains the most extensively studied clinical application area for rare-earth nanomaterials, reflecting the convergence of imaging, bio sensing, and phototherapeutic functions that tumour management particularly demands. Hydroxyapatite nanoparticles doped with rare-earth ions have been reviewed as a versatile theranostic platform spanning bone-tumour imaging, drug delivery, and localized therapy, benefiting from the intrinsic

osteoconductivity of the hydroxyapatite host in skeletal malignancies. Emerging microneedle-based hybrid carbon–lanthanide nanotheranostic platforms allow minimally invasive, localized delivery of both diagnostic and therapeutic payloads directly to superficial tumours such as early-stage melanoma, combining transdermal drug delivery engineering with rare-earth luminescent tracking. Beyond direct tumour treatment, customized lanthanide nanobiohybrid phototheranostic strategies originally developed for infection management illustrate the underlying versatility of rare-earth photonic platforms, which can be readily reconfigured toward oncological targets by substituting the targeting ligand and payload while retaining the same luminescent transduction chemistry.

### 4.6 Wearable and Point-of-Care Healthcare Devices

The combination of high sensitivity, background-free readout, and compatibility with simple optical excitation sources has made rare-earth luminescent nanomaterials attractive components for wearable and point-of-care (POC) diagnostic devices intended to extend precision medicine beyond centralized laboratories. Smartphone-integrated up conversion luminescence readers have been demonstrated for rapid detection of viral RNA and other biomarkers, exploiting the compact excitation and detection hardware achievable with NIR laser diodes and consumer camera sensors. Microneedle-based platforms that combine rare-earth nanomaterials with skin-penetrating microstructures enable minimally invasive, continuous biomarker sampling and localized therapeutic delivery in a wearable format suitable for chronic disease monitoring. Coordination-chemistry approaches to biomolecule-functionalized up converting nanomaterials continue to refine the interface between rare-earth photonics and point-of-care hardware, supporting the broader goal of decentralized, rapid, and quantitative diagnostic testing that is central to the practical realization of precision healthcare.

## 5. Challenges, Future Perspectives and Conclusion

### 5.1 Toxicity and Biocompatibility Challenges

Despite generally favourable biocompatibility profiles relative to heavy-metal-based quantum dots, rare-earth nanomaterials are not toxicologically inert, and a growing body of literature published since 2023 has clarified the mechanisms underlying their potential adverse effects. Rare-earth oxide nanoparticles have been shown to undergo biotransformation within biological environments, in some cases forming needle-like secondary structures that disrupt lysosomal membranes and trigger pro-inflammatory cytokine release, pyroptosis, and pneumoconiosis-like pulmonary pathology; genetic modulation of the lysosomal enzyme SMPD1 has been identified as a controllable factor influencing this biotransformation pathway. Reactive-oxygen-species-mediated, dose-dependent cytotoxicity has similarly been documented for rare-earth oxide nanoparticles investigated for antimicrobial applications, alongside a tendency toward aggregation in biological media that can alter both toxicological and pharmacokinetic behaviour. Systematic reviews of cerium dioxide nanoparticle bio distribution in murine models further confirm that toxicological outcomes are highly dependent on particle size, surface coating, dose, and route of administration, underscoring the need for standardized, harmonized testing protocols before broader clinical consideration. Disruption of gut and mucosal microbiota

balance by rare-earth oxide nanoparticles has also been reported, adding a further dimension to the toxicological profile that must be considered for orally or systemically administered formulations.

**5.2 Clinical Translation and Regulatory Considerations**

The translation of rare-earth nanomaterials from preclinical proof-of-concept studies to approved clinical products remains limited, constrained by several interlinked factors. Batch-to-batch reproducibility of nanoparticle size, doping ratio, and surface chemistry is difficult to guarantee at manufacturing scale, yet is essential for consistent pharmacokinetics and regulatory approval. Comprehensive, long-term bio distribution and clearance data in large-animal and eventually human studies remain sparse relative to the volume of in vitro and rodent literature, leaving important safety questions around chronic low-dose exposure and organ accumulation only partially answered. Existing regulatory frameworks for nanomedicines, developed primarily around lipid- and polymer-based nanoparticles, do not yet provide fully tailored guidance for inorganic lanthanide-based materials, creating uncertainty for developers navigating approval pathways. Progress on layer-by-layer surface engineering that promotes rapid renal excretion represents a promising design response to these concerns, since materials that are efficiently cleared from the body inherently reduce the risk profile associated with long-term tissue retention.

**5.3 Sustainable Nanomaterial Design**

The supply of rare-earth elements is geographically concentrated and subject to considerable geopolitical and environmental scrutiny, which raises sustainability concerns for any biomedical technology intended for large-scale clinical deployment. Green synthesis methods that replace toxic organic solvents and surfactants with plant-derived, biopolymer-based, or aqueous-phase precursors reduce both the environmental footprint of nanoparticle manufacture and the burden of residual toxic capping agents on the final biomedical product. Designing nanomaterials for efficient renal or hepatobiliary clearance after their diagnostic or therapeutic function has been fulfilled further reduces the long-term environmental and physiological burden associated with rare-earth accumulation, aligning biomedical nanomaterial design with broader principles of sustainable and circular material use. Continued development of low-dopant, high-brightness architectures that achieve strong luminescence with minimal rare-earth content would additionally help reconcile the growing biomedical demand for these materials with the finite and strategically sensitive nature of global rare-earth reserves.

**5.4 Future Research Opportunities**

Several research directions are likely to shape the next phase of development in this field. Data-driven and machine-learning-assisted approaches to nanomaterial design are beginning to be applied to predict optimal host–dopant combinations and shell architectures for targeted photo physical outcomes, potentially accelerating a design process that has historically relied on iterative empirical optimization. Integration of rare-earth luminescent sensing with wearable and digital health infrastructure, including smartphone-based readers and cloud-connected diagnostic platforms, offers a route toward continuous, longitudinal patient monitoring that extends

precision medicine beyond discrete clinical encounters. Expansion of multiplexed, multimodal theranostic platforms capable of simultaneously reporting multiple biomarkers while delivering spatially and temporally controlled therapy represents a further natural extension of current single-modality systems. Finally, continued mechanistic toxicology research, particularly regarding long-term biotransformation pathways and their genetic determinants, will be essential to establishing the safety evidence base required for eventual regulatory approval and routine clinical use.

**Conclusion**

Rare-earth doped nanomaterials occupy a distinctive position within the broader nanomedicine landscape, combining tunable, background-free luminescence with genuine multimodal and theranostic versatility. Advances between 2023 and 2026 in two-dimensional architectures, multilayer shell engineering, green synthesis, and NIR-II-optimized probe design have substantially expanded both the performance and the biomedical scope of these materials, extending their relevance from oncology into infectious disease, neurodegeneration, and regenerative medicine. At the same time, unresolved questions surrounding long-term toxicity, biotransformation, manufacturing reproducibility, and regulatory pathways continue to constrain clinical translation. Addressing these challenges through sustainable design, rigorous toxicological characterization, and closer alignment with regulatory expectations will determine whether rare-earth doped nanomaterials fulfil their considerable promise as a foundational technology for precision medicine and advanced, decentralized healthcare delivery.

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# NUTRIENT INTAKE OF PREGNANT WOMEN IN KASHMIR BY PRE-PREGNANCY BMI: A COMPARISON WITH ICMR GUIDELINES

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## Introduction

Nutritional status of the mother as determined by pre-pregnancy maternal weight & Body Mass Index (BMI) influences the mother and her child throughout the reproductive cycle, beginning before conception and continuing through the entire course of gestation as well as during the period of lactation. Amongst various factors that determine fetal growth and pregnancy outcome, one such factor and the indicator of the overall fetal growth and pregnancy status is gestational weight gain and obviously it is determined and influenced by maternal dietary/nutrient intake during the entire course of gestation (Paturi *et al.,* 2008).

During the period of gestation, energy and other nutrient both macro as well as micro requirements are increased to support increased maternal metabolism, red-cell mass expansion & supply essential nutrients to the growing fetus (Grieger and Clifton, 2014). Poor nutrition during gestation is an important indicator of greater short- & long-term health risk of both the mother and child. (Branca *et al.,* 2015; Kattula *et al.,* 2014).

Balanced diet supports maternal health during gestation, delivery and breastfeeding and so is the proper nutrition during the period of gestation considered important for the wellbeing of both mother and fetus (Paturi *et al.,* 2008), the resultant impact of maternal nutrition affecting the growth and development of the fetus and subsequent health outcomes in the offspring are proved beyond doubt.

## Objectives

- To assess daily intake of energy, protein, fat, calcium and iron among pregnant women in Kashmir through 24hrs dietary recall method.
- To compare nutrient intake across different pre-pregnancy BMI categories
- To evaluate adequacy of nutrient intake with respect to ICMR 2010 RDA for pregnancy.

## Methodology

The methodology undertaken in the present study is as follows:

- **Data Source and Collection:** In the present study both the primary as well assecondary sources of data were used to obtain the desired information.
- **Primary Data:** The present study was conducted in a hospital-based setting. Pregnant women attending the department of Obstetrics and Gynecology SKIMS hospital situated in Srinagar district of Kashmir Valley (UT of J&K) were selected for data collection. From among all pregnant women attending the OPD, randomly every 3$^{rd}$ pregnancy was

included. A structured questionnaire and an interview schedule were devised and used for collecting the primary information from the Selected Pregnant Women.The Questionnaire was prepared in accordance with the latest standards. A non stretchable measuring tape and a digital weighing scale were also used to collect information regarding the height and weight of the respondents. Every enrolled pregnant woman had two follow up visit to obtain necessary information as per the objectives of the study. At the time of registration during the first visit maternal pre-pregnancy weight and height were recorded in order to assess the pre-pregnancy nutritional status of the pregnant women. Second Visit, towards the end of 2nd trimester, the data was collected regarding Dietary history (24-hour recall method and FFQ) of the selected pregnant women was also recorded.

- **Secondary Data:** Data collected from secondary sources represented the information obtained from books, published research papers, medical and public health journals and latest information from internet etc.
- **Data Analysis**: For analysis of data in the present study, Microsoft Excel and statistical package SPSS version 22 was used. After collecting the data, coding of the responses of the questions was imperative for statistical analysis. Every item of the questionnaire was coded and was entered into Microsoft-Excel sheet. The data thus generated was tabulated and presented as per the objectives of the study. The data was analyzed using mean, standard deviation and subjected to various tests of significance like Pearson chi-square, ANOVA, t test. Tests were considered significant for p value <0.05. Some of the observations are presented in graphic forms also.

**Results**

**Table 1: Energy Intake (24hrs dietary Recall) as per Pre –Pregnancy Nutritional Status of the Respondents (BMI Kg/m$^2$) (N=400)**

| Variable | BMI (Kg/m$^2$) | N | Mean Intake | ±SD | %AD** | Min. | Max. | F | df | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Energy (kcal/d)** | **<18.5 (Under weight)** | 50 | 1622.79 | 395.22 | 72.08 | 1100.86 | 2600.22 | 12.35 | 3,396 | ≤ 0.0001* |
| | **18.5-22.9 (Normal Weight)** | 227 | 1995.96 | 482.23 | 88.70 | 1000.22 | 2980.11 | | | |
| | **23-24.9 (Over weight)** | 86 | 2107.07 | 484.72 | 93.64 | 1150.23 | 2890.33 | | | |
| | **≥25 (Obese)** | 37 | 2073.39 | 436.57 | 92.15 | 1344.20 | 2666.32 | | | |

* Significant at 5%

Note: Body Mass Index (BMI kg/m$^2$) for Asian population (WHo 2004)
SDI (Suggested Daily Intake):-2250kcal/d as per RDA given by ICMR 2010

$$** \ \%AD \ (\text{Percent Adequacy}) \ = \frac{\text{Mean Intake}}{\text{Suggested Intake}} \times 100$$

It is quite clear from the table 1 that all of the respondents as per their pre pregnancy nutritional status (BMI kg/m$^2$) were taking less energy as per SDI. Highest mean intake of energy was found among overweight individuals followed by obese and healthy respondents. Among all the four categories of BMI, underweight individuals were taking lowest mean energy intake. Mean intake of 1622.79± 395.22kcal/d was observed among underweight respondents (N=50) with a maximum intake of 2600.22, which is higher than the SDI and a min. of 1100.86.Percent adequacy (%AD) among them was 72.08 that is more than half of the RDA for energy intake.1995.96±482.23kcal/d mean energy intake was found among healthy weight(N=227) respondents, with %AD among them was 88.70.Mean intake of 2107.07±484.72 was found among overweight individuals followed by a mean intake of 2073±436.57 among obese respondents with %AD of 93.64 and 92.15 of the SDI respectively. Maximum intake of 2890.33and 2666.32 was found among them respectively. The mean difference among the respondents with respect to energy intake among four categories of BMI Kg/m$^2$ was found to be statistically significant (p-value ≤0.0001).

**Table 2: Protein Intake (24hrs Dietary Recall) as perPre-Pregnancy Nutritional Status of the Respondents (BMI Kg/m$^2$) (N=400)**

| Variable | BMI (Kg/m$^2$) | N | Mean Intake | ±SD | %AD ** | Min. | Max. | F | df | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Protein (gm/d)** | **<18.5 (Under weight)** | 50 | 49.44 | 12.75 | 63.38 | 30.22 | 78.44 | 7.73 | 3,396 | ≤ 0.0001* |
| | **18.5-22.9 (Normal Weight)** | 227 | 60.65 | 17.06 | 77.76 | 30.05 | 100.82 | | | |
| | **23-24.9 (Over weight)** | 86 | 62.12 | 16.36 | 79.64 | 32.32 | 88.77 | | | |
| | **≥25 (Obese)** | 37 | 62.21 | 16.11 | 79.76 | 32.58 | 89.77 | | | |

*Significant at 5%

**Note:** Body Mass Index (BMI kg/m$^2$) for Asian population (WHO 2004)
SDI (Suggested Daily Intake):- 78gms/d as per RDA given by ICMR 2010

$$** \ \%AD \ (\text{Percent Adequacy}) \ = \frac{\text{Mean Intake}}{\text{Suggested Intake}} \times 100$$

Perusal of the 2 table shows proteins intake of the respondents as per their pre-pregnancy nutrition status. It is quite clear from the table that all of the respondents as per their pre pregnancy nutritional status (BMI kg/$m^2$) were taking lessprotein as per SDI given by the ICMR. Highest mean intake of protein was found among obese individuals followed by overweight and healthy respondents. Among all the four categories of BMI, underweight individuals were taking lowest mean protein intake. Mean intake of 49.44±12.75gm/d was observed among underweight respondents (N=50) with a maximum intake of 78.44gm/d and a min. of 30.22gm/d. Percent adequacy (%AD) among them was 63.38. Mean protein intake of 60.65±17.06gm/d was found among healthy weight (N=227) respondents, with %AD among them was 77.76. Mean intake of 62.21±16.11gm/d was found among obese individuals followed by a mean intake of 62.12±16.36gm/d among overweight respondents with %AD of 79.76 and 79.64 of the RDA respectively. Maximum intake of 89.77and 88.77 gm/d was found among them respectively. The mean difference with respect to protein intake among four categories of BMI Kg/$m^2$ was found to be statistically significant (p-value ≤0.0001).

**Table 3: Fat Intake (24hrs Dietary Recall) as perPre-Pregnancy Nutritional Status of the Respondents (BMI Kg/$m^2$) (N=400)**

| Variable | BMI (Kg/$m^2$) | N | Mean Intake | ±SD | %AD ** | Min. | Max. | F | df | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Fat (gm/d)** | **<18.5 (Under weight)** | 50 | 33.10 | 6.69 | 110.33 | 15 | 40 | 6.78 | 3,396 | ≤ 0.0001* |
| | **18.5-22.9 (Normal Weight)** | 227 | 37.60 | 6.78 | 125.33 | 15 | 45 | | | |
| | **23-24.9 (Over weight)** | 86 | 38.31 | 7.69 | 127.70 | 10 | 45 | | | |
| | **≥25 (Obese)** | 37 | 37.97 | 7.30 | 126.56 | 15 | 40 | | | |

*Significant at 5%

**Note:** Body Mass Index (BMI kg/$m^2$) for Asian population (WHO 2004)

SDI (Suggested Daily Intake):-30gm/d as per RDA given by ICMR 2010.

$$** \text{ \%AD (Percent Adequacy)} = \frac{\text{Mean Intake}}{\text{Suggested Intake}} \times 100$$

Table 3 shows average Intake of Fats on daily basis as per Pre-Pregnancy Nutritional Status of the Respondents is given, the mean intake of Fats (gm/d) by the respondents in all the four categories of BMI (kg/$m^2$) wasmore than the Suggested daily Intake (SDI). Mean Intake of fats 33.10± 6.69gm/d among underweight (N=50) respondents, which is slightly more than the SDI for fats of 30gm/d, with %AD110.33. Maximum fat intake per day among them was 40gm and minimum was 15gm. Mean intake of 37.60±6.78gm/d and 37.97±7.31gm/d of fats with %AD of

125.33 and 126.56 was seen among healthy (N=227) and obese (N=37) respondents respectively. Minimum fat intake among them was 15gm/d, whereas maximum intake was 45gm/d and 40gm/d respectively. Slightly more mean intake i.e. 38.31±7.69gm/d of fats was found among overweight respondents (N=86) with %AD of 127.7. Minimum fat intake among them was 10gm/d and maximum 45gm/d. The different between the mean fat intake among respondents as per pregnancy nutritional status (BMI kg/m$^2$) was found to be statistically significant. (p-value≤ 0.0001)

**Table 4: Calcium Intake (24hrs Dietary Recall) as perPre-Pregnancy Nutritional Status of the Respondents (BMI Kg/m$^2$) (N=400)**

| Variable | BMI (Kg's/m$^2$) | N | Mean Intake | ±SD | %AD ** | Min. | Max. | F | df | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Calcium (mg/d)** | **<18.5 (Under weight)** | 50 | 507.29 | 165.78 | 42.27 | 207.83 | 1583.68 | 8.28 | 3,396 | ≤ 0.0001 * |
| | **18.5-22.9 (Normal Weight)** | 227 | 748.69 | 364.81 | 62.39 | 309.22 | 1529.00 | | | |
| | **23-24.9 (Over weight)** | 86 | 774.89 | 374.94 | 64.57 | 230.40 | 1716.39 | | | |
| | **≥25 (Obese)** | 37 | 831.52 | 415.80 | 69.29 | 207.83 | 1716.39 | | | |

*Significant at 5%

**Note:** Body Mass Index (BMI kg/m$^2$) for Asian population (WHO 2004)

SDI (Suggested Daily Intake):- 1200mgs/d as per RDA given by ICMR 2010.

$$** \text{ \%AD (Percent Adequacy)} = \frac{\text{Mean Intake}}{\text{Suggested Intake}} \times 100$$

Perusal of the table 4 shows calcium intake of the respondents as per their pre-pregnancy nutrition status. It is quite clear from the table that all of the respondents as per their pre pregnancy nutritional status (BMI kg/m$^2$) were taking less calciumas per SDI. Highest mean intake of calcium was found among obese individuals followed by overweight and healthy respondents. Among all the four categories of BMI, underweight individuals were taking lowest mean calcium intake. Mean intake of 507.29± 165.78mg/d was observed among underweight respondents (N=50) with a maximum intake of 1583.68mg, which is higher than the RDA and a min. of 207.83mg, which is very much less than RDA for calcium. Percent adequacy (%AD) among them was 42.27 that is less than half of the RDA for calcium intake. Mean calcium intake of 748.69±364.81 mg/d was found among healthy weight(N=227) respondents, with %AD among them was 62.39. Mean intake of 831.52±415.80mg was found among obese individuals

followed by a mean intake of 774.89±374.94mg/d among overweight respondents with %AD of 69.29 and 64.57 of the RDA respectively. Maximum intake of 1716.39 was found among them. The mean difference with respect to calcium intake among four categories of BMI Kg/$m^2$ was found to be statistically significant (p-value ≤0.0001).

**Table 5: Iron Intake (24hrs Dietary Recall) as perPre-Pregnancy Nutritional Status of the Respondent(BMI Kg/$m^2$) (N=400)**

| Variable | BMI (Kg/$m^2$) | N | Mean Intake | ±SD | %AD ** | Min. | Max. | F | df | p-value |
|---|---|---|---|---|---|---|---|---|---|---|
| **Iron (mgs/d)** | **<18.5 (Under weight)** | 50 | 8.61 | 3.41 | 24.6 | 5.64 | 21.22 | 6.38 | 3,396 | ≤ 0.0001* |
| | **18.5-22.9 (Normal weight)** | 227 | 12.06 | 6.39 | 34.46 | 5.98 | 34.80 | | | |
| | **23-24.9 (Over weight)** | 86 | 13.54 | 8.04 | 38.68 | 6.11 | 44.12 | | | |
| | **≥25 (Obese)** | 37 | 12.83 | 6.03 | 36.66 | 6.33 | 28.14 | | | |

*Significant at 5%

**Note:** Body Mass Index (BMI kg/$m^2$) for Asian population (WHO 2004)

SDI (Suggested Daily Intake):- 35mgs/d as per RDA given by ICMR 2010.

$$** \ \%\text{AD (Percent Adequacy)} = \frac{\text{Mean Intake}}{\text{Suggested Intake}} \times 100$$

Mean intake of iron (mgs/d) by the respondents (Table 5) in all the four categories of BMI (kg/$m^2$) was much less than the Suggested daily Intake (SDI). Minimum iron consumption among the respondents was 5.64mg/d and maximum were 44.12 mg/d. Mean Intake of iron was 8.61±3.4mg/d among underweight (N=50) respondents with % AD (Percent Adequacy) of only 24.6, which is quite less than the SDI for iron. Maximum iron intake per day among them was 21.22mg and minimum was 5.64mg/d. Mean intake of 12.06±6.39mg/d with %AD of 34.46 was seen among healthy (N=207) respondents. Min. iron intake among them was 5.98mg/d, whereas maximum intake was 34.80 mgs/d. Highest mean intake i.e. 13.54±8.04 mg/d of iron was found among overweight respondents (N=86), followed by mean intake of 12.83±6.3 mg/d among obese individuals with %AD of 38.68 and 36.66 respectively. Minimum iron intake among them was 6.11mg/d and 6.33mg/d respectively. Maximum intake of 28.14 and 44.12mg/d of iron was found among obese and overweight respondents respectively. The different between the mean iron intake as per pregnancy nutritional status (BMI kg/$m^2$) was found to be statistically significant (p-value≤ 0.0001)

**Discussion**

In the present study it is revealed thatPregnant women in Kashmir have sub-optimal intake of most of the nutrients as per ICMR 2010 guidelines, with critical deficit in calcium and iron and excess fat intake. Pre-pregnancy BMI did not protect against poor nutrient intake.

In the present study mean intake of energy and protein among respondents was 1980.69±487.74kcal/d and 59.73±16.74gm/d respectively, that is equivalent to 88% and 76.57% of 2250kcal/d and 78gm/d of recommended energy and protein intake for sedentary pregnant women respectively. Mean intake of fat among respondents was more than the SDI, with %AD of 124.06. Mean intake of calcium and iron among respondents was lower than the recommended intake. Lowest mean intake was found with respect to iron. Only 12.02±6.60mg/d mean intake of iron was seen among the respondents with %AD as low as 34.34, much less than half of the SDI.Similar results observed by Andersen *et al.* (2003) in India revealed that the pregnant women's diet was insufficient in energy and all other nutrients except fat (24 hrs. recall) compared with the Indian recommended allowances. Kumar *et al.* (2013) in his study also revealed that the intake of energy, protein, iron and calcium was low in pregnant woman when compared to Recommended Daily Allowances (RDA) and they did not increase their dietary intake in the entire course of gestation. A study conducted by Rehman & Sing (2016) revealed that nutritional intake (24 hours recall) of pregnant women with respect to iron, calcium and folic acid was much lower than recommended dietary allowances (RDA) given by the ICMR.

Lee *et al.* (2013) found that the intakes of energy, fat, protein and carbohydrate on daily basis when compared to RDA were higher in women living in Caribbean and Central South America as compared to African and Asian pregnant women. Micronutrients like folate, iron and calcium intakes were inadequate and below the recommended dietary requirements. Cereal based dietary pattern on daily basis was followed by most of the women across the region.

**Conclusion**

The present study reveals that despite high fat intake by Kashmiri pregnant women, had inadequate intake of macro as well as micro nutrients irrespective of pre-pregnancy BMI.The low calcium and iron intake is a major concern and at the same time excess fat intake may contribute to excessive gestational weight gain in overweight women. The lack of variation by BMI suggests dietary habits of Kashmiri pregnant women are uniformly poor across groups, indicating urgent need for nutrition counseling.

**Recommendations**

While pre-pregnancy measures can be both of social and medical nature to ensure that young healthy adult female is ready to enter reproductive life, however, the measures during pregnancy will be mainly of medical nature to yield desired pregnancy outcome. Some of the modifiable and doable measures are recommended as under:

**Measures Recommended in Pre-Pregnancy Stage**

Details of various pre-pregnancy measures remain beyond the scope of present recommendations yet following few important needs a mention.

- Pre-pregnancy weight of mother being an important variable affecting birth weight of neonate, the women of reproductive age group need to be counseled regarding its significance in this context.
- A complete and comprehensive care of girl child right from her childhood, through adolescence and adult hood to ensure that she becomes a healthy adult girl is of paramount importance.
- Effort to encourage, promote and ensure female literacy is highly advisable.
- Measures to Improve overall socio-economic status of women and ensuring women empowerment for right decision at right time will take care of women's health and nutrition in pregnancy.
- Ensuring utilization of services provided under Nutrition intervention program directed towards young females and at risk population as to improve pre-conceptual maternal nutritional status may be more beneficial than those during pregnancy.
- Sensitizing adult female through mass awareness and education on role and importance of pregnancy weight gain for mother and newborn.
- Popularizing concept of postponement of first pregnancy after marriage and spacing between the pregnancies appears to be a feasible and achievable goal to ensure better reproductive health in absence of increasing age at marriage as well repeated conceptions especially in rural areas.

**Measures Recommended in Pregnancy**

- Nutritional care during the course of pregnancy contributes significantly to an adequate nutritional status preventing negative impacts on maternal and neonatal health resulting from low or excess gestational weight gain than the recommended.
- In order to ensure a thorough pregnancy monitoring, prenatal care should begin during the first trimester (before 13 weeks). It is equally very much important to emphasize that not only is early prenatal care imperative but regular care at the same time is just as fundamental. For low-risk pregnancy, women seeking all prenatal visits, provides better opportunity to keep an eye on gestational weight gain and undertake nutrition education for them so that they will adopt healthy eating habits
- Ensure all pregnant mothers are registered early in the pregnancy and remain under regular medical supervision for better pregnancy care.
- Ensure all 'at risk' and 'high risk' mothers are identified to provide special care during pregnancy and at delivery.
- Ensure balanced dietary intake and nutrition supplementation throughout the pregnancy by regular counseling and awareness activities.
- Ensure institutional deliveries for better pregnancy outcome.

- Ensure to meet all the essential nutrient needs within calorie limits, choose a variety of nutrient-dense foods across and within all the major food groups in recommended amounts as specified by the ICMR.
- Pregnant women should be advised regarding recommended weight gain ranges during the antenatal visits.
- Ensure that a pregnant woman maintains ideal gestational weight gain throughout pregnancy.
- Ensure that pregnancy progresses normally during antennal period and all deliveries are conducted in an institution for better intra natal and newborn out come.

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# SYNTHESIS, CHARACTERIZATION, AND PHOTOCATALYTIC AND BIOMEDICAL APPLICATIONS OF GELATIN–SODIUM ALGINATE–$TiO_2$ NANOCOMPOSITES

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## 1. Introduction

The integration of inorganic nanoparticles into biopolymer matrices has become a frontier in materials science due to the essential requirement of sustainable nanomaterials with multiple functions that can solve various environmental and biological problems (Paradisi *et al.,* 2024; Yang *et al.,* 2026). In this regard, the design of gelatin-sodium alginate matrices presents an ideal option for encapsulating $TiO_2$ nanoparticles based on the use of the natural polymers' biocompatibility and hydrophilicity for optimizing the photodegradation of recalcitrant dye pollutants as well as drug delivery (Ammouchi *et al.,* 2026; Nemr *et al.,* 2024). Through the provision of effective structural architecture, such composite matrices have been able to effectively prevent the formation of nanoparticles, hence ensuring that there is sufficient surface area for effective photocatalytic activity (Sawut *et al.,* 2023). In addition, the use of gelatin and alginate as a composite scaffold ensures that immobilization of $TiO_2$ is done through synergy in such a way that there is improved thermal stability of the materials used (Bukit *et al.,* 2024; Huang *et al.,* 2024). These biopolymer composites do not only overcome the limitations posed by inorganic composite materials through their green synthesis but also have adjustable physicochemical properties necessary for the development of green nanotechnology (Lam *et al.,* 2024), (Kumar *et al.,* 2023). These hybrid systems take advantage of the role of the polymeric network in preventing agglomeration of $TiO_2$, thus ensuring that there are maximum available reactive sites for degradation and bioactivity (Jiang *et al.,* 2023). The intrinsic hydrophilic and biodegradable nature of alginate, together with the superior cell adhesion properties of gelatin, make these composites effective for drug delivery and tissue engineering applications (Nandhini *et al.,* 2024; Wu *et al.,* 2023). This class of biopolymer-nanofiller composites can be considered a revolutionary move towards sustainable functional materials with applications in highly-efficient environmental cleanup procedures as well as in advanced medical treatments (Pamula *et al.,* 2025; Shrimali *et al.,* 2025). The use of these bionanocomposites in industry and clinics helps to avoid all difficulties that arise during the post-treatment process of using unmodified semiconductors (Ainali *et al.,* 2021), creating an innovative system that contributes both to the improvement of reactive charge separation and mechanical properties (Ksenija *et al.,* 2024). The shift towards such systems is mainly motivated by the critical need for replacing conventional petroleum-based polymers with bio-polymers demonstrating higher functional flexibility

(Phadtare *et al.,* 2024). In addition, the application of these materials in scalable environmental and health care solutions helps to solve the problem of the increasing amount of plastics and to move towards Net Zero society (Castro-Dominguez *et al.,* 2025). All these developments comply with the basic tenets of Green Chemistry through employing natural resources to create highly-performing materials (Silva *et al.,* 2021).

## 2. Literature Review

Recent studies have focused on developing biopolymer–$TiO_2$ hybrid materials using techniques such as the sol–gel method, ultrasonication, and microwave-assisted synthesis to achieve better control over morphology and improve photocatalytic performance (Ahmad *et al.,* 2023). Natural polymers such as gelatin and sodium alginate provide a versatile structural framework that helps overcome the mechanical limitations of pure $TiO_2$ while enhancing its stability and biocompatibility (Alshangiti *et al.,* 2023). Incorporating $TiO_2$ nanoparticles into these porous polymeric matrices also reduces the rapid recombination of photogenerated electron–hole pairs and simplifies catalyst recovery after water treatment, thereby improving the degradation efficiency of persistent organic pollutants (Elgarahy *et al.,* 2023).

Beyond environmental applications, these nanocomposites have attracted considerable attention in the biomedical field. Their porous architecture and abundant surface functional groups support controlled drug delivery, tissue regeneration, and localized antibacterial activity (Ruan *et al.,* 2023). However, despite these promising developments, relatively few studies have explored multifunctional systems that simultaneously optimize photocatalytic efficiency and biological performance for both environmental remediation and biomedical applications (Shaikh *et al.,* 2024). Most existing investigations examine these properties independently, leaving the synergistic potential of biopolymer-based $TiO_2$ composites largely unexplored (Cantarella *et al.,* 2021; Мансуров *et al.,* 2023).

This gap highlights the need for systematic studies that examine how $TiO_2$ loading influences both photocatalytic activity and cellular compatibility (Arif *et al.,* 2022; Benkhira *et al.,* 2024). Understanding this relationship is essential for designing multifunctional materials that can efficiently remove organic pollutants while maintaining favorable biological properties. In this context, the present study investigates the incorporation of $TiO_2$ into a gelatin–sodium alginate matrix to achieve effective methyl red dye degradation together with the biological characteristics required for tissue engineering applications (Yarahmadi *et al.,* 2024).

Another important aspect that has received limited attention is the role of cross-linking density in determining material performance. Cross-linking not only affects the diffusion of pollutants and therapeutic molecules but also influences the mechanical strength and structural stability of the hydrogel network (Babić *et al.,* 2023). Therefore, a better understanding of how calcium-ion cross-linking controls mass transport, scaffold integrity, and overall functionality is essential for the rational design of multifunctional biomaterials (Folorunso *et al.,* 2024). An ideal hydrogel should retain its structural integrity under aqueous conditions while closely mimicking the

extracellular matrix to promote cell attachment, proliferation, and migration (Santhamoorthy & Kim, 2025).

Designing such dual-functional scaffolds requires balancing two important but often competing characteristics: a high surface area for efficient photocatalytic degradation and excellent biocompatibility to support cell–material interactions (Orisawayi *et al.,* 2024; Rana *et al.,* 2023). Although alginate-based hydrogels possess excellent biocompatibility, they generally lack sufficient cell-adhesive functional groups, which can limit cellular attachment (Murab *et al.,* 2022). The addition of gelatin compensates for this limitation by providing bioactive motifs that promote cell adhesion and proliferation while complementing the photocatalytic and antibacterial functions of $TiO_2$ nanoparticles (Urruela-Barrios *et al.,* 2026).

**3. Methodology**

The $TiO_2$–gelatin–sodium alginate nanocomposite is commonly synthesized using a one-step sol–gel method, in which $TiO_2$ nanoparticles are uniformly dispersed within an aqueous gelatin–sodium alginate solution (Mirek *et al.,* 2022; Siddiqui *et al.,* 2022). The resulting polymer network is stabilized through ionic cross-linking with calcium chloride, while the gelatin component is further strengthened by glutaraldehyde vapor treatment. At the same time, the alginate phase undergoes ionic gelation in a 2 wt% $CaCl_2$ ethanolic solution. This dual-cross-linking approach enhances the structural integrity of the hydrogel, minimizes nanoparticle leaching, and maintains the high surface area required for efficient photocatalytic activity (Ghiṭman *et al.,* 2021; Wan *et al.,* 2022).

The successful formation of the nanocomposite is confirmed through a range of structural characterization techniques. X-ray diffraction (XRD) is employed to verify the crystalline nature of the embedded $TiO_2$ nanoparticles (Bobu *et al.,* 2024), whereas Fourier-transform infrared (FTIR) spectroscopy is used to identify the interactions between the functional groups of gelatin, sodium alginate, and $TiO_2$ (Liang *et al.,* 2026). In addition, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) provide detailed information on the morphology, pore structure, and distribution of nanoparticles within the hydrogel matrix, confirming their homogeneous dispersion (Tutar *et al.,* 2024).

The photocatalytic performance of the prepared nanocomposite is evaluated by monitoring the degradation of model organic dyes under UV irradiation. The decrease in dye concentration is measured using UV–Vis spectroscopy at predetermined time intervals, enabling the determination of degradation efficiency and reaction kinetics (Nguyen *et al.,* 2024). To optimize the photocatalytic process, the effects of operational parameters such as initial dye concentration, catalyst dosage, and solution pH are systematically investigated (Verma *et al.,* 2025).

The biological properties of the hydrogel are assessed through a series of in vitro biocompatibility studies. Cell viability and proliferation are determined using the MTT assay to examine whether the incorporation of $TiO_2$ nanoparticles influences the cytocompatibility of the gelatin–alginate scaffold (Naeini *et al.,* 2025; Priya *et al.,* 2024). Cellular behavior is further investigated using NIH3T3 fibroblast cells to evaluate cell attachment, proliferation, and long-

term metabolic activity (Hajishoreh *et al.,* 2023). Similarly, HEK293T cells cultured in DMEM/F12 medium are employed to assess the cytotoxicity of scaffold extracts prepared in phosphate-buffered saline through MTT-based metabolic activity measurements (Abdullaev *et al.,* 2023).

The hemocompatibility of the fabricated scaffolds is examined using hemolysis assays to ensure that the nanocomposite does not induce adverse interactions with blood components under physiological conditions (Serafin *et al.,* 2023). Mechanical characterization includes the evaluation of swelling behavior, viscoelastic properties, and compressive modulus to determine whether the scaffold possesses sufficient strength while retaining the flexibility required to mimic the native extracellular matrix (Ghanbari, Sadjadinia, *et al.,* 2022; Ghanbari, Salavati-Niasari, *et al.,* 2022; Patel *et al.,* 2021; Sangkatip *et al.,* 2023). These properties are essential for maintaining structural stability during long-term tissue engineering applications.

The combined structural, photocatalytic, biological, and mechanical evaluations demonstrate the potential of $TiO_2$-integrated gelatin–alginate nanocomposites as multifunctional materials for both environmental remediation and regenerative medicine (DIANSARI *et al.,* 2026; Kasi *et al.,* 2023). Future investigations should focus on understanding the long-term biodegradation behavior of these scaffolds to ensure that their degradation rate matches the pace of new tissue formation (ZhengYue *et al.,* 2023). In vivo studies involving histological analysis in rodent models will also be important for evaluating immunological responses and confirming the safety of these materials for clinical applications (Rajan *et al.,* 2025).

