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Harnessing Living Therapeutic Materials: Integrating Microbial Engineering with Biocompatible Nano scaffolds

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Abstract

Background: *The Living Therapeutic Materials (LTMs) represent a notable advancement in the fields of Nano, biomedical, and pharmaceutical research. These materials integrate engineered microorganisms with biocompatible matrices, enabling innovative therapeutic functions, particularly in the realm of sustained drug delivery. This review focuses on the current state of LTMs, highlighting preclinical challenges, safety considerations, and recent advancements in the field. The exploration of LTMs seeks to elucidate their potential applications and the obstacles that must be addressed to fully harness their capabilities in therapeutic contexts.*

Methods: *This review orchestrates recent literature concerning microbe-based Live Therapeutic Materials (LTMs) through in vitro, ex vivo, and in vivo studies. It evaluates various methodologies and performance metrics, focusing on critical aspects such as biocompatibility, immune activation, cytotoxicity, pharmacokinetics, and therapeutic efficacy. The review aims to assess common practices in the field, including advanced therapy medicinal products and live biotherapeutic products (LBPs), highlighting trends and advancements in the development and application of these innovative therapeutic strategies.*

Results: *The LTMs are shown to produce significant therapeutic outcomes, including functions such as the controlled release of drugs, tissue repair, and immune modulation. Despite these advancements, there are persistent challenges that need to be addressed, particularly concerning genetic stability, regulatory classification, and numerous other issues that impact their use and development.*

Conclusion: *LTMs are a complex yet promising framework for the creation of next-generation medicines. Although these treatments have a lot of potential advantages, there are a lot of obstacles that must be overcome to guarantee their effectiveness and safety in clinical settings.*

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Introduction

The Living Therapeutic Materials (LTMs) are integrated engineered microorganisms with biocompatible matrices to enable innovative therapeutic functions, particularly in sustained drug delivery. They represent a notable advancement in the fields of biomedical and pharmaceutical research [1]. They perform various functions such as sensing, drug delivery, and tissue repair. They are categorized into various types based on the living entity they carry within their non-living matrix. In this context, many of these are called “biological engines": bacterial mammalian cell, yeast, fungal, algal, and herbal bioactive eluting therapeutic matrices.

Carriers, supports, or matrices for living materials are basically of three different types: (i) Hybrid materials (HLMs) are prepared by the combination of cells with abiotic scaffolds such as hydrogels, polymers, or inorganic nanoparticles; (ii) bioengineered materials (Bio-ELMs), scaffold-free materials where the cells themselves produce the entire structural framework, such as an engineered bacterial biofilm; and (iii) surface-coated materials, wherein cells or colonies are wrapped in protective "shells" (e.g., cell membranes or mineral layers) to increase their survival and reduce immune clearance in the body [2]. Research outcomes in this advanced technical field are being used in various medical fields for on-demand drug delivery (insulin for diabetes or cytokines for tumors), biosensing and diagnostics, and regenerative scaffolds (to heal chronic wounds or repair damaged organs) [3].

The broader historical trajectory of drug delivery systems showed the emergence of LTMs, as shown in Fig.1

- The Era of the Inception of Liposomes (1970s–1980s): The inception of liposomes marks the first major advancement in controlled drug delivery. Doxil® for cancer chemotherapy [4].
- The Inception of Nanoparticles (1990s–2000s): The tunable chemistry of polymeric nanoparticles as passive carriers to improve the sustained and targeted delivery (Poly (lactic-co-glycolic acid) (PLGA) nanoparticles) [5].
- The debut of smart nanocarriers (2010s): The nanocarriers responsive to pH, temperature, redox state, or enzymatic activity to bring the possibility of site-specific release helped the precision treatment of cancer and inflammatory disorders [6].
- Biohybrid era (2020s–present): Utilization of stimuli beyond response by living cells. The paradigm shift of passive to active delivery with the convergence of biological intelligence via therapeutic platforms [7].

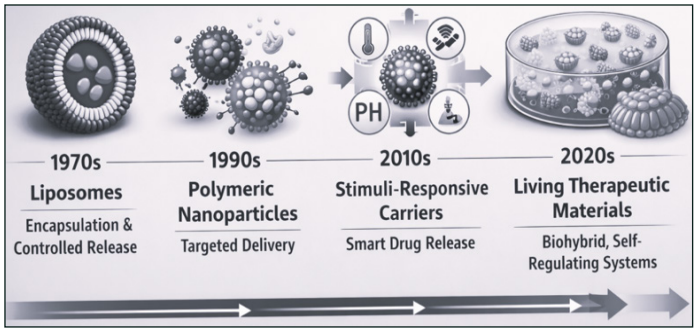

Figure 1: Timeline of development of Drug Delivery Systems Leading to LTMs

The current review focused on the illustration of the transformative potential of LTMs as advanced biological technical platforms of bioengineering, pharmaceuticals, and nanotechnology with an innovative regulatory compliance knowledge domain platform. The therapeutic promise, safety considerations assessment, and clinical translational capacity and scalability have been highlighted with a comprehensive research review [8].