Additional biological assessments, including prothrombin time and platelet adhesion tests, can further verify the hemocompatibility of the developed scaffolds and support their potential use in vascular tissue engineering (Atef *et al.,* 2025). Likewise, immersion in simulated body fluid provides valuable information on the bioactivity of the scaffold by assessing its ability to induce surface mineralization, an important characteristic for bone regeneration (Mohammadpour *et al.,* 2021). Incorporating hydroxyapatite or bioactive glass into the hydrogel matrix may further enhance osteoblast differentiation and mineralized matrix formation, thereby improving its suitability for orthopedic applications (Zare *et al.,* 2022).

Recent advances have also highlighted the importance of rheological properties when developing hydrogel-based bioinks for 3D bioprinting. Parameters such as viscosity, shear-thinning behavior, and print fidelity play a critical role in fabricating complex, patient-specific scaffolds with high structural accuracy (Kakarla *et al.,* 2021; Ramirez *et al.,* 2021). Achieving an appropriate balance between $TiO_2$ loading and polymer cross-linking density is equally important to maintain scaffold stability while preventing the release of unreacted components that could affect cytocompatibility (Zhou *et al.,* 2023).

The hydrogel is formed through ionic cross-linking of alginate chains with calcium ions together with the thermal or chemical solidification of gelatin, resulting in a highly porous and mechanically stable network that promotes cellular attachment and proliferation (Eldeeb *et al.,* 2022). SEM and TEM analyses reveal an interconnected porous architecture that facilitates

nutrient transport and cellular infiltration, further supporting the scaffold's suitability for tissue engineering applications (Khan & Hossain, 2025).

$TiO_2$ nanoparticles into the biopolymer matrix, while Fourier-transform infrared (FTIR) spectroscopy reveals the interfacial interactions and functional group bonding between the gelatin–alginate polymer chains and the inorganic $TiO_2$ phase. In addition to structural characterization, rheological analysis is performed to evaluate the viscosity and shear-thinning behavior of the hybrid bioink, both of which are essential for achieving reliable extrusion during 3D bioprinting (Li *et al.,* 2021; Zhu *et al.,* 2022). These measurements ensure consistent filament extrusion and excellent shape fidelity throughout scaffold fabrication (Skopińska-Wiśniewska *et al.,* 2024; Hadad *et al.,* 2023). The bioink also exhibits rapid viscoelastic recovery, with more than 70% of its original viscosity restored within 10 seconds after extrusion, indicating good structural recovery following deposition (Koutsomarkos *et al.,* 2026). The mechanical stability of the fabricated scaffolds is further assessed through compressive strength and swelling kinetics studies to verify their ability to maintain structural integrity under physiological conditions.

**3.1 Materials and Synthesis**

The $TiO_2$–gelatin–sodium alginate nanocomposite is typically synthesized using a sol–gel method, where gelatin functions as a thermo-responsive polymer to provide initial structural stability, while sodium alginate forms an ionically cross-linked network in the presence of calcium chloride (Najafi *et al.,* 2023; Soufivand *et al.,* 2023). $TiO_2$ nanoparticles are first dispersed into the polymer precursor solution using high-shear homogenization to achieve a uniform distribution before cross-linking. This preparation method combines the ionic gelation capability of alginate with the cell-adhesive characteristics of gelatin, resulting in a nanocomposite with improved mechanical strength and biological performance (Mainardi *et al.,* 2021; Sarah *et al.,* 2024). The concentration of $TiO_2$ is carefully adjusted to regulate the crystalline structure of the nanoparticles as well as the pore architecture of the hydrogel scaffold (Bhattacharyya *et al.,* 2022).

After homogeneous mixing, the solution is cooled under controlled conditions to induce gelatin gelation, followed by treatment with calcium chloride to initiate ionic cross-linking of the alginate chains (Kakapour *et al.,* 2024). The hybrid network is subsequently stabilized either by storage at 4 °C or by applying suitable chemical cross-linking agents to strengthen the polymer matrix and improve its structural integrity (Pazhouhnia *et al.,* 2024). The fabricated scaffolds are then freeze-dried to preserve their three-dimensional porous architecture, which plays an important role in facilitating nutrient transport, waste removal, and cellular infiltration (Barrulas & Corvo, 2023). Careful optimization of the post-cross-linking conditions is essential to ensure that the scaffold maintains sufficient mechanical stability during cell growth while undergoing gradual and controlled biodegradation (Kaliampakou *et al.,* 2023).

The ratio of gelatin to sodium alginate also has a significant influence on the rheological behavior of the hydrogel. Adjusting the polymer composition allows the viscoelastic and shear-thinning properties of the bioink to be tailored for extrusion-based fabrication techniques,

thereby improving printability and dimensional stability during scaffold production (Chowdhury *et al.,* 2024).

The photocatalytic performance of the developed nanocomposite is evaluated using model organic dyes such as methylene blue and acid red under UV-light irradiation (Al-Harby *et al.,* 2024). During the experiment, the scaffold is immersed in the dye solution, and samples are collected at regular intervals to monitor the degradation process. The reduction in dye concentration is determined using UV–Vis spectrophotometry by measuring changes in absorbance at the characteristic wavelength of the dye. These measurements provide information on the photocatalytic efficiency of $TiO_2$ through the generation of reactive oxygen species responsible for pollutant degradation.

The biological performance of the scaffold is assessed through standard cytocompatibility studies using mesenchymal stem cells to evaluate cell attachment, proliferation, and viability over a 14-day culture period (Wen *et al.,* 2022; Wierzbicka *et al.,* 2024). These studies are complemented by in vitro biodegradation experiments, in which the gradual mass loss of the hydrogel is monitored to determine whether its degradation rate is compatible with the process of new tissue formation (Tomić *et al.,* 2023). Hemocompatibility and hemolysis assays are also performed to verify that the material does not induce undesirable blood-related or inflammatory responses following physiological exposure (Ghanbari *et al.,* 2021). In addition, histological and biochemical analyses are carried out to confirm the absence of harmful degradation products, thereby supporting the overall safety of the material for future biomedical applications (Xu *et al.,* 2022).

The suitability of these hybrid hydrogels for tissue engineering is largely attributed to their ability to mimic the native extracellular matrix and promote favorable interactions between cells and the scaffold (Serafin *et al.,* 2023). The negatively charged alginate network contributes to rapid water absorption and swelling, whereas gelatin provides bioactive cell-binding sites that enhance cell adhesion and proliferation (Alston *et al.,* 2026). Together, these complementary properties create a stable microenvironment that supports tissue regeneration and long-term cellular growth (Łabowska *et al.,* 2021; Segneanu *et al.,* 2025). The incorporation of $TiO_2$ nanoparticles further enhances the functionality of the scaffold by introducing photocatalytic and antimicrobial properties while maintaining the excellent biocompatibility of the gelatin–alginate matrix (Maksoud *et al.,* 2022; Sharma *et al.,* 2023).

The structural characteristics of the nanocomposite are confirmed using a combination of analytical techniques. X-ray diffraction (XRD) verifies the crystalline phase of $TiO_2$ within the polymer matrix, while Fourier-transform infrared (FTIR) spectroscopy identifies the intermolecular interactions and hydrogen bonding between gelatin, sodium alginate, and $TiO_2$ nanoparticles (Ghanbari *et al.,* 2021). Field-emission scanning electron microscopy (FESEM) is used to examine the surface morphology and pore interconnectivity of the scaffold, ensuring that the porous structure is suitable for cellular infiltration and nutrient transport (Soltani *et al.,* 2024). Transmission electron microscopy (TEM) further provides information on the size,

morphology, and spatial distribution of $TiO_2$ nanoparticles within the polymer network (Aliabad *et al.,* 2025). Collectively, these characterization techniques confirm the structural uniformity and stability of the nanocomposite, supporting its potential application in advanced tissue engineering and skin regeneration (Wathoni *et al.,* 2026).

The wound-healing potential of the developed nanocomposite is evaluated through a series of in vitro studies. Moisture vapor transmission rate (MVTR) and swelling measurements are performed to determine the scaffold's ability to maintain a moist environment, which is essential for effective wound healing (Saberian *et al.,* 2024). The antimicrobial activity of the scaffold is further assessed using inhibition zone assays against common pathogenic bacteria to confirm the antibacterial properties imparted by the incorporated metal oxide nanoparticles (Kuddushi *et al.,* 2025). Mechanical strength tests are also conducted to evaluate the scaffold's resistance to physiological stresses while ensuring that its degradation rate remains compatible with the tissue remodeling process (Baniasadi, 2025; Gigimon *et al.,* 2025).

The development of multifunctional wound dressings is aimed at promoting faster tissue repair while reducing pain and discomfort during healing (Keerthana *et al.,* 2026). Improved cell attachment, enhanced cell-to-cell interactions, and increased proliferation observed in comparable nanoparticle-loaded polymer systems further demonstrate the regenerative potential of these hybrid scaffolds (Ibrahim *et al.,* 2024). In addition, scanning electron microscopy (SEM) provides detailed information on the surface morphology, phase distribution, and nanoparticle dispersion within the composite matrix, all of which significantly influence its mechanical strength and biological performance (Li *et al.,* 2024).

The $TiO_2$–gelatin–alginate bionanocomposite is synthesized using a sol–gel process in which $TiO_2$ nanoparticles are uniformly dispersed in an aqueous gelatin–alginate solution before controlled cross-linking is carried out to stabilize the polymer network (Huang *et al.,* 2024; Youssef *et al.,* 2021). An evaporation-induced self-assembly process is employed to produce transparent, crack-free films with uniformly distributed nanoparticles throughout the biopolymer matrix (Kędzierska *et al.,* 2022). Calcium chloride is then introduced to ionically cross-link the alginate chains, forming a stable three-dimensional network with gelatin that enhances the structural integrity of the scaffold (El-Lakany *et al.,* 2025; Jiang *et al.,* 2023).

The degree of cross-linking plays an important role in determining the mechanical properties, swelling behavior, and release characteristics of the incorporated nanoparticles, which ultimately influence both the antibacterial activity and regenerative performance of the scaffold (Bal-Öztürk *et al.,* 2024). Calcium ions interact with the carboxylate groups of alginate through the well-known "egg-box" mechanism, producing a stable network that regulates moisture vapor transmission, water uptake, and mechanical stability (Silva *et al.,* 2025). This ionic gelation strategy also facilitates the formation of a robust hydrogel with properties well suited for biomedical applications (Wathoni *et al.,* 2024). The final performance of the scaffold is strongly influenced by the polymer composition and $TiO_2$ content, as these parameters govern mechanical strength, degradation behavior, and cellular compatibility (Çakıcı, 2021; Hong *et al.,* 2024).

Photocatalytic activity is evaluated by immersing the nanocomposite in aqueous dye solutions, such as methylene blue, followed by controlled light irradiation (Bukit *et al.,* 2024). The concentration of the remaining dye is measured at regular intervals using UV–Vis spectroscopy to determine degradation efficiency and photocatalytic reaction kinetics (Yang *et al.,* 2026). Process variables, including $TiO_2$ loading and irradiation time, are optimized to maximize degradation efficiency while maintaining catalyst stability during repeated treatment cycles (Kumar *et al.,* 2023). The effective immobilization of $TiO_2$ within the calcium alginate matrix minimizes nanoparticle loss and enables consistent photocatalytic performance over multiple reuse cycles (Ammouchi *et al.,* 2026). In addition, $TiO_2$ provides active photocatalytic sites that promote the generation of reactive oxygen species, thereby enhancing dye mineralization and reinforcing the multifunctional nature of the composite.

Titanium-assisted cross-linking has also been reported to improve the structural stability of alginate-based materials by strengthening the interaction between metal ions and the polymer network (Yamashita & Tanaka, 2026). The characteristic "egg-box" arrangement of alginate facilitates ionic interactions between calcium ions and the uronic acid residues, producing a highly stable hydrogel structure (Amrabadi *et al.,* 2023). Structural characterization using X-ray diffraction (XRD) confirms the crystalline $TiO_2$ phase within the composite, whereas Fourier-transform infrared (FTIR) spectroscopy reveals the chemical interactions between the inorganic nanoparticles and the gelatin–alginate polymer matrix (Sawut *et al.,* 2023). XRD analysis further distinguishes the anatase and rutile phases of $TiO_2$, while FTIR spectra demonstrate the interactions between the alginate carboxylate groups and the hydroxyl-rich surface of $TiO_2$ nanoparticles, confirming the successful integration of the inorganic and organic components (Lam *et al.,* 2024).

**3.2 Characterization**

The morphology and microstructure of the $TiO_2$–gelatin–alginate nanocomposite are investigated using scanning electron microscopy (SEM) and transmission electron microscopy (TEM), which provide detailed information on the distribution and incorporation of $TiO_2$ nanoparticles within the polymer scaffold (Alsubhe, 2025). TEM analysis further reveals the interface between the inorganic nanoparticles and the surrounding biopolymer matrix, demonstrating that the $TiO_2$ particles are uniformly embedded throughout the interconnected network with minimal particle agglomeration (Paradisi *et al.,* 2024).

The crystalline structure of the incorporated $TiO_2$ nanoparticles is examined using X-ray diffraction (XRD), while Fourier-transform infrared (FTIR) spectroscopy is employed to identify the characteristic functional groups and the intermolecular interactions between gelatin, sodium alginate, and $TiO_2$ (Hossain *et al.,* 2025; Ma *et al.,* 2024). These analyses confirm the successful incorporation of the nanoparticles and indicate the formation of hydrogen-bonding interactions that contribute to the stability of the composite. Field-emission scanning electron microscopy (FESEM), operated at an accelerating voltage of 3 kV, provides high-resolution images of the

scaffold surface and further demonstrates a uniform morphology with only limited nanoparticle aggregation (Navaei *et al.,* 2025).

The elemental composition and spatial distribution of titanium within the scaffold are verified using energy-dispersive X-ray spectroscopy (EDS), confirming that the nanoparticles are evenly dispersed throughout the polymer matrix (Tordi *et al.,* 2023). Mechanical performance is evaluated through uniaxial compression testing, whereas thermogravimetric analysis (TGA) is used to determine the thermal stability of the scaffold under elevated temperatures. Together, these analyses provide valuable information regarding the structural robustness of the nanocomposite and its suitability for long-term biomedical applications (Makar *et al.,* 2023).

The biological performance of the developed scaffold is assessed through cytocompatibility studies using established mammalian cell lines. Cell viability and proliferation are quantified using MTT or XTT colorimetric assays to determine whether the incorporation of $TiO_2$ nanoparticles affects the intrinsic biocompatibility of the gelatin–alginate matrix (Byun *et al.,* 2023; Babić *et al.,* 2023). Hemocompatibility is also evaluated through hemolysis studies to ensure that the scaffold does not produce adverse interactions with blood cells following physiological exposure.

In addition, the surface characteristics of the nanocomposite are investigated by measuring the zeta potential in aqueous media. This analysis provides information on the colloidal stability of the nanoparticles and the surface charge of the scaffold, both of which influence protein adsorption, cellular attachment, and subsequent tissue integration (Phan *et al.,* 2023). Collectively, these structural, mechanical, physicochemical, and biological evaluations provide a comprehensive understanding of the performance of $TiO_2$–gelatin–alginate nanocomposites and support their potential use in advanced biomedical applications.

**3.3 Biomedical Assays**

The biological performance of gelatin–alginate/$TiO_2$ nanocomposites is evaluated through a series of in vitro studies to determine their suitability for tissue engineering applications. Cell viability and proliferation are investigated using periodontal ligament fibroblast cells to examine their interaction with the fabricated scaffold (Nandhini *et al.,* 2024). Cellular metabolic activity is quantified using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, which confirms that the incorporation of $TiO_2$ nanoparticles supports cell growth without causing significant cytotoxicity (Zohoori *et al.,* 2025).

The antimicrobial activity of the nanocomposite is also evaluated against common pathogenic microorganisms. The $TiO_2$-containing scaffold exhibits larger inhibition zones than the control samples, demonstrating its potential as an antimicrobial wound dressing material (Alshangiti *et al.,* 2023). To further assess biological safety, hemolysis assays and live/dead staining are performed to determine the compatibility of the scaffold with mammalian cells. The results indicate that the gelatin–alginate matrix maintains its structural integrity while minimizing adverse cellular responses, supporting its suitability for biomedical applications (Dutta *et al.,* 2023).

These biological evaluations provide valuable insight into the therapeutic potential of the developed nanocomposite by demonstrating that the incorporation of $TiO_2$ nanoparticles enhances the interaction between the scaffold and surrounding biological tissues. Nevertheless, additional in vivo investigations are required to evaluate long-term safety, systemic toxicity, and regenerative performance under physiological conditions (Ali *et al.,* 2025). Extended culture studies are equally important for understanding scaffold degradation behavior and the stability of cell–material interactions over prolonged periods, both of which are critical for successful tissue regeneration (Kalva *et al.,* 2024).

Hemocompatibility represents another essential requirement for wound-healing materials. A low hemolysis percentage, generally below 10%, is considered necessary to ensure that the scaffold does not adversely affect blood components or interfere with nitric oxide-mediated tissue repair mechanisms (Zhao *et al.,* 2023). Cellular responses are further investigated by monitoring mitochondrial activity through the enzymatic reduction of tetrazolium salts into formazan crystals, providing a reliable measure of cell viability and metabolic function (Ghiṭman *et al.,* 2021).

The incorporation of $TiO_2$ nanoparticles also contributes to improved protein adsorption on the scaffold surface, which facilitates cell attachment and promotes cellular proliferation (Rocha *et al.,* 2022). In addition, $TiO_2$ reinforces the structural stability of the gelatin–alginate network by strengthening the cross-linked polymer matrix, thereby creating a favorable environment for sustained cell growth (Ibrahim *et al.,* 2024). The antioxidant properties of $TiO_2$ further help reduce oxidative stress at the wound site, supporting tissue regeneration and accelerating the healing process (Bień *et al.,* 2025).

Evidence from in vivo studies, including hematological and histological analyses, suggests that similar $TiO_2$-based composites exhibit good biocompatibility without inducing significant toxicity or inflammatory responses, highlighting their potential for future clinical applications (Zeng *et al.,* 2023). Building on these findings, future research should focus on integrating these nanocomposites with advanced 3D bioprinting technologies to fabricate patient-specific scaffolds that closely mimic the architecture of the native extracellular matrix (Nosrati *et al.,* 2021). Precise control over pore size, pore interconnectivity, and scaffold geometry can improve nutrient transport, vascularization, and tissue regeneration (Babić *et al.,* 2025). Optimizing these structural features may also allow the degradation rate of the scaffold to better match the natural stages of tissue healing, thereby reducing the need for additional surgical interventions (Zhao *et al.,* 2023).

Overall, gelatin–alginate/$TiO_2$ nanocomposite scaffolds provide a stable and biocompatible microenvironment that supports cell migration, proliferation, and tissue integration (Nosrati & Heydari, 2024). Their porous architecture also enables the controlled release of therapeutic agents, which may further enhance wound healing and facilitate the development of personalized treatment strategies tailored to the biological requirements of individual patients (Urruela-Barrios *et al.,* 2026).

## 4. Results

The structural and morphological properties of the $TiO_2$–gelatin–sodium alginate nanocomposite are investigated using scanning electron microscopy (SEM), which reveals a highly porous and interconnected network within the hydrogel matrix. This porous architecture is particularly important because it facilitates nutrient transport, promotes cellular infiltration, and supports tissue regeneration (Cheah *et al.,* 2023; Naeini *et al.,* 2025). The uniform distribution of $TiO_2$ nanoparticles throughout the scaffold is further confirmed by energy-dispersive X-ray spectroscopy (EDS), demonstrating successful incorporation of the nanoparticles while minimizing particle agglomeration and preserving the nanofibrous structure of the composite (Aboomeirah *et al.,* 2022; Liu *et al.,* 2025).

Fourier-transform infrared (FTIR) spectroscopy provides additional evidence of the interactions between the polymer matrix and $TiO_2$ nanoparticles. The characteristic absorption bands indicate the formation of coordination interactions between the carboxyl groups of sodium alginate and the titanium atoms, confirming the successful integration of the inorganic nanoparticles within the biopolymer network (Su *et al.,* 2025). These molecular interactions contribute to the structural stability of the scaffold and enhance its overall performance.

The photocatalytic efficiency of the nanocomposite is evaluated through the degradation of methylene blue under UV irradiation. More than 85% of the dye is degraded within four hours, demonstrating the high photocatalytic activity of the incorporated $TiO_2$ nanoparticles. In addition to its photocatalytic performance, the scaffold exhibits effective antibacterial activity against *Staphylococcus aureus*, indicating its potential for wound dressing and other biomedical applications (Tran *et al.,* 2024).

Biocompatibility studies using L929 fibroblast cells show excellent cellular compatibility, with cell viability assays indicating sustained metabolic activity throughout the culture period (Jadbabaei *et al.,* 2021). These findings are consistent with previous studies reporting that bioactive polymeric nanofibrous scaffolds can closely mimic the native extracellular matrix, thereby promoting cell adhesion, proliferation, and tissue regeneration (Çerçi *et al.,* 2024).

The incorporation of sodium alginate also improves the liquid absorption capacity of the scaffold, allowing it to retain sufficient moisture to support the migration of fibroblasts during the re-epithelialization stage of wound healing (Abid *et al.,* 2024). Cell adhesion and spreading observed after three days of culture further demonstrate that the scaffold surface provides a favorable environment for rapid cellular attachment and integration with surrounding tissues (Irantash *et al.,* 2024).

The structural stability of the scaffold is maintained through appropriate post-fabrication cross-linking, which helps preserve the integrity of the hydrogel during prolonged exposure to physiological conditions (Dip *et al.,* 2025). Owing to their excellent biocompatibility, low toxicity, and favorable structural characteristics, gelatin–sodium alginate/$TiO_2$ nanocomposites represent promising candidates for a wide range of biomedical applications, particularly in tissue engineering and advanced wound healing (Kadhim *et al.,* 2024; Zhou *et al.,* 2025).

Future research should focus on enhancing the functionality of gelatin–sodium alginate/$TiO_2$ nanocomposites by incorporating bioactive molecules such as growth factors and antimicrobial peptides. These modifications could further improve the biological response of the scaffold and increase its effectiveness in the treatment of chronic and infected wounds. Recent studies have demonstrated that the combined action of $TiO_2$ nanoparticles and the biopolymeric matrix exhibits broad-spectrum antimicrobial activity, highlighting the potential of these nanocomposites for advanced wound management (Mathew *et al.,* 2025; Pamula *et al.,* 2025).

Biological evaluations have consistently shown that these hybrid scaffolds provide a favorable environment for cell growth while maintaining low cytotoxicity. Cell viability studies indicate that the materials support cellular proliferation, making them suitable candidates for dermo-epidermal grafts and other tissue engineering applications (Alven *et al.,* 2023; Moradi *et al.,* 2023). Similar findings have been reported in previous studies, where gelatin–alginate-based nanocomposites promoted cell attachment, proliferation, and overall biocompatibility without inducing toxic effects (Eldeeb *et al.,* 2022; Mohammadpour *et al.,* 2021). Improved cell adhesion and spreading observed in these systems further demonstrate that the scaffold surface provides a supportive microenvironment for long-term tissue regeneration (Hajishoreh *et al.,* 2023).

The structural integrity of the nanocomposite is supported by X-ray diffraction (XRD) analysis, which confirms the successful incorporation of $TiO_2$ nanoparticles within the polymer network and indicates the formation of a stable composite structure (Benkhira *et al.,* 2024). Complementary characterization studies suggest that strong interactions between the nanoparticles and the gelatin–alginate matrix contribute to the stability of the scaffold during immersion in simulated body fluids, an important consideration for biomedical applications (Bobu *et al.,* 2024). In addition, the scaffold exhibits tunable swelling behavior and controlled degradation, enabling it to maintain mechanical stability while gradually integrating with regenerating tissue (Folorunso *et al.,* 2024).

The biomimetic properties of the gelatin–alginate matrix also promote fibroblast proliferation, which plays a key role in tissue repair and helps reduce the likelihood of excessive scar formation (Wu *et al.,* 2022). Unlike certain metallic nanomaterials that may raise toxicity concerns, $TiO_2$-based nanocomposite scaffolds demonstrate excellent antimicrobial activity while maintaining good biocompatibility, making them attractive candidates for clinical tissue engineering (Angarita *et al.,* 2024). The combination of structural support, antimicrobial performance, and biological compatibility enables these materials to regulate local inflammatory responses and accelerate wound healing (Dam *et al.,* 2023).

Natural polymer-based composite dressings offer advantages beyond conventional wound coverings by functioning as both protective barriers and bioactive therapeutic systems (Gobi *et al.,* 2021). Because gelatin and sodium alginate closely resemble the native extracellular matrix, these materials provide a promising platform for the development of personalized regenerative therapies (Tosyalı *et al.,* 2025). The incorporation of $TiO_2$ nanoparticles further enhances this

platform by combining mechanical flexibility with photocatalytic and antimicrobial properties, thereby improving the overall functionality of the wound dressing (Tiwari *et al.,* 2025).

The effectiveness of these nanocomposites is also influenced by their highly porous and interconnected microstructure. Scanning electron microscopy demonstrates that $TiO_2$ nanoparticles are uniformly distributed throughout the polymer matrix with minimal aggregation, ensuring consistent structural and functional performance (Li *et al.,* 2023). This porous architecture facilitates cell infiltration while enabling the controlled release of incorporated therapeutic agents, thereby supporting sustained tissue regeneration (Babić *et al.,* 2024). The adjustable pore structure also contributes to mechanical properties that closely resemble those of the native extracellular matrix.

Beyond biomedical applications, the incorporation of $TiO_2$ nanoparticles significantly enhances the photocatalytic performance of the scaffold because of their large surface area and high reactivity. These properties enable efficient degradation of organic dyes, demonstrating the dual functionality of the material for both tissue engineering and environmental remediation (Prakash *et al.,* 2023). Kinetic studies further indicate that $TiO_2$ nanoparticles promote rapid oxidative degradation of organic pollutants while maintaining high catalytic activity during repeated use (Wang *et al.,* 2024). The sustained photocatalytic performance, together with the inherent biocompatibility of the gelatin–alginate hydrogel, highlights the potential of this multifunctional nanocomposite as a platform capable of supporting wound healing while simultaneously providing localized decontamination in biomedical and environmental applications (Ranatunga *et al.,* 2026).

The immobilization of $TiO_2$ nanoparticles within the gelatin–sodium alginate hydrogel is primarily achieved through strong hydrogen-bonding interactions between the hydroxyl groups of the biopolymers and the nanoparticle surface. These interactions effectively prevent nanoparticle leaching, thereby improving the long-term stability and therapeutic performance of the scaffold (Kurian *et al.,* 2024). In addition to enhancing structural stability, the immobilized nanoparticles reduce the risk of nanoparticle-related cytotoxicity while improving the mechanical strength of the hydrogel, making it suitable for use under dynamic physiological conditions (Kasi *et al.,* 2023; Li *et al.,* 2021).

The properties of the hydrogel can be further tailored by adjusting the $TiO_2$ content and the gelatin-to-alginate ratio, both of which influence the cross-linking density of the polymer network (Nguyen *et al.,* 2024). Optimizing these parameters allows the scaffold to achieve an appropriate balance between mechanical strength, swelling behavior, and structural flexibility. The resulting porous architecture facilitates the diffusion of nutrients, oxygen, and metabolic waste while providing sufficient surface area for effective cell attachment and cell–matrix interactions (Abdullaev *et al.,* 2023; Sayed, 2023).

The highly interconnected pore structure also contributes to improved photocatalytic performance by promoting the diffusion of reactive oxygen species generated during UV irradiation. As a result, the nanocomposite exhibits enhanced degradation of organic dyes,

demonstrating its potential for environmental remediation applications (Surkatti *et al.,* 2024). This photocatalytic activity is accompanied by strong antibacterial performance, as in vitro studies have shown effective inhibition of common wound-associated pathogens (Jomaa *et al.,* 2024; Owda *et al.,* 2021).

The antibacterial activity of the scaffold is largely attributed to the generation of reactive oxygen species by $TiO_2$ nanoparticles. These reactive species damage bacterial cell membranes, disrupt essential cellular processes, and inhibit biofilm formation, thereby reducing microbial colonization and promoting a cleaner wound environment (Jing *et al.,* 2024; Wang *et al.,* 2022). Similar observations have been reported in in vivo studies, where $TiO_2$-based hydrogel dressings accelerated wound closure and regulated the inflammatory response, facilitating the transition from the inflammatory phase to the proliferative stage of tissue repair (Bhubhanil *et al.,* 2021).

Another important characteristic of the hydrogel is its high swelling capacity, which enables efficient absorption of wound exudate while supporting the sustained release of endogenous growth factors that are essential for dermal regeneration (Farazin *et al.,* 2021; Kuddushi *et al.,* 2023). This combination of moisture retention and controlled biological activity creates a favorable environment for tissue repair and healing.

The interactions between $TiO_2$ nanoparticles and the gelatin–sodium alginate matrix are further confirmed by Fourier-transform infrared (FTIR) spectroscopy. Shifts in the characteristic amide and carboxylate absorption bands indicate the formation of stable interactions between the polymer functional groups and the nanoparticle surface (Abdullah *et al.,* 2025). These molecular interactions contribute to the enhanced thermal stability and improved compressive strength reported for similar $TiO_2$-based hydrogel nanocomposites, further supporting their suitability for biomedical applications (Berardis *et al.,* 2026; Zhang *et al.,* 2023).

## 5. Discussion

The enhanced photocatalytic degradation efficiency observed in the gelatin–sodium alginate/$TiO_2$ nanocomposite can be attributed to the combined contribution of the biopolymer matrix and $TiO_2$ nanoparticles. Unlike pristine $TiO_2$ or conventional polymer–$TiO_2$ composites, the gelatin–alginate network creates a favorable microenvironment that improves charge separation while facilitating the diffusion of dye molecules to the active catalytic sites (Li *et al.,* 2024; Zhu *et al.,* 2023). The interconnected biopolymer framework supports efficient electron transport by acting as a bridge for photogenerated charge carriers, thereby reducing the probability of electron–hole recombination. As a result, a greater number of reactive oxygen species are generated and retained near the pollutant molecules, leading to more effective oxidation and degradation processes (Feng *et al.,* 2026). The prolonged lifetime of these charge carriers ultimately contributes to the superior photocatalytic performance of the composite compared with pure $TiO_2$ (Wang *et al.,* 2022).

In addition to improving photocatalytic activity, the gelatin–alginate matrix provides excellent structural stability by uniformly dispersing and firmly anchoring the $TiO_2$ nanoparticles within the hydrogel network. This immobilization minimizes nanoparticle aggregation and preserves a

large number of accessible active sites throughout repeated degradation cycles. Consequently, many previously reported nanoparticle-loaded hydrogel systems, particularly solvent-cast polymer composites that often experience structural degradation or reduced catalytic efficiency during prolonged use (Huang *et al.,* 2024).

The incorporation of sodium alginate into the gelatin matrix significantly improves the swelling behavior of the hydrogel, creating a hydrated three-dimensional network that facilitates the diffusion of nutrients, metabolites, and other biologically relevant molecules. This enhanced swelling capability offers a clear advantage over conventional rigid nanocomposite scaffolds, which often exhibit limited mass transport under physiological conditions (Huang *et al.,* 2023; Kuddushi *et al.,* 2025). As a result, the gelatin–alginate hydrogel provides a more favorable environment for both photocatalytic reactions and biomedical applications.

An additional benefit of this composite system is the effective immobilization of $TiO_2$ nanoparticles within the biopolymer network. Unlike conventional powdered photocatalysts, which are difficult to recover after use and may contribute to secondary environmental contamination, the hydrogel matrix securely retains the nanoparticles while preserving their catalytic activity. This structural arrangement not only simplifies catalyst recovery but also enhances the sustainability and practical applicability of the material for repeated wastewater treatment processes.

The uniform distribution of $TiO_2$ throughout the hydrogel, together with its core–shell-like architecture, increases the accessible surface area available for pollutant adsorption and photocatalytic reactions. Consequently, the composite exhibits improved adsorption-assisted photocatalytic performance compared with many previously reported immobilized catalyst systems (Khalil *et al.,* 2021). Importantly, the hydrogel maintains high degradation efficiency over repeated operating cycles, achieving more than 95% pollutant removal while retaining its structural integrity. This excellent reusability effectively addresses one of the major drawbacks of powdered semiconductor photocatalysts, namely the difficulty of nanoparticle recovery and the gradual loss of catalytic performance during repeated use (Wan *et al.,* 2022; Yang *et al.,* 2025).

Beyond environmental remediation, the combination of photocatalytic functionality with a biocompatible gelatin–alginate matrix broadens the potential applications of the composite. The material offers opportunities for integrating water purification with biomedical technologies, particularly in wound healing and tissue engineering, where antibacterial activity and controlled therapeutic performance are highly desirable (Monroy *et al.,* 2025; Мансуров *et al.,* 2023). By simultaneously providing efficient catalytic activity, mechanical stability, and reusability, this multifunctional hydrogel successfully addresses the long-standing challenge of balancing photocatalytic efficiency with practical usability. These characteristics make it a promising platform for sustainable wastewater treatment as well as advanced biomedical applications (Ksenija *et al.,* 2024).

Future research should focus on optimizing the cross-linking density of the gelatin–alginate network to achieve more precise control over drug release kinetics and mechanical properties. The incorporation of stimuli-responsive components, such as pH-, temperature-, or light-sensitive polymers, could further enable localized and on-demand therapeutic delivery. In addition, comprehensive biocompatibility and long-term cytotoxicity studies are required to ensure that $TiO_2$ nanoparticles remain securely immobilized within the hydrogel matrix. Such improvements would enhance antibacterial performance while maintaining the safety profile necessary for prolonged clinical use in wound management and regenerative medicine (Deng *et al.,* 2021; Mirek *et al.,* 2022).

**Conclusion**

The gelatin–sodium alginate/$TiO_2$ nanocomposite demonstrates considerable potential as a multifunctional material by combining efficient photocatalytic activity with desirable biological properties. The integration of $TiO_2$ nanoparticles within the gelatin–alginate matrix enables effective degradation of organic dye pollutants while simultaneously providing a biocompatible platform suitable for wound healing applications (Li *et al.,* 2021; Yang *et al.,* 2026). The adopted cross-linking strategy produces a structurally stable composite that exhibits both environmental and biomedical functionality, highlighting its promise as a sustainable material for wastewater treatment and regenerative medicine (Ammouchi *et al.,* 2026).

Although the current findings demonstrate encouraging performance, further investigations are required to expand the practical applications of this material. Future studies should explore the incorporation of photo-responsive release systems capable of regulating the delivery of therapeutic agents in response to external light stimulation. Such an approach could improve treatment precision by enabling controlled, site-specific drug release for advanced wound care (Kumar *et al.,* 2023; Wu *et al.,* 2022). Equally important is the evaluation of the long-term biological performance of these hydrogels under physiological conditions. Detailed in vitro and in vivo studies are necessary to confirm that their antimicrobial and antioxidant activities remain effective throughout the tissue regeneration process without compromising biocompatibility (Lu *et al.,* 2022; Pranantyo *et al.,* 2024).

The successful translation of this technology from laboratory-scale research to industrial and clinical practice will also depend on a comprehensive assessment of its sustainability. Life cycle assessment, cost analysis, and large-scale manufacturing studies should be undertaken to determine the environmental and economic feasibility of the proposed green synthesis route (Gao *et al.,* 2025). In addition, integrating biosensing elements into the hydrogel network could further enhance its functionality by enabling continuous monitoring of wound conditions, such as infection-associated pH changes. Coupling these sensing capabilities with light-triggered therapeutic activation may facilitate timely antimicrobial intervention and improve wound management outcomes (Ke *et al.,* 2025).

Overall, the gelatin–sodium alginate/$TiO_2$ nanocomposite provides a versatile platform that bridges environmental remediation and biomedical engineering. Its combination of

photocatalytic efficiency, biocompatibility, structural stability, and potential for intelligent therapeutic design positions it as a promising candidate for future sustainable technologies in both wastewater treatment and advanced healthcare applications.

Advances in stimuli-responsive hydrogel systems have created new opportunities to improve the treatment of chronic and complex wounds. By responding to external stimuli such as light, pH, or temperature, these materials can provide controlled therapeutic action that is better suited to the dynamic conditions of the wound microenvironment (Baniasadi, 2025; Wathoni *et al.,* 2026). Such adaptability has the potential to enhance treatment efficacy while supporting the natural healing process.

The integration of these smart hydrogels with three-dimensional (3D) printing technologies represents another important step toward personalized medicine. Patient-specific scaffolds can be fabricated with customized architectures and compositions that closely match the biological and pathological characteristics of individual wounds. This approach may improve tissue regeneration by accommodating the heterogeneous microenvironments commonly associated with chronic, non-healing wounds (Ruan *et al.,* 2023; Geng *et al.,* 2023).

Looking ahead, the combination of multi-omics analyses with advanced manufacturing technologies is expected to accelerate the clinical translation of these multifunctional nanocomposites. A deeper understanding of the molecular mechanisms involved in wound healing, together with precise scaffold fabrication, will support the development of more effective and personalized therapeutic strategies (Wu *et al.,* 2026).

Overall, the integration of photocatalytic activity with regenerative biomaterial design provides a unique platform capable of addressing both environmental and biomedical challenges. By combining efficient pollutant degradation with properties that promote tissue repair, these multifunctional scaffolds offer promising opportunities for sustainable environmental remediation while advancing the development of next-generation personalized healthcare technologies (Qi *et al.,* 2021; Siebert *et al.,* 2021; Yang *et al.,* 2022).

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# MATHEMATICAL MODELLING OF DISEASE PROGRESSION AND THERAPEUTIC RESPONSE IN PRECISION MEDICINE

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**Abstract**
Mathematical modelling has become an indispensable component of contemporary biomedical research, offering a quantitative language through which the progression of disease and the response of patients to therapy can be described, predicted, and optimized. This chapter presents a synthesis of the principal modelling frameworks employed in disease progression and therapeutic response research, spanning ordinary and partial differential equation models, stochastic formulations, agent-based simulations, and network-theoretic representations of disease. Particular attention is given to how these mechanistic frameworks intersect with precision medicine, where the objective shifts from describing population-averaged behaviour to characterizing individual trajectories using patient-specific parameters. The chapter further examines pharmacokinetic and pharmacodynamic modelling as the quantitative backbone of therapeutic optimization, and it discusses the growing integration of artificial intelligence and machine learning with mechanistic models to build hybrid, data-driven predictive systems, including digital twins. Representative equations, including logistic and Gompertz growth laws, reaction-diffusion formulations, and compartmental pharmacokinetic models, are presented with full variable definitions and clinical interpretation. The chapter concludes with a discussion of current methodological challenges, including parameter identifiability, data scarcity, and model validation, and outlines future directions for the field, emphasizing multiscale and hybrid mechanistic-AI approaches as the most promising route toward clinically actionable precision models.

**Keywords:** Mathematical Modelling, Disease Progression, Precision Medicine, Pharmacokinetics-Pharmacodynamics, Tumour Growth Models, Artificial Intelligence.

## 1. Introduction

The transition of medicine from empirically guided, population-averaged practice toward a precision paradigm rests on the ability to represent disease as a dynamic, quantifiable process rather than a static diagnostic label. Mathematical modelling supplies exactly this capability: by encoding biological mechanisms, physiological constraints, and pharmacological interactions into systems of equations, researchers can simulate how a disease evolves and how a given patient is likely to respond to a proposed intervention before that intervention is administered.

Precision medicine seeks to match the right treatment to the right patient at the right time, a goal that depends on inferring individual-level parameters from limited and noisy clinical

observations. Mathematical models provide the structural scaffold onto which such parameters can be estimated, tested for identifiability, and used for forward simulation. Disease progression modelling (DPM), broadly defined, integrates mathematical functions with pathophysiological principles to quantitatively describe the time course of a condition (Barrett, Nicholas, Azer, & Corrigan, 2022). When coupled with pharmacokinetic-pharmacodynamic (PK/PD) formulations, these models extend naturally into therapeutic optimization. This chapter surveys the principal classes of mathematical models used in disease progression and therapeutic response research, discusses their application within precision medicine, and examines the growing role of artificial intelligence (AI) in extending and hybridizing mechanistic frameworks.

## 2. Fundamentals of Disease Progression Modelling

Disease progression models describe how a measurable indicator of disease status, such as tumour volume, viral load, biomarker concentration, or a composite clinical score, changes over time under the influence of underlying biological mechanisms and interventions. Three properties distinguish a useful model from a purely descriptive curve fit: mechanistic grounding, in which terms correspond to identifiable physiological or pathological processes; parsimony, whereby the number of free parameters remains commensurate with the information content of the available data; and predictive validity, meaning the model generalizes to unseen patients or time points rather than merely reproducing the calibration data.

Model development typically follows an iterative cycle comprising problem formulation, selection of an appropriate mathematical structure, parameter estimation, validation, and, where intended for clinical decision support, prospective testing (Figure 1). Sensitivity analysis and identifiability assessment are essential within this cycle, since many disease models contain parameters that cannot be uniquely determined from sparse clinical sampling, a limitation that becomes acute when moving from population-level to individual-level, precision-medicine applications.

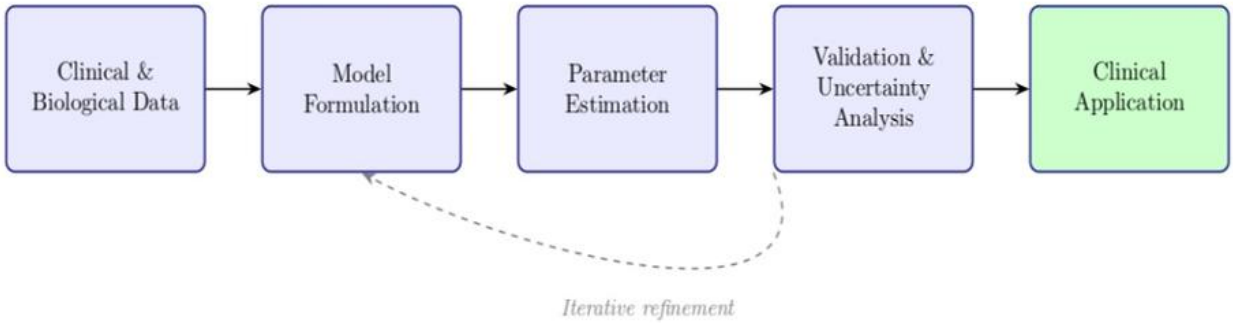


**Figure 1. Workflow for mathematical disease progression modelling**

## 3. Mathematical Models Used in Disease Progression

### 3.1 Ordinary Differential Equations

Ordinary differential equation (ODE) models describe the temporal evolution of one or more state variables that are assumed to be well mixed in space, such as total tumor burden, circulating

drug concentration, or the size of an infected cell population. Two of the most widely used growth laws in this category are the logistic and Gompertz models.

The logistic growth model is expressed as:

$$\frac{dN}{dt} = rN(1 - \frac{N}{K})$$

Where N(t) denotes the size of the population of interest (for example, the number of tumor cells) at time t, r is the intrinsic growth rate, and K is the carrying capacity, the maximum sustainable population size given resource constraints. The logistic model captures the biologically realistic phenomenon of decelerating growth as a population approaches an environmental limit, and it has been shown to yield more favorable predicted responses to therapy than exponential or Gompertz alternatives under certain immune-mediated treatment scenarios.

The Gompertz growth model, widely applied to describe tumor kinetics, takes the form:

$$\frac{dV}{dt} = V[\alpha - \beta \ln(\frac{V}{V_0})]$$

Where V(t) is tumour volume at time t, $V_0$ is the initial volume, α is the initial specific growth rate, and β describes the rate at which growth decelerates as the tumour expands. Unlike the logistic law, the Gompertz model imposes a continuously slowing but formally unbounded deceleration, a property found empirically to fit longitudinal tumour volume data from murine models more accurately than exponential or logistic alternatives (Vaghi *et al.,* 2020). A notable finding from population-level analyses is a strong correlation between the two Gompertz parameters, motivating a reduced, single-parameter formulation that improves the precision of individualized tumor-age predictions from sparse late-stage measurements (Vaghi *et al.,* 2020).

**3.2 Partial Differential Equations**

When spatial heterogeneity cannot be neglected, for example in the invasion of tumour cells into surrounding tissue or the diffusion of a therapeutic agent through an organ, partial differential equation (PDE) models are required. The reaction-diffusion equation is the canonical formulation:

$$\frac{\partial u}{\partial t} = D\nabla^2 u + f(u)$$

where u(x, t) represents the local density of the biological quantity of interest (such as cell density or drug concentration) at spatial position x and time t, D is the diffusion coefficient governing spatial spread, $\nabla^2$ is the Laplacian operator describing diffusive flux, and f (u) is a reaction term capturing local proliferation, decay, or interaction dynamics, often itself taking a logistic or Gompertzian form. Reaction-diffusion models have been applied to describe glioma invasion, wound healing, and the spatial distribution of immune infiltrate within a tumour microenvironment, allowing imaging-derived spatial data to be incorporated directly into model calibration.

### 3.3 Stochastic Models

Deterministic ODE and PDE models describe the expected or average trajectory of a system but do not capture the intrinsic randomness arising from finite population sizes, molecular noise, or unpredictable environmental fluctuations. Stochastic models address this limitation by treating state variables as random processes, often formulated through a master equation or an equivalent stochastic differential equation. In tumour growth modelling, coupling multiplicative and additive noise into a Gompertzian framework and analyzing the resulting Fokker-Planck equation allows characterization of the steady-state probability distribution of tumour size and the mean first-passage time to clinically relevant thresholds, offering insight into the variability of disease trajectories across a patient population (He *et al.,* 2022). Stochastic formulations are valuable in precision medicine because they yield prediction intervals rather than single-point forecasts, better reflecting genuine forecast uncertainty.

### 3.4 Agent-Based Models

Agent-based models (ABMs) simulate a system as a collection of discrete, autonomous agents, such as individual cells, immune effectors, or patients, each governed by local rules and capable of interacting with other agents and with their environment. Emergent, population-level behaviour arises from the aggregation of these individual-level interactions rather than being imposed a priori through an aggregate equation. This bottom-up structure makes ABMs well suited to modelling heterogeneous patient populations and complex adaptive systems, including tumour-immune interplay or the spread of infectious and chronic disease through networks of interacting individuals. Hybrid frameworks coupling ABMs with ODE or PDE components have been used to capture immune-response dynamics across molecular, cellular, and systemic scales simultaneously, illustrating the value of multiscale integration when no single formalism can represent all levels of biological organization.

### 3.5 Network Models

Network, or graph-theoretic, models represent a system as a set of nodes connected by edges encoding interactions, whether contacts between individuals in an epidemic, molecular interactions within a signalling pathway, or comorbidity relationships among diseases in a patient population. Epidemic processes on complex networks have been studied extensively, revealing that network topology, including the presence of highly connected hub nodes, fundamentally alters the threshold conditions and speed of disease spread compared with the homogeneous-mixing assumption underlying classical compartmental models such as the susceptible-infectious-recovered (SIR) framework (Pastor-Satorras, Castellano, Van Mieghem, & Vespignani, 2015). In precision medicine, network approaches extend beyond epidemiology to biomedical knowledge graphs integrating molecular, cellular, pharmacological, and clinical data, enabling mechanistic inference across biological scales.

## 4. Mathematical Modelling of Therapeutic Response

Modelling the response of a disease to therapy requires linking a pharmacological exposure to a measurable biological or clinical effect. Pharmacokinetic (PK) models describe the time course

of drug concentration in the body, most commonly through compartmental formulations. A one-compartment model with first-order elimination is written as:

$$\frac{dC}{dt} = -k_e . C$$

Where C (t) is the plasma drug concentration at time t and $k_e$ is the first-order elimination rate constant, which is related to the drug half-life through $t_{\frac{1}{2}} = \frac{\ln(2)}{k_e}$ Pharmacodynamic (PD) models then relate this concentration to a pharmacological effect, most frequently through the $E_{max}$ model:

$$E = \frac{E_{max} . C}{(EC_{50} + C)}$$

Where E is the observed pharmacological effect, $E_{max}$ is the maximum achievable effect, C is the drug concentration, and $EC_{50}$ is the concentration producing half of the maximal effect. Integrated PK/PD modelling combines these components to characterize the complete time course of the dose-response relationship, an approach central to optimizing dosing regimens in oncology, infectious disease, and immunology, and one that underlies regulatory initiatives such as the US Food and Drug Administration's Project Optimus, which promotes model-informed dose optimization in oncology drug development.

In oncology specifically, tumour growth-inhibition models extend the growth laws of Section 3.1 by adding a drug-dependent killing term:

$$\frac{dV}{dt} = V[\alpha - \beta \ln\left(\frac{V}{V_0}\right)] - \kappa C(t) V$$

Where κ is a drug-sensitivity parameter that determines the strength of the drug-induced tumour-killing effect, and C (t) is the drug concentration at time t. Fitting such combined growth-and-treatment models to serial imaging or biomarker data allows estimation of patient-specific values of κ, providing a quantitative basis for distinguishing responders from non-responders earlier than possible from raw volumetric measurements alone. Comparative model-selection studies using large clinical immunotherapy datasets show that classical growth models differ substantially in their ability to describe real patient tumour trajectories, underscoring the importance of rigorous validation before such frameworks guide treatment decisions. PK/PD modelling of therapeutic antibodies has further addressed challenges specific to biologics, including target-mediated drug disposition and resistance, extending mechanistic modelling into large-molecule therapeutics (Tang & Cao, 2021).

**5. Mathematical Modelling in Precision Medicine**

Precision medicine reframes the modelling problem from characterizing an average patient to inferring individual-specific parameters from a patient's own longitudinal data. This shift imposes distinct demands. Models must typically be re-parameterized using a population approach, most often nonlinear mixed-effects modelling, in which population-level parameter distributions are estimated jointly with individual deviations, allowing new patients to be characterized from sparse data by borrowing statistical strength from the broader population

(Vaghi *et al.,* 2020). Model outputs must also be translated into decision-relevant quantities, such as the probability that a patient's disease will progress within a specified interval, or the dose adjustment required to maintain a target exposure. Uncertainty must be explicitly propagated and communicated, since decisions based on an overconfident point prediction carry different risk than those informed by a calibrated probability distribution.

Bayesian network approaches have been proposed as a practical tool for individualized risk prediction, integrating diverse real-world clinical variables into a single interpretable probabilistic framework (Arora *et al.,* 2019). Disease progression models have also found application in guiding neurodegenerative disease research, where temporal realignment techniques align patients along a common, data-derived disease timeline despite differing ages of onset, providing a mechanistically informed basis for staging patients and selecting trial candidates. Precision medicine therefore does not require abandoning mechanistic modelling in favour of purely statistical approaches, but rather demands that mechanistic structure be embedded within a statistical framework capable of handling inter-individual variability.

**6. Integration of Artificial Intelligence with Mathematical Modelling**

Mechanistic mathematical models offer interpretability and grounding in known biology, but they can struggle to capture the full complexity of high-dimensional clinical, imaging, and omics data. Artificial intelligence and machine learning methods excel at extracting patterns from such heterogeneous data, but they often function as opaque predictors that are difficult to interpret mechanistically and may generalize poorly outside their training range. The integration of these two paradigms, commonly described as hybrid or mechanistic-AI modelling, seeks to combine the complementary strengths of both approaches (Figure 2).

Several integration strategies have emerged. Machine learning can estimate patient-specific parameters of an otherwise mechanistic model directly from multimodal data, replacing conventional but data-hungry population pharmacokinetic estimation procedures. Neural networks can serve as surrogate models approximating the output of computationally expensive PDE or agent-based simulations, enabling real-time decision support. Machine learning applied to genome-scale metabolic models has provided mechanistic understanding of how genotype relates to phenotype, supporting the design of targeted and combination therapies (Bhinder, Gilvary, Madhukar, & Elemento, 2021). Digital twins, continuously updated virtual, patient-specific replicas integrating multimodal biological, clinical, and lifestyle data, represent an emerging synthesis of mechanistic and AI-driven modelling aimed at simulating an individual patient's disease trajectory in near real time. Explainability remains a central concern. Systematic reviews of explainable AI in digital health for precision medicine emphasize that transparency and interpretability are essential for clinical trust and regulatory acceptance, motivating continued interest in hybrid architectures where a mechanistic ‘skeleton’ constrains and interprets the outputs of otherwise data-driven components (Allen, 2024)

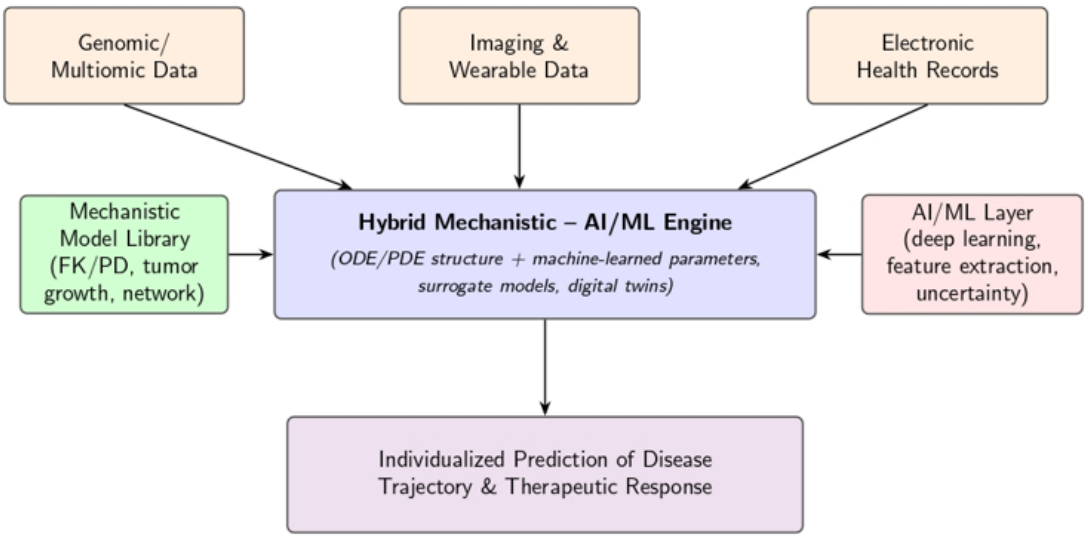


**Figure 2. Hybrid mechanistic-AI framework for precision medicine**

**Table 1: Comparison of mathematical modelling approaches for disease progression.**

| Modelling Approach | Typical Applications | Advantages | Limitations |
|---|---|---|---|
| Ordinary differential equations (logistic, Gompertz) | Tumour growth kinetics; population dynamics of pathogens | Analytically tractable; few parameters; well-established fitting methods | Assumes spatially homogeneous, well-mixed populations |
| Partial differential equations (reaction-diffusion) | Tumour invasion; drug diffusion in tissue; wound healing | Captures spatial heterogeneity and infiltration patterns | Computationally intensive; requires spatial imaging data |
| Stochastic models | Small-population dynamics; variability in individual trajectories | Quantifies uncertainty; yields prediction intervals | Complex analytical treatment; parameter estimation challenges |
| Agent-based models | Immune - tumour interaction; infectious and chronic disease spread | Captures emergent behaviour; models heterogeneous agents | High computational cost; sensitive to unobserved assumptions |
| Network models | Epidemic spread; comorbidity and molecular interaction networks | Represents topology and connectivity explicitly | Requires detailed interaction/contact data |
| Hybrid mechanistic - AI models | Individualized digital twins; surrogate modelling; parameter inference | Combines interpretability with high-dimensional pattern recognition | Requires large, high-quality multimodal datasets; explainability challenges |

Table 1 summarizes the principal mathematical modelling approaches discussed in this chapter, together with their characteristic applications and their main advantages and limitations for disease progression and therapeutic response modelling.

**7. Current Challenges and Future Directions**

Despite substantial progress, several methodological challenges continue to constrain clinical translation. Parameter identifiability remains a persistent obstacle: many mechanistic models contain more free parameters than can be reliably estimated from the sparse, irregularly sampled data typical of clinical practice, particularly at the individual-patient level rather than in a well-controlled experimental cohort. Data scarcity and heterogeneity across institutions further complicate calibration and validation, especially for rare diseases or novel therapeutic modalities. Model validation itself is nontrivial, since a model that fits retrospective data well may fail to generalize prospectively, and rigorous external validation across independent cohorts remains comparatively rare in the published literature.

The integration of mechanistic and AI-driven components introduces additional challenges around explainability, reproducibility, and regulatory acceptance, since hybrid models must satisfy both the interpretability expectations of mechanistic pharmacometrics and the performance expectations of modern machine learning. Joint workshops convened by regulatory agencies and academic centers have highlighted the need for trustworthy AI frameworks tailored to precision medicine, echoing broader concerns about the key barriers to delivering measurable clinical impact with AI-based tools in routine practice (Kelly, Karthikesalingam, Suleyman, Corrado, & King, 2019).

Looking forward, multiscale frameworks that couple molecular, cellular, and organ-level dynamics within a single computational structure offer a route toward mechanistic digital twins integrating multiomic and imaging data. Continued development of population-based and Bayesian estimation methods will improve the feasibility of individualizing complex mechanistic models from limited clinical data, while closer collaboration between mathematical modellers, clinicians, and regulatory scientists remains essential to translate increasingly sophisticated models into demonstrable improvements in patient outcomes.

**Conclusion**

Mathematical modelling provides a rigorous, mechanistically grounded language for describing how disease evolves and how patients respond to therapy, forming a natural quantitative foundation for precision medicine. Ordinary and partial differential equation models, stochastic formulations, agent-based simulations, and network representations each capture complementary aspects of disease biology, from well-mixed population dynamics to spatial invasion, individual-level heterogeneity, and system-wide connectivity. Pharmacokinetic and pharmacodynamic models extend this framework to therapeutic optimization, while the growing integration of artificial intelligence with mechanistic structures is enabling increasingly personalized, data-rich predictive tools, including digital twins. Realizing the full clinical potential of these approaches will depend on addressing persistent challenges in parameter identifiability, data quality, and

model validation, and on continued interdisciplinary collaboration between mathematicians, biomedical scientists, and clinicians.

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# MULTI-OMICS APPROACHES FOR DECIPHERING: MICROBIAL FUNCTIONS AND METABOLISM

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**Abstract**
Microorganisms drive nearly every biogeochemical process on Earth and underpin health, disease, agriculture and industrial biotechnology, yet a single omics layer rarely explains how a microbial genome is translated into a functioning metabolic phenotype. Multi-omics approaches integrate genomics, transcriptomics, proteomics and metabolomics — together with their community-level counterparts, metagenomics, metatranscriptomics and metaproteomics — to provide a systems-level view of microbial function. This chapter reviews the principal omics technologies used to interrogate microorganisms and microbial communities, describes computational strategies for integrating heterogeneous multi-omics datasets, surveys key bioinformatic tools and databases, and highlights applications in human and environmental microbiology, industrial biotechnology and agriculture. Current limitations, including data heterogeneity, incomplete reference databases and the challenge of establishing causality, are discussed alongside emerging solutions such as long-read sequencing, single-cell multi-omics and artificial-intelligence-based data fusion. Twenty-two illustrative figures, tables and literature references are provided to orient readers new to the field and to guide experimental and analytical design.

**Keywords:** Multi-Omics, Metagenomics, Metatranscriptomics, Metaproteomics, Metabolomics, Microbial Metabolism, Systems Biology, Data Integration.

## 1. Introduction

Microorganisms are the most metabolically diverse organisms on the planet, capable of exploiting an extraordinary range of energy sources, electron donors and acceptors, and carbon substrates. This versatility underlies their central role in biogeochemical cycling, symbiosis with plants and animals, bioremediation, and industrial fermentation. For more than a century, microbial physiology was studied largely through pure-culture biochemistry; however, the recognition that the vast majority of environmental microorganisms cannot be readily cultured shifted the field toward culture-independent, sequence-based approaches.

The first culture-independent surveys relied on 16S ribosomal RNA gene sequencing to catalogue microbial diversity, an approach that revealed the immense phylogenetic breadth of the microbial world but offered little insight into function. The advent of high-throughput 'omics' technologies — genomics, transcriptomics, proteomics and metabolomics — transformed

microbiology from a descriptive to a functional and predictive science. Each omics layer captures a different molecular tier of the cell: the genome defines metabolic potential, the transcriptome reflects regulatory response, the proteome represents the functional machinery actually deployed, and the metabolome captures the biochemical outcome of all upstream processes.

No single omics layer is sufficient to explain microbial function, because genes that are present are not necessarily expressed, transcripts are not always translated, and enzyme abundance does not guarantee catalytic flux. Multi-omics approaches address this gap by simultaneously or sequentially profiling several molecular layers from the same biological sample and integrating the resulting datasets computationally. This chapter provides a structured overview of multi-omics strategies for deciphering microbial functions and metabolism, covering single-organism and community-level (meta-omics) approaches, integration methodology, key bioinformatic resources, and representative applications.

## 2. The Omics Layers: An Overview

Multi-omics studies of microorganisms typically combine four core molecular layers — genomics, transcriptomics, proteomics and metabolomics — each measured either from a single isolate (pure-culture omics) or directly from a mixed microbial community (meta-omics). Table 1 summarises the molecular target, principal technology and typical functional readout of each layer.

**Table 1: Overview of the principal omics layers used to study microbial function and metabolism**

| Omics Layer | Molecular Target | Principal Technology | Functional Readout |
|---|---|---|---|
| Genomics / Metagenomics | DNA (genome or community gene pool) | Whole-genome / shotgun metagenome sequencing (Illumina, Nanopore, PacBio) | Metabolic potential; taxonomy; gene inventory |
| Transcriptomics / Metatranscriptomics | mRNA (total or community transcripts) | RNA-seq, microarrays | Which genes are actively expressed under given conditions |
| Proteomics / Metaproteomics | Expressed proteins | LC-MS/MS shotgun and targeted proteomics | Functional machinery actually synthesised; enzyme abundance |
| Metabolomics / Metabonomics | Small-molecule metabolites (<1500 Da) | LC-MS, GC-MS, NMR spectroscopy | Metabolic phenotype; substrate use and end products |
| Epigenomics / Phenomics (emerging) | DNA methylation; growth/physiological traits | Bisulfite/Nanopore methylation sequencing; high-throughput phenotyping | Regulatory state and observable phenotype |

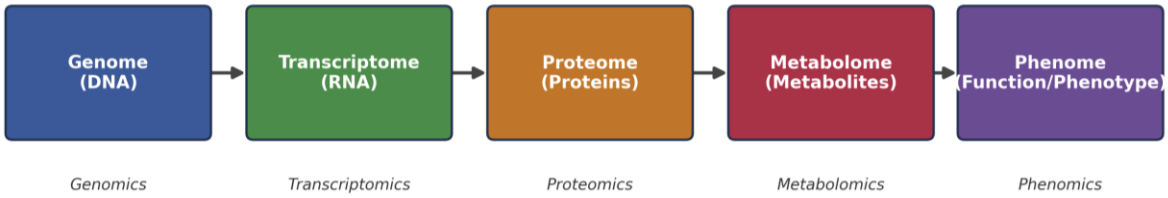


**Figure 1: The multi-omics cascade linking genotype to phenotype in a microbial cell. Each molecular layer is interrogated by a dedicated high-throughput technology**

Applied to single isolates, these layers reveal the physiology of a defined strain under controlled conditions. Applied to whole communities — as metagenomics, metatranscriptomics, metaproteomics and community metabolomics — they reveal the collective functional capacity and activity of an ecosystem without the need for cultivation, an approach often termed "meta-omics" [1,2]. The two scales are complementary: pure-culture multi-omics establishes causal, mechanistic links between genotype and phenotype, while meta-omics captures the emergent, ecosystem-level functions that arise from microbial interactions [3].

## 3. Genomics and Metagenomics: Defining Metabolic Potential

Whole-genome sequencing of pure isolates provides the metabolic blueprint of a microorganism, enabling reconstruction of complete biosynthetic and catabolic pathways, prediction of nutritional requirements, and identification of biotechnologically relevant gene clusters such as those encoding secondary metabolites or biodegradative enzymes. Comparative genomics across strains further reveals the pangenome — the core genes shared by all strains of a species and the accessory genes that confer niche-specific traits [4].

Metagenomics extends this principle to entire microbial communities by sequencing total community DNA directly from an environmental or host-associated sample, bypassing the need for cultivation. Amplicon metagenomics (typically 16S rRNA gene sequencing) provides taxonomic profiles at low cost, whereas shotgun metagenomics sequences all genomic DNA present, yielding gene catalogues, functional potential, and — through metagenome assembly and binning — metagenome-assembled genomes (MAGs) representing uncultured taxa [5,6]. Landmark efforts such as the Human Microbiome Project and the Sargasso Sea and Tara Oceans surveys demonstrated that shotgun metagenomics could catalogue millions of previously unknown genes and reconstruct genomes of organisms that had never been cultured [7,8].

### 3.1 Strengths and Limitations

- Strength: reveals full metabolic potential and taxonomic composition without cultivation bias.
- Strength: enables discovery of novel enzymes, biosynthetic gene clusters and uncultured lineages.
- Limitation: DNA presence does not indicate gene expression or activity.
- Limitation: assembly and binning are challenging in highly diverse or strain-heterogeneous communities.

**4. Transcriptomics and Metatranscriptomics: Capturing Regulatory Response**

RNA-sequencing of a pure culture quantifies genome-wide transcript abundance, exposing which genes are switched on or off under a given growth condition, stress, or interaction. This has been central to understanding regulatory networks, operon structure, small regulatory RNAs, and condition-specific virulence or stress-response programmes in model and non-model bacteria [9].

Metatranscriptomics applies the same principle to whole communities, sequencing total community RNA (after depletion of the highly abundant ribosomal RNA) to reveal which of the many genes encoded in the metagenome are actually being transcribed at the moment of sampling [10]. Because transcript levels change within minutes in response to environmental cues, metatranscriptomics captures a dynamic snapshot of community activity that metagenomics, reflecting cumulative genomic potential, cannot provide. It has been widely used to study active nutrient cycling guilds in soil and ocean ecosystems, and to identify actively transcribing taxa in the human gut during dietary or pharmacological interventions [11].

**4.1 Strengths and Limitations**

- Strength: distinguishes metabolically active taxa from dormant or dead community members.
- Strength: reveals real-time regulatory and stress responses.
- Limitation: mRNA is short-lived and technically challenging to preserve and extract from environmental samples.
- Limitation: transcript abundance does not always correlate with protein abundance or enzymatic activity.

**5. Proteomics and Metaproteomics: The Functional Machinery**

Proteins are the primary functional effectors of the cell, and mass-spectrometry-based proteomics — typically bottom-up shotgun proteomics using liquid chromatography tandem mass spectrometry (LC-MS/MS) — directly measures which proteins are present and in what relative abundance. Quantitative proteomics of microbial isolates has clarified the composition of central metabolic enzymes, secretion systems, and stress-response regulons under defined culture conditions [12].

Metaproteomics extracts and identifies proteins directly from environmental or host-associated communities, matching mass spectra against protein databases derived from paired metagenomic or reference sequences. Because it measures the functional gene products actually deployed by the community, metaproteomics is considered one of the most direct readouts of in situ microbial activity, and has been used to quantify the contribution of specific taxa to nutrient cycling in activated sludge, soils, and the human gut microbiome [13,14].

**5.1 Strengths and Limitations**

- Strength: measures the actual functional machinery of the cell or community, closest to phenotype among the sequence-based omics.

- Strength: can quantify taxon-specific functional contributions in complex communities.
- Limitation: requires comprehensive, well-annotated protein sequence databases for accurate spectral matching.
- Limitation: lower sensitivity than genomics/transcriptomics for low-abundance taxa or proteins.

**6. Metabolomics and Metabonomics: The Biochemical Endpoint**

Metabolomics comprehensively profiles the small-molecule metabolites — sugars, amino acids, organic acids, lipids, and secondary metabolites — present in a cell, supernatant, or environmental matrix, using nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry coupled to gas or liquid chromatography [15]. Because metabolite pools represent the net outcome of upstream gene expression, translation, and enzymatic flux, metabolomics is regarded as the omics layer closest to the observable phenotype.

In microbiology, metabolomics is used to define nutrient utilisation profiles, identify fermentation products and secondary metabolites of biotechnological interest, and characterise the biochemical dialogue between microorganisms and their hosts, such as short-chain fatty acid production by the gut microbiota and its systemic metabolic effects [16]. Community-level ("meta-metabolomics") approaches profile the extracellular metabolite pool of an entire microbial ecosystem, revealing cross-feeding interactions and metabolic handoffs between taxa that cannot be inferred from genomic potential alone [17].

**6.1 Strengths and Limitations**

- Strength: directly reflects metabolic phenotype and functional output.
- Strength: sensitive to environmental and physiological state changes.
- Limitation: many metabolite features remain structurally unidentified ("dark metabolome").
- Limitation: does not attribute metabolites to specific community members without complementary data.

**7. Multi-Omics Data Integration Strategies**

The central analytical challenge of multi-omics research is integrating datasets that differ in scale, dynamic range, noise structure and biological turnover. Broadly, integration strategies fall into three, often complementary, categories: vertical (statistical) integration of paired samples, network-based integration that links molecular features across layers, and pathway- or knowledge-based integration that projects multi-omics data onto curated metabolic pathway maps [18,19]. Increasingly, machine-learning and deep-learning methods are used to fuse heterogeneous omics matrices into a single latent representation that captures cross-layer covariation [20].

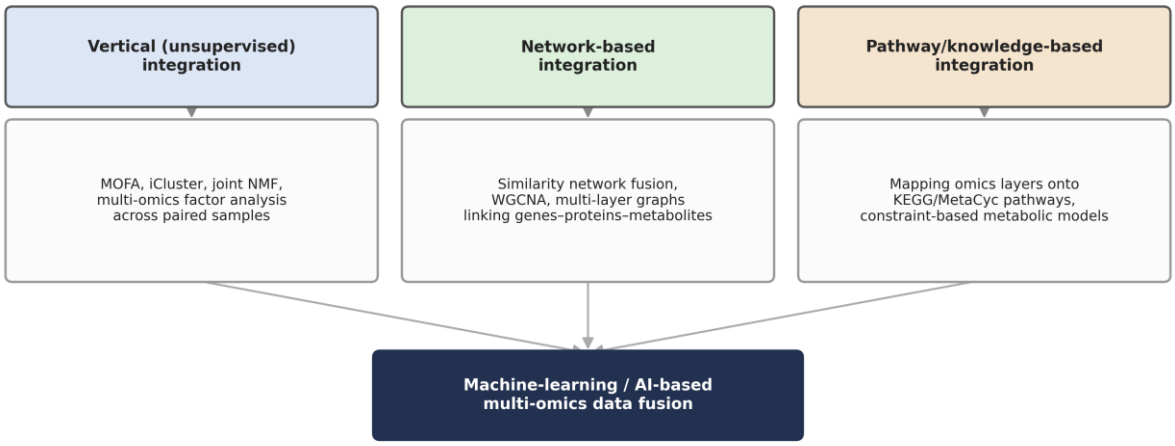


**Figure 2. Principal strategies for integrating multi-omics datasets, ranging from statistical factor models to pathway-based mapping and machine-learning fusion**

**Table 2: Common statistical and computational strategies for multi-omics data integration.**

| Integration Approach | Representative Methods/Tools | Typical Use Case |
|---|---|---|
| Vertical / statistical integration | MOFA+, iCluster, joint non-negative matrix factorisation, canonical correlation analysis | Identifying latent factors that jointly explain variation across paired omics layers from the same samples |
| Network-based integration | WGCNA, similarity network fusion, multi-layer/graph-based models | Building gene–protein–metabolite co-abundance or regulatory networks |
| Pathway/knowledge-based integration | KEGG Mapper, MetaCyc/Pathway Tools, genome-scale metabolic models (GSMMs), flux balance analysis | Mapping multi-omics measurements onto curated pathways and predicting metabolic fluxes |
| Machine-learning / AI-based fusion | Autoencoders, random forest, multi-omics deep learning frameworks | Predictive modelling, biomarker discovery, and dimensionality reduction across large, heterogeneous datasets |

Genome-scale metabolic models (GSMMs) deserve particular mention as an integration framework specific to microbial metabolism: reconstructed from an annotated genome, a GSMM encodes all known biochemical reactions of an organism as a stoichiometric matrix, which can be constrained using transcriptomic, proteomic or metabolomic data (through methods such as flux balance analysis) to predict condition-specific metabolic flux distributions [21]. This approach directly links genomic potential to a quantitative, testable metabolic phenotype and is widely used in both single-organism and community-scale (multi-species) metabolic modelling.

## 8. Bioinformatic Tools and Databases

A mature ecosystem of open-source software and curated databases supports each stage of multi-omics analysis, from raw sequence quality control through pathway-level interpretation. Table 3 lists representative tools organised by omics layer and analytical function; this is illustrative rather than exhaustive, and tool choice should be guided by data type, community complexity, and computational resources.

**Table 3: Representative bioinformatic tools and databases used across the multi-omics analysis pipeline [22–24]**

| Analytical Stage | Representative Tools/Databases | Function |
|---|---|---|
| Sequence QC & assembly | FastQC, Trimmomatic, MEGAHIT, metaSPAdes | Quality filtering and de novo assembly of genomic/metagenomic reads |
| Genome/MAG binning & QC | MetaBAT2, MaxBin2, CheckM | Recovery and quality assessment of metagenome-assembled genomes |
| Taxonomic profiling | Kraken2, MetaPhlAn, QIIME2 | Assigning taxonomy to reads or amplicon sequences |
| Gene prediction & annotation | Prodigal, Prokka, eggNOG-mapper | Identifying and functionally annotating open reading frames |
| Transcriptomic quantification | HISAT2/STAR, Salmon, DESeq2/edgeR | Read alignment, transcript quantification and differential expression analysis |
| Proteomic identification | MaxQuant, Mascot, DIAMOND | Peptide-spectrum matching and protein quantification |
| Metabolomic processing | XCMS, MZmine, MetaboAnalyst | Peak detection, alignment and statistical analysis of MS/NMR data |
| Pathway & network databases | KEGG, MetaCyc, STRING | Curated metabolic pathways and protein–protein interaction networks for functional interpretation |
| Genome-scale metabolic modelling | COBRApy, CarveMe, ModelSEED | Reconstruction and flux simulation of metabolic networks |

## 9. Applications in Deciphering Microbial Functions and Metabolism

Integrated multi-omics pipelines, illustrated schematically in Figure 3, have been applied across essentially every branch of microbiology, from single-strain physiology to whole-ecosystem functional ecology.