Review of Literature

Engineered Microorganisms

Escherichia coli Nissle 1917 is regarded as the framework that has been widely used in the development of LTMs due to its probiotic safety properties, ease of genetic handling, and also the tendency to occupy sites in the inflamed gut tissue. The organism has been tailored to stimulate mucosal repair, produce antioxidants, and provide site-specific delivery of medications. [9-12]. *Lactobacillus* species, especially *Lactobacillus reuteri* and *Lactobacillus plantarum*, are well-integrated into the human gut ecosystem, capable of adhering to mucus and producing therapeutic amounts of lactic acid [13-15]. Cyanobacteria like *Synechococcus elongatus* PCC 7942 and *Synechocystis* PCC 6803 produce oxygen by means of photosynthesis and, therefore, find their use in treating hypoxic tumors and ischemic wounds [16-17]. *Bacillus subtilis is* Gram-positive and produces antifungal compounds, making this bacterium useful for long-term skin microbiome therapies because of its inherent adaptation to the skin ecosystem [18]. *Shewanella oneidensis* MR-1 is electroactive and consumes lactate anaerobically via extracellular electron transport, thus being able to disrupt the metabolism of tumor cells beyond current chemotherapies [19-20].

The yeast *Saccharomyces cerevisiae* is capable of macrophage engagement via mannose receptors and engineered to become a producer of acetaldehyde for killing tumor cells [21-22]. *Chlamydomonas reinhardtii is* a motile green alga with micro-robotics capabilities for drug delivery based on the sustained locomotion of the microbe itself [23]. *Chlorella vulgaris* is a photosynthetic, non-motile algae that generates oxygen and increases photodynamic sensitization in both wound and tumor microenvironments [24]. *Komagataeibacter sucrofermentans*, a cellulose-producing bacterium that synthesizes bacterial cellulose scaffolds *in-situ,* and when co-cultured with photosensitizer-modified glucose, it produces a live wound dressing with intrinsic light-triggered bactericidal action [14]. Together, all of these above-mentioned organisms represent the entire range of microbial kingdoms that are relevant to therapeutic material engineering, as summarized and shown in Fig. 2.

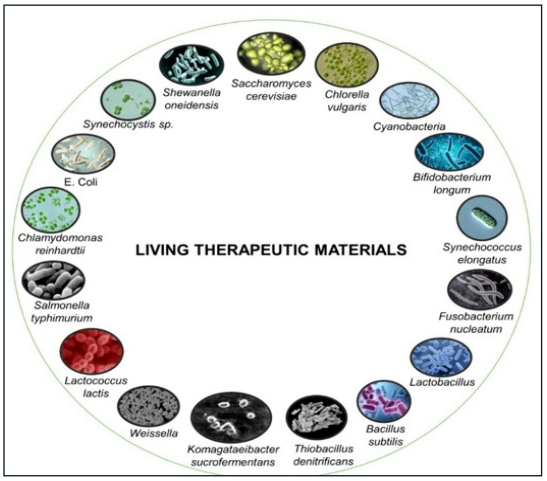

Figure 2: Microorganisms explored so far by different research reports

Biocompatible Matrices

The scaffolds used for living materials protect microbes from immune attack and physical stress, control the localization and maintenance of microbes at the site of action, control the release of products, and can impart mechanical or adhesive properties. Thus, a wide range of scaffold materials is employed owing to their different biological activities.

One of the scaffold types widely used is hydrogels, possessing a high-water content, porosity, and mechanical properties similar to those of soft tissues. In one example, alginate hydrogels containing cyanobacteria are used in photodynamic therapy to ensure bacterial viability while allowing light and oxygen penetration [25-27]. Pluronic F-127 is a thermosensitive polymer whose solution transforms into a gel at body temperature. The ability to create an injectable solution that gels in the place of application makes it attractive for use in skin wound treatment and other dermal LTMs [28]. Metal organic frameworks (MOFs), especially ZIF-8 and ZIF-90, allow obtaining a material able to degrade under acidic conditions, releasing its content only in specific regions, such as tumors and inflamed tissues. For example, ZIF-8 can be used as cages for oral delivery of yeasts and enzymes, activating them after reaching the destination point [29]. Liposomes attached to bacterial surfaces allow the delivery of two therapeutic agents simultaneously: small molecules and living bacteria [30]. Hyaluronic acid-containing scaffolds functionalized with thiol moieties or decorated with ROS-scavenging nanoparticles improve targeting to mucus-coated tissues [31].

Comparison with Conventional Systems

The comparison between LTMs and conventional drug delivery systems is that the kinetic basis of conventional nanoparticles lies in the ability of pre-loaded drugs to become exhausted, leading to the cessation of therapy. The solution to this problem involves continuous production by LTMs, whereby microbial systems act as biological drug factories in response to environmental stimuli [32]. The engineering of *Escherichia coli* (EcN), which are capable of detecting hypoxic tumors using lysis circuits and then self-destructing to release antigens that transform macrophages from an immunosuppressive M2 phenotype to an antitumor M1 phenotype, is an achievement that static therapies have failed to achieve [33]. Additionally, photosynthetic cyanobacteria within GelMA scaffolds produce oxygen upon exposure to light, overcoming tumor hypoxia to sustain the photodynamic process, unlike oxygen transporters [16-17]. Bacterial hydrogels engineered to synthesize curli nanofibers, for example, can regenerate their own matrix in a nutrient-rich environment, thus offering a prolonged period of action as compared to static scaffolds [34].

Methodology (Evaluation)

In vitro Assays

Classical toxicology assays are simple, reproducible, reliable, and cost-effective methods widely used for assessing cell viability and toxicity. This method remains important in both research and regulatory applications. The assays widely used are 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction, lactate dehydrogenase (LDH) release, resazurin reduction, Neutral Red Uptake (NRU), and total protein or biomass quantification, which are classified based on biological endpoints and serve as the foundation of routine cytotoxicity evaluation [35]. Tetrazolium-based assays used redox reactions to evaluate the metabolic activity of living cells. The tetrazolium salts are reduced by NADH/NADPH-dependent oxidoreductase enzymes into coloured formazan products, which enter metabolically active cells. The quantity of formazan produced correlates with the number of viable cells, and after solubilization, it can be quantified spectrophotometrically if insoluble derivatives are formed [36]. *In vitro* investigations primarily focused on assessing the interactions between LTMs and mammalian cells under controlled laboratory conditions.

Two-dimensional (2D) cell culture models are primarily used to evaluate cytotoxicity (MTT, XTT, and LDH assays), cellular uptake, oxidative stress, intracellular trafficking, hemocompatibility, and drug-release kinetics under controlled laboratory conditions [37]. However, these 2D models have some limitations as they fail to accurately reproduce the structural complexity, extracellular matrix interactions, tissue polarity, and multicellular organization present in native tissues. To overcome these limitations, *advanced 3D in vitro infection models*, such as spheroids, organoids, organ-on-chip systems, and 3D bio printed models, have emerged as powerful alternatives [38]. *In vitro* model systems provide reproducible, rapid, and cost-effective platforms for mechanistic studies and high-throughput screening. Evaluations typically take place using immune and tissue-derived cells, which include cytokine production, oxidative stress, inflammasome signalling, macrophage polarization, cell viability, and bacterial-material interactions [39]. Chitosan–cellulose hydrogels are assessed for swelling behaviour, pH-responsive drug release, mechanical stability, and antimicrobial efficacy, primarily utilizing breast cancer and fibroblast cell models to evaluate bio evaluation [40]. Functional components such as nanoparticles, nanofibers, bioactive agents, peptides, essential oils, and anticancer drugs were integrated into polymeric matrices to enhance mechanical properties, drug delivery, responsiveness, and biological performance [41]. *Probiotic Living Materials (PLMs)* are promising next-generation living therapeutics that combine genetically engineered probiotics with biomaterial matrices to enable safe, targeted, and precise disease treatment. Their development involves bottom-up and top-down fabrication approaches, genetic engineering of probiotic strains, hydrogel encapsulation, microencapsulation, 3D bioprinting, and nanoparticle integration [42].

Ex Vivo Tissue Explant Models, Immune Activation Profiling

Ex vivo studies utilize isolated tissues and organs, which provide a similar physiological environment and reduce dependence on animal experimentation. It helps in assessing tissue penetration, permeability, retention, metabolism, and localized toxicity while preserving native tissue architecture [37].

To evaluate immune activation, cytokine release, cellular infiltration, and biomaterial interactions, *ex vivo* models use human blood samples, tissue explants,

precision-cut tissue slices, and isolated organ-specific immune cells [39]. *Ex vivo* hydrogel formulations evaluate antimicrobial performance, oxidative stress reduction, and localized therapeutic retention in tissue-mimicking environments using bioactive compounds such as essential oils, honey, probiotics, or nanomaterials [40].

3D tumor and Patient-Derived Explant (PDE) Models use tumor spheroids, Xeno spheres, and patient-derived tissues to evaluate therapeutic efficacy. These models are generated through controlled cell culture and spheroid formation, and the efficacy is assessed using the lactate dehydrogenase (LDH) release [43].