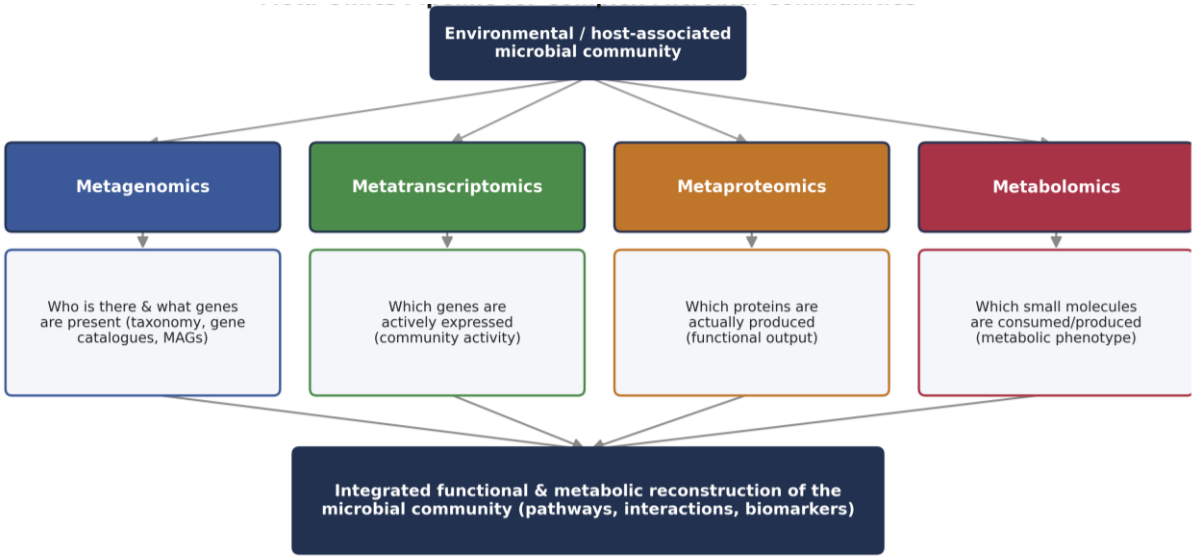


**Figure 3: A typical meta-omics pipeline for complex microbial communities, combining metagenomics, metatranscriptomics, metaproteomics and metabolomics to reconstruct community-level function**

### 9.1 Human and Animal Microbiome Research

Multi-omics studies of the human gut microbiome have linked shifts in microbial gene content, transcriptional activity, protein expression and metabolite output (notably short-chain fatty acids, bile acid derivatives and tryptophan metabolites) to host metabolic and immune phenotypes, informing biomarker discovery in inflammatory bowel disease, type 2 diabetes and colorectal cancer [16,25].

### 9.2 Environmental and Climate Microbiology

In soils, sediments and oceans, meta-omics has clarified the microbial drivers of carbon, nitrogen and sulphur cycling, identifying which taxa are transcriptionally and proteomically active in specific biogeochemical transformations rather than merely genomically present [11].

### 9.3 Industrial Biotechnology

Multi-omics guides rational strain engineering for biofuel, enzyme and commodity-chemical production by pinpointing rate-limiting metabolic steps, unexpected regulatory bottlenecks, and byproduct-forming pathways that are not evident from genome sequence alone [21].

### 9.4 Agriculture and Plant–Microbe Interactions

Multi-omics profiling of the rhizosphere and phyllosphere microbiome has identified taxa and metabolic pathways associated with plant growth promotion, nutrient acquisition and biocontrol of pathogens, supporting the development of microbial inoculants.

### 9.5 Bioremediation

Combining metagenomics with metatranscriptomics and metabolomics has been used to track the induction of biodegradative pathways in contaminated soils and wastewater, confirming in situ pollutant transformation rather than mere genetic capacity for degradation.

## 10. A Generalised Multi-Omics Experimental Workflow

Despite differences across study systems, most multi-omics investigations of microbial function follow a common experimental and analytical arc: biomolecule extraction from the same or

matched biological samples, layer-specific high-throughput measurement, bioinformatic processing and quantification, and finally computational integration to generate testable biological hypotheses (Figure 5). Careful experimental design — including consistent sampling time points, adequate replication, and, where possible, extraction of multiple biomolecule classes from a single sample aliquot — is essential to preserving the biological correspondence between omics layers that integration methods depend upon [18].

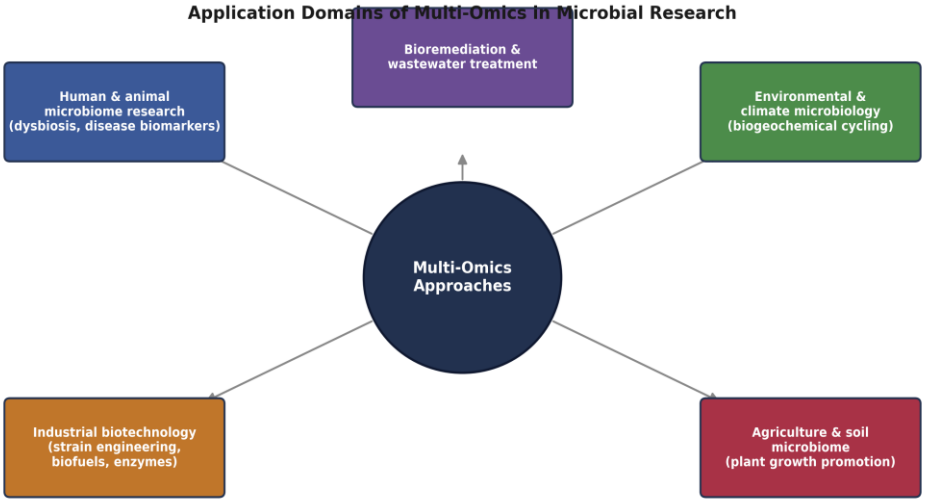


**Figure 4: Major application domains of multi-omics approaches in microbial research**

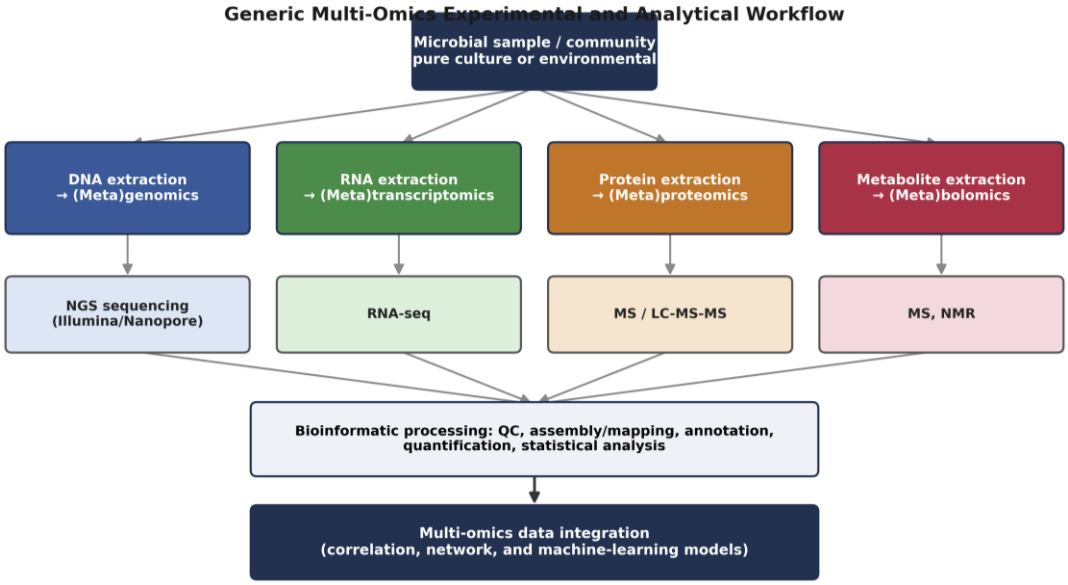


**Figure 5: Generic experimental and computational workflow for a multi-omics study of microbial function, from sample to integrated biological insight**

## 11. Challenges and Limitations

Despite substantial technical progress, several challenges continue to constrain multi-omics microbiology (Table 4). Foremost among these is data heterogeneity: genomic, transcriptomic, proteomic and metabolomic datasets differ in dimensionality, dynamic range, missingness patterns and noise structure, complicating direct statistical comparison [19]. Reference database

incompleteness disproportionately affects environmental samples containing large fractions of uncultured, taxonomically novel organisms, reducing annotation confidence for metagenomic, metatranscriptomic and metaproteomic data alike. Furthermore, correlation between omics layers does not establish causality, and experimental validation (for example, through targeted gene knockouts or isotope-tracing flux experiments) remains necessary to confirm mechanistic links suggested by multi-omics correlation [3].

**Table 4: Key challenges in multi-omics microbiology and emerging mitigation strategies**

| Challenge | Description | Emerging Mitigation |
|---|---|---|
| Data heterogeneity | Different scales, distributions and noise structures across omics layers | Layer-specific normalisation; probabilistic and Bayesian integration frameworks |
| Reference database gaps | Uncultured and taxonomically novel organisms are poorly represented in reference databases | Expanded MAG catalogues; long-read sequencing; culturomics efforts |
| Cost and technical complexity | Multiple platforms, expertise and sample material required | Multi-omics extraction kits; shared core facilities; cloud-based pipelines |
| Correlation vs. causation | Statistical association across layers does not confirm mechanism | Targeted genetic manipulation; isotope-labelling flux studies; gnotobiotic models |
| Standardisation & reproducibility | Variable protocols hinder cross-study comparison | Community standards (e.g., MIxS, minimum reporting guidelines); standardised pipelines |
| Single-cell resolution | Bulk omics obscures strain- and cell-level heterogeneity | Single-cell genomics/transcriptomics; Raman-activated cell sorting |

## 12. Future Perspectives

Several developments are poised to extend the resolution and interpretability of multi-omics microbiology. Long-read sequencing technologies (e.g., Oxford Nanopore, PacBio HiFi) are improving metagenome assembly contiguity and enabling direct detection of epigenetic modifications alongside sequence [6]. Single-cell and spatial multi-omics methods are beginning to resolve functional heterogeneity within nominally clonal populations and to map metabolic activity onto spatial structure in biofilms and host tissues. Artificial-intelligence-based integration frameworks, including deep-learning autoencoders and foundation models trained on large multi-omics compendia, promise to identify cross-layer patterns beyond the reach of conventional statistics [20]. Finally, combining multi-omics with genome-scale metabolic modelling of multi-species communities is increasingly used to predict, rather than merely describe, the metabolic consequences of community perturbation, moving the field from correlative description toward predictive, engineerable microbial ecology.

## Conclusion

Multi-omics approaches have transformed microbiology from a discipline constrained by cultivability into one capable of comprehensively characterising the genetic potential, regulatory

activity, functional machinery and metabolic output of microorganisms and the communities they form. By integrating genomics, transcriptomics, proteomics and metabolomics — at both the single-organism and community scale — researchers can move beyond static gene inventories to a dynamic, mechanistic understanding of microbial function. Continued advances in sequencing depth, mass spectrometry sensitivity, computational integration methods and single-cell resolution will further sharpen this picture, cementing multi-omics as the central methodological framework for deciphering microbial functions and metabolism across human health, environmental and industrial contexts.

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# TRANSFORMING HEALTHCARE: PRECISION MEDICINE AND PHARMACEUTICAL INNOVATION

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**Abstract**

Precision medicine is shifting healthcare from a population-based model to one that takes into account genetic, molecular, environmental, lifestyle, and clinical distinctions. It seeks to identify preventive, diagnostic, and therapeutic treatments appropriate for physiologically significant patient populations. Genomics, transcriptomics, proteomics, metabolomics, pharmacogenomics, artificial intelligence (AI), nanotechnology, biotechnology, and digital health are all hastening this transformation. Pharmaceutical sciences are important because precision medicine necessitates targeted medications, biomarker-guided therapies, biologics, gene and cell therapies, RNA therapeutics, and personalized drug delivery systems. Pharmacogenomics can assist forecast variation in efficacy and toxicity, whilst companion diagnostics aid in the selection of patients who are likely to benefit from specific treatments. AI and machine learning are being more widely employed in target selection, molecular design, virtual screening, pharmacokinetic/pharmacodynamic prediction, biomarker discovery, and clinical trial optimization. Nanomedicine has the potential to improve target selection, bioavailability, controlled release, and therapeutic index. Data quality, privacy, algorithmic bias, regulatory science, cost, clinical validation, infrastructure, and equal access are all major challenges.

**Keywords**: Precision Medicine, Pharmacogenomics, Pharmaceutical Innovation, Biomarkers, Targeted Therapy, Artificial Intelligence, Nanomedicine, Drug Delivery, Biologics, Gene Therapy, RNA Therapies, Precision Cancer.

## Introduction

Although modern medicine has achieved significant breakthroughs, persons with the same diagnosis can have vastly different disease susceptibility, progression, drug metabolism, efficacy, and side responses. Genetic diversity, epigenetics, age, gender, environment, lifestyle, microbiota, and comorbidities can all influence results. Precision medicine addresses this heterogeneity by transitioning from a "one-size-fits-all" strategy to biologically defined patient groupings. It does not necessitate a one-of-a-kind drug for each individual; rather, it seeks clinically significant subgroups for which a certain technique is more likely to be effective.

This strategy is altering pharmaceutical development. In addition to identifying active molecules, precision development considers which molecular target causes disease, which patients have the relevant change, which biomarker predicts response, what doses is appropriate, how the therapy should be delivered, and how response or toxicity should be monitored.

**Evolution of Precision Medicine:**

This change can be summarized as follows:

1. Conventional treatment includes disease diagnosisstandard treatment, clinical monitoring, and treatment change.
2. Precision is achieved through patient characterisation, molecular profiling, biomarker identification, patient stratification, targeted intervention, continuous monitoring, and adaptive treatment.

Molecular biology, recombinant DNA technologies, high-throughput screening, genomic sequencing, and bioinformatics laid the groundwork. The Human Genome Project and next-generation sequencing have increased the ability to study genetic factors of disease and medication response.

**Precision Medicine Versus Personalized Medicine**

The terms are frequently used interchangeably, although precision medicine typically stresses segmentation into physiologically, ecologically, or therapeutically significant subgroups. As a result, it does not require the creation of a completely unique product for each individual.

**Major Components of Precision Medicine**

| Component | Key Information | Pharmaceutical Relevance |
|---|---|---|
| Genomics | Genetic variants | Target identification; pharmacogenomics |
| Transcriptomics | Gene-expression patterns | Disease classification |
| Proteomics | Protein profiles and interactions | Biomarker/target discovery |
| Metabolomics | Metabolic signatures | Disease characterization |
| Epigenomics | DNA methylation, histone/chromatin changes | Novel targets |
| Microbiome | Microbial composition | Drug-response prediction |
| Clinical/lifestyle data | Phenotype, history, diet, exposure, records | Patient stratification |
| Imaging/wearables | Structural and continuous physiological data | Diagnosis and real-time monitoring |

Precision medicine combines multiple information streams to create a multimodal patient profile. Clinical data may consist of medical and family history, test results, imaging, food, physical activity, environmental exposure, prescription history, electronic health records, and continuous monitoring.

**Biomarkers and Patient Stratification**

Biomarkers are detectable biological traits that indicate normal or diseased processes, as well as therapeutic reactions. They are critical to precision medication development because they assist identify relevant individuals and increase clinical trials' ability to detect treatment effects.

- Diagnostic biomarkers aid in illness detection.
- Prognostic indicators predict disease progression regardless of treatment.

- Predictive biomarkers can identify patients who are more likely to respond to medication.
- Pharmacodynamic indicators reflect a drug's expected biological effects.
- Safety biomarkers indicate potential toxicity.

**Pharmaceutical Innovation in Precision Medicine**

Precision medicine is pushing innovation across the pharmaceutical development pipeline.

- **Targeted Small-Molecule Therapies:** Targeted small-molecule therapies are intended to selectively act on certain molecular targets, such as enzymes, receptors, kinases, or signaling cascades, which contribute to disease progression. They are particularly successful when the molecular changes causing a disease are fully understood. Targeted small compounds that interfere with certain disease-associated pathways can decrease inappropriate cellular activity while perhaps decreasing the consequences on healthy tissues.

  Examples include kinase inhibitors, enzyme inhibitors, receptor modulators, and other pathway-specific small compounds used to treat cancer, inflammatory illnesses, and metabolic diseases.
- **Monoclonal Antibodies:** Monoclonal antibodies (mAbs) are highly specialized biological treatments that identify and bind to specific antigens, receptors, or signaling molecules implicated in disease processes. Their high target specificity allows for selective control of pathogenic pathways, which has considerably advanced the therapy of cancer, autoimmune disorders, and inflammatory illness.

  Examples of monoclonal antibody medicines include trastuzumab targeting HER2 in cancer, rituximab targeting CD20 in B-cell malignancies, pembrolizumab and nivolumab targeting PD-1 in cancer immunotherapy, and adalimumab and infliximab targeting TNF-α in autoimmune disorders.
- **Antibody Drug Conjugates:** ADCs are targeted biopharmaceutical therapeutics that combine a monoclonal antibody with a highly effective cytotoxic or therapeutic payload via a chemical linker. The antibody specifically recognizes and binds to a specific antigen expressed on sick cells, allowing preferential delivery of the payload to the target cell.

  Examples of ADCs include trastuzumab emtansine and trastuzumab deruxtecan (HER2-targeted), brentuximab vedotin (CD30-targeted), sacituzumab govitecan (Trop-2-targeted), enfortumab vedotin (Nectin-4-targeted), and gemtuzumab ozogamicin (CD33-targeted.
- **Bispecific and Multispecific Therapeutics:** Bispecific and multispecific therapies are biological compounds that are created to interact with two or more separate molecular targets. Unlike traditional medicines that target a single cell, these drugs can connect several cells, disrupt multiple signaling pathways, and boost immune responses. As a result, they broaden the scope of molecularly focused therapy and open up new possibilities for precise treatment.

Examples include blinatumomab (CD19×CD3), teclistamab (BCMA×CD3), mosunetuzumab (CD20×CD3), glofitamab (CD20×CD3), and emicizumab (FIXa×FX), demonstrating the potential of multi-target therapeutics in oncology and other diseases.

- **Gene, RNA, and Cell Therapies:** Gene, RNA, and cell therapies are cutting-edge precision medicine methods that address disease at the genetic, molecular, or cellular level. These treatments can alter faulty genes, modulate gene expression, or employ living cells as therapeutic agents.
- **Genetic Therapy**: Gene therapy is the process of inserting, replacing, silencing, or altering genetic material to fix or compensate for disease-related genetic defects. Examples: Luxturna (voretigene neparvovec), Zolgensma (onasemnogene abeparvovec, Casgevy (exagamglogene autotemcel)
- **RNA Therapeutics:** RNA-based treatments, which include mRNA, siRNA, antisense oligonucleotides, and microRNA-based methods, affect protein production or gene expression. Examples: Patisiran is a siRNA treatment that targets transthyretin (TTR), Nusinersen is an antisense oligonucleotide used in spinal muscular atrophy.

**Nanotechnology and Precision Drug Delivery**

Nanotechnology has become an important component of precision medicine because it enables therapeutic agents to be engineered, protected, transported, and released at specific biological sites**.** Many conventional drugs are limited by poor aqueous solubility, low bioavailability, rapid degradation, short circulation time, and nonspecific distribution. Nanocarrier-based systems can overcome several of these limitations by improving drug stability, pharmacokinetics, biodistribution, and controlled release.

**Major Nanocarrier Platforms**

- **Liposomes and Lipid Nanoparticles** – Facilitate the delivery of poorly soluble drugs, nucleic acids, and biological therapeutics.
- **Polymeric Nanoparticles** – Provide controlled and sustained drug release and can be functionalized for targeted delivery.
- **Solid Lipid Nanoparticles and Nanostructured Lipid Carriers** – Improve the solubility, stability, and bioavailability of poorly water-soluble drugs.
- **Dendrimers and Nanogels** – Highly tunable systems capable of incorporating therapeutic molecules and targeting ligands.
- **Metallic Nanoparticles** – Include gold and other metal-based systems with applications in drug delivery, imaging, diagnosis, and therapeutic intervention.

**Targeting Strategies**

- **Passive targeting** exploits physiological or pathological characteristics of diseased tissues to promote nanocarrier accumulation.
- **Active targeting** involves the attachment of ligands such as antibodies, peptides, aptamers, or other recognition molecules that selectively interact with receptors expressed on target cells.

- **Stimulus-responsive targeting** enables drug release in response to specific biological or external stimuli, including pH, enzymes, temperature, light, redox conditions, or ultrasound.

**Precision Drug-Delivery Pathway**

Therapeutic Molecule
↓
Nanocarrier Formulation
↓
Surface Functionalization / Targeting
↓
Systemic Circulation
↓
Target-Site Accumulation
↓
Cellular Uptake
↓
Controlled Drug Release
↓
Target-Specific Pharmacological Action
↓
Therapeutic Response

**Applications of Precision Medicine**

1. **Precision Oncology:** Cancer is one of the most sophisticated examples of precision medicine because malignancies at the same anatomical site might have distinct genetic drivers. Genomic and molecular characterisation can reveal changes in tumor behavior and therapy response.
2. **Liquid Biopsy:** Liquid biopsy can give molecular information from body fluids by analyzing circulating tumor-derived material. Potential applications include early detection, molecular characterisation, response monitoring, resistance detection, and recurrence monitoring.
3. **Precision Medicine for Cardiovascular Disease:** Cardiovascular disease is the outcome of interplay between genetic, metabolic, environmental, and behavioral variables. Precision methods may aid in cardiovascular risk prediction, pharmacogenomic drug selection, personalized lipid management, individualized antihypertensive therapy, the detection of inherited cardiovascular illnesses, and biomarker-based risk stratification.
4. **Precision Medicine for Diabetes and Metabolic Disorders:** Diabetes is a varied disease, with variations in insulin secretion, insulin sensitivity, hereditary predisposition, progression, and therapeutic response. Precision methods can help with molecular classification, risk prediction, treatment response prediction, antidiabetic drug selection,

individualized diet, and continuous metabolic monitoring. Future techniques could include genetic, metabolomic, microbiome, lifestyle, and glucose monitoring data.

5. **Precision Medicine for Infectious Disease:** Pathogen genomic sequencing can uncover genetic changes related with antibiotic resistance and aid in epidemic investigation. Host factors have an impact on both susceptibility and treatment response. Pathogen sequencing, quick molecular diagnostics, antimicrobial resistance prediction, precision antibiotic selection, host-directed therapy, and individualized vaccination techniques are all examples of applications.
6. **Digital Health and Wearable Technologies:** Wearable technologies can continuously collect physiological data such as heart rate, physical activity, sleep, oxygen saturation, electrocardiographic signals, and glucose levels. When combined with electronic health records, genomes, laboratory data, and imaging, these data can aid in AI-based risk prediction, patient stratification, individualized intervention, and continuous monitoring. The integrated model includes wearable sensors, EHR, genomics, laboratory data, and imaging. It then progresses to cloud/data infrastructure, AI and machine learning, risk prediction and patient stratification, personalized intervention, and continuous monitoring.

**Challenges of Precision Medicine**

- The cost of genomic testing, advanced biologics, gene treatments, diagnostics, and sophisticated delivery systems can be high.
- Large molecular datasets necessitate computing infrastructure and specialist knowledge.
- Strong security and control are necessary for genomic and clinical data to ensure privacy.
- Algorithmic bias: Underrepresented communities can impact model reliability.
- Statistical associations may not guaranty clinical usefulness.
- Regulation: Products can include medications, diagnostics, equipment, software, and biological components.
- Infrastructure requirements include molecular laboratories, bioinformatics, data systems, trained workers, and decision-support technologies.
- Health equity: Benefits should not be limited to those with more financial or technological means.

**Conclusion**

Precision medicine represents a significant shift in healthcare. Rather than presuming identical responses among patients with the same illness, it acknowledges biological and environmental variables that influence disease and treatment outcome. Pharmaceutical sciences are central to this transformation: pharmacogenomics supports rational drug and dose selection; biomarkers identify responsive populations; targeted therapies address disease-associated pathways; biologics, gene, RNA, and cell therapies address increasingly specific mechanisms; and nanotechnology improves delivery and targeting.

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# AI-DRIVEN NANOPHARMACEUTICAL SYSTEMS FOR PRECISION DRUG DELIVERY

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## 1. Introduction

The fast progress of nanotechnology, artificial intelligence (AI), and precision medicine is revolutionizing the development of new pharmaceutical treatments. Traditional delivery systems usually suffer from the drawbacks of poor solubility, bioavailability, non-selective biodistribution, fast clearance, systemic toxicity and inability to achieve the desired therapeutic concentration at the target area. Nanopharmaceutical systems are one of the solutions that can help to overcome these obstacles through enhancing drug solubilization, protection, controlled release, increased cell penetration and targeted delivery. Nevertheless, there are many factors that make the formulation of nanopharmaceuticals difficult as they significantly affect the performance of these particles, including but not limited to size, surface charge, morphology, composition, drug loading, loading efficiency, release profile and biological interactions.

One of the computational technologies that could assist in solving these difficulties is artificial intelligence (AI). AI includes machine learning (ML), deep learning (DL), generative models, natural language processing and other methods that can be used for pattern recognition and analysis in high dimensional and voluminous datasets. In the field of pharmaceutical research, there is an increasing tendency towards using such computational technologies in the fields of drug discovery, drug formulation, pharmacokinetic modeling, toxicity and therapeutic response prediction. In conjunction with nanotechnology, AI can greatly improve the design and optimization process of nanocarriers.

Nanopharmaceutical formulation design with the assistance of AI can exploit formulation ingredients, manufacturing process parameters, physicochemical properties, biological information, and pharmacokinetics to predict critical quality attributes and improve formulation performance. Machine learning-based models can potentially predict properties including nanoparticle size, polydispersity index, encapsulation efficiency, drug loading, stability, drug release profile, cellular uptake, biodistribution and toxicity. The ability to do so can speed up formulation design and allow researchers to find good formulations before performing many experiments.

An interesting future direction involves moving from predictive modeling to AI-supported inverse design. In traditional formulation design, researchers choose materials and process parameters and test the resulting formulation. In inverse design, researchers define the desired

therapeutic and physicochemical properties and use computational tools to find suitable nanocarrier materials and process parameters that will lead to these properties.

The connection between AI and nanomedicine becomes especially important in relation to precision drug delivery, which is defined as the provision of the drug dosage based on the biological properties of patients. In other words, patient information such as genomic data, proteomic information, clinical data, imaging data and pharmacokinetics can be integrated into the AI system for choosing the right drug-delivery system. Moreover, the assistance of AI in disease-specific target identification, predictions of the interaction between drugs and nanocarriers, optimization of drug dosage and reaction prediction may help in developing more precise therapy.

One should note that the development of smart and adaptive drug-delivery systems is another tendency in nanomedicine. Stimuli-responsive nanocarriers may be sensitive to changes in pH, temperature, enzymes, redox state, and even to external stimuli. Thus, when combining such nanocarriers with AI, biosensors, monitoring, and feedback loops, one can use closed-loop drug delivery with the dynamic adjustment of the drug release.

However, there are still many challenges that should be taken into account in order to ensure the clinical translation of AI-driven nanopharmaceutical systems. They include the lack of homogenous datasets, their quality, data interpretability, reproducibility, validation, nanoparticle toxicity, scalability, manufacturing quality assurance, regulatory uncertainty, and the necessity to have computational and experimental standardization. Furthermore, the predictions produced by using AI algorithms should be validated via the appropriate experimental studies in the laboratory, pre-clinical and clinical setting.

Thus, the integration of AI with nanotechnology is a promising approach to the development of data-driven, targeted, adaptive, and personalized drug-delivery systems. In this chapter, the basic concepts of nanopharmaceutical systems will be described as well as the application of AI in formulations and nanocarriers design, nanopharmaceutical performance prediction, targeted and stimuli-responsive delivery enabled by AI and some new applications of AI in the field of personalized medicine.

## 2. Fundamentals of Nanopharmaceutical Systems

Nanopharmaceutical systems constitute an important category within pharmaceutical nanotechnology that involves the use of nanoparticles in order to enhance the delivery, efficiency and safety of pharmaceutical agents. The small size and tunable surface properties of nanocarriers could be harnessed in modifying the physicochemical and biological properties of drugs through enhanced solubility, stabilization, circulation time, cellular internalization and controlled drug release. The performance of a nanopharmaceutical system is determined by the nature of the components used, the particle size and shape, surface properties, drug loading capacity, drug release pattern, and interactions with biological systems. Therefore, rational design and characterization are key in order to produce reliable nanomedicines. Artificial

Intelligence could also be integrated into the field of nanopharmaceuticals in understanding the complex relationship between formulation variables and therapeutic efficacy.

**2.1 Definition and Principles of Nanopharmaceutics**

Nanopharmaceutics can be defined as the application of the principles of nanoscale science and engineering to the design, development, characterization, and application of pharmaceutical products and drug delivery systems. Nanopharmaceuticals typically employ nanoscale structures or materials to manipulate the way a pharmaceutical agent will be delivered and behave biologically. A key tenet of nanopharmaceutics is to make use of the special physicochemical properties of nanoscale materials to optimize the delivery of drugs. The reduction in particle size could result in the increased surface area, which could help with increased dissolution and interaction of the drug with the biological interface. Nanoscale carriers could provide protection to drugs, especially to those that are inherently unstable, like peptides, proteins, nucleic acids, and some small molecules.

The second principle is controlled and targeted delivery. Drugs can be released slowly by nanocarriers and/or triggered to be released in response to physiological changes. Nanocarriers' surfaces could be decorated with targeting ligands, antibodies, peptides, aptamers, etc., resulting in more selective binding to specific cell types or tissues. The approach of passive targeting relies on the altered physiological conditions, such as increased vascular permeability, while active targeting uses specific ligand-receptor interactions.

Nanopharmaceutical systems can incorporate different types of drugs possessing various physicochemical properties, e.g., hydrophobic, hydrophilic, water-insoluble, biological macromolecules, genetic materials. Depending on the material used to create the carrier and its design, nanopharmaceuticals could affect drug bioavailability, pharmacokinetics, and even therapeutic index. For precision drug delivery, optimization of the system is required based on a number of variables at once. Therefore, the development of nanopharmaceutical systems is a multidimensional problem, offering great opportunities for machine learning applications.

**2.2 Major Types of Nanocarriers**

- Liposomes – Vesicles formed out of phospholipids which can enclose hydrophilic and lipophilic compounds.
- Polymeric Nanoparticles – Nanoparticles formed from biodegradable polymers such as PLGA, PLA and PCL.
- Solid Lipid Nanoparticles (SLNs) – Nanocarriers comprised of solid lipids providing controlled drug release and increased stability.
- Nanostructured Lipid Carriers (NLCs) – The second generation lipid carriers containing a mixture of solid and liquid lipids.
- Nanoemulsions – Oil-in-water or water-in-oil nano dispersions suitable for poorly soluble drugs.

- Nanogels – Three-dimensional, cross-linked polymeric networks having high ability to absorb water.
- Dendrimers – Highly-branched, tree-like polymers having precise nanoscale structure and various functional groups on the surface.
- Metallic and Inorganic Nanoparticles – Comprises gold, silver, iron oxides, silica and others.
- Polymeric Micelles – Amphiphilic assemblies of polymers having hydrophobic core and hydrophilic shell which are useful in solubilization of poorly soluble drugs.
- Exosomes and Extracellular Vesicles – Natural nanocarriers which can transport various drugs, proteins, nucleic acids, and other biomolecules.
- Protein-based Nanoparticles – Nanocarriers prepared using proteins such as albumin, gelatin and casein.
- Nanocrystals – Pure drugs in nanoparticles range stabilized using surfactants and polymers.

**2.3 Critical Quality Attributes of Nanopharmaceuticals**

Critical Quality Attributes (CQAs) refer to the measurable physical, chemical, biological and functional characteristics that impact the quality, safety, efficacy and effectiveness of nanopharmaceutical formulations.

| **Critical Quality Attribute** | **Description & Significance** |
|---|---|
| 1. Particle Size | Determines cellular uptake, distribution, drug release, circulation time, and penetration into tissues. Nanoparticles have a size scale of nanometers. |
| 2. Polydispersity Index (PDI) | Represents the uniformity of the size distribution of particles. Lower PDI usually denotes a more physically stable system. |
| 3. Surface Charge (Zeta Potential) | Affects stability, aggregation, protein adsorption, cell interactions and biological distribution. |
| 4. Encapsulation Efficiency (EE%) | Denotes the percent of the starting drug that gets encapsulated by the nanocarrier. Higher encapsulation efficiency can improve the efficacy of the drug formulation. |
| 5. Drug Loading (DL%) | Represents the proportion of the drug contained compared to the entire mass of the nanocapsule formulation. The parameter is important in ensuring that the required amount of medication is delivered. |
| 6. Stability | Includes stability in physical, chemical, and biological terms when stored. Alterations in particle size, PDI, zeta potential, drug concentration, or aggregation may impact product quality. |
| 7.Drug-Release Characteristics | Explain the rate and mechanism of drug delivery from the nanocarrier. It will help in improving efficacy and minimize the need for frequent dosing. |

## 3. Artificial Intelligence in Nanopharmaceutical Development

Artificial Intelligence (AI) technology has become an effective means of boosting the speed of design, development, optimization, and evaluation of nanopharmaceutical systems. The conventional development of nanopharmaceuticals has been known to involve multiple trials due to the many variables involved, which include lipid or polymer type, amount of excipients, properties of the drugs being used, mode of formulation and other parameters such as environmental factors. ML and DL algorithms are capable of modeling complex relationships in data obtained from formulations.

### 3.1 Machine Learning and Deep Learning

Through machine learning, computer systems have the ability to learn from experimental and historical data sets without programming every single prediction. In relation to nanopharmaceuticals, machine learning algorithms will allow for linking formulation and processing parameters to critical quality attributes, which include particle size, PDI, zeta potential, encapsulation efficiency, drug loading, and drug release. Examples of machine learning algorithms are random forest, support vector machine, artificial neural networks, gradient boosting and regression models.

Deep learning is a more sophisticated version of machine learning whereby multi-layer neural network models are used to extract complex nonlinear relationships from large and high dimensional data sets. Deep learning is important when there are many parameters relating to the formulation, physicochemical, biological, and processing aspects in the nanopharmaceutical data set. Such models may help in predicting formulation performance and material/process parameter combinations that would not be easy to determine through statistics.

### 3.2 AI-Assisted Formulation Design

Artificial intelligence will help in rationally selecting the formulation components like drugs, polymers, lipids, surfactants, stabilizers, and targeting ligands. On the basis of past formulation information, artificial intelligence can predict the combinations that will result in desired characteristics of the nanocarrier. Thus the process of trial and error in the formulation will be reduced.

In addition, AI-assisted formulation design could use predictive modeling with Design of Experiment (DoE). Instead of trying many formulations experimentally, AI could select the best formulations for experimental work. And the experimental results would be used to refine the model and make another round of predictions, formulations and experiments.

### 3.3 Prediction of Nanoparticle Physicochemical Properties

The physicochemical properties of the nanoparticles greatly affect the stability, biodistribution, cellular uptake, drug delivery, and efficacy of these particles. The AI algorithms can predict the physicochemical properties like particle size, PDI, surface charge, morphology, crystallinity, drug load and encapsulation efficiency depending on the input variables.

For instance, these algorithms can determine the connection between the concentrations of polymer and surfactant, ratio of solvents, mixing conditions, and preparation methods and the

corresponding physicochemical properties of the nanoparticles. Therefore, this prediction approach helps to determine the key parameters of the formulation at the early development stage.

### 3.4 AI-Based Prediction of Drug Loading and Encapsulation Efficiency

DL and EE are critical CQAs since they are related to the amount of therapeutic substance present in the nanocarrier. DL and EE levels are influenced by various parameters such as the solubility of drugs, properties of polymers or lipids used, ratio of drugs to carriers, surfactants levels, mode of preparations, and processing conditions.

Artificial intelligence can analyze such parameters and predict the expected DL and EE levels before the extensive experimental optimization. Artificial intelligence can assist researchers in identifying formulation parameters that would result in high drug loading and reduce the loss of drugs during processing.

### 3.5 Prediction of Particle Size and Polydispersity

Particle size and polydispersity index are some of the key CQAs in the formulation of nanopharmaceuticals. These affect drug delivery, cell uptake, colloidal stability, distribution in the body and the efficacy of the drug formulation. The AI models are able to predict particle size and PDI based on parameters including lipids/polymer concentration, surfactant content, mixing or homogenization process, sonication time, solvent, temperature, and fabrication methods.

Once the predictive models have been developed, one can use them to establish experimental parameters that can yield nanoparticles of desired size and size distribution.

### 3.6 AI-Based Optimization of Formulation Variables

Nanopharmaceuticals have multiple interrelated parameters which make the optimization using traditional techniques difficult. Optimization by AI will allow evaluation of multiple formulations and processes parameters in parallel to find out the most effective combination of parameters for CQAs maximization.

Parameters for optimization can include such factors as drug/carrier ratio, concentration of polymer or lipid, concentration of surfactant, pH, solvent ratio, mixing rate, homogenization pressure, sonication time and temperature. Multi-objective AI optimization is possible in cases when several CQAs have to be optimized at once, such as small particle size, low PDI, high encapsulation efficiency, high drug content and controlled drug delivery.

Thus, AI enables shifting the nanopharmaceutical development from being mostly trial-and-error based into a data-driven predictive and iterative process.

## 4. AI-Driven Design and Optimization of Nanocarriers

The application of AI for nanocarrier design can be considered a radical transition from traditional hit-or-miss formulation development to a data-driven and rationale-based approach. Nanocarrier behavior is affected by many factors, including material composition, drug properties, ratios in the formulation, manufacturing method and process parameters. AI and machine learning can combine all these factors and provide information about the optimal combination of factors that will give the desired critical quality attributes (CQA).