Ex vivo biofilm infection models use human skin, pig corneas, extracted teeth, and pig lungs to study chronic infections, biofilm formation, and antimicrobial efficacy in physiologically relevant environments. These models bridge the gap between simplified in vitro systems and complex *in vivo* studies, as they provide a biologically relevant and ethically advantageous alternative [44-47].

In Vivo Animal Models for Pharmacokinetics, Biodistribution, Therapeutic Efficacy

In vivo studies were conducted primarily in mice, rats, pigs, and dogs, as animal models formed the necessary components of preclinical evaluation for living therapeutic materials [48]. Innate immune responses are investigated using additional vertebrate models, such as zebrafish, which is useful in detecting biodistribution, developmental toxicity, and real-time imaging of host-material interactions [39]. These studies evaluate pharmacokinetics, biodistribution, therapeutic efficacy, tumor regression, systemic toxicity, and ADME (absorption, distribution, metabolism, and excretion) profiling. For real-time tracking of nanocarriers, imaging techniques such as Magnetic Resonance Imaging (MRI), Positron Emission Tomography (PET), and fluorescence imaging are also employed [37]. *In-vivo* methodologies of chitosan–cellulose hydrogel systems in animal models focus on assessing the therapeutic efficacy, biodegradation, wound healing performance, tissue regeneration capacity, and biocompatibility [40].

In-vivo biofilm models are the most clinically relevant systems for studying biofilm infections, antimicrobial efficacy, drug penetration, and PK/PD relationships within a living host. The evaluation of biofilm formation, bacterial burden, host responses, and therapeutic outcomes under physiologically relevant conditions can be done using lung, wound, ocular, urinary tract, endocarditis, and implant-associated infection models [44].

Integration with Advanced Therapy Medicinal Products (ATMPs) and Live Biotherapeutic Products (LBPs)

Live biotherapeutic products (LBPs) consist of well-defined formulations containing single or multiple microbial strains and use phage- and microbiome-based therapeutics, which offer promising strategies for combating pathogenic bacteria and antimicrobial resistance. It requires rigorous quality control, manufacturing validation, clinical evaluation, and risk–benefit analysis [49]. Microbial metabolites can modulate key anticancer immune pathways, including T-cell activation, macrophage polarization, antigen presentation, and immune checkpoint responses. These metabolites may enhance the effectiveness of existing therapies such as immune checkpoint inhibitors by reshaping the tumor microenvironment and reducing systemic toxicity [50].

CRISPR-based microbiome modulation enables precise editing of gut microbes, selectively eliminating harmful bacteria while preserving beneficial microbiota and restoring immune homeostasis. It can combine with synthetic biology and multi-omics approaches, engineer smart probiotics, sense disease signals, deliver targeted therapeutics, and support personalized microbiome-based treatments [51-52]. The future success of personalized ATMPs depends on strong collaboration between academia, hospitals, clinicians, engineers, and pharmaceutical manufacturers. Combining AI-driven automation with patient-centred clinical research can accelerate the development of safer, more effective, and individualized therapies, ultimately improving patient access to transformative medicines [53] .To facilitate the successful clinical translation of LTMs and personalized ATMPs, a comprehensive evaluation pipeline progressing from *in vitro* studies to *ex vivo* and *in vivo* validation is required, as illustrated in Fig. 3.

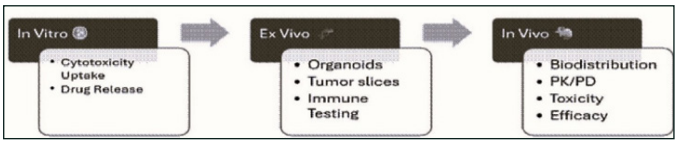

Figure 3: Workflow of LTM evaluation pipeline (*In vitro → Ex vivo → In vivo*)

Therapeutic Applications

Living therapeutic materials (LTMs) integrate viable microorganisms or mammalian cells with engineered biomaterial scaffolds to create responsive systems that can sense disease cues and release bioactive agents in a controlled, localized manner [54]. These advances in synthetic biology and biomaterials enable precise spatiotemporal control of therapeutic activity for targeted drug delivery, tissue repair, immune modulation, and cancer therapy, improving efficacy while reducing systemic toxicity.

Controlled Drug Release

Living Therapeutic Materials (LTMs) are engineered living materials that continuously produce and deliver therapeutic drugs directly at the disease site, minimizing systemic toxicity and avoiding chemotherapy-related side effects [54]. Bacteria with therapeutic properties are summarized in Table 1 [54].