### 4.1 Conventional Trial-and-Error Formulation versus AI-Driven Design

Conventional nanocarrier design is usually based on the step-by-step laboratory experimentation process where the variables in the formulation are adjusted until desirable performance is achieved. While DoE optimizes the assessment process, traditional methods still entail significant expenditure of time, material and experimentation effort whenever there is an interaction between many variables.

By comparison, AI-based design makes use of historic and experimental data sets to create a link between the input formulation variables and the desired output. AI models are capable of predicting the particle size, PDI, encapsulation efficiency, drug loading, zeta potential and drug release properties even prior to experimental design.

### 4.2 Machine-Learning-Based Formulation Optimization

Machine learning can detect nonlinear dependencies between formulation factors and the performance of nanocarriers. Various algorithms, such as random forests, support vector machines, artificial neural networks, gradient boosting and Gaussian processes, can be used to train a machine learning algorithm based on experimental data obtained from the formulation process.

For instance, an ML algorithm can estimate the impact of the concentration of the polymer, concentration of the lipid, surfactant concentration, drug/polymer ratio, solvent mixture, mixing speed and time required for the formulation on the size of particles and efficiency of the encapsulation process.

### 4.3 Quantitative Structure–Property Relationships

Quantitative Structure-Property Relationships (QSPR) are used to find correlations between chemical structure and physical properties. QSPR modeling in the nanopharmaceutical field can be applied in order to find connections between molecular structure and properties like solubility, permeability, hydrophobicity, interaction between the active agent and carrier, and encapsulation properties of the agent.

Using QSPR with machine learning can help predict the influence of the molecular structure on the properties of nanocarriers.

### 4.4 AI-Assisted Selection of Nanocarrier Materials

Choice of the right materials for the nanocarriers is an important part of formulation design. AI can examine large databases that contain data on polymers, lipids, surfactants, phospholipids, stabilizers, targeting moieties and drug substances and find the right combinations of materials.

For instance, AI can figure out whether the specific drug will be more suitable to be combined with a lipid, polymer, dendrimer, or vesicle nanocarrier. The choice of materials may be done considering such characteristics as drug solubility, compatibility, biodegradability, loading, stability, delivery and so forth.

### 4.5 Multi-Objective Optimization

Nanocarrier design is not usually the process of optimizing any single criterion. There could be a situation in which several criteria have to be optimized, including small particle size, low PDI,

good encapsulation efficiency, high drug load, proper surface charge, stability and controllable drug delivery.

Multi-criteria optimization using AI technology allows analyzing these conflicting criteria all together and finding the optimal combination of formulations. The result obtained is not one optimal formulation but rather a set of Pareto optimal formulations from which the researcher may choose based on his/her particular therapeutic target.

**4.6 Bayesian Optimization**

Bayesian optimization is especially valuable for experiments that are costly, time-consuming, or restricted by the number of iterations. Bayesian optimization utilizes the results of prior experiments to construct a probability model of the design space and determine the next experiment that will bring the biggest gain or information.

For nanocarrier development, the sequential recommendation of formulation and process parameters using Bayesian optimization is possible. Following an experiment, the new data is used by the algorithm to update the predictions and select the next formulation to be tested. Consequently, a fairly small number of experiments may efficiently explore a complicated formulation space.

**4.7 Generative AI and Inverse Design of Nanocarriers**

However, Generative AI opens a new avenue wherein models can create new formulation or material candidates rather than predict the characteristics of the available formulations only. In case of the inverse design, the researchers specify their objectives, like particle size, drug-loading capacity and so on, and the models find out the material formulations or formulation process which will deliver the desired results.

Thus, Generative Models could be used to discover new polymers, lipids, excipients, nanocarrier structures and even formulation processes. By integrating Generative AI with the molecular simulation, QSPR, machine learning and experimental validation, one could facilitate the process of discovering new generation nanocarriers.

**5. AI for Targeted and Precision Drug Delivery**

Targeted drug delivery refers to the delivery of therapeutic drugs to a selective cell, tissue, organ, or disease site with reduced delivery to normal tissues. Nanocarriers like liposomes, polymeric nanoparticles, lipid nanoparticles, nanogels and dendrimers offer targeted delivery systems due to the fact that their size, surface properties, composition, and targeting ligands can be tailored. AI can facilitate this process through the incorporation of various types of information on molecules, biology, physicochemistry and medicine.

**5.1 Target Identification Using AI**

These models allow large-scale genomic, proteomic, transcriptomic and clinical data analysis to discover molecular targets associated with diseases. In nanomedicine, such models can be used to find out receptors, proteins, biomarkers, or signaling pathways that are abundant in disease-afflicted cells but are less abundant in normal tissues.

Target discovery through artificial intelligence can thus help select molecular targets that can be targeted by active nanocarriers.

**5.2 Prediction of Drug–Nanocarrier Interactions**

The interaction between a drug and the nanocarrier plays a critical role in drug loading, encapsulation efficiency, stability, drug release and bioactivity. Artificial intelligence can model molecular properties and experimental data to predict interactions between the therapeutic molecule and the polymer, lipid, protein, or other components of the nanocarrier.

The predictions can help identify the suitable drug-carrier systems and avoid unnecessary experimentation. AI can also be used for predicting the effect of surface chemical modification or alteration of nanocarrier components on drug retention and release.

**5.3 AI-Guided Ligand/Receptor Selection**

The process of active targeting often relies on the addition of ligands, such as antibodies, peptides, aptamers, carbohydrates, or any other targeting molecules, to the surface of the nanoparticles. The AI may determine the relationship between a particular ligand and receptor and provide predictions regarding the potential affinity and specificity of the interaction.

In addition, the application of AI to the ligand selection can be helpful when trying to identify targeting molecules that demonstrate high affinity to receptors associated with disease development.

**5.4 Tumor-Targeted Nanomedicine**

Targeted nanomedicine with AI assistance has several key applications, including cancer treatment. Cancerous tumors have significant molecular and biological diversity, which makes universal targeting techniques difficult. AI could analyze genomic, proteomic, imaging, and clinical information about the tumor and determine specific markers of that particular tumor and the appropriate nanocarriers strategy.

AI can help choose the nanocarrier size, surface features, targeting agents, drug cocktails, and release mechanism depending on tumor parameters. It could potentially increase selectivity of drug delivery and reduce toxicity of the process. AI can also help create personalized nanomedicine techniques for individual patients with different tumor profiles.

**5.5 Cell-Specific and Tissue-Specific Delivery**

AI can be useful in designing nanocarriers that can interact specifically with certain cell types and/or tissues. Through the analysis of receptor expression, cellular uptake, tissue distribution, and physicochemical features of the nanocarriers, AI models can be developed to determine which formulation features would be ideal for certain biological targets.

This method can find use in various areas such as brain delivery, liver targeting, immune cell targeting, pulmonary delivery, cardiovascular disease treatment and cancer therapy. The use of AI together with biological data sets can aid in designing better nanocarriers.

**5.6 Personalized Nanocarrier Selection**

Personalized nanomedicines are those that use information about the patient’s disease, molecular characteristics, pharmacokinetics, and therapeutic needs to choose or design a particular type of

nanocarrier. The use of artificial intelligence will allow integration of the patient's data along with other information to determine the best composition, dosage form, and release rate of the nanocarrier.

Thus, the use of AI, nanotechnology, molecular diagnosis, and personal data could provide an opportunity for the implementation of precision drug delivery when nanocarriers are customized for each person depending on their biological characteristics.

## 6. Applications in Disease-Specific Precision Therapy

Systems based on nanopharmaceuticals that leverage the power of artificial intelligence (AI) hold great promise for disease-specific precision medicine, as various parameters of nanocarriers can be customized based on disease biology and needs. From among the promising applications, some of the notable ones are those in cancer, neurology, and genetics.

### 6.1 Cancer Therapy

The field of nanomedicine used in cancer treatment through artificial intelligence is one of the most thoroughly studied areas. Cancer-targeting nanoparticles can be programmed to target receptors or biomarkers overexpressed on cancer cells. Artificial intelligence can process molecular and clinical data sets to find proper targets and design the best possible combination of nanoparticle size, surface properties, targeting molecules and cargo.

Artificial intelligence can assist in the development of personalized nanomedicine by analyzing genomic, proteomic, imaging, and clinical information specific for the patient's tumor. This can lead to the development of nanocarriers tailored to the molecular profile of the tumor. Moreover, artificial intelligence can help in designing nanoparticles with multiple therapies onboard.

### 6.2 Neurological Disorders

The delivery of drugs to the brain is a difficult process owing to the existence of the blood-brain barrier (BBB). The nanocarriers can be designed in such a way that would make their transportation through or interaction with the BBB easier and enable their delivery to certain regions within the brain.

The AI can help find the appropriate targeting ligands and also establish the correlations between the physicochemical features of the nanocarriers and their transportation through the BBB.

### 6.3 Infectious Diseases

Nanotechnology is an area where there exist chances of delivering the antimicrobial drugs to the site of infection. Antimicrobial-loaded nanocarriers would help in achieving targeted delivery and thus increased stability and localized delivery of drugs. AI technology can be used to analyze various data from formulation and biology aspects for optimizing the composition of the nanocarriers.

The optimized delivery of such nanocarriers would prove beneficial for developing new nanocarriers for the infections that are difficult to treat.

### 6.4 Genetic and RNA Therapeutics

Nanocarriers have been developed as effective delivery tools for fragile genetic medicines that need to be protected against degradation and efficient delivery into the cell cytoplasm. Both

mRNA and siRNA can be delivered within lipid or polymer nanoparticles in order to facilitate cellular uptake and cytoplasm delivery.

AI technology can help predict interaction between nucleic acids and carriers, optimize lipid or polymer composition, and find formulations that would be more stable, provide efficient encapsulation, cellular uptake, and release. In a similar manner, nanocarriers can deliver guide RNA, mRNA, or other molecular components for CRISPR or any other gene editing systems to the target cells.

In summary, AI combined with nanotechnology can help develop targeted disease-specific and personalized treatments, especially in cases when conventional drug delivery methods fail to address biological obstacles.

## 7. Challenges, Regulatory Issues and Future Perspectives

Despite the vast possibilities that lie in the field of nanopharmaceuticals developed through artificial intelligence, there is still an issue with the transfer of this science from the level of computer prediction into something that will be used clinically.

### 7.1 Data Quality and Availability

Training of AI models largely depends on the quality, amount, consistency, and diversity of training data. Data used for training the models in nanopharmaceutics is likely to be less than other types of data sets since they are usually smaller and heterogeneous in nature. Differences in data collection and reporting may decrease model accuracy.

### 7.2 Model Interpretability

Very complex artificial intelligence and deep learning algorithms can act as “black boxes,” which makes it hard to know why a certain formulation or prediction was made by the algorithm. The methods of explainable artificial intelligence should be used to find out what factors led to such a prediction by the model.

### 7.3 Reproducibility and Validation

The accuracy of AI predictions needs to be tested through independent experimental validation. Variations in apparatus, starting materials, lab environment, and process could impact the properties of nanocarriers. It is necessary for models to go through thorough validation both internally and externally through independent experiments prior to application in pharmaceuticals.

### 7.4 AI Bias and Dataset Limitations

Models built using small and/or inadequate datasets may generate algorithmic biases and may not work well when used with new chemical structures, materials, or patients with diseases different from those included in the training set. Diversity in the dataset and proper data processing can reduce this risk.

### 7.5 Nanotoxicity and Long-Term Safety

Nanocarriers may have biological behavior which is different from normal drug delivery systems. Their properties such as size, surface characteristics, composition, breakdown products and tissue accumulation may affect their toxicity. AI can be used to make predictions for toxicity

and development of better drug formulations but computational predictions do not substitute for the full testing of toxicology and pharmacology and clinical trials.

**7.6 Regulatory Challenges**

AI implementation raises a series of regulatory questions related to the validation of models used, the origin of the data used, updates of the software, changes in algorithms, and responsibility for AI-enabled decisions. Nanopharmaceuticals have their own regulatory challenges as well. Common regulatory strategies will be needed in assessing the AI-enabled products.

**7.7 Standardization of AI–Nanomedicine Workflows**

Standardized processes are required for collecting data, characterizing nanoparticles, developing, validating, testing, and performance assessment of the models. Having standardized data formats and standardized protocols for experiments would increase the compatibility between different laboratories and make model comparisons easier.

**7.8 Intellectual Property and Data Privacy**

In addition to large proprietary databases, new algorithms, formulations and synthetic materials, there can be an instance where the use of artificial intelligence in the drug discovery process may give rise to a number of legal questions such as ownership of discoveries made, patentability, data sharing, confidentiality and privacy issues, especially patient data.

**7.9 Future of Autonomous Nanopharmaceutical Development**

Future possibilities may lie in autonomous nanopharmaceutical development systems that incorporate elements such as artificial intelligence, robotics, high throughput experimentation, analysis and decision-making. These systems may operate by predicting formulations, conducting experiments, analyzing outcomes and updating the model with little or no human involvement.

**Conclusion**

Combining AI and nanotechnology has become a promising methodology for creating the future generation of nanopharmaceutical systems. AI-based technologies can expedite the process of formulation development, facilitate predictions of important quality factors, optimize the composition and manufacturing process parameters of nanocarriers and minimize reliance on traditional experimental approaches. The use of machine learning, deep learning, QSPR, Bayesian optimization and generative AI provide different methodologies for analyzing complicated dependencies between formulation parameters and the efficacy of therapy.

The combination of AI and nanotechnology is associated with numerous possibilities in relation to the development of precision medicine. AI can help in identifying target diseases, ligands and receptors, predicting interactions between drugs and nanocarriers, selecting appropriate nanocarriers and optimizing targeted drug delivery. All these capabilities can help create nanopharmaceutical systems that are tailored to specific diseases, particular tissues, cells and even individual patients.

Even with such advances, however, effective translation will involve the overcoming of a number of challenges associated with issues such as data quality, interpretation of models, reproducibility, toxicity, regulatory compliance, standardization, intellectual property rights and data privacy. Predictions made using artificial intelligence should be validated through clinical trials and experiments to ensure their safety and effectiveness.

In the future, the combination of AI, automation, high throughput experimentation, advanced nanomaterials and digital technologies will allow for increasingly autonomous and adaptive design of nanopharmaceuticals. These systems will learn from the results of experiments and clinical trials and tailor the formulation process based on pre-determined therapeutic goals. Thus, AI-based nanotechnology holds great promise for transforming drug delivery from traditional formulation development into predictive, personalized and precision patient-centric nanomedicine.

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# GASTRORETENTIVE DRUG DELIVERY SYSTEMS

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**Abstract**

Gastro-retentive drug delivery systems (GRDDS) are advanced oral drug delivery systems designed to prolong the residence time of dosage forms in the stomach. These systems improve the bioavailability of drugs that are absorbed primarily in the stomach or upper small intestine, have a narrow absorption window, or exhibit pH-dependent solubility. GRDDS employ various mechanisms such as floating, high-density, bioadhesion, expansion, raft formation, magnetic retention, and superporous hydrogels to delay gastric emptying and provide controlled or sustained drug release. By increasing gastric retention time, GRDDS enhances therapeutic efficacy, reduces dosing frequency, improves patient compliance, and enables site-specific drug delivery for the treatment of gastric disorders. Due to these advantages, GRDDS has become an important strategy in the development of novel oral controlled-release dosage forms.

**Keywords:** GRDDS, Floating, Controlled Relese, Non-Floating, Novel Drug.

## Introduction

Gastro-retentive drug delivery systems (GRDDS) are an advanced oral drug delivery approach developed to increase the retention time of dosage forms in the stomach. The main aim of these systems is to provide controlled and site-specific drug release in the stomach and upper gastrointestinal tract, thereby improving both local and systemic therapeutic effects.

In recent years, controlled release oral formulations have gained considerable attention due to their ability to maintain therapeutic drug concentrations for prolonged periods while reducing dosing frequency (2).

Drugs with short biological half-lives are rapidly eliminated from the body, making frequent administration necessary to achieve the desired therapeutic effect. Sustained-release formulations were introduced to overcome this drawback by releasing the drug gradually over an extended duration, ensuring consistent plasma drug levels (3).

GRDDS are specially designed to remain in the stomach for an extended period after administration, allowing the drug to be released in a controlled manner at the desired absorption site. However, variations in gastric emptying time (GET) and limited gastric residence time (GRT) may reduce the effectiveness of these systems by causing premature passage of the dosage form into the intestine (4).

Increasing the gastric residence time enhances drug absorption, improves the bioavailability of drugs that are poorly soluble at higher intestinal pH, minimises drug loss, and extends the duration of drug release. In addition, GRDDS are useful for the treatment of gastric and upper intestinal disorders, such as peptic ulcers, by providing prolonged local drug action (5).

**Advantages of GRDDS**

- Prolonged gastric residence time: GRDDS remain in the stomach for an extended period, allowing sustained and controlled drug release.
- Reduced dosing frequency: Sustained drug release decreases the need for frequent administration, thereby improving patient compliance (6).
- Improved bioavailability: They enhance the absorption of drugs that are primarily absorbed in the stomach or upper small intestine.
- Improved solubility of pH-dependent drugs: Drugs that are more soluble in acidic conditions exhibit better dissolution and absorption (7).
- Enhanced therapeutic efficacy: By maintaining therapeutic drug concentrations for a longer duration, GRDDS improve treatment outcomes.
- Localised drug delivery: GRDDS are beneficial for treating gastric diseases such as peptic ulcers and Helicobacter pylori infections by providing prolonged local drug action (8).

**Disadvantages of GRDDS**

- Not suitable for all drugs: Drugs unstable in acidic gastric fluid or intended for colonic absorption are not ideal candidates for GRDDS.
- Requirement of sufficient gastric fluid: Some GRDDS require an adequate amount of gastric fluid to float or swell effectively (6).
- Dependence on gastric conditions: Gastric emptying time varies among individuals and is influenced by food intake, age, posture, and disease conditions, affecting system performance.
- Unsuitable in certain gastrointestinal disorders: Patients with gastric obstruction, severe motility disorders, or delayed gastric emptying may not benefit from these systems (7).
- Risk of dose dumping: Failure of the controlled-release mechanism may result in rapid drug release, increasing the risk of adverse effects.
- Complex formulation and higher manufacturing cost: Designing GRDDS often requires specialised polymers and advanced formulation techniques, making production more expensive (8).

**Modulation of GI Transit Time**

Modulation of gastrointestinal (GI) transit time is an important strategy in gastro-retentive drug delivery systems (GRDDS). It aims to prolong the residence of the dosage form in the stomach, thereby improving drug absorption, bioavailability, and therapeutic efficacy. Several

physiological and anatomical factors influence GI transit time and should be considered during the design of GRDDS.

It includes:

- Anatomy of Gastrointestinal Tract
- Dynamics of the GI Tract
- GI Transit
- Ileocecal Junction
- Colon and Gut Flora
- GI Mucus
- Anatomy of Gastrointestinal Tract

The gastrointestinal tract extends from the mouth to the anus and consists of the oral cavity, pharynx, oesophagus, stomach, small intestine, large intestine, rectum, and anus. The stomach serves as a temporary reservoir where food is mixed with gastric secretions before entering the small intestine. The GI wall is composed of four layers: mucosa, submucosa, muscularis externa, and serosa. Understanding GI anatomy is essential for designing effective gastro-retentive dosage forms because drug retention and absorption depend on the structure and function of different GI regions (9).

**Dynamics of the GI Tract**

The movement of food and dosage forms through the GI tract is controlled by coordinated muscular contractions known as peristalsis and segmentation. During fasting, the stomach exhibits a cyclic motor pattern called the migrating motor complex (MMC), which clears indigestible materials every 90–120 minutes. After food intake, this pattern changes to continuous contractions, delaying gastric emptying and increasing gastric residence time. These physiological changes significantly affect the performance of GRDDS (10).

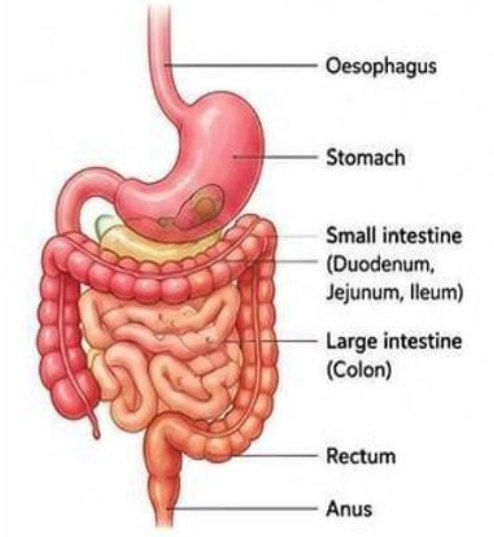


**Figure 1: Anotomy off GI**

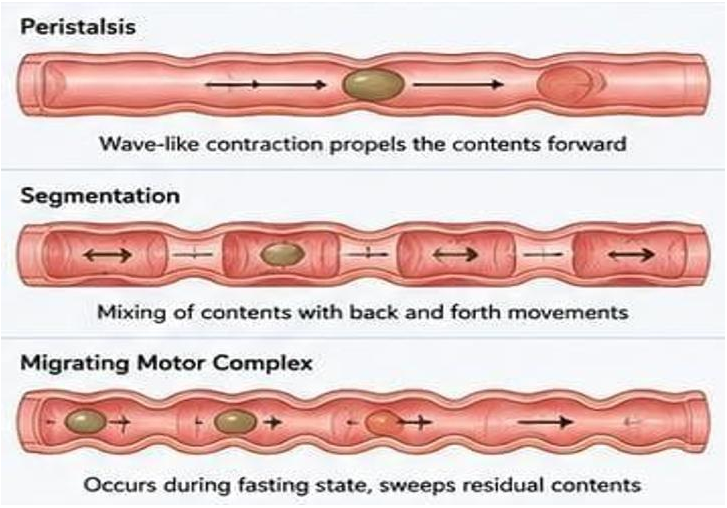


**Figure 2: Dynamics of the GI tract**

**GI Transit**

GI transit time refers to the time required for a drug or food to pass through the gastrointestinal tract. Gastric emptying time varies depending on age, gender, diet, disease state, emotional stress, posture, and the physical characteristics of the dosage form. Since unpredictable gastric emptying can reduce drug absorption, modulation of GI transit is a key objective in gastro-retentive drug delivery (11).

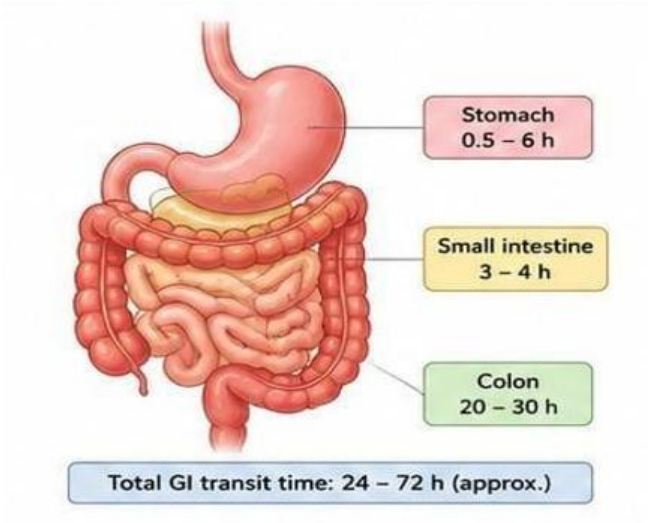


**Figure 3: GI transit**

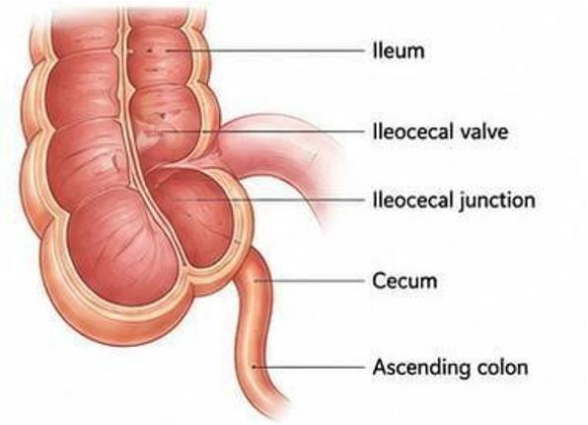


**Figure 4: Ileocecal junction**

**Ileocecal Junction**

The ileocecal junction is located between the ileum and the colon and acts as a physiological valve that regulates the movement of intestinal contents into the large intestine. It prevents the backflow of colonic contents into the small intestine and contributes to maintaining an appropriate intestinal transit time. Although it has limited direct influence on gastric retention, it plays an important role in the overall gastrointestinal transit of oral dosage forms (12).

**Colon and Gut Flora**

The colon contains a large population of microorganisms that play a significant role in the metabolism of drugs, polymers, and dietary substances. Gut flora can degrade certain drug delivery systems and influence drug release and absorption. Therefore, the interaction between drug formulations and colonic bacteria should be considered while designing controlled-release systems intended to reach the lower gastrointestinal tract (13).

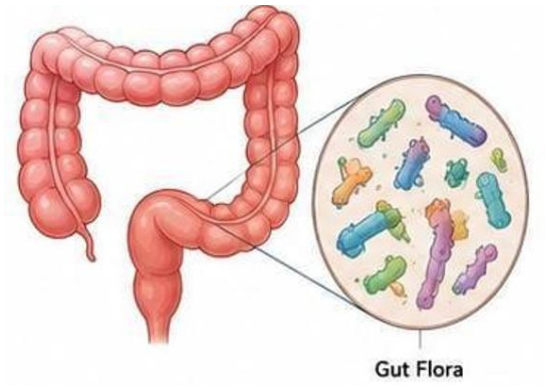


**Figure 5: Colon and gut flora**

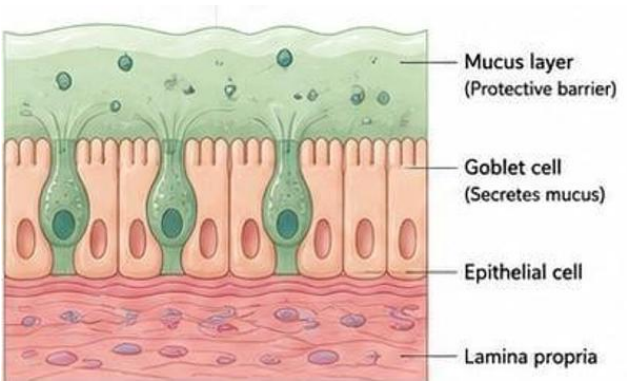


**Figure 6: GI Mucus**

**GI Mucus**

The gastrointestinal tract is lined with a mucus layer that protects the epithelium from mechanical damage, digestive enzymes, and acidic gastric contents. Mucoadhesive gastro-retentive systems utilize this mucus layer to increase the residence time of the dosage form by adhering to the gastric mucosa. The thickness and turnover rate of mucus influence the effectiveness of mucoadhesive formulations (14).

**1. Approaches to Gastric Retention:**

- **Low-Density Systems (Floating Systems)**
  - **Effervescent Floating Systems**
    - **Volatile Liquid Containing Systems**
      - Intragastric Floating Gastrointestinal Drug Delivery System
      - Inflatable Gastrointestinal Delivery System
      - Intragastric Osmotically Controlled Drug Delivery System
    - **Gas-Generating Floating Systems**
      - Floating System with Ion-Exchange Resins
      - Floating Capsules
      - Floating Pills
  - **Non-Effervescent Floating Systems**
    - Hydrodynamically Balanced Systems (HBS)
    - Alginate Beads
    - Microballoons (Hollow Microspheres)
    - **Layered Tablets**
      - Single-Layer Floating Tablets
      - Double-Layer Floating Tablets
- **High-Density Systems (Non-Floating Systems)**

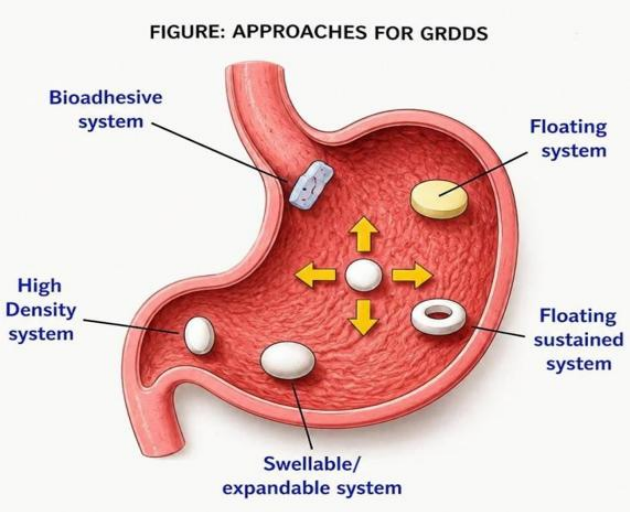


**Figure 7: Approaches to gastric retension**

**Low-Density Systems (Floating Drug Delivery Systems)**

- **Effervescent / Volatile & Gas-Generating Systems**
  - **Volatile Liquid Containing Systems**
    - Intragastric Floating Gastrointestinal Drug Delivery System
    - Inflatable Gastrointestinal Delivery System
    - Intragastric Osmotically Controlled Drug Delivery System
  - **Gas-Generating Floating Systems**
    - Floating System with Ion-Exchange Resins
    - Floating Capsules
    - Floating Pills
- **Non-Effervescent Floating Systems**
  - Hydrodynamically Balanced Systems (HBS)
  - Alginate Beads
  - Microballoons (Hollow Microspheres)
  - **Layered Tablets**
    - Single-Layer Floating Tablets
    - Double-Layer Floating Tablets

Low-density or floating drug delivery systems (FDDS) are gastro-retentive dosage forms with a density lower than that of gastric fluid (approximately 1.004 g/cm³). These systems float on the gastric contents without interfering with the normal gastric emptying process. While floating, the dosage form releases the drug in a controlled manner, resulting in prolonged gastric residence time, improved bioavailability, and enhanced therapeutic efficacy for drugs absorbed mainly in the stomach or upper small intestine (15).

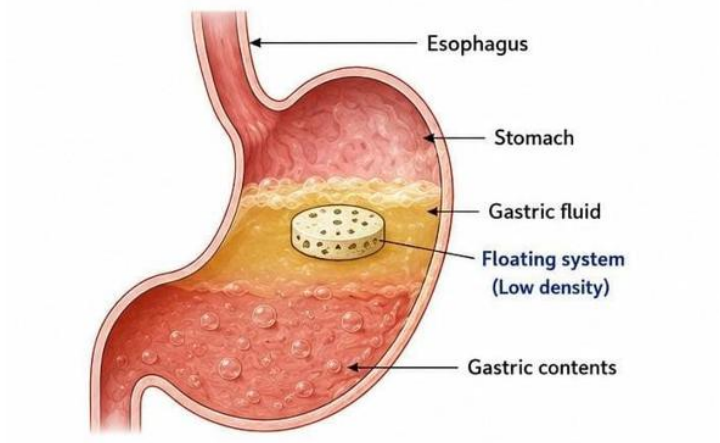


**Figure 8: Low density system**

**Effervescent Floating Systems**

Effervescent floating systems contain gas-generating agents such as sodium bicarbonate, calcium carbonate, citric acid, or tartaric acid. When these agents come into contact with gastric fluid, carbon dioxide is produced and becomes trapped within the polymer matrix. This decreases the density of the dosage form, causing it to float on gastric fluid (16).

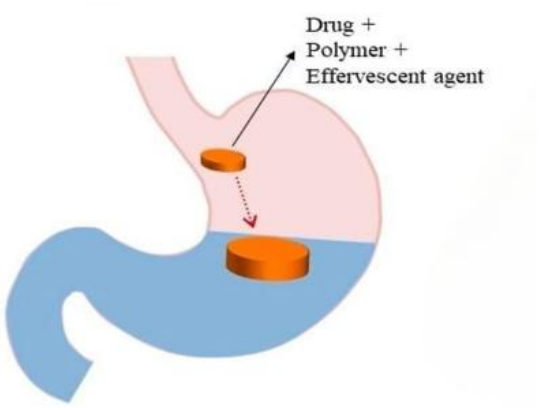


**Figure:9: Effervescent floating system**

**Volatile Liquid Containing Systems**

Volatile liquid containing systems use a chamber filled with a volatile liquid, such as ether or cyclopentane. At body temperature, the liquid vaporizes and inflates the chamber, reducing the density of the system and allowing it to float in the stomach for an extended period (17).

**Intragastric Floating Gastrointestinal Drug Delivery System**

This system consists of a drug reservoir and a floating chamber containing a volatile liquid. After oral administration, body temperature causes the liquid to vaporize, inflating the floating chamber. As a result, the system remains buoyant in the stomach and releases the drug gradually over an extended period.

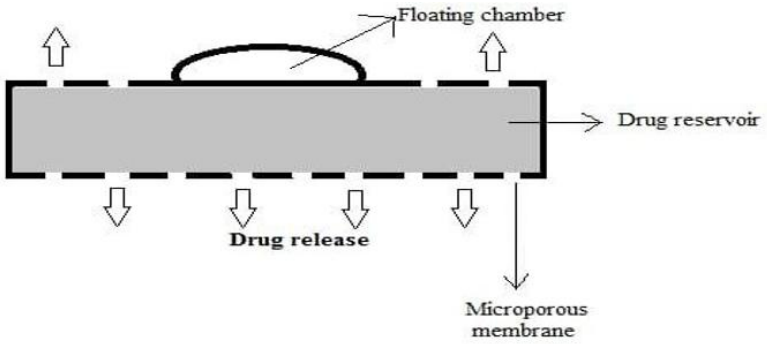


**Figure 10: Intragastric Floating Gastrointestinal Drug Delivery System**

**Inflatable Gastrointestinal Delivery System**

Inflatable gastrointestinal delivery systems contain a balloon-like chamber filled with a volatile liquid. Once inside the stomach, the chamber expands due to vaporization of the liquid, increasing the size of the dosage form and preventing its passage through the pylorus. After complete drug release, the system deflates and is naturally eliminated from the body.

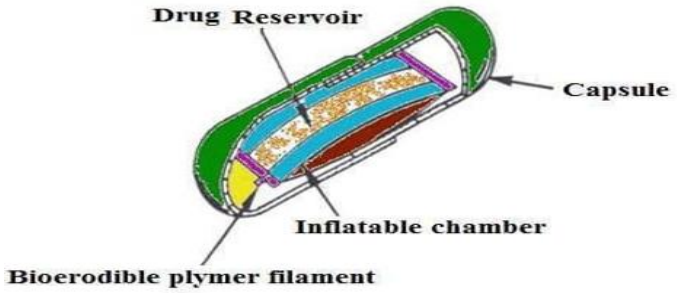


**Figure 11: Inflatable Gastrointestinal Delivery System**

**Intragastric Osmotically Controlled Drug Delivery System**

This system combines osmotic pressure with floating technology. Water enters through a semipermeable membrane, generating osmotic pressure that controls drug release. At the same time, a floating chamber maintains buoyancy, allowing prolonged gastric retention and controlled drug delivery.

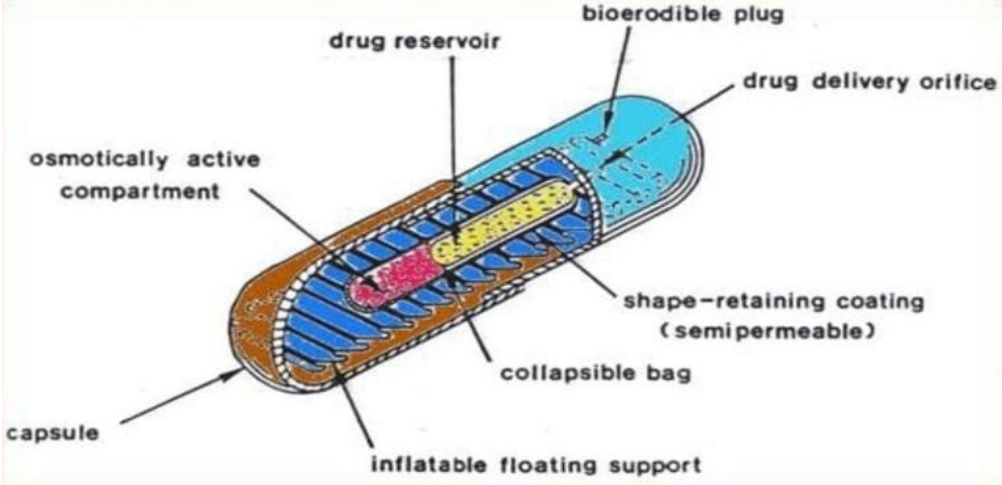


**Figure 12: Intragastric Osmotically Controlled Drug Delivery System**

**Gas-Generative Floating Systems (18)**

Gas-generative systems contain gas-forming agents that react with gastric acid to produce carbon dioxide. The generated gas becomes trapped within the hydrated polymer matrix, decreasing the density of the dosage form and enabling it to float.

**Floating System with Ion-Exchange Resins**

These systems contain ion-exchange resins loaded with bicarbonate ions. When exposed to gastric acid, carbon dioxide is released and trapped inside the dosage form, providing buoyancy. Such systems maintain prolonged gastric residence while ensuring controlled drug release.

**Floating Capsules**

Floating capsules are hard or soft gelatin capsules containing gas-generating agents and swellable polymers. After ingestion, the capsule absorbs gastric fluid, generates carbon dioxide, and floats on the stomach contents while slowly releasing the drug.

**Floating Pills**

Floating pills consist of an inner drug core surrounded by a polymeric membrane containing gas-generating agents. The produced carbon dioxide is retained within the membrane, decreasing the density of the pill and allowing it to float in the stomach until the drug is completely released.

**Non-Effervescent Floating Systems (19)**

Non-effervescent floating systems do not produce gas. Instead, they contain swellable polymers such as hydroxypropyl methylcellulose (HPMC), sodium alginate, carbopol, or polyethylene oxide. These polymers absorb gastric fluid, swell, and form a gel barrier with low density, enabling the dosage form to float.

**Hydrodynamically Balanced System (HBS)**

Hydrodynamically balanced systems contain gel-forming polymers that rapidly hydrate upon contact with gastric fluid. The swollen gel maintains a density lower than gastric fluid, allowing the dosage form to float while providing sustained drug release.

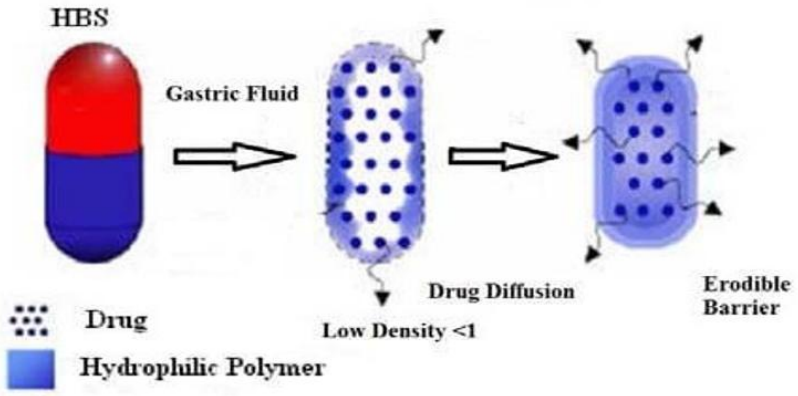


**Figure 13: Hydrodynamically Balanced System (HBS)**

**Alginate Beads**

Alginate beads are prepared by ionotropic gelation using sodium alginate and calcium chloride. The porous structure of the beads reduces their density, enabling them to float in the stomach. They are widely used for sustained drug delivery and gastric retention.


**Figure 14: Alginate Beads**

**Microballoons (Hollow Microspheres)**

Microballoons are hollow spherical particles with an internal cavity. Their low density enables them to float on gastric contents for several hours. They provide prolonged drug release, increased gastric residence time, and improved bioavailability.

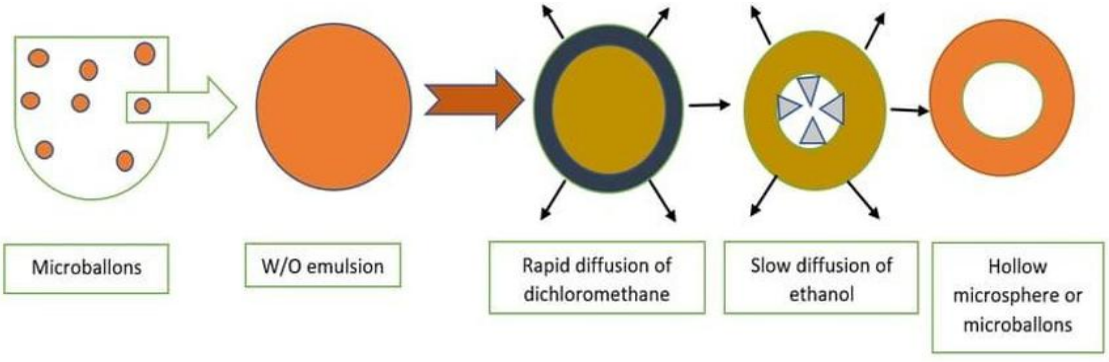


**Figure 15: Microballoons**

**Layered Tablets**

Layered floating tablets are composed of one or more layers designed to provide buoyancy and controlled drug release.

**Single-Layer Floating Tablets**

Single-layer floating tablets contain both the drug and gas-generating agents within a single matrix. Upon contact with gastric fluid, the tablet swells, generates gas, floats, and releases the drug in a controlled manner.

**Double-Layer Floating Tablets**

Double-layer floating tablets consist of an immediate-release layer and a sustained-release floating layer. The first layer provides rapid therapeutic action, whereas the second layer maintains prolonged drug release and gastric retention.

**High-Density Systems (Non-Floating Systems)**

- Bioadhesive Systems
- Magnetic Systems
- Expandable, Unfoldable and Swellable Systems
- Raft-Forming Systems
- Superporous Hydrogel Systems

**High-Density Systems (Non-Floating Systems)**

High-density or non-floating gastro-retentive systems are designed with a density greater than that of gastric fluid (usually more than 2.5 g/cm³). Due to their high density, these dosage forms sink to the bottom of the stomach and resist gastric emptying. This prolonged gastric retention enhances drug absorption and improves bioavailability, particularly for drugs absorbed in the stomach or upper small intestine (20).

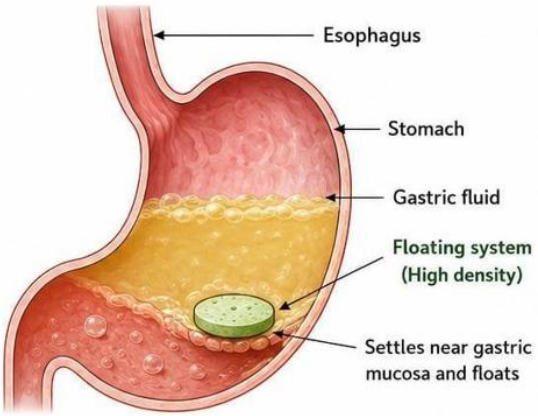


**Figure 16: High-Density Systems**

**Bioadhesive Systems**

Bioadhesive (mucoadhesive) systems contain polymers that adhere to the gastric mucosal surface through physical or chemical interactions. The adhesion prolongs the gastric residence time of the dosage form, allowing sustained drug release and improved therapeutic efficacy. Common bioadhesive polymers include carbopol, chitosan, polycarbophil, sodium alginate, and hydroxypropyl methylcellulose (HPMC) (21).

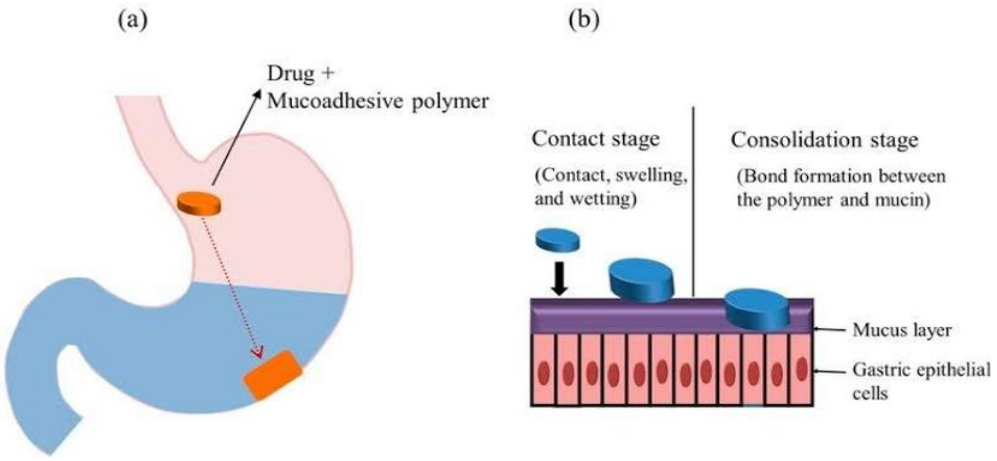


**Figure 17: Bioadhesive Systems**

**Magnetic Systems**

Magnetic systems consist of a dosage form containing a small internal magnet. An external magnet is placed over the stomach to hold the dosage form in the gastric region for an extended period. Although this approach effectively increases gastric retention, its clinical application is limited due to difficulties in correctly positioning and maintaining the external magnet (22).

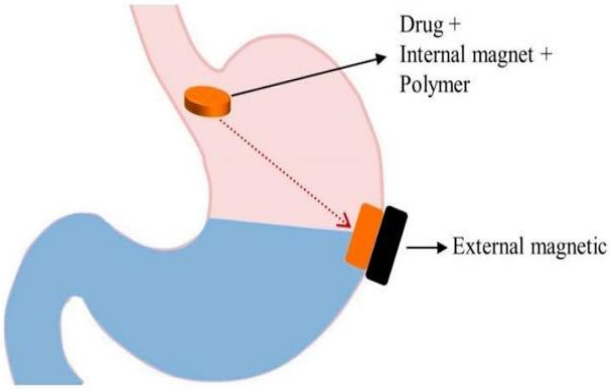


**Figure 18: Magnetic Systems**

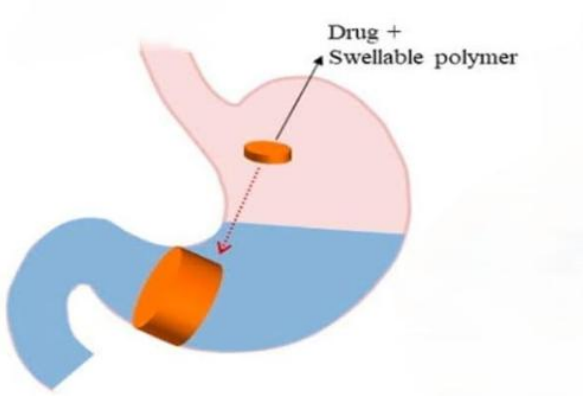


**Figure 19: Expandable, Unfoldable and Swellable Systems**

**Expandable, Unfoldable and Swellable Systems**

These systems are designed to increase in size after reaching the stomach. The dosage form unfolds or swells by absorbing gastric fluid, becoming larger than the pyloric opening. This prevents its passage into the small intestine and prolongs gastric residence time. After complete drug release, the system gradually decreases in size and is eliminated naturally (23).

**Raft-Forming Systems**

Raft-forming systems are mainly used for the treatment of gastroesophageal reflux disease (GERD). Upon contact with gastric fluid, polymers such as sodium alginate react with bicarbonate to form a viscous gel (raft) containing entrapped carbon dioxide. This floating gel acts as a barrier over the gastric contents, prolonging gastric retention and reducing acid reflux while providing sustained drug release (24).

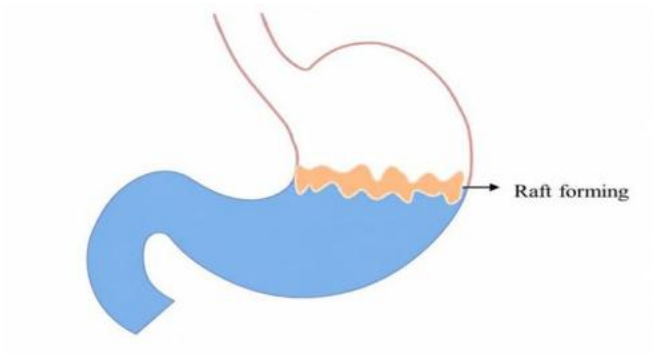


**Figure 20: Raft-Forming Systems**

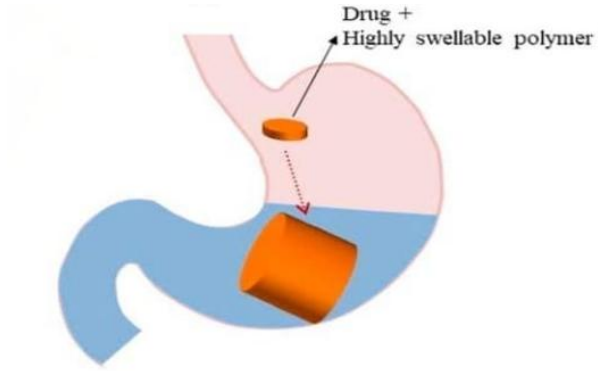


**Figure 21: Superporous Hydrogel Systems**

**Superporous Hydrogel Systems**

Superporous hydrogels possess interconnected pores that rapidly absorb large amounts of gastric fluid. Within a few minutes, they swell to several times their original size while maintaining sufficient mechanical strength. The enlarged hydrogel remains in the stomach for an extended period, allowing controlled drug release and improved bioavailability (25).

**Application of GRDDS**

- Improves bioavailability of drugs having narrow absorption window in stomach or upper small intestine.
- Provides controlled and sustained drug release for prolonged therapeutic effect.
- Useful for drugs that are unstable in intestinal/colonic environment.
- Enhances solubility and absorption of drugs with low solubility at higher pH.
- Provides site-specific delivery for gastric disorders like H. pylori infection and peptic ulcer.
- Reduces dosing frequency and improves patient compliance (26).

**Conclusion**

Gastro-retentive drug delivery systems (GRDDS) are an effective approach for improving the oral delivery of drugs that require prolonged gastric residence. By retaining the dosage form in the stomach for an extended period, GRDDS enhances drug bioavailability, provides sustained and site-specific drug release, and improves therapeutic efficacy. Various approaches such as floating, high-density, bioadhesive, expandable, raft-forming, magnetic, and superporous hydrogel systems have been developed to overcome the limitations of conventional oral dosage forms. Overall, GRDDS offers significant advantages for drugs with a narrow absorption window, pH-dependent solubility, or local action in the stomach, making it a promising strategy in modern controlled drug delivery.

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# IMMUNOPROPHYLAXIS THROUGH VACCINES: CURRENT CONCEPTS AND EMERGING INNOVATIONS

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## What Are Vaccines?

A vaccine is a biological preparation that provides active acquired immunity to a particular infectious disease. It contains an agent that resembles a disease-causing microorganism — often made from weakened or killed forms of the microbe, its toxins, or one of its surface proteins. When administered, the vaccine stimulates the immune system to recognize the agent as foreign, destroy it, and develop immunological memory. Upon future exposure to the virulent pathogen, the immune system responds more rapidly and effectively.

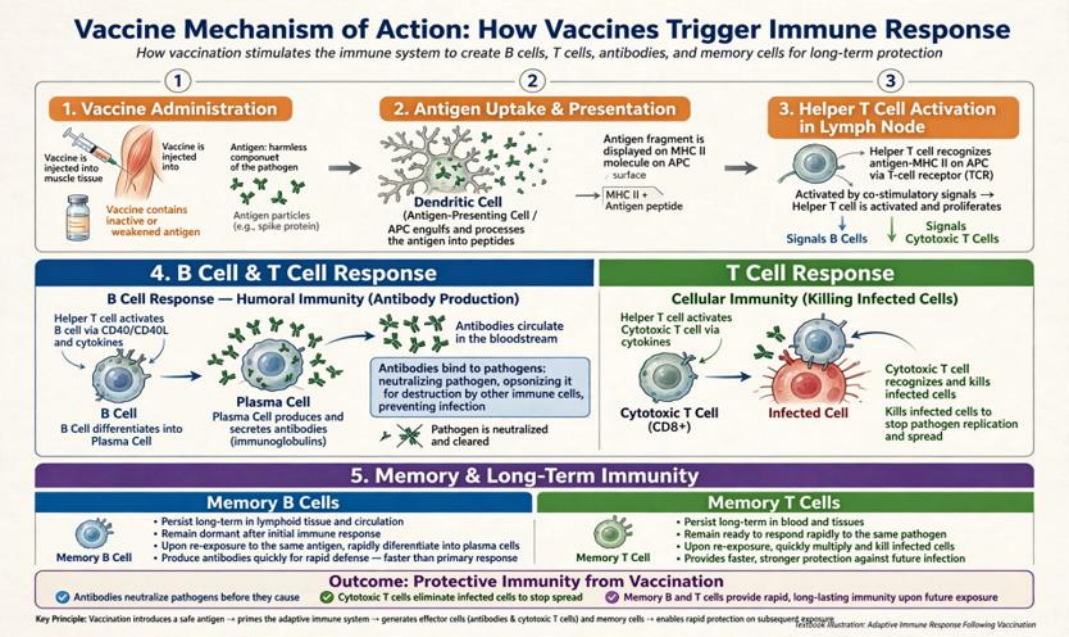


**Figure 1: Mechanism of Vaccine Action - How vaccines train immune system**

The principle was first scientifically demonstrated by Edward Jenner in 1796 against smallpox and later established by Louis Pasteur through his germ theory.

## 2. Need of Vaccines

Vaccines are essential for individual and public health due to the following reasons:

- **Prevention of Infectious Diseases**: They are the most effective method to prevent high-mortality diseases like polio, tetanus, measles, tuberculosis.
- **Herd Immunity**: When a large percentage of population is vaccinated, transmission chain is broken, protecting even unvaccinated individuals.
- **Eradication of Diseases**: Smallpox has been eradicated and polio is near eradication solely due to global vaccination programs.

- **Reduction of Healthcare Burden:** Vaccination reduces hospitalization, antimicrobial use, and prevents long-term complications.
- **Economic Benefits:** It is far more cost-effective than treating the disease.
- **Control of Emerging Infections:** In pandemics like COVID-19, rapid vaccine development is the key to containment.

**3. Types of Vaccines**

Based on the nature of antigen and technology used, vaccines are classified into 6 major types:

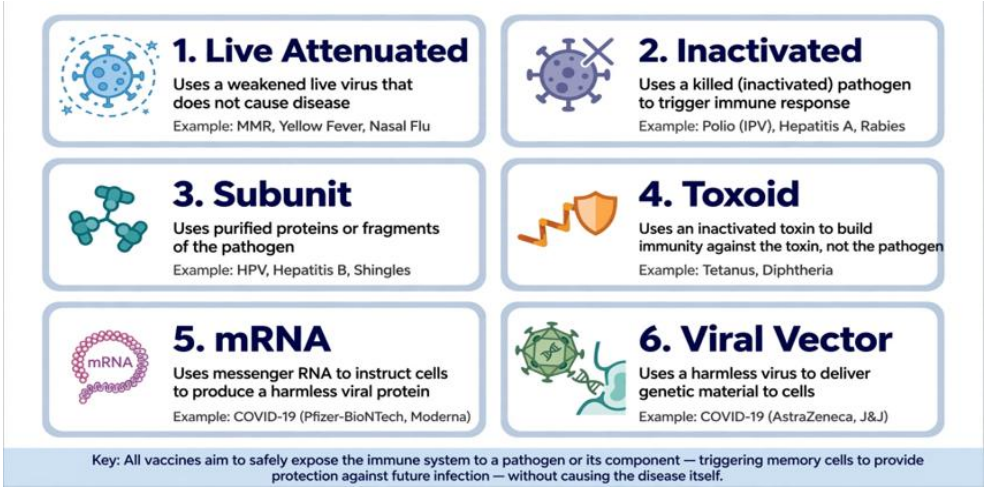


**Figure 2: Types of vaccines**

Table 1: **Types of vaccines**

| Sr.No. | Type | Principle | Example | Advantage / Limitation |
|---|---|---|---|---|
| 1. | Live Attenuated | Live pathogen weakened in lab so it cannot cause disease | BCG, MMR, Oral Polio, Rotavirus | Strong, lifelong immunity with 1-2 doses. Not suitable for immunocompromised. |
| 2. | Inactivated / Killed | Pathogen killed by heat/chemicals | IPV, Rabies, Hepatitis A | Safer, but requires boosters, weaker immunity. |
| 3. | Subunit / Conjugate | Only a part (protein/polysaccharide) of pathogen | Hepatitis B, HPV, Acellular Pertussis | Very safe, targeted. Requires adjuvant. |
| 4. | Toxoid | Inactivated bacterial toxin | Tetanus, Diphtheria | Protects against toxin-mediated diseases. |
| 5. | Viral Vector | Harmless virus used to deliver genetic code of antigen | AstraZeneca COVID-19, Ebola vaccine | Strong cellular immunity. |
| 6. | mRNA / DNA | mRNA instructs host cells to make antigen | Pfizer-BioNTech, Moderna COVID-19 | Rapid production, highly effective. Needs cold chain. |

**4. Preparation of Vaccines**

Vaccine production is a complex, multi-stage process conducted under GMP (Good Manufacturing Practice) conditions.

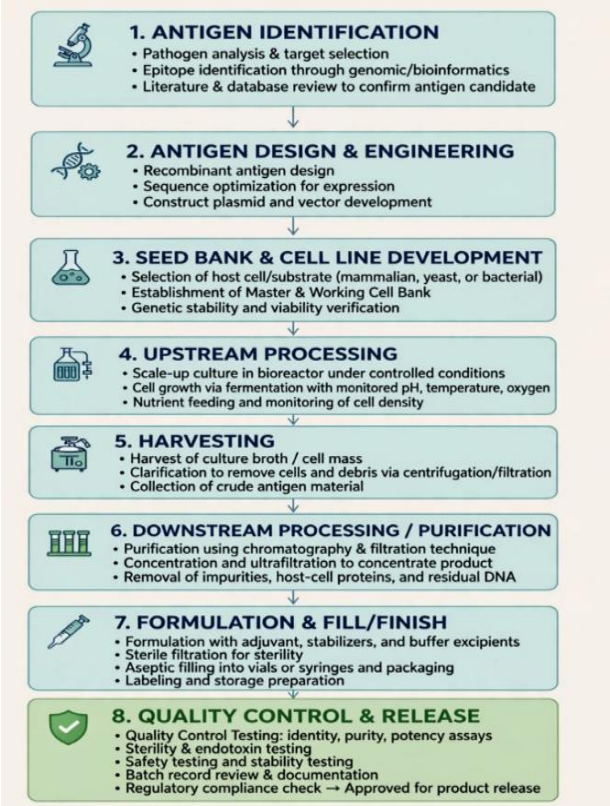


**Figure 3: Preparation of Vaccines Flowchart**

**General Steps**

i. **Antigen Identification**: Genomic and proteomic analysis to identify immunogenic proteins.
ii. **Antigen Production**: Growing pathogen in cell cultures (egg, yeast, mammalian cells like Vero cells) or recombinant expression in E. coli/Yeast.
iii. **Inactivation/Attenuation**: For killed vaccines - formaldehyde or heat. For live vaccines - serial passage.
iv. **Purification**: Removal of cell debris, using chromatography, ultrafiltration.
v. **Formulation**: Mixing purified antigen with adjuvant (e.g., Alum to boost immune response), stabilizers (gelatin, sorbitol), preservatives (thiomersal).
vi. **Quality Control**: Testing for sterility, potency, safety, identity, and stability as per WHO/Pharmacopoeia norms.
vii. **Fill and Finish**: Filling into vials/syringes under sterile conditions.

**5. Vaccine Administration**

Route of administration influences immune response:

- **Intramuscular (IM):** Most common. Deltoid or thigh. Eg. Hepatitis B, COVID-19, Tetanus.
- Good vascularity ensures rapid absorption.
- **Subcutaneous (SC):** Fatty tissue under skin. Eg. MMR, Varicella.

- **Intradermal (ID):** Into dermis. Eg. BCG, Rabies (ID regimen). Requires skilled technique.
- **Oral**: Eg. OPV, Rotavirus, Typhoid Ty21a. Induces mucosal immunity (IgA).
- **Intranasal:** Eg. Live attenuated Influenza vaccine. Mimics natural infection route.
- **Cold Chain**: Most vaccines require 2-8°C storage. mRNA vaccines require -20°C to -80°C. Break in cold chain leads to potency loss.
- **Dosing Schedule**: Primary doses + booster doses to maintain memory cell levels. Eg. DPT at 6,10,14 weeks and booster at 16-24 months.

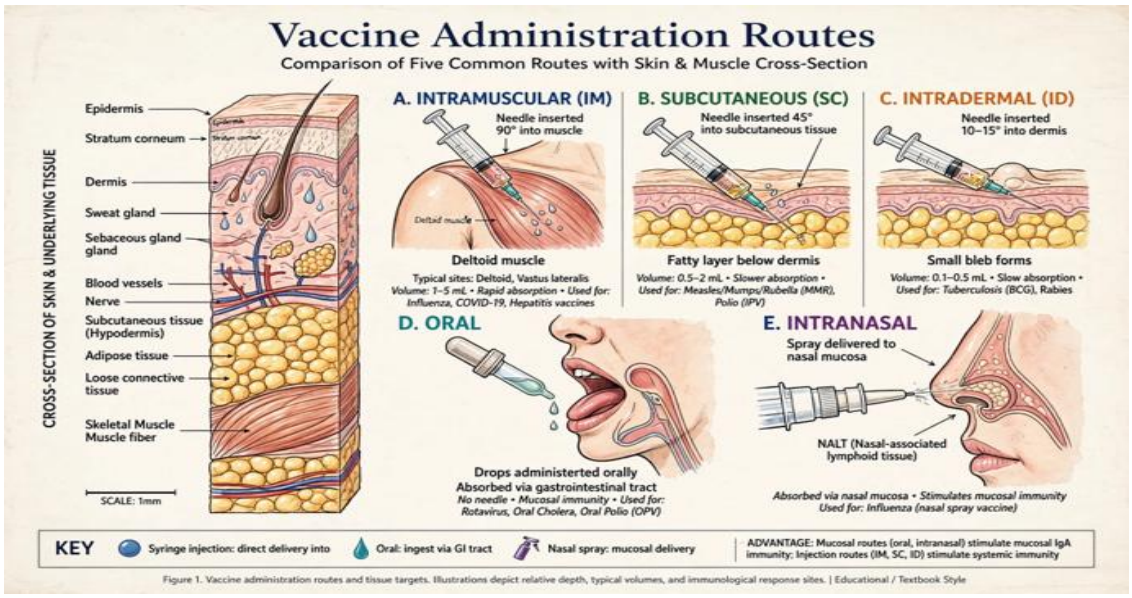


**Figure 4: Vaccine administration routes**

## 6. New Vaccine Strategies

Modern vaccinology is moving beyond conventional methods:

- **Reverse Vaccinology**: Using genome sequence of pathogen to predict antigens in silico without culturing the organism.
- **mRNA Vaccine Technology**: Synthetic mRNA enclosed in lipid nanoparticles. Fast to design, no live virus needed. Future for cancer vaccines.
- **DNA Vaccines**: Plasmid DNA encoding antigen. Stable, easy to produce.
- **Virus-Like Particles (VLPs):** Empty shells that look like virus but have no genome. Eg. HPV vaccine.
- **Nanoparticle-based Vaccines**: Antigens displayed on nanoparticles for better uptake by dendritic cells.
- **Edible Vaccines**: Transgenic plants (banana, tomato) expressing antigens. Cost-effective, needle-free.
- **Universal Vaccines**: Aim for broad protection against variants, e.g., universal influenza vaccine targeting conserved stalk region of hemagglutinin.
- **AI and Structural Biology**: AI predicts antigen-antibody interaction to design better immunogens.

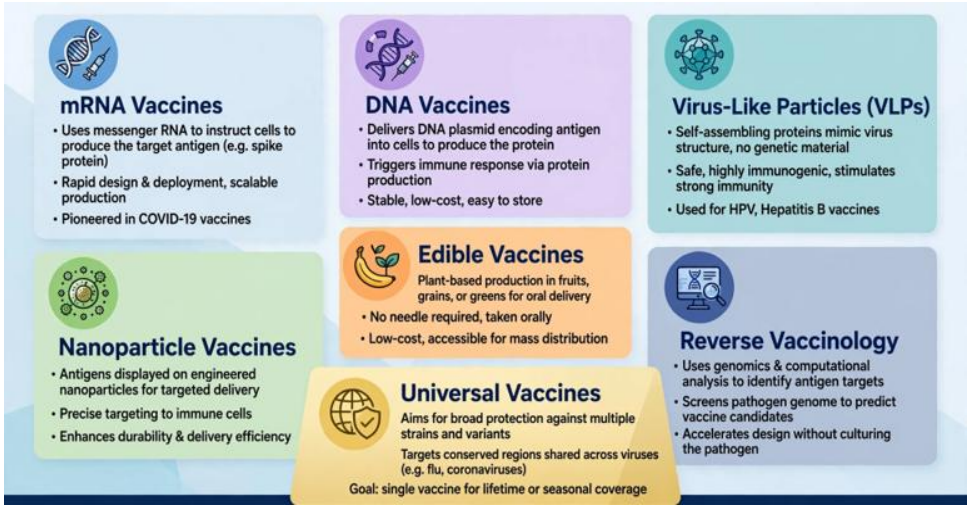


**Figure 5: Next-generation vaccine platforms 2024-2026**

## 7. Advantages and Disadvantages

**Advantages:**

- Provides long-lasting protection and immunological memory
- Prevents mortality, disability and complications
- Leads to herd immunity and disease eradication
- Safe compared to risk of natural infection
- Reduces antimicrobial resistance by reducing infections
- Cost-effective public health intervention

**Disadvantages / Limitations**:

- Mild side effects: fever, pain at injection site, allergic reactions (rare)Live vaccines contraindicated in immunocompromised, pregnant women
- Requires strict cold chain maintenance
- Multiple doses/boosters needed for some vaccines
- Vaccine hesitancy due to myths and misinformation
- Development takes long time and high cost for new pathogens
- Some vaccines have limited efficacy and duration (e.g., influenza needs annual update)

## Conclusion

Vaccines remain the greatest achievement of preventive medicine. From Pasteur's first rabies vaccine to the mRNA vaccines developed in less than a year for COVID-19, the field has evolved enormously. For a Microbiologist, understanding vaccine design, production, and immunology is critical to tackling emerging and re-emerging infectious diseases.

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# ADVANCES IN NUCLEAR MEDICINE FOR THE DIAGNOSIS OF BENIGN THYROID DISEASES

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## 1. Introduction

Benign thyroid disorders account for a vast majority of endocrine consultations and include Graves disease, toxic multinodular goiter, autonomously functioning thyroid adenoma, thyroiditis, congenital hypothyroidism and a variety of benign nodular enlargement. These manifestations can be structural, biochemical or physiological and a full diagnosis should involve more than one type of diagnosis. Nuclear medicine is still relevant in this context since it clearly visualizes thyroid function and answers questions that morphology cannot.

The thyroid's physiological suitability for radionuclide imaging is very good. Follicular cells concentrate iodide through the sodium-iodide symporter and in the gland organizes the material before it is fed into thyroid hormone precursors. This physiological process can be studied in a variety of ways: trapping, organization, total gland avidity and regional functional autonomy. Nuclear medicine adds a functional map to the conventional anatomical map provided by ultrasonography (1, 3, 8, 9). The practical questions thyroid nuclear medicine addresses are clinical ones. Is thyrotoxicosis due to increased synthesis, destructive release of hormone, or exogenous hormone intake? Is a suppressed-TSH nodule autonomously functioning? Is a child with congenital hypothyroidism caused by agenesis, ectopy, or dyshormonogenesis? Does the gland have enough iodine avidity for radioiodine treatment? And this is why radionuclide investigations are still relevant in the clinical field despite the widespread use of high-resolution ultrasound and fine-needle aspiration cytology.

This chapter is written in a textbook style and reviews the principles, techniques, syndrome-based applications, pitfalls, and future directions of nuclear medicine in benign thyroid disease. Each section closes by linking its central concept to the next part of the chapter so that the discussion progresses in a logical clinical sequence rather than in the format of a research paper.

In summary, thyroid nuclear medicine is best understood as a functional extension of endocrine diagnosis. The physiological basis of this functional perspective forms the subject of the next section.

## 2. Foundations of Thyroid Nuclear Medicine

Thyroid scintigraphy is based on the tracer. Technetium-99m pertechnetate is trapped in the thyroid by the sodium-iodide symporter but is not organified. It is attractive to the eye because of its low cost and fast acquisition of images, and is often used in routine thyroid scintigraphy. Iodine-123 is also trapped but is additionally organified, making it a closer physiological

analogue to stable iodine and very useful when uptake measurement and/or imaging is necessary, or ectopic tissue evaluation is needed. Iodine-131 is also trapped and organified but its beta emission is high and greater radiation burden restricts its routine diagnostic role; it is used mainly for treatment and selected dosimetric applications. (1-3,8,11)

The lesson is that the same symptom complex does reflect very different thyroid physiology. Diffuse autoimmune stimulation, focal autonomy, destructive thyroiditis, iodine contamination, and exogenous hormone use can all lead to thyrotoxicosis, but they produce different radionuclide patterns because the underlying pathophysiology is not the same. The scan is meaningful only if the physiological behavior of the tracer is interpreted alongside the biochemical and clinical profile of the patient. (1,3,4)

Another important idea we need to remember is that the thyroid scan should not be seen as a test of structure in the narrow radiological sense. Its real value is in pattern recognition anchored to physiology: diffuse uptake signals global stimulation, patchy uptake signals heterogeneous autonomy, focal uptake indicates a dominant autonomous region, and absent uptake usually indicates failed trapping or suppressed function. Once this mindset is established, routine studies and unusual findings can be understood in a more coherent manner. (1, 2, 9)

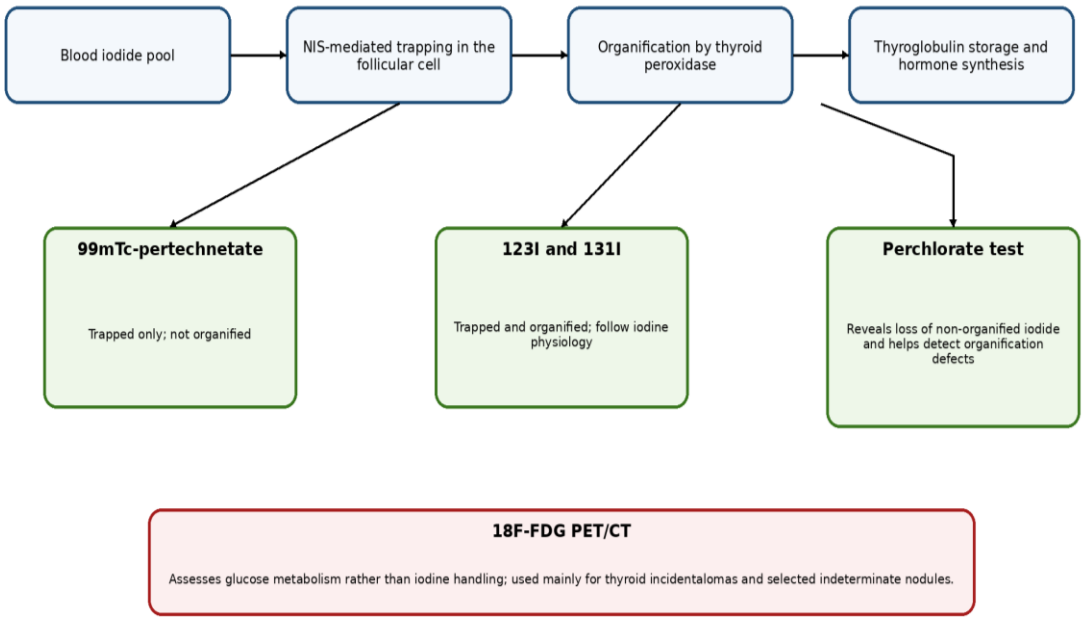


**Figure 1: Thyroid iodide handling and the principal diagnostic tracers used in benign thyroid disease**

In summary, the meaning of any thyroid scan depends first on what the tracer is designed to show. The main clinical techniques therefore become much easier to understand when approached from this radiopharmaceutical foundation.3. CORE DIAGNOSTIC TECHNIQUES3.1 Thyroid scintigraphyThe thyroid scintigraphy is still the primary imaging in benign thyroid nuclear medicine. Normal study shows two fairly symmetrical lobes with homogeneous tracer distribution and little background activity. From this background, clinically important patterns of disease emerge. Graves disease typically presents as diffuse increased

uptake, toxic multinodular goiter as patchy heterogeneous uptake, and toxic adenoma as a focal hot nodule with suppression of the surrounding gland. However, if thyroiditis or drug use is present in the body, the uptake is typically low or absent. Scintigraphy is also useful for demonstrating ectopic functioning thyroid tissue. (1-4, 8-11)3.2 Radioiodine uptake testingRadioiodine uptake test complements scintigraphy and provides a quantitative measure of gland avidity. Its primary clinical application is in thyrotoxicosis in which high uptake indicates active hormone secretion and low uptake indicate destructive release, iodine contamination or exogenous hormone use. Uptake values are also useful for radioiodine treatment planning. It is important to follow an approach based on the locally established normal values as dietary iodine intake, instrumentation and protocol timing will be very important in determining the measured values. (3,4,7,8)

**Table 1: Major radiopharmaceuticals used in benign thyroid imaging**

| Radio-pharmaceutical | Physiological Basis | Main Use | Key Strength | Main Limitation |
|---|---|---|---|---|
| 99mTc-pertechnetate | Trapping only | Routine scintigraphy | Fast and widely available | Not organified |
| 123I-iodide | Trapping and organification | Scintigraphy and uptake | Closest to iodine physiology | Cost and availability |
| 131I-iodide | Trapping and organification | Therapy and selected dosimetry | Direct theranostic value | Higher radiation burden |
| 18F-FDG | Glucose metabolism | Incidentalomas and selected nodules | Whole-body metabolic information | Low specificity |

### 3.3 Ultrasonography as a Complementary Modality

Ultrasonography is the primary structural examination of the thyroid but should not be seen as a competitor to scintigraphy. Ultrasound describes gland size, nodular architecture, calcification, and suspicious structural features, while scintigraphy determines whether the tissue is functioning normally, autonomously, or not. This complementarity is especially important when TSH is suppressed and the clinician must decide whether a nodule is hyperfunctioning or whether multinodular disease contains significant autonomous tissue. (2,5,9,12)

### 3.4 Potassium Perchlorate Testing

The perchlorate discharge test has a special but conceptually important role. In normal thyroid physiology trapped iodide is rapidly organified. If organification is defective perchlorate administration results in non-organified iodide and does not keep the retained activity. Thus the performance of the test helps to confirm an organification defect in selected children with congenital hypothyroidism and suspected dyshormonogenesis. This test is not a routine adult study and remains a fascinating example of dynamic physiology in nuclear medicine (6, 10, 13).