Table 1: Engineered Living Therapeutic Materials (LTMs) for antibiotic and anticancer drug delivery (57)

Bacterium	Therapeutic Function
Salmonella typhimurium VNP20009	Tumor colonization and drug delivery
Escherichia coli Nissle 1917	Production of anticancer proteins and nanobodies
Clostridium novyi-NT	Selective destruction of hypoxic tumor tissue
Bifidobacterium longum	Tumor-targeted gene delivery
Listeria monocytogenes (attenuated)	Cancer vaccine platform

Tissue Repair

Living therapeutic materials (LTMs) enhance tissue regeneration by integrating viable cells into engineered scaffolds that release growth factors, matrix components, and antimicrobials in response to disease cues. They include engineered bacteria in dressings (e.g., *Lactococcus lactis* and *Bacillus subtilis*), MSC-laden hydrogels, curli-producing *Escherichia coli* nanofiber scaffolds, and bacterial cellulose from *Komagataeibacter xylinus*, which together promote angiogenesis, antimicrobial protection, tissue ingrowth, and improved wound healing, including in diabetic wounds [54].

Immune Modulation

Living therapeutic materials enable site-specific immune modulation by delivering anti-inflammatory cytokines (IL-10, IL-4, and TGF-β) through engineered cells that respond to local inflammatory signals, avoiding systemic immunosuppression [55]. Genetically modified *Lactococcus lactis* secreting IL-10 treats Crohn's disease and IBD, with a landmark clinical trial confirming the safety and feasibility of live engineered bacteria for cytokine delivery in humans [56].

Cancer Therapy

Cancer treatment exemplifies sophisticated LTM use since bacteria preferentially colonize hypoxic tumor microenvironments inaccessible to conventional drugs [57]. Engineered *Escherichia coli* Nissle 1917 and hydrogel-embedded *Salmonella typhimurium* deliver anti-PD-L1 nanobodies, cytokines, and toxins locally within tumors, while TRAIL-secreting bacteria induce targeted apoptosis, sparing healthy tissue. *Escherichia coli* with synchronized lysis circuits reduces tumor growth, and *Clostridium novyi*-NT spores cause localized necrosis with systemic immune activation [58]. New microbial LTMs use CRISPR regulation, quorum sens-

ing, tumor-responsive biosensors, and kill switches to enhance precision and biosafety for cancer immunotherapy. Bacterial species with demonstrable tumor-targeting properties are in various stages of development by reputed universities across the world as demonstrated by Table 2.

Table 2: Bacterial Species with Demonstrable Tumor-Targeting Properties

Engineer ed Organism	Therapeutic Application	Key Therapeutic Molecules / Mechanism	Developing Institution / Organization	Development Stage	Ref.
Lactococcus lactis (engineered, NZ9000)	Antibiotic delivery – MDR *S. aureus*	Phage endolysin, VAPGH lysins, nisin	University of Kuopio/Turku (Finland)	Preclinical (*in vitro MDR S. aureus*)	(74)
E. coli Nissle 1917 (EcN::mcmA)	Antibiotic delivery – *Salmonella* suppression	Microcin MccM (siderophore–microcin), MccH47	South China Agricultural University (Guangzhou)	Preclinical (*in vitro*, iron-limited)	(75)
Bacillus subtilis (in Pluronic thermogel)	Antibiotic/antifungal – skin infection, wound healing	Antifungal agents, surfactin (biofilm disruption)	Mizrahi group, Ben-Gurion (Israel)	Preclinical (mouse skin fungal)	(76)
Engineered E. coli in core–shell hydrogel	Stimuli-responsive protein delivery	Environmental-stimuli-triggered protein release	MIT; Patrick Doyle group	Preclinical (*in vitro*/controlled release)	(77)
Salmonella VNP20009 (attenuated, purI/msbB)	Anticancer – tumor colonization, cytotoxic proteins	Hypoxic tumor targeting, cytotoxic protein delivery	John Hopkins University	Phase I (completed; no regression)	(78)
E. coli Nissle 1917/ *Salmonella* + SLC	Anticancer – pulsatile nanobody/ protein release	SLC (quorum-sensing lysis): HlyE, mCCL21, CDD-iRGD; +5-FU	UCSD & MIT; Jeff Hasty, Tal Danino, Sangeeta Bhatia	Preclinical(mouse tumors;tumor regression + chemo)	(58)
C.novyi-NT (attenuated anaerobe)	Anticancer – hypoxic tumor germination, necrosis, immune activation	Spore-based therapy; haemorrhagic necrosis, lysis, systemic immunity	Johns Hopkins /Dana Farber / MSK; Scott Ferrone, Steven Hope, Janku	Phase1b (NCT01924689); canine sarcoma (partial/complete), human myosarcoma (near complete)	(79)

Recent living therapeutic materials combine tumor-targeting bacteria with hydrogels or biomaterial scaffolds to improve retention at tumor sites, extend therapeutic exposure, and enhance anticancer efficacy. They include *Escherichia coli* Nissle 1917 with programmed lysis circuits delivering anti-PD-L1 nanobodies and toxins, hydrogel-encapsulated *Salmonella typhimurium* for sustained immune activation, and TRAIL-expressing bacterial scaffolds that selectively induce cancer cell apoptosis while sparing healthy tissue. Engineering strategies for various cell types are summarized in Table 3.