### 3.5 Patient Preparation and Acquisition Considerations

Prepared patients increase diagnostic reliability and safety. The clinician should document the exposure of the patient to iodinated contrast, amiodarone, iodine-containing supplements, and

antithyroid medication because all these can affect gland avidity. Before radioiodine treatment is administered in women of childbearing age, the patient's pregnancy status should be considered, and lactation precautions should be made known. And as one is taking the image, the position of the patient, the neck extension, and the chances of contamination from saliva or esophageal activity all need to be taken into account. These seemingly simple details have a direct influence on interpretation and often can be the key to a diagnosis and how accurate a scan is. (3,4,7,11)

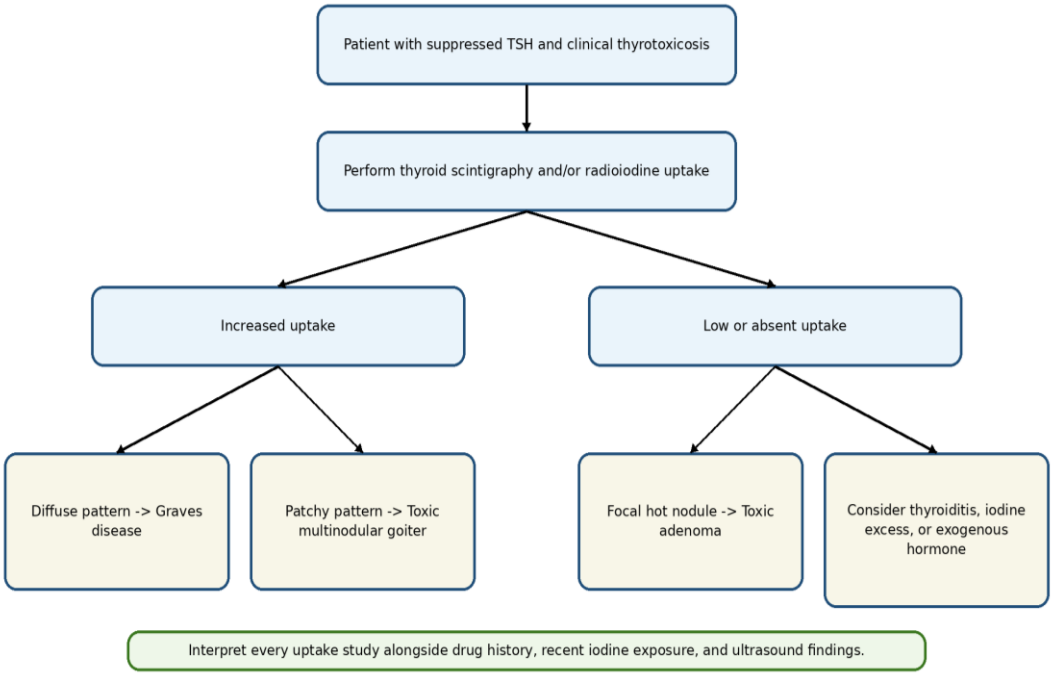


**Figure 2: Functional approach to a patient with suppressed TSH and suspected thyrotoxicosis**

**Table 2: Comparative profile of the principal diagnostic techniques**

| Technique | What it Measures | Most Useful Indication | Important Caveat |
|---|---|---|---|
| Scintigraphy with 99mTc | Regional trapping | Autonomy, thyrotoxicosis, ectopic tissue | Does not assess organification |
| Scintigraphy with 123I | Trapping and organification | Uptake studies, congenital disorders | Longer protocol |
| Radioiodine uptake test | Global iodine avidity | Differentiate synthesis from release; therapy planning | Use local reference ranges |
| Ultrasonography | Structure and morphology | Nodules and gland anatomy | Cannot determine function |
| Perchlorate discharge test | Integrity of organification | Selected congenital hypothyroidism | Specialized, not routine |

**Table 3: Practical preparation points before thyroid nuclear medicine studies**

| Preparation Issue | Why It Matters | Practical Note |
|---|---|---|
| Recent iodinated contrast or supplements | May suppress uptake and mislead interpretation | Document history before scheduling |
| Antithyroid medication | Can alter trapping and therapy planning | Follow indication-specific withdrawal guidance |
| Pregnancy status | Affects safety and test selection | Exclude pregnancy before radioiodine use |
| Lactation | Influences tracer precautions | Provide tracer-specific breastfeeding advice |
| Positioning and motion | Affects image quality and symmetry | Use comfortable extension and stable positioning |

In summary, the major diagnostic methods of thyroid nuclear medicine are complementary and clinically focused. Their full significance becomes clearest when they are applied to specific benign disorders.

## 4. Nuclear Medicine in Common Benign Thyroid Disorders

### 4.1 Graves Disease and Diffuse Toxic Goiter

In Graves disease, thyroid-stimulating immunoglobulins activate the TSH receptor and produce diffuse hormone synthesis throughout the gland. The typical radionuclide pattern is a diffusely enlarged or normal-sized gland with diffusely increased uptake and fairly homogeneous distribution. This is especially important when differentiation from thyroiditis is required or nodularity is hindering clinical examination. Uptake studies also provide a functional baseline before radioiodine treatment. (1,3,4)

### 4.2 Toxic Adenoma and Toxic Multinodular Goiter

Autonomous thyroid tissue has a distinct scintigraphic pattern. A toxic adenoma demonstrates focal intense uptake within the autonomous nodule and suppression of the remaining parenchyma, whereas toxic multinodular goiter reveals multiple heterogeneous foci of increased uptake. These appearances confirm endogenous hormone overproduction, map the distribution of autonomy, and help explain mild or intermittent biochemical hyperthyroidism in older patients. (2,4,5,9).

### 4.3 Thyroiditis, Iodine Excess, and Exogenous Hormone Use

Subacute, painless and postpartum thyroiditis are destructive illnesses in which preformed hormone leaks out of damaged follicles. The radionuclide hallmark is low or no uptake. The same pattern may follow exposure to iodinated contrast, occur with iodine-rich medications like amiodarone or be observed for exogenous thyroid hormone use. While the scan alone does not indicate which low-uptake cause exists, it certainly clarifies the fundamental physiological difference between synthesis and release in the body. (1, 3, 4, 8)

### 4.4 Congenital Hypothyroidism and Dyshormonogenesis

Scintigraphy makes a unique contribution to congenital hypothyroidism by showing whether the thyroid is absent, ectopic, eutopic, or present with suspected dyshormonogenesis. In combination with ultrasonography, it improves etiologic classification and helps guide family counseling. When the gland is present but hormone synthesis is defective, the perchlorate discharge test can demonstrate abnormal organification. (6,10,13)

### 4.5 Benign Nodules and Thyroid Incidentalomas

When TSH is suppressed or low-normal, scintigraphy is very informative in nodular thyroid disease because it tells if the index lesion is hyperfunctioning. A truly hyperfunctioning nodule is rarely malignant and a nonfunctioning lesion needs to be stratified by ultrasound and cytology when appropriate. With more and more oncologic FDG PET/CT, focal thyroid incidentalomas are common and there is a whole new functional and metabolic context for thyroid evaluation. (2, 5, 12, 14-16)

### 4.6 Special Clinical Situations

Some clinical settings are especially important to note because radionuclide evaluation is very nuanced. In pediatric practice, scintigraphy is especially helpful for congenital hypothyroidism and in some cases of ectopic thyroid tissue, but the need for treatment and radiation stewardship must always be considered. In pregnancy, diagnostic radioiodine is almost always off-limits and imaging should be carried out to maintain maternal-fetal safety. In lactation, interruption protocols and tracer-specific precautions are necessary. Similarly, in older individuals with multinodular goiter, a scan may be of benefit, since biochemical hyperthyroidism is subtle and the burden of autonomous tissue may be considerable. These cases are indicative that thyroid nuclear medicine is physiologically informative but context-dependent as well. (4,6,10)

**Table 4: Characteristic scintigraphic patterns in common benign thyroid disorders**

| Clinical Disorder | Typical Radionuclide Pattern | Principal Implication |
|---|---|---|
| Graves disease | Diffuse increased uptake | Global receptor-mediated hyperfunction |
| Toxic multinodular goiter | Patchy heterogeneous uptake | Multifocal autonomy |
| Toxic adenoma | Focal hot nodule with suppressed background | Solitary autonomous function |
| Thyroiditis | Low or absent uptake | Destructive hormone release |
| Ectopic thyroid | Functioning tissue outside the normal bed | Defines ectopy |
| Dyshormonogenesis | Preserved trapping with abnormal organification | Consider perchlorate testing |

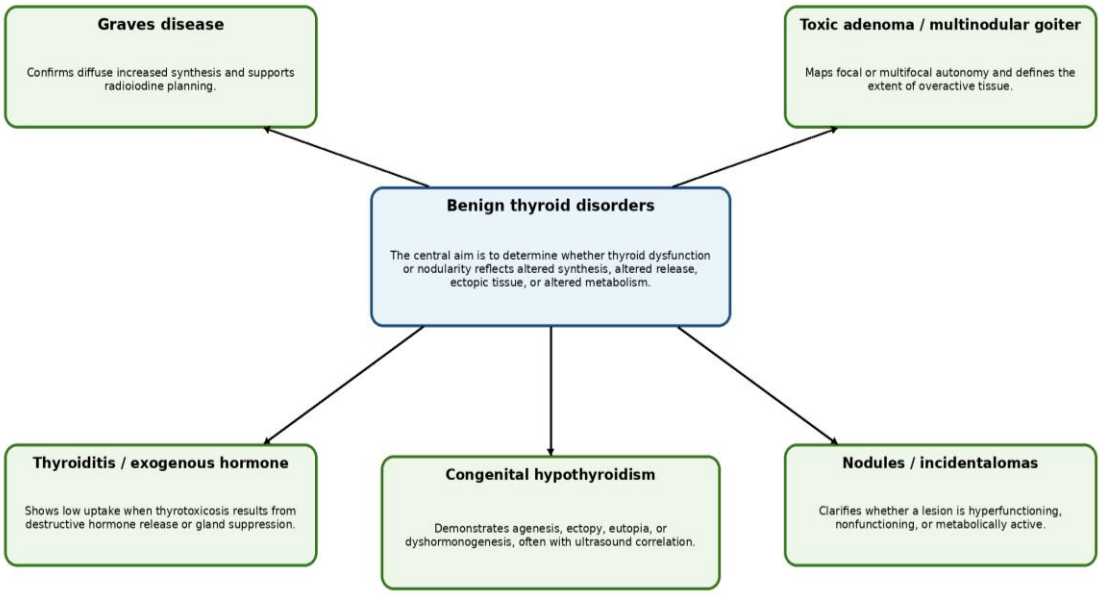


**Figure 3: Major benign thyroid disorders and the characteristic diagnostic role of nuclear medicine**

In summary, the usefulness of radionuclide studies becomes most tangible when interpretation is anchored to a defined clinical syndrome. This syndrome-based perspective leads naturally to the contribution of PET, SPECT/CT, and digital image analysis.

## 5. PET, Hybrid Imaging, and Emerging Digital Developments

### 5.1 Fluorodeoxyglucose PET/CTFluorine-18 Fluorodeoxyglucose

PET/CT is not a routine first-line test for benign thyroid disease since it reflects glucose metabolism, not iodine handling. Its significance lies mainly in incidental thyroid uptake observed during PET for non-thyroid causes and selected indeterminate nodules. Diffuse uptake tends to accompany autoimmune or inflammatory thyroid disease, while focal uptake should be evaluated for thyroid disease due to a meaningful but nonspecific malignancy risk.The development of thyroid incidentalomas on cross-sectional and oncologic imaging has also broadened the scope of nuclear medicine. Diffuse FDG uptake is associated with inflammatory or autoimmune thyroid disease, while focal uptake will require further structural and functional evaluation. The clinician should not be tempted to associate FDG avidity with malignancy; rather, it is a metabolic fingerprint that should be integrated with sonographic appearance, cytology, and thyroid scintigraphy in low-TSH patients. (14-16)

### 5.2 SPECT/CT, Quantification, and Artificial Intelligence

Hybrid imaging extends the anatomical precision of conventional scintigraphy. SPECT/CT is particularly effective when planar studies show ambiguous localization, ectopic tissue is suspected, or functional and structural information must be integrated in the same examination. Quantitative SPECT/CT has shown promise in Graves disease and dosimetric applications by providing reproducible activity measures. Artificial intelligence approaches, especially deep learning models, are being explored as tools for automated classification and decision support in thyroid scintigraphy. (3,17,18)

**Table 5: Hybrid and emerging imaging modalities in benign thyroid diagnostics**

| Modality | Primary Advantage | Current Role in Benign Disease | Main Limitation |
|---|---|---|---|
| FDG PET/CT | Whole-body metabolic survey | Incidental thyroid uptake; selected nodules | Limited specificity |
| SPECT/CT | Improved localization | Ectopic tissue and equivocal planar foci | Greater complexity |
| Quantitative SPECT/CT | Potential reproducibility | Research and dosimetry | Limited standardization |
| AI-assisted analysis | Pattern recognition support | Emerging workflow support | Needs external validation |

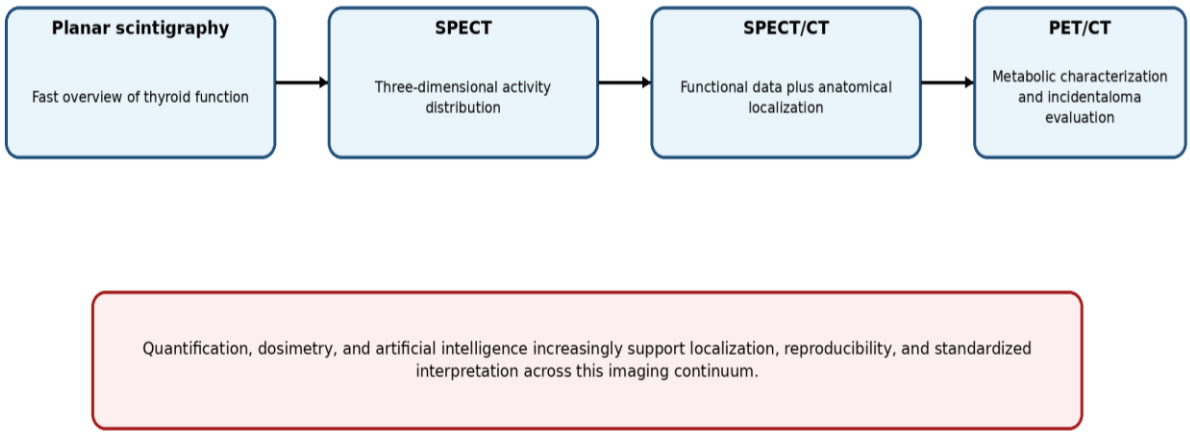


**Figure 4: Imaging continuum from planar scintigraphy to hybrid and quantitative imaging**

In summary, hybrid imaging and digital methods refine rather than replace conventional thyroid scintigraphy. Their practical importance becomes especially clear when diagnosis is linked to treatment with radioiodine.

**6. Diagnostic-Therapeutic Continuity and Radioiodine**

One of the distinguishing strengths of thyroid nuclear medicine is its theranostic continuity. The same iodine-handling pathway that allows diagnostic scintigraphy and uptake testing also permits targeted treatment with iodine-131. Scintigraphic pattern recognition helps define whether disease is diffuse or focal, while uptake data and clinical context assist in selecting an appropriate therapeutic strategy. (1,2,19)

Radioiodine therapy is well established for Graves disease, toxic multinodular goiter and toxic adenoma. The clinical objective can be different from one condition to the next, but the main idea is selective irradiation of iodine-avid thyroid tissue. Early adverse effects are related to follicle injury and transient hormone release, and long-term follow-up is concerned with detecting hypothyroidism and maintaining stable biochemical control. Eye disease in Graves disease is of particular interest because orbitopathy may worsen in susceptible individuals. (1,2,4,19)

Follow-up after radioiodine therapy is an important component of the diagnostic-therapeutic continuum. Clinical review and thyroid function tests are necessary to assess symptom control, detect transient hormone release after treatment, and identify late hypothyroidism. In toxic multinodular goiter and toxic adenoma, persistence or recurrence of hyperthyroidism may suggest residual autonomous tissue; in Graves disease the expected trajectory may be progression to hypothyroidism that requires lifelong levothyroxine replacement. Therefore, the theranostic approach does not end with treatment delivery; it extends into structured endocrine follow-up. (1,2,4,19)

In summary, the role of radioiodine therapy illustrates how diagnosis and treatment are linked by the same underlying thyroid physiology. Appreciation of this continuity sharpens awareness of the pitfalls and safety considerations that govern responsible use.

**7. Limitations, Pitfalls, and Safety Considerations**

Most pitfalls in thyroid nuclear medicine are understandable due to tracer biology or poor preparation. A history with iodinated contrast, iodine-rich medication or dietary supplements can lead to low radioiodine uptake and misleading results. Antithyroid drugs, levothyroxine treatment, and TSH suppression may influence the appearance of the gland's function. Technical artifacts may arise from motion, poor neck positioning, low count studies, or contamination from salivary and esophageal activity (3,7,8,11).

Radiation exposure from routine diagnostic studies is relatively small, but the principles of justification and optimization are still necessary. Pregnancy is an important contraindication to radioiodine procedures and lactation needs to be taken seriously. The most effective safeguard against error is multimodality integration: the scan should be interpreted along with serum TSH, thyroid hormone concentrations, iodine history, medication use, ultrasound findings and, where necessary, cytology. (3, 4, 8, 12)

**Table 6: Common causes of misleading thyroid nuclear medicine results**

| Source of Error | Typical Effect | Practical Response |
|---|---|---|
| Recent iodinated contrast | Depressed radioiodine uptake | Delay or reinterpret the study |
| Amiodarone or iodine-rich drugs | Low or unpredictable uptake | Review medication history |
| Antithyroid medication | Altered trapping and therapy planning | Use supervised withdrawal guidance |
| Levothyroxine or TSH suppression | Reduced endogenous stimulation | Interpret with current TSH |
| Salivary or esophageal activity | False focal uptake | Repeat images or use SPECT/CT |
| Motion or poor positioning | Apparent asymmetry or focality | Reposition and reacquire |
| Inappropriate reference range | False uptake classification | Use locally validated norms |

The principle of justification is really important for thyroid imaging as the thyroid gland is radiosensitive and most patients are young women. The radiation exposure from well-selected diagnostic studies is generally modest, no radionuclide test should be requested without a clear clinical question. When imaging does proceed, optimization of the administered activity, adherence to guideline-based protocols, and careful explanation to the patient enhance safety and quality. (3,11)

In summary, most limitations of thyroid nuclear medicine are manageable when the study is properly indicated, correctly prepared, and interpreted in clinical context. These practical constraints frame the future direction of the field.

**Future Directions and Conclusion**

In benign thyroid nuclear medicine, the future is less about creating new tracers than getting better at using the existing data. Quantitative SPECT/CT can increase reproducibility and open up new methods of dosimetry and functional phenotyping. Hybrid imaging will also further refine localization in ectopic tissue, retrosternal goiter, and nodular autonomy. Machine learning tools will be able to automate pattern recognition, quality control, and standardized reporting in centers with high imaging volume.

At the same time, new technology means clinical judgment is not less important. The lasting power of nuclear medicine in benign thyroid disease is that it can show you how the thyroid gland behaves (traps, organizes, releases, or metabolizes). And this functional perspective can serve as a complement to ultrasound and laboratory testing in a way that no purely structural approach will ever replace that of this kind of structural diagnosis. Thyroid nuclear medicine is a unique and clinically meaningful discipline that is still one in a class of its own because it puts physiology at the center of diagnosis.

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# FRONTIERS IN CONTRAST-ENHANCED ULTRASOUND (CEUS): EXPANDING CLINICAL APPLICATIONS AND FUTURE INNOVATIONS

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## 1. Introduction

Ultrasonography is an integral part of modern diagnostic medicine thanks to its portability, real-time imaging, low cost and freedom from ionizing radiation. Traditional B-mode imaging provides high resolution anatomic information in many superficial and abdominal organs, and Doppler imaging gives direction and velocity information for macroscopic blood flow. Both methods are less sensitive when the clinical question is based on very slow flow, capillary perfusion, small-vessel architecture, or subtle differences in vascular behaviour between tissues. Contrast-enhanced ultrasound (CEUS) was developed to address this gap by adding a blood-pool contrast agent that significantly enhances the acoustic signal from the vascular compartment.

The modern ultrasound contrast agents are stabilized gas-filled microbubbles of a few micrometres in diameter and are small enough to pass through the pulmonary circulation after peripheral intravenous injection and remain intravascular rather than in the interstitium. This is a fundamental feature that sets CEUS apart from most extracellular CT and MRI contrast agents and makes it especially suitable for tissue vascularity. Microbubbles under ultrasound field expand and contract in a nonlinear manner and produce harmonic and subharmonic echoes that can be detected by contrast-specific pulse sequences [1-3]. The result is a dynamic evolution of enhancement that can be observed from the arterial arrival of contrast through portal, venous and late stages in time. Large-scale clinical experience also provides a favorable safety profile [4].The clinical benefit of CEUS is not that vessels look brighter. Its greatest advantage is that it has temporal resolution. A focal lesion can be tracked as contrast enters, spills out into microvasculature and is washed away. These kinetic patterns are often more informative than one-dimensional images. In the liver, the approach is standardized enough to fit international practice guidelines and CEUS Liver Imaging Reporting and Data System (LI-RADS) for patients at risk of hepatocellular carcinoma (HCC) [5]. Outside the liver, other applications are assessment of renal masses, abdominal organ trauma, vascular disease, inflammatory complications, pancreatic and splenic abnormalities, endocavitary studies as well as a few pediatric applications [6].

## 2. Foundations of Contrast-Enhanced Ultrasound

### 2.1 Microbubble Physics and Nonlinear Signal Generation

Microbubbles are quite efficient acoustic scatterers since gas is very compressible compared to blood and tissue. Even at very low pressure, the volume of a microbubble oscillates in response

to the compression and rarefaction of the ultrasound wave. The oscillation is nonlinear in the sense that the echo is filled with frequencies not present (or only weakly present) in the transmission pulse. Contrast-specific imaging modes can take advantage of this difference by pulse inversion or amplitude modulation or other multipulse techniques to cancel linear tissue echoes while keeping the nonlinear signal from microbubbles. Tissue suppression provides a rather dark background against which even small concentrations of microbubbles can be observed.

Mechanical index (MI) is an important measure of acoustic output with respect to ultrasound frequency. CEUS is usually performed with a low MI to prevent premature destruction of microbubbles and to detect the enhancement. If the acoustic pressure is deliberately increased, microbubbles could be disrupted. However, this is not always undesirable; for example, a high-energy flash from the imaging plane can clear the contrast and replenishment kinetics can provide information about perfusion.

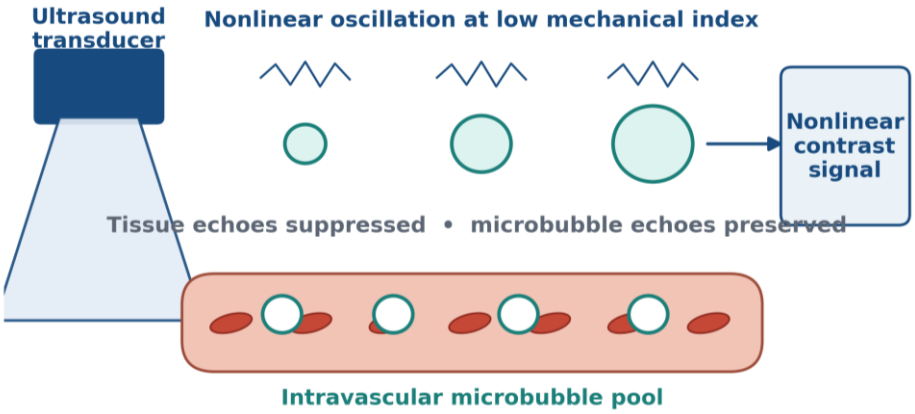


**Figure 1: Acoustic basis of CEUS: low-mechanical-index insonation preserves intravascular microbubbles while contrast-specific pulse sequences isolate their nonlinear echoes**

### 2.2 Microbubble Contrast Agents and Safety

Clinically used ultrasound contrast agents are suspended gas-filled microbubbles stabilized by phospholipid, lipid, or protein shells. Their erythrocyte-scale size and transport route allow them to escape the vessel after peripheral injection while remaining in the vascular compartment. With poorly soluble gases such as sulfur hexafluoride and perfluorocarbons, they continue to persist; shell composition contributes to stability and acoustic properties. Product availability and labeled indications vary geographically; perfluorobutane has a delayed Kupffer cell phase in the liver when approved [1-3].

The safety of microbubble agents is very good, but it should not be regarded as an absence of risk. A large multicenter study of 463,434 examinations with sulfur hexafluoride microbubbles found that the overall adverse event rate is 0.034%, and that very few serious reactions occur [4]. It is safe to screen for previous hypersensitivity, adhere to product-specific contraindications, have trained personnel, and have resuscitation equipment at hand immediately. Biological effects

of ultrasound depend on acoustic pressure, exposure duration, and local tissue conditions, and the principle of the lowest acoustic output is still in place for diagnosis.

**Table 1: Representative microbubble contrast agents used in contemporary ultrasound practice**

| Agent / Trade Name | Gas Core | Shell | Distinctive Practical Feature |
|---|---|---|---|
| Sulfur hexafluoride (SonoVue/Lumason) | SF6 | Phospholipid | Widely used for abdominal CEUS; purely intravascular vascular-phase behavior |
| Perflutren lipid microspheres (Definity/Luminity) | C3F8 | Lipid | Established ultrasound-enhancing agent, especially in echocardiography |
| Perflutren protein-type A (Optison) | C3F8 | Human albumin | Cardiac chamber opacification and endocardial border enhancement |
| Perfluorobutane (Sonazoid) | C4F10 | Phospholipid | Vascular phases plus delayed Kupffer-cell phase in the liver |

### 2.3 Image Acquisition, Mechanical Index, and Contrast Phases

Bolus administration is common in routine diagnostic CEUS. Immediately after injection and saline flush, the operator keeps the lesion or organ of interest in its field of view and starts a timer. In the liver it is often interpreted according to arterial, portal venous and late phase and the precise timing depend on the cardiac output, injection technique and agent. The behavior of the arterial phase is crucial to determine hypervascularity and vascular architecture and washout is measured by comparing lesion enhancement with surrounding parenchyma over time [5]. Continuous infusion may be used if the contrast concentration is stable, most notably in perfusion quantification, but is not adopted for clinical lesion characterization.

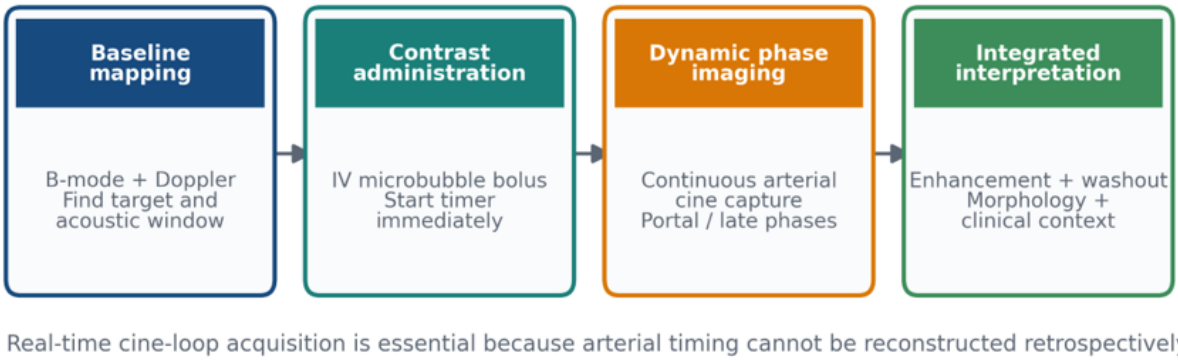


**Figure 2: Practical CEUS examination sequence from baseline mapping through timed dynamic acquisition and integrated interpretation**

Image optimization is essential. The region of interest should be adequately visualized on baseline ultrasound before contrast injection, focal depth should be appropriate, gain should be set to suppress background tissue noise without losing weak microbubble signal and the transducer should be as stable as possible during dynamic imaging. Excessive acoustic output

can destroy microbubbles and create false washout while a very low signal level can obscure subtle enhancement. Repeated sweeps through an organ may be necessary, but continuous observation of a suspicious lesion is preferable during diagnostically important phases [3, 5].

**Table 2: Fundamental CEUS phases and their interpretive value**

| Phase | Approximate Timing* | Main Information Obtained |
|---|---|---|
| Arterial | about 10-30 s | Arterial vascularity, enhancement intensity, vessel architecture and early hyperenhancement |
| Portal venous | about 30-120 s | Persistence of enhancement and onset of washout relative to background parenchyma |
| Late | beyond about 120 s | Degree and timing of washout; residual lesion conspicuity |
| Postvascular / Kupffer | agent dependent | Delayed parenchymal uptake with selected agents such as perfluorobutane |

## 3. Enhancement Kinetics as a Dynamic Tissue Biomarker

The contrast pattern of a lesion is better understood as a sequence rather than a single appearance. The first question to be answered is whether enhancement is absent or low, similar or larger than the surrounding reference tissue and the second is when that relationship changes. If a lesion is hyperenhancing during arterial inflow and later isoenhancing, then it will wash out gradually until it is clearly hypoenhancing. The timing and intensity of washout often take more weight than enhancement at one particular point. Hence, continuous observation is the difference between CEUS and techniques where only a few pre-determined post-contrast phases are sampled [3, 5].

Interpretation of enhancement architecture is also necessary. Peripheral nodular enhancement, spoke-wheel arterial filling, rim enhancement, chaotic intralesional vessels, nonenhancing necrosis, and clearly defined perfusion defects are all manifestations of different biological processes. These vascular signatures are never interpreted in isolation because in chronic cancer, the lesion morphology, patient age, background organ disease, cancer history, and laboratory results, as well as the pretest probability of malignancy, determine the meaning of the CEUS pattern. In a cirrhotic liver, a normal kidney, and a recently traumatized spleen, the same enhancement may have different meaning.

## 4. Clinical Applications of CEUS

CEUS is currently in use in many organ systems but the diagnostic task varies from clinical setting to clinical setting. In some cases the lesion characterization is the key, in others perfusion detection, active bleeding, tissue viability assessment, treatment response or guidance of an intervention. The method is best when the expected vascular behavior can be compared to a known reference tissue in real time. The non-hepatic guidance of international organizations

emphasizes that the indication, technical approach and interpretation should be organ specific rather than being reduced to a single rule [6].

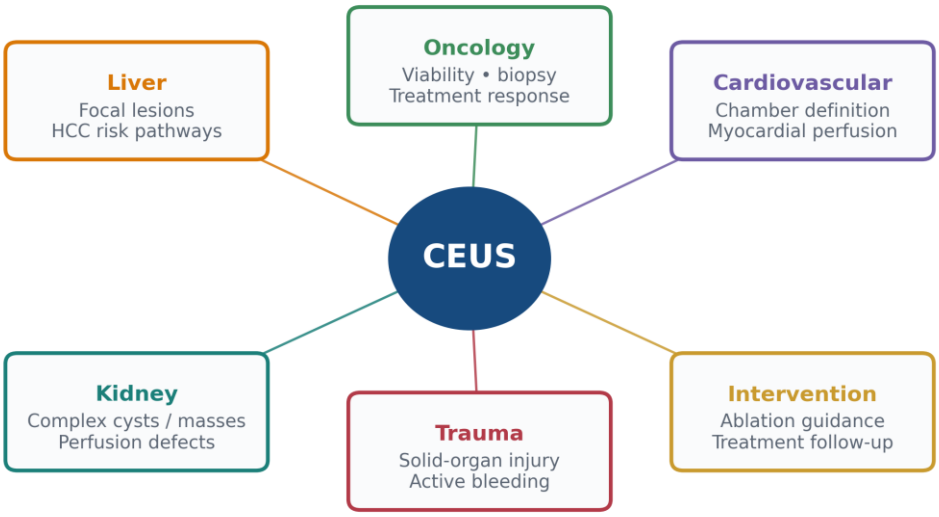


**Figure 3: Major clinical domains in which CEUS contributes focused information about tissue perfusion, lesion vascularity, bleeding, or treatment response**

### 4.1 Hepatic Imaging

The liver is the most validated organ of CEUS. This dual blood supply creates diagnostically helpful temporal phases: lesions can be assessed during arterial inflow (compared to portal venous enhancement) and later washout detected. WFUMB and other societies agree on the terminology and interpretation of focal liver lesions (FLLs) including benign lesions, HCC, and non-HCC malignancies [5]. It is advantageous to monitor enhancement continuously because the timing and degree of washout may help to distinguish lesions that look similar on baseline gray-scale ultrasound.

A typical hemangioma typically exhibits peripheral, discontinuous nodular enhancement with progressive centripetal fill-in. Frequent focal nodular hyperplasia usually exhibits rapid arterial hyperenhancement (sometimes a spoke-wheel vascular pattern), and thus persistent isoenhancement. Malignant lesions typically show washout more frequently, but the onset and intensity of washout are often different. In HCC, arterial phase hyperenhancement followed by late-onset and mild washout are common patterns in the appropriate high-risk population, whereas early or marked washout is seen in non-HCC malignancy (metastasis or intrahepatic cholangiocarcinoma) [5, 7-9].

CEUS LI-RADS has standard categories for observations in patients at risk for HCC. The system is capable of describing basic features such as arterial phase hyperenhancement and washout behavior, which reduce ambiguity in reporting and align with clinical practice in liver imaging [8]. Validation studies have shown good diagnostic performance, especially for small nodules of 20 mm or less, although no imaging algorithm completely eliminates clinical context or multidisciplinary review [9]. CEUS can also clarify indeterminate observations detected on CT or MRI, particularly when arterial timing on cross-sectional imaging was not optimal.

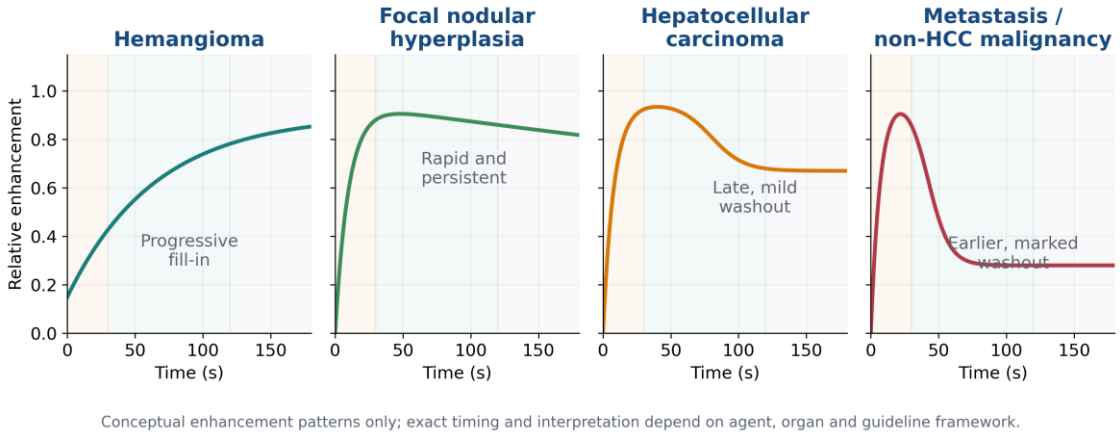


**Figure 4: Conceptual enhancement patterns of selected focal liver lesions. The curves illustrate typical tendencies and are not diagnostic thresholds**

### 4.2 Oncology and Image-Guided Intervention

CEUS is important in cancer diagnosis, biopsy planning, treatment and response. Tumors have vascular networks that vary in vessel density, structure, permeability, flow rate and response to therapy. Microbubbles are intravascular and so CEUS gives the view of the perfused vascular compartment. Hyperenhancement might be due to the increased vascularity of the tumor compared to the normal tissue and heterogeneous or not improved tissue can be a sign of necrosis, fibrosis or poorly perfused tissue [10]. Common grayscale imaging does not provide clear clues to the part of a complex mass that can be incorporated into the microbubble [10].

**Table 3: Typical CEUS patterns of selected focal liver lesions**

| Lesion | Arterial Phase | Portal / Late Phase | Interpretive Emphasis |
|---|---|---|---|
| Hemangioma | Peripheral discontinuous nodular enhancement | Progressive centripetal fill-in; often persistent enhancement | Characteristic benign vascular filling pattern |
| Focal nodular hyperplasia | Rapid homogeneous or spoke-wheel hyperenhancement | Usually isoenhancing without significant washout | Benign hypervascular lesion pattern |
| Hepatocellular carcinoma | Arterial phase hyperenhancement, often non-rim | Typically late and mild washout in appropriate high-risk patients | Use structured CEUS LI-RADS criteria |
| Metastasis | Rim or heterogeneous arterial enhancement may occur | Earlier and/or marked washout common | Washout strongly supports malignancy |
| Intrahepatic cholangiocarcinoma | Peripheral or heterogeneous arterial enhancement | Often early or marked washout | Can mimic HCC if timing and washout quality are ignored |

One of the most practical oncologic applications is intervention. A lesion on non-enhanced ultrasound without a visible lesion on CEUS may be visible enough to enable the biopsy needle or ablation applicator to focus on viable enhancing tissue and away from necrotic areas. CEUS can be performed as part of a procedure with no cumulative radiation and therefore can be used to confirm coverage of treatment and to assess residual enhancement [11]. Not only diagnostic but also procedural and can shorten the time interval between diagnosis and intervention for some cases.