Table 3: Remarkable Translational Examples of Living Therapeutic Materials

Organism/Cell Type	Engineering Strategy	Therapeutic Application	Ref.
Lactococcus lactis	IL-10 secretion	Crohn's disease, IBD	(56)
E. coli Nissle 1917	Synchronized lysis circuit	Tumor-targeted cancer therapy	(80)
Salmonella Typhimurium VNP20009	Tumor colonization	Anticancer drug delivery	(78)
Clostridium novyi-NT	Hypoxia-selective germination	Solid tumor destruction	(79)
Bifidobacterium longum	Therapeutic gene delivery	Cancer therapy and immune modulation	(81)
Komagataeibacter xylinus	Antimicrobial peptide secretion	Infected wound treatment	(76)
Komagataeibacter xylinus	Bacterial cellulose production	Wound healing scaffolds	(82)
Mesenchymal Stem Cells (MSCs)	Growth factor secretion	Bone, cartilage, and skin regeneration	(83)

Pre-Clinical Challenges

Genetic Stability of Engineered Microbes

The efficiency of LTM treatments relies heavily on the longevity of gene circuit activity during the process. The rapid replication rate of bacteria leads to high selection pressure on the plasmid, which allows for faster growth of bacteria without plasmids, which would ultimately result in the deactivation of the LTM [59]. Although chromosomal integration reduces this problem, other issues arise, such as insertional mutagenesis and context sensitivity, as focused under the Genetic instability in Table 4.

The stability of synthetic gene circuits, especially those involving layering, feedback systems, and quorum sensing, in *in vivo* conditions of stress, fluctuating pH levels, and variation in nutrient availability, is understudied [32]. The *Escherichia coli* Nissle 1917 (EcN) platform for tumor treatment provides a concrete example of such a problem. The hypoxia-activated Pvhb-lysis system that utilizes the bacteriophage protein PhiX174E increases the complexity of the genetic circuit, which should be tested extensively due to the risk of mutations that could impair the circuit's activity. Such mutations would affect the promoter and regulatory genes and reduce the effectiveness of the therapy [12]. This concern is directly linked to the Mitigation strategies summarized under Genetic instability in Table 4.

Host Immune Response and Risk of Rejection

In-vitro tests alone cannot provide the full scope and complexity of interactions between living therapeutic matrices (LTMs) and host immunity systems. Gram-negative bacteria such as *Escherichia coli* Nissle 1917 (EcN) secrete LPS, and while even endotoxin-free strains (ClearColi) can induce different immune reactions depending on the background of the donor, Yanamandra et al. showed in PBMC experiments that pro-inflammatory donors (with spontaneous high levels of IL-2 release) have a higher IFN-γ response and cytotoxicity for bacterial hydrogels than quiescent ones, indicating that the immune response to LTMs can be very personalized and unpredictable based on safety statistics in the population [59]. As Table 4 shows the patient-specific immune responses, which represents this diversity.

Rodents as experimental subjects do not show this diversity because of their uniform immune systems.

Immune isolation by hydrogel encapsulation provides only limited protection. While the physical barriers created by scaffolds prevent bacteria from contacting immune system cells, their products can easily diffuse to nearby tissues. The effectiveness of immune isolation is dependent on hydrogel pore diameter, composition, degradation status, and elasticity changes over time. Balancing between immune isolation and permeability is one of the major goals of material engineering for scaffold development, as highlighted under the Host immunogenicity and scaffold degradation in Table 4 [32].

Cytotoxicity and Unintended Systemic Effects

Confined localization of live microorganisms in the treatment sites is imperative but difficult to achieve. Systemic translocation of bacteria into the bloodstream is harmful, especially in immunocompromised individuals, who form a major part of the target group for LTMs. Although mucoadhesive and biofilm-based coating techniques proposed by Wang and Yang increase the site-specific localization, there is insufficient quantification of the translocation hazard in different clinical settings [9,11,60]. his issue is summarized under the Bacterial translocation in table 4.

In case of intravenous delivery of systemically active LTMs, like phage-nanoparticle conjugates introduced by Zheng *et al.*, there are several potential hazards, including blood cell toxicity, complement activation, and liver damage. Intravenously administered phage nanoparticles do not affect the counts of blood cells, immunoglobulin, or the function of the liver and kidneys in pigs, which is a promising observation. However, the results are only based on one animal species and one treatment protocol; thus, further toxicological testing, which should involve multiple species, repeated doses, and reproductive tests, among others, is necessary [61]. These concerns align with the Cytotoxic microbial products that are mentioned in Table 4.

Manufacturing Reproducibility and Scalability

Challenges for the LTMs' scale-up process differ from those involved in the production of conventional pharmaceuticals. The live cells have to maintain cell viability, metabolism, and genetic information through the processes of fermentation, isolation, incorporation in the scaffold, sterilization (often done at cold temperatures), and preservation. All these processes entail different factors, which may lead to a decrease in effectiveness [32]. These risks have been mentioned under the Manufacturing variability in Table 4.

Another challenge in LTM development is presented by thermosensitive hydrogels. An example is Pluronic F-127, which gels around 37°C, meaning that the suspension of bacteria must stay in a liquid state before being applied to the site where it solidifies [28]. This issue is linked to the Scaffold degradation altering containment in Table 4.

Table 4: Preclinical Challenges in LTM Development with Mitigation Strategies

Challenge	Mechanism of Concern	Current Mitigation Approaches	Ref.
Genetic instability	Plasmid loss under selection pressure; promoter silencing	Chromosomal integration; auxotrophic selection; kill-switch coupling	(32)
Host immunogenicity	LPS, bacterial antigens triggering innate immune response	Endotoxin-attenuated strains (Clear Coli); hydrogel encapsulation; anti-inflammatory scaffold additives	(59)
Bacterial translocation	Migration beyond target site to systemic circulation	Mucoadhesive coatings; biofilm encapsulation; hydrogel containment	(9) (28)
Cytotoxic microbial products	Secreted toxins, ROS-generating metabolites affecting host tissue	Lysis circuits limiting bacterial density; attenuated strains	(12) (16)

Manufacturing variability	Lot-to-lot differences in viable cell count, metabolic activity	Defined fermentation protocols; potency assays; cold-chain optimization	(32)
Scaffold degradation altering containment	Hydrogel erosion releasing bacteria into surrounding tissue	Cross-linking optimization; biodegradation profiling	(32)
Patient-specific immune responses	Inter-individual variability in inflammatory background	Pre-screening for IL-2 levels; personalized formulation	(59)

Safety Deliberations

Risk of Uncontrolled Microbial Proliferation

One of the main safety considerations associated with LTM therapy relates to the possibility that the delivered microbes will grow in excess of their functional purpose, leading to infection. The strains that have been classified as harmless may still be dangerous when considering immunocompromised patients who would likely stand to gain the most from using LTM technology to treat their disease. The strains selected for treatment purposes are highly attenuated. For example, strain *Salmonella typhimurium* VNP20009 uses deletions in purI and msbB, making it LPS-deficient and auxotrophic, whereas EcN is a much safer choice compared with wild-type *E. coli* [33].

Containment Strategies

Two major biological strategies are used to control microorganisms in LTMs:

- Auxotrophy: This process involves engineering microorganisms such that they rely on an external nutrient for their survival. The external nutrient is usually either an artificial amino acid or a metabolic precursor not found in mammals. In case the engineered organisms escape the scaffold, they will not survive since they require the external nutrient [32].
- Genetic kill switches: These are genetically engineered circuitries that lead to programmed cell death when certain triggers, such as lack of oxygen, particular molecules, or quorum-sensing thresholds, are reached. An example is the hypoxia-induced lysis circuit used in the D@T@E-PL strategy, where the cancer bacteria die off in regions of normal oxygen concentration while surviving in regions of hypoxic conditions [12].
- The main benefit of this technique is that the biology of the cancer tumor itself acts as the trigger for retaining bacteria in hypoxic regions.

Ethical Concerns

The use of these “living implants” by patients raises a corresponding issue of ethics as well. Informed consent for using these living implants should include explanations concerning the genetically engineered status of these organisms, their inability to reproduce, and the temporary nature of their therapeutic effect [62].

Regulatory Ambiguity

Living Therapeutic Materials (LTMs) integrate microbes with biomaterial scaffolds, defying proper classification. They can simultaneously resemble: (i) Biologics: via microbial metabolism or genetic programming, (ii) Devices: through the scaffold’s mechanical or immunomodulatory roles, (iii) Combination/ATMPs: when integrated for regenerative or diagnostic purposes.

Examples include *Escherichia coli* Nissle 1917 in Pluronic F127 hydrogels for immune testing and magnetic alginate hydrogels carrying diagnostic EcN for intestinal retention [59,63]. The key stages involved in the clinical translation of LTMs, from design and preclinical assessment to clinical trials and market approval, are summarized in Fig. 4.