**4.3 Cardiovascular and Vascular Imaging**

Ultrasound enhancement agents have been available since the beginning of echocardiography. By enhancing left ventricular opacification and endocardial border definition, contrast can increase confidence in chamber assessment when baseline acoustic windows are poor. As proposed in the 2018 American Society of Echocardiography guideline update, these applications could include better assessment of ventricular volumes and ejection fraction, detection of intracardiac abnormalities such as apical thrombus, and chosen use in stress echocardiography and myocardial perfusion [12]. The concept is somewhat different from abdominal CEUS, but the agent physics is the same: circulating microbubbles can produce a contrast signal in the cardiovascular blood pool. CEUS has vascular applications in atherosclerotic disease and is able to better delineate the lumen and plaque-induced neovascularity [13].

Myocardial contrast echocardiography can assess perfusion at the microvascular level. Once a microbubble is destroyed by a high-energy impulse, the rate and extent of replenishment in myocardium is the key to microvascular blood flow and it is relevant to ischemic heart disease, but this is not the case in all regions and laboratories. Contrast could also enhance stress echocardiography by providing a better visualization of the endocardial border, resolution of wall motion, and perfusion information in experienced centers [12].

**4.4 Trauma and Emergency Imaging**

CEUS is particularly useful in trauma imaging for focused evaluation of solid organs— liver, spleen, kidneys. A normally perfused organ is likely to be enhanced homogeneously whereas laceration, contusion, infarction or devascularized tissue is likely to be a focal or geographic nonenhancing defect. Active bleeding is called extravasation or progressive pooling of microbubbles outside the expected vascular boundaries. Pseudoaneurysms and other vascular complications can also be dynamically shown. These capabilities enable CEUS to provide functional information that traditional B-mode ultrasound and standard FAST examination cannot reliably provide [6, 14].

CT is still the most popular reference for many adults who suffer high-energy polytrauma, as it provides a comprehensive assessment of the whole body, anatomical images of bones and deep structures and surveys multiple compartments at once. CEUS is therefore complementary, not competitive. It is particularly desirable for hemodynamically stable patients who need targeted follow-up, for children who need radiation reduction and when a solid organ injury that was

previously reported needs to be assessed in a serial manner without repeated CT. Pediatric trauma literature has shown the ability to detect and follow abdominal organ injury with CEUS and international evidence supports its use in appropriate patients [14].

**4.5 Renal, Breast, Pancreatic, Splenic, and Pediatric Applications**

Renal imaging is the more established non-hepatic application. Microbubbles are particularly useful when the clinical question is to enhance a cyst wall, septum, mural nodule or solid component. CEUS can detect very small amounts of vascularity without exposing the patient to iodinated or gadolinium-based contrast, which is particularly important in patients with renal impairment. Studies of histopathologic validation have shown high diagnostic utility for renal mass assessment, but classification frameworks and local regulatory indications differ [15]. CEUS also helps in discriminating perfused and nonperfused parenchyma, renal infarction or trauma and select vascular complications.

**Table 4: Selected clinical applications of CEUS across organ systems**

| Organ / System | Focused Clinical Question | Primary CEUS Contribution | Important Limitation |
|---|---|---|---|
| Liver | What is the vascular phenotype of a focal lesion? | Continuous arterial enhancement and washout characterization; structured HCC pathways | Requires an adequate acoustic window and target visualization |
| Kidney | Does a cyst wall, septum, nodule, or solid component enhance? | Sensitive detection of microvascular enhancement without renal excretion of contrast | Deep location, calcification, or bowel gas may obscure lesions |
| Trauma | Is solid-organ tissue perfused and is there active bleeding? | Depicts nonperfused injury, pseudoaneurysm, and microbubble extravasation | Does not replace whole-body CT in unstable or high-energy polytrauma |
| Oncology / intervention | Where is viable tumor and has treatment eliminated perfusion? | Biopsy targeting, ablation guidance, early perfusion response | Target must remain sonographically accessible |
| Cardiovascular | Are chamber borders or myocardial perfusion inadequately seen? | Improves endocardial definition and selected perfusion assessment | Requires dedicated expertise and indication-specific protocols |
| Breast / other superficial organs | Can vascular behavior refine an indeterminate ultrasound finding? | Adjunct characterization and treatment-response information | Usually complementary to established multimodality pathways |

Breast CEUS is an evolving adjunct, not a replacement for mammography, ultrasound and MRI. Malignant breast lesions have disordered neovascularity and rapid enhancement, heterogeneous perfusion, penetrating vessels and other differences from benign masses. Reviews have pointed towards the potential for lesion characterization, response to neoadjuvant therapy and selection of viable tissue for biopsy [16]. However, protocol variation, overlap of benign and malignant enhancement patterns and performance of conventional breast imaging have made CEUS a selective and complementary method.

**5. Quantitative CEUS and Perfusion Analysis**

CEUS visual interpretation is very effective in many applications, but still has to depend on the observer experience and qualitative judgment. Quantitative CEUS (qCEUS) attempts to convert dynamic enhancement into numerical measures of perfusion by placing a region of interest in the target tissue and tracking it over time to obtain a time-intensity curve. Depending on the acquisition and model, parameters may include arrival time, time to peak, peak enhancement, wash-in slope, area under the curve, mean transit time and washout rate [10]. These are surrogate variables for tissue blood volume, blood flow and vascular transit that do not include histologic measurements.

The acquisition should be standardized for qCEUS to work. Bolus volume and injection speed affect peak concentration, cardiac output determines arrival time, respiratory motion can shift the lesion to the area of interest, acoustic output affects microbubble destruction and signal intensity may not be directly comparable between machines and software packages. Motion compensation and normalization to a reference tissue can help with reproducibility, but it doesn't eliminate all other sources of variation. Therefore, qCEUS is most robust when used in longitudinal comparison between patients, in a very matched way, rather than an isolated one-way measurement.

**Table 5: Common quantitative CEUS parameters and physiologic interpretation**

| Parameter | What it Represents | Typical Clinical or Research Use |
|---|---|---|
| Arrival time | Interval from injection to detectable enhancement | Transit delay and timing of vascular inflow |
| Time to peak | Time from arrival or injection to maximum signal | Speed of regional perfusion |
| Peak enhancement | Maximum signal intensity in the region of interest | Relative blood-volume-related enhancement |
| Wash-in slope | Rate of signal increase during inflow | Relative perfusion and microvascular inflow |
| Area under the curve | Integrated signal over a defined interval | Composite measure of enhancement exposure |
| Mean transit time | Average duration of microbubble passage through tissue | Microvascular transit behavior |
| Washout rate | Rate of signal decline after the peak | Retention and clearance pattern |

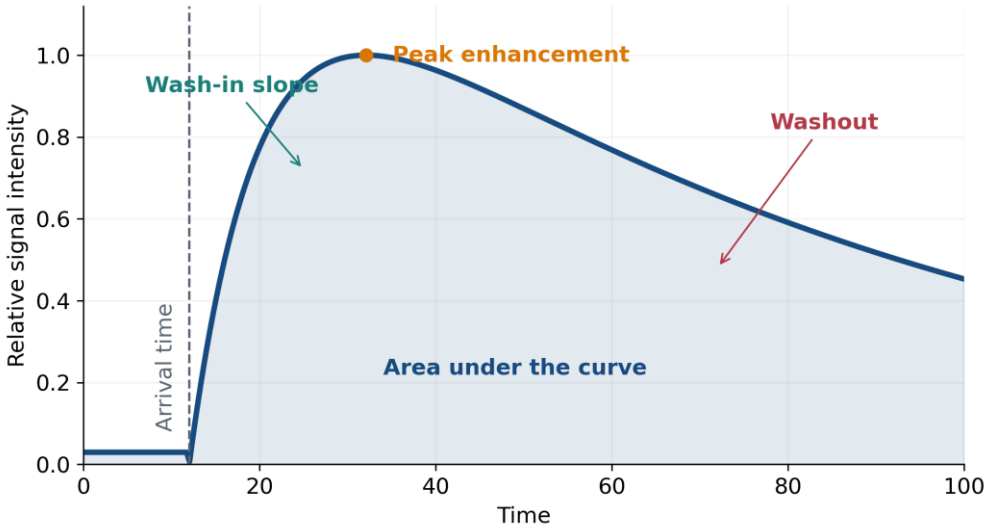


**Figure 5: Schematic time-intensity curve illustrating commonly derived quantitative CEUS perfusion parameters**

The time-intensity curve is the one used in most qCEUS applications. After correction for motion and selection of lesion and reference region, the signal intensity is plotted against time. The rising part shows the difference between arrival and wash-in, the peak is the maximum recorded enhancement, and the descending part is the washout or redistribution. Destruction-replenishment techniques use a short high energy pulse to destroy microbubbles in the imaging plane and then study the rate at which circulating bubbles refill the tissue. If well controlled, these techniques can provide physiologic information. However, they are system- and protocol-dependent and are not interchangeable [10].

**6. CEUS in Intervention and Treatment Monitoring**

The vascular information used in CEUS is also in real-time to improve targeting. A mass that is indistinct on gray-scale ultrasound could be discernible during arterial enhancement and the operator can target a needle to the right type of tissue. This is particularly important for chemotherapy, radiotherapy, ablation or spontaneous necrosis where a lesion may have perfused and nonperfused components. CEUS can be combined with fusion imaging so that a target defined on CT or MRI is co-registered with real-time ultrasound to minimize sampling of an unrepresentative area [11].

After thermal ablation, the treated zone should not show any internal enhancement after devascularization. If nodular or irregular enhancement remains outside the zone, it is a concern for a viable tumor and might be possible to return immediately to the same session depending on the procedure and local protocol. CEUS can be performed without ionizing radiation and without renal excretion of contrast so it is well suited for a short-interval procedural reevaluation. Post-treatment gas, acoustic shadowing, deep targets and very large treatment zones may in general reduce visualization at first so CEUS should be evaluated within the whole multimodality follow-up approach [5, 11].

## 7. Advances and Innovations in CEUS

### 7.1 Improved Acquisition, Fusion Imaging, and Three-Dimensional CEUS

Three dimensional and matrix-array CEUS can reduce dependence on a single imaging plane and fusion imaging co-registers live ultrasound with prior CT, MRI or PET to better locate lesions. Motion compensation and automated tracking are particularly important for quantitative analysis because respiratory displacement can move a region of interest away from the target. These capabilities are being combined into integrated acquisition, navigation, quantification and reporting workflows with standardized service development and operator training [17].

### 7.2 Artificial Intelligence and Machine Learning

AI is ideally suited for CEUS because the analysis leads to high-dimensional spatiotemporal information. A human observer generally incorporates some of this information: the time of enhancement, the degree of enhancement, the washout, the structure of lesions and comparison with background tissue. Machine learning systems can in principle analyze complete cine loops, extract quantitative temporal information, detect subtle textural features and incorporate these into clinical aspects. Such techniques could reduce interobserver variability and support less experienced operators without requiring expert involvement.

A recent systematic review of AI for liver CEUS found 41 original papers published through early 2024, most of them focusing on the classification of focal liver lesions. Although the accuracies were generally high, the review also noted that the algorithm has some limitations: hand-selected images, relatively small or single-center cohorts, limited external validation and considerable heterogeneity in methodology [18]. An algorithm that does well on a curated set of images from one scanner may not work for multiple pieces of equipment, acquisition settings, patient populations or lesion prevalence.

### 7.3 Targeted Molecular Imaging and Theranostic Microbubbles

The initial ultrasound contrast agents were unstable and destroyed very quickly. Poorly soluble gases and stabilizing shells now contribute to intravascular persistence and a robust nonlinear response at clinically acceptable acoustic pressures [1, 2]. The next step is functionalization. By attaching ligands to the microbubble surface, researchers can design agents to attach to endothelial targets for angiogenesis, inflammation, thrombosis, or other disease processes. Molecular ultrasound therefore can assess not only where blood flows but also which vascular biomarkers are expressed [19].

Microbubbles can interact with vascular walls and cell membranes when they are exposed to appropriate ultrasound fields. Stable oscillation can induce microstreaming and shear forces, while more energetic cavitation may temporarily increase membrane permeability. This phenomenon, commonly known as sonoporation, has stimulated a lot of research into ultrasound-assisted delivery of drugs, genes, nanoparticles, and other therapeutic payloads [19, 20]. The attraction is spatial control: the therapeutic effect can be concentrated where the ultrasound beam is applied, potentially increasing local exposure but reducing systemic toxicity.

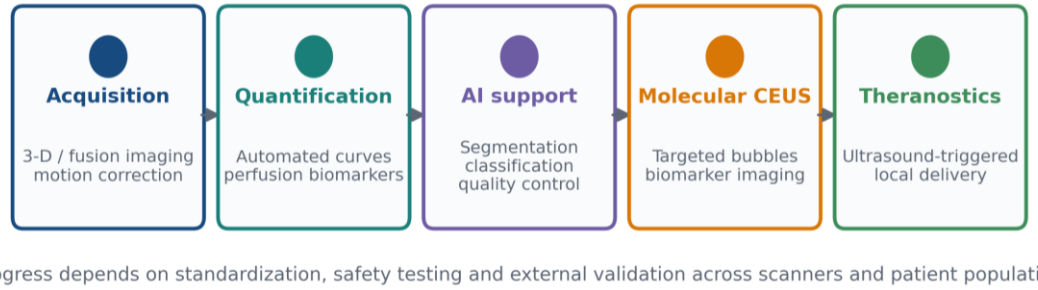


**Figure 6: Converging technological directions in CEUS, from improved acquisition and quantification to AI-supported interpretation, molecular targeting, and theranostic delivery.8. Limitations, Standardization, and Quality Assurance**

The benefits of CEUS are great but it has its limitations that need to be taken into account. The most important is operator dependence. The quality of diagnostic information depends on probe location, target location in the imaging plane, injection timing, acoustic output, patient respiration, and the ability of the observer to spot enhancement patterns in real time. Unlike CT or MRI, which can easily be reviewed in retrospect, a poorly acquired CEUS arterial phase cannot always be reconstructed after the event. Training, protocol discipline, cine-loop storage, and quality assurance are essential [3, 17].

**Table 6: Major limitations of CEUS and practical strategies to reduce their impact**

| Limitation | Why it Matters | Practical Response |
|---|---|---|
| Operator dependence | Acquisition and interpretation occur in real time | Formal training, structured protocols, cine storage, double reading during learning |
| Restricted acoustic window | Depth, obesity, ribs, bowel gas, lung, or dressings may obscure target | Optimize patient position and transducer; use CT/MRI when the target is inaccessible |
| Brief arterial opportunity | Poor timing can miss decisive enhancement | Prepare target before injection, start timer immediately, record uninterrupted cine |
| Platform variability | Proprietary pulse sequences and analysis software alter quantitative values | Use standardized local settings and avoid assuming cross-platform equivalence |
| Limited whole-body survey | CEUS is a focused examination | Use CT or MRI for comprehensive staging or high-energy polytrauma |
| Regulatory variability | Approved agents and indications differ geographically | Follow local product labeling, institutional policy, and current professional guidance |

Standardization remains imperfect. Different contrast agents have different physical properties and regulatory indications. Scanner manufacturers use proprietary algorithms and display scales. Quantitative values can vary with acoustic output, gain, depth, transducer, bolus technique, and software model. This is especially true for qCEUS and AI, where a model trained on one platform may not be transferable to another. International guidelines provide a common conceptual framework, but local protocols still need validation and internal quality control [5, 6].

Acoustic access has a physical limitation that no contrast agent can completely overcome. Ultrasound attenuation increases with depth and bowel gas, ribs, lung, obesity, dressings, wounds, and patient immobility may prevent adequate visualization. Deep retroperitoneal structures or lesions more than several centimeters from the transducer can be challenging and complete staging of malignancy usually requires cross-sectional imaging. CEUS should be selected when the target is accessible and the clinical question is focused instead of reflexively as a universal replacement for CT or MRI.

Quality assurance should include both operator technique and system performance. A CEUS service is backed by documented injection protocols, consistent mechanical-index settings, standardized phase timing, cine-loop storage, organ-specific terminology, and training in artifact recognition and emergency preparedness. Local audit is useful for the most part because many failures are due to the poor visualization of the target, incorrect timing, and/or suboptimal settings rather than the contrast agent itself [3, 17].

**9. CEUS within Contemporary Imaging Pathways**

The way to position CEUS is not to ask whether it is better than CT or MRI in general but if it will answer a specific clinical question faster and safely. CEUS has real-time temporal resolution in continuous time, can be performed at the bedside, is repeatable, and can provide direct blood-pool information without ionizing radiation. CT is fast to get coverage of the whole body, a good spatial view and access to deep structures in detail. MRI has soft tissue contrast, multiparametric characterization and whole organ assessment without ionizing radiation, but it is more expensive and may be restricted by motion, patient tolerance, implants or contrast.

**Table 7: Practical comparison of CEUS, contrast-enhanced CT, and contrast-enhanced MRI**

| Feature | CEUS | CT | MRI |
|---|---|---|---|
| Temporal sampling | Continuous real-time observation | Discrete phases, rapid whole-body coverage | Discrete or dynamic phases; longer acquisition |
| Ionizing radiation | No | Yes | No |
| Contrast behavior | Intravascular microbubbles | Predominantly extracellular iodinated agent | Agent dependent; extracellular or hepatobiliary |
| Bedside availability | High when equipment/expertise available | Low | Very low |
| Deep / whole-body survey | Limited by acoustic window | Excellent | Excellent but slower |
| Intervention guidance | Excellent for sonographically accessible targets | Useful but radiation-based | Limited for routine real-time guidance |
| Best conceptual role | Focused perfusion, lesion characterization, intervention, repeat follow-up | Comprehensive anatomy, trauma, staging | Multiparametric soft-tissue and whole-organ characterization |

The absence of ionizing radiation and the non-reaction of microbubbles in the renal system are very attractive for serial examinations or iodinated contrast exposure is unwanted. CEUS does not alleviate the need for clinical triage at all. A deeply positioned lesion that cannot be visualized on baseline ultrasound cannot be rescued by contrast enhancement, and a patient who needs whole-body staging still prefers CT or MRI. The best answer is to use all of these modalities to answer the question, and CEUS is often a bedside, follow-up or interventional procedure [5, 6].

**10. Future Perspectives**

The next decade of CEUS development will probably be one of convergence. Hardware improvements will increase sensitivity to weak contrast signals at depth; three-dimensional and matrix-array techniques might reduce dependence on a single imaging plane; fusion imaging will bring the real-time benefits of ultrasound with previously acquired CT or MRI; and automated motion correction will improve quantitative analysis. At the same time, structured reporting systems and training standards will make performance more reproducible across institutions.

AI will play an important role in the acquisition and interpretation. In future systems, rather than just classifying an individual lesion after images are taken, it will be possible to provide live feedback on whether the arterial phase was captured well, whether the lesion was in the field of view, and if it is scientifically appropriate for quantification. Algorithms can automatically determine arrival time, segment the lesion by respiration, compare enhancement with reference tissue and suggest diagnostic categories. The scientific challenge is to show that scanners, operators, patient populations and disease prevalence can be generalized [18].

A more advanced future for CEUS will depend on the convergence of technology and standards. Better transducers, automated quality assessment, externally validated AI and clinically tested molecular or therapeutic agents must preserve the bedside simplicity of ultrasound but add reproducible quantitative information and demonstrable patient benefit [18-20].

**11. Chapter Summary**

Contrast-enhanced ultrasound has become a clinically significant extension of ultrasonography that allows bedside access and continuous visualization of the microvascular compartment. It has the best evidence in liver imaging, but there is also much to offer in renal lesions, trauma, oncology, cardiovascular imaging, pediatric practice, treatment monitoring, and image-guided intervention [5, 6, 8]. CEUS should be viewed as a regular physiologic assessment. The target should be visible, the acoustic window sufficient, the mechanical index low, and the cine acquisition uninterrupted in the relevant stages. Next, it can be interpreted as enhancement architecture and washout with respect to the tissue. Quantification, AI, targeted agents, and theranostic microbubbles can be further improved, so long as standardization is considered safe and externally proven.

**Conclusion**

Contrast-enhanced ultrasound (CEUS) is rapidly becoming the next major development in conventional ultrasonography based on real-time monitoring of tissue perfusion and microvascular architecture and trends with high performance and high accuracy and flexibility

with low radiation exposure. Increasingly, contrast-enhanced ultrasound in hepatic imaging, oncology, cardiovascular assessment, trauma, renal and breast imaging and image-guided treatment is being used in the clinic as well as within health care. Although the limitations like operator dependence, limited penetration in deep structures, short contrast duration, protocol and regulatory issues remain, technological advances in quantitative perfusion analysis, targeted and theranostic microbubbles, artificial intelligence and interpretation of the image are constantly improving CEUS and expanding the diagnostic scope. With the standardization and expansion of its use and the introduction of a more reliable and data-rich imaging system into clinical practice, CEUS will be a very important tool in precision imaging, treatment monitoring and patient-centered diagnosis.

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# TUBERCULOSIS: A PERSISTENT GLOBAL HEALTH CHALLENGE WITH A SPECIAL FOCUS ON INDIA

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## 1. Introduction

Tuberculosis (TB) remains one of the most important infectious diseases affecting global health. It is caused predominantly by *Mycobacterium tuberculosis*, an airborne bacterium that most frequently affects the lungs but may involve almost any organ, including the lymph nodes, bones, kidneys, meninges, and other tissues. Pulmonary and laryngeal TB can be transmitted from person to person through the air when an infectious individual coughs, speaks, sneezes, sings, or otherwise generates respiratory aerosols. The risk of transmission is particularly high in crowded, poorly ventilated environments [1].

TB is especially challenging because it is both preventable and curable, yet it continues to cause substantial morbidity and mortality. In 2024, an estimated 10.7 million people developed TB globally and approximately 1.23 million people died from the disease, including about 150,000 people living with HIV [1,2]. The persistence of TB reflects not only biological factors but also delayed diagnosis, treatment interruption, antimicrobial resistance, undernutrition, poverty, stigma, comorbidities, and unequal access to healthcare.

The global response to TB therefore requires more than effective antimicrobial therapy. It requires timely diagnosis, uninterrupted treatment, preventive interventions, social protection, strengthened health systems, community participation, and sustained investment in research and innovation. This chapter reviews the contemporary global TB burden, with particular emphasis on India, and examines the epidemiological, biological, socioeconomic, diagnostic, therapeutic, and programmatic factors that continue to influence TB control.

## 2. The Global Burden of Tuberculosis

According to the World Health Organization (WHO) *Global Tuberculosis Report 2025*, approximately 10.7 million people developed TB in 2024. This included an estimated 5.8 million men, 3.7 million women, and 1.2 million children and young adolescents. The estimated global incidence was 131 cases per 100,000 population [1]. Approximately 5.8% of incident TB cases occurred among people living with HIV [1].

TB caused an estimated 1.23 million deaths in 2024, including approximately 1.08 million deaths among people without HIV and 150,000 deaths among people living with HIV [2]. Although mortality has declined from the pandemic-affected period, the pace of reduction remains insufficient to achieve the targets established under the WHO End TB Strategy [2,3].

The geographical distribution of TB is highly unequal. In 2024, approximately 34% of incident TB cases occurred in the WHO South-East Asia Region, followed by 27% in the Western Pacific Region and 25% in the African Region [1]. Thirty high-TB-burden countries accounted for approximately 87% of all incident cases. Eight countries—India, Indonesia, the Philippines, China, Pakistan, Nigeria, the Democratic Republic of the Congo, and Bangladesh—collectively accounted for approximately two-thirds of the global total [1].

India represented approximately one-quarter of the global TB burden in 2024, making its national TB programme critically important to global progress [1]. Encouragingly, the global TB incidence rate decreased by approximately 1.7% between 2023 and 2024. Nevertheless, the cumulative reduction in incidence between 2015 and 2024 was only about 12%, considerably below the 50% reduction that had been established as the 2025 milestone of the End TB Strategy [1,3].

The WHO End TB Strategy aims for an 80% reduction in TB incidence and a 90% reduction in TB mortality by 2030 compared with 2015 levels, together with elimination of catastrophic costs for TB-affected households [3]. Current trends indicate that substantially accelerated action will be required to reach these objectives.

**3. Tuberculosis in India**

India has the largest absolute burden of TB worldwide and therefore occupies a central position in the global TB response. Estimating the country's TB burden is complex because not every person with TB is diagnosed or notified through routine surveillance. WHO therefore uses a country-specific estimation approach incorporating information from India's national TB prevalence survey, case notifications, mortality data, and other relevant sources [1,4].

There is evidence of meaningful progress. WHO estimates indicate that India's TB incidence rate declined from approximately 237 cases per 100,000 population in 2015 to 195 per 100,000 in 2023, representing a reduction of approximately 17.7%. During the same period, estimated TB mortality declined from 28 to 22 deaths per 100,000 population, a reduction of approximately 21.4% [5].

India's response is coordinated through the National Tuberculosis Elimination Programme (NTEP), which replaced the Revised National Tuberculosis Control Programme (RNTCP) in 2020. The national strategic approach has been organised around four broad pillars: **Detect, Treat, Prevent, and Build** [6,7]. These pillars encompass early diagnosis, appropriate treatment, prevention of disease and transmission, community engagement, health-system strengthening, digital surveillance, and social support.

The programme has also intensified engagement with private healthcare providers. This is particularly important because a substantial proportion of people with TB seek care outside government facilities. In 2023, the private sector contributed approximately 32% of India's total TB notifications, reflecting substantial progress in public-private collaboration and notification practices [8]. National reporting subsequently recorded more than 25.5 lakh TB notifications in 2024, demonstrating continued improvement in case detection and reporting [9].

The Indian strategy has increasingly emphasised active case finding and reaching populations that may otherwise remain outside routine services. In December 2024, the Government of India launched a 100-day intensified TB elimination campaign covering 347 districts in 33 states and union territories. The campaign focused on active case finding, improved access to diagnostic services, nutritional support, and reduction of diagnostic delays [5,10].

Although India had set an ambitious national goal of TB elimination by 2025, the persistence of substantial disease burden demonstrates that elimination requires sustained action beyond a single target year. Continued progress will depend on maintaining political commitment, strengthening primary healthcare, improving case detection, ensuring treatment completion, and addressing the social determinants of TB.

## 4. Risk Factors and Underlying Determinants

TB disease is determined by an interaction between exposure to *M. tuberculosis*, host immunity, nutritional status, comorbidities, and environmental and socioeconomic circumstances. Although approximately one-quarter of the world's population is estimated to have been infected with TB bacteria, only a minority of infected individuals develop TB disease during their lifetime. The risk is substantially higher among people with impaired immunity and certain underlying health and social conditions [11].

### 4.1 Undernutrition

Undernutrition is one of the major determinants of TB globally and is particularly important in high-burden settings such as India. Inadequate nutritional status can impair immune function and increase the probability that TB infection will progress to active disease. Undernutrition may also adversely affect treatment tolerance, recovery, and survival. Consequently, nutritional assessment and support are increasingly recognised as important components of comprehensive TB care [1,5].

### 4.2 Diabetes Mellitus

Diabetes mellitus is an established risk factor for TB disease. Metabolic abnormalities and impaired immune responses associated with diabetes can increase susceptibility to TB and may complicate diagnosis and treatment. The coexistence of diabetes and TB is particularly relevant to India because of the country's substantial diabetes burden. Integration of TB and diabetes screening and management is therefore an important component of a comprehensive TB-control strategy [11].

### 4.3 HIV Infection

HIV infection substantially increases the likelihood of progression from TB infection to active disease because of impaired cellular immunity. HIV-associated TB may also present with atypical clinical and radiological manifestations and can be associated with increased mortality. In 2024, approximately 619,000 people living with HIV developed TB globally [1]. Effective HIV testing, antiretroviral therapy, TB preventive treatment, and coordinated TB-HIV services are therefore essential.

### 4.4 Tobacco Use

Tobacco smoking damages respiratory defence mechanisms and is associated with increased susceptibility to TB and adverse treatment outcomes. Tobacco control and smoking cessation should consequently form part of integrated TB prevention and care, particularly in populations where tobacco use is common [11].

### 4.5 Social and Environmental Conditions

TB transmission and disease progression are strongly influenced by social and environmental conditions. Household crowding, inadequate ventilation, poverty, homelessness, occupational exposure, migration, incarceration, and limited access to healthcare can increase exposure and delay diagnosis. These factors demonstrate why TB cannot be addressed exclusively through clinical interventions. Improvements in nutrition, housing, social protection, occupational safety, and access to primary healthcare are also important components of TB control [1,3].

## 5. Diagnosis and Treatment

Early diagnosis is fundamental to TB control because undiagnosed infectious individuals can continue transmitting the organism. WHO recommends the use of rapid diagnostic technologies, particularly molecular tests, as an important component of modern TB diagnostic services. Such tests can detect *M. tuberculosis* and, in appropriate platforms, identify resistance to key drugs much more rapidly than conventional culture-based approaches [12].

Rapid molecular diagnostics have become increasingly important in national TB programmes. In 2024, approximately 54% of people newly diagnosed with TB globally had initially undergone a rapid diagnostic test, compared with 48% in 2023 [13]. WHO has also recommended newer near-point-of-care diagnostic approaches to bring testing closer to communities and reduce delays between presentation and treatment [14].

Drug-susceptible TB can generally be cured with an appropriate combination of anti-TB medicines administered for the recommended duration. Treatment success depends not only on the pharmacological efficacy of the regimen but also on adherence, management of adverse effects, uninterrupted drug availability, and appropriate clinical follow-up [1].

Incomplete or interrupted treatment can contribute to relapse, treatment failure, and the emergence or amplification of drug resistance. Accordingly, effective TB programmes must combine diagnosis with patient-centred treatment support. Important interventions include contact investigation, TB preventive treatment for eligible contacts and risk groups, infection prevention and control, adherence support, nutritional assistance, and social protection [6,7].

## 6. Drug-Resistant Tuberculosis

Drug-resistant TB represents one of the most serious threats to long-term TB control. Multidrug-resistant TB (MDR-TB) refers to TB caused by organisms resistant to at least rifampicin and isoniazid, while rifampicin-resistant TB (RR-TB) refers to resistance to rifampicin, with or without resistance to other anti-TB medicines. MDR/RR-TB is associated with greater diagnostic and therapeutic complexity than drug-susceptible disease [1].

WHO estimated that approximately 390,000 people developed MDR/RR-TB globally in 2024. Of these, approximately 164,545 people were reported to have received treatment, corresponding to roughly 42% of the estimated number requiring treatment [13]. This substantial gap demonstrates the continuing challenge of identifying and treating drug-resistant disease.

India contributes the largest national share of global MDR/RR-TB cases. In 2024, approximately 32% of the estimated global MDR/RR-TB burden was attributed to India [15]. Consequently, improvements in India's drug-resistance surveillance, rapid testing, treatment access, and treatment outcomes have implications far beyond its national borders.

Treatment of drug-resistant TB has undergone major changes in recent years. Shorter, all-oral regimens incorporating newer drugs have increasingly replaced older, prolonged regimens that often depended on injectable agents. Nevertheless, successful implementation requires rapid resistance testing, appropriate regimen selection, monitoring for adverse events, reliable drug supplies, and comprehensive patient support [13,15].

**7. Prevention and Control Strategies**

Effective TB control requires simultaneous action at individual, household, community, and health-system levels. Important strategies include:

- Early identification and prompt treatment of infectious TB cases;
- Systematic investigation of household and other close contacts;
- TB preventive treatment for eligible individuals at increased risk of progression to disease;
- Appropriate airborne infection prevention and control measures in healthcare and congregate settings;
- BCG vaccination, particularly for protection against severe forms of TB in children;
- Nutritional assessment and support;
- Tobacco-cessation interventions;
- Screening and management of HIV, diabetes, and other important comorbidities;
- Decentralised and accessible diagnostic services;
- Uninterrupted availability of quality-assured anti-TB medicines;
- Digital notification and surveillance systems; and
- Community engagement to reduce stigma and improve treatment adherence [1,6,7,16].

BCG vaccination remains an important component of childhood TB prevention. Its greatest benefit is protection against severe childhood forms of TB, whereas its protection against adult pulmonary TB—the principal source of community transmission—is more variable [16].

India has implemented many of these interventions through NTEP. Contact investigation, TB preventive treatment, airborne infection control, nutritional support, digital case notification, private-sector engagement, and community-based interventions have increasingly become integrated into the national response [6,7].

The 100-day intensified TB campaign launched in December 2024 represents another example of India's effort to accelerate case detection and reach underserved populations. The campaign was designed to bring diagnostic services closer to communities and reduce delays in diagnosis and treatment [5,10].

**8. Social and Economic Consequences**

TB affects far more than physical health. Patients may experience loss of income, interruption of education, reduced productivity, transportation expenses, treatment-related costs, social isolation, and stigma. These effects can be particularly severe among households dependent on daily wages or informal employment.

WHO defines catastrophic total costs associated with TB as the combined direct medical, direct non-medical, and indirect costs exceeding 20% of annual household income or expenditure. Evidence from national surveys demonstrates that a substantial proportion of TB-affected households experience such financial hardship [17]. The pooled estimate from available national surveys in the WHO's 2025 analysis was approximately 47%, while model-based estimates for low- and middle-income countries suggested that approximately 55% of TB-affected households experienced catastrophic total costs [17].

The economic relationship between TB and poverty is therefore bidirectional. Poverty can increase exposure and vulnerability through inadequate nutrition, crowded housing, occupational risks, and barriers to healthcare. Conversely, TB can deepen poverty by reducing household income and increasing direct and indirect expenses.

India has increasingly incorporated nutritional and social-support interventions into its TB programme. Such interventions recognise that successful TB treatment depends not only on providing medicines but also on addressing the circumstances that influence whether patients can initiate, continue, and complete treatment [6,9].

**9. Persistent Challenges**

Despite substantial progress, several barriers continue to slow the global response to TB.

- **Delayed diagnosis:** People with TB may experience prolonged periods between symptom onset, healthcare consultation, diagnosis, and treatment initiation. These delays increase the possibility of transmission and may worsen individual outcomes [12,14].
- **Drug resistance:** MDR/RR-TB requires sophisticated diagnostic and therapeutic capacity, and global treatment coverage remains substantially below the number of people estimated to need treatment [13,15].
- **Socioeconomic inequality:** Poverty, malnutrition, insecure employment, migration, overcrowded housing, and limited healthcare access continue to influence TB risk and treatment outcomes [1,17].
- **Comorbidities:** HIV infection, diabetes, undernutrition, and tobacco use may increase susceptibility to TB and complicate clinical management [11].

- **Stigma:** Fear of discrimination and social consequences can discourage individuals from seeking testing or disclosing their diagnosis, thereby delaying care.
- **Financing gaps:** Global TB programmes continue to face major funding shortages. WHO estimated that approximately US$22 billion annually was required for TB prevention, diagnosis, treatment, and care, while only about US$5.9 billion was available in 2024 [13].

These challenges indicate that technological progress alone will not be sufficient. Political commitment, domestic financing, international cooperation, health-system strengthening, community participation, and attention to social determinants must accompany biomedical advances.

**10. Looking Ahead**

The future of TB control will depend increasingly on shortening the interval between infection, disease development, diagnosis, and treatment. Diagnostic innovations that can be deployed closer to communities may help identify cases earlier, particularly among populations with limited access to conventional laboratory services [14].

Further expansion of rapid molecular diagnostics, improved resistance testing, shorter and safer treatment regimens, digital surveillance, and community-based care will be important. At the same time, TB programmes must deliberately address populations that may be missed by conventional services, including migrants, people experiencing homelessness, residents of remote areas, prisoners, and other socially or economically marginalised groups [1,3].

India provides an important example of large-scale TB programme implementation. The country's experience illustrates the importance of combining government health services with private providers, laboratories, community organisations, digital surveillance platforms, and social-support mechanisms. Continued engagement with communities is particularly important for addressing stigma and ensuring that people complete treatment [6,8].

Research remains essential. Priority areas include improved vaccines, more sensitive and accessible diagnostics, shorter treatment regimens, new anti-TB medicines, better approaches to drug-resistant disease, and strategies for preventing progression from TB infection to active disease. Although BCG remains valuable for preventing severe childhood TB, a more effective vaccine capable of preventing pulmonary TB across age groups would have major implications for global TB elimination [16].

**Conclusion**

Tuberculosis remains a major global health challenge despite being a preventable and curable disease. In 2024, an estimated 10.7 million people developed TB and approximately 1.23 million died from the disease [1,2]. India contributed approximately one-quarter of global incident TB cases, making progress within the country essential to achieving global TB targets [1].

India has nevertheless demonstrated important advances. The decline in estimated incidence and mortality, expansion of case notification, increasing participation of private providers, wider

availability of molecular diagnostics, nutritional and social-support interventions, and intensified community-based case finding all represent significant progress [5,8-10].

However, TB elimination cannot be achieved through antimicrobial treatment alone. Undernutrition, poverty, diabetes, tobacco use, HIV infection, stigma, treatment interruption, drug resistance, and unequal access to healthcare continue to influence the course of the epidemic. Effective TB control must therefore combine high-quality clinical care with social protection, community engagement, strengthened surveillance, and action on the underlying determinants of disease.

The global experience demonstrates that TB elimination is technically possible but requires sustained political commitment, adequate financing, scientific innovation, and health systems capable of reaching people before disease becomes advanced or transmission becomes prolonged. For India and other high-burden countries, continued progress will depend on maintaining these efforts while adapting them to changing epidemiological, social, and technological circumstances.

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