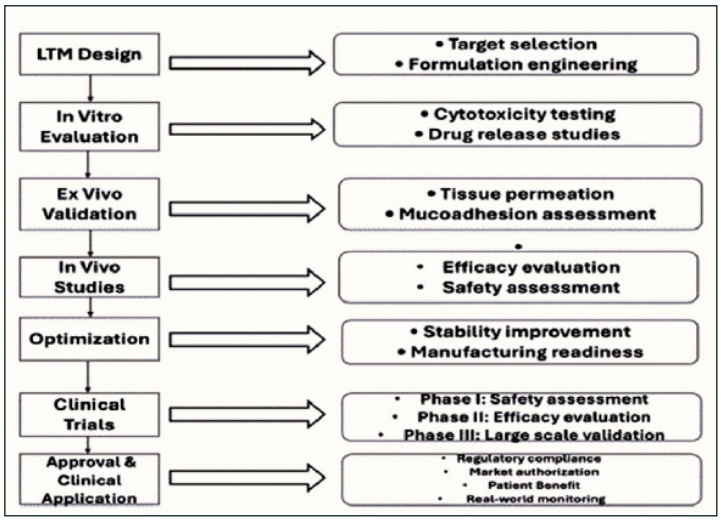

Figure 4: Roadmap for clinical translation of LTMs

Recent Advancements in Living Therapeutic Materials

Living therapeutic materials (LTMs) emerge at the interface of synthetic biology, advanced biomaterials, and integrated biosensing, enabling programmable therapeutic systems with fine spatiotemporal control [64]. Synthetic gene circuits enable embedded cells to sense disease-relevant inputs, perform logic-level computation, and execute therapeutic programs, while stimuli-responsive hydrogels regulate molecular transport, cell fate, and matrix mechanics *in situ* [65].

Programmable Gene Circuits

Programmable gene circuits enable living cells within LTMs to sense, compute, and actuate therapeutic responses controllably [64]. These circuits process disease-relevant inputs and trigger outputs like cytokine secretion or apoptosis only under pathological conditions. Synchronized lysis circuits in *Escherichia coli* coordinate pulsatile drug release and self-limiting growth within tumors, achieving significant tumour regression [58]. Programmable gene circuit strategies applied to living therapeutic materials for targeted diseases are shown in Table 5.

Table 5: Representative Programmable Gene Circuit Strategies Applied to Living Therapeutic Materials

Sensing feature	Host system in LTM	Disease	Property	Ref.
Quorum-sensing synchronized lysis	Engineered *E. coli*	Oncology	Density-dependent lysis, pulsatile protein release	(85)
AND-gate inflammatory sensor	Engineered cells	Autoimmune/ inflammatory	Anti-inflammatory cytokines only with 2 pro-inflammatory inputs	(86)
Inducible, drug-responsive cassette	Designer stem/immune cells	Regenerative medicine, cancer	On-demand growth factors/ checkpoint modulators via small molecules	(87)
Safety kill switch (suicide circuit)	Bacterial /mammalian	Oncology, inflammation, wound healing	Controlled cell ablation on off-target activity or treatment end	(88)

Smart, stimuli-responsive hydrogels are key biomaterial matrices for LTMs, providing dynamic microenvironments responding to local biochemical or physical cues. They reversibly change swelling, porosity, or network structure in response to pH, temperature, ROS, glucose, enzymes, or mechanical forces, modulating drug release, cell behaviour, and matrix stiffness in real time. Stimuli-sensitive hydrogels optimize wound healing by promoting moisture retention, cellular migration, and mechanical protection while enabling on-demand

release of antimicrobial or pro-regenerative factors [66]. Stimuli-responsive matrices coupled with engineered microbes enable "sense-and-respond" LTMs [67]. Stimuli-responsive hydrogel strategies relevant to living therapeutic materials are highlighted in Table 6.

Table 6: Stimuli-responsive Hydrogel Strategies Relevant to Living Therapeutic Materials

Stimulus	Response	LTM application	Ref.
pH (acidic chronic wound milieu)	Increased swelling and accelerated drug release at low pH	Chronic wound dressings with enhanced antimicrobial and pro-regenerative delivery	(66)
Reactive oxygen species (ROS)	ROS-triggered bond cleavage and release of antioxidants or drugs	Anti-inflammatory LTMs that neutralize oxidative damage and modulate local immunity	(66)
Glucose (diabetic wound milieu)	Glucose-dependent crosslink instability or charge change	Diabetic wound hydrogels that adjust insulin or growth factor release based on glucose level	(66)
Enzymes (e.g., matrix metalloproteinases)	Enzyme-mediated degradation of peptide crosslinks	Regenerative scaffolds that remodel in synchrony with tissue repair and cell invasion	(89)

Digital Health Integration

Integration of LTMs with digital health technologies and biosensors enables closed-loop, personalized therapy. Biosensing systems provide quantitative readouts of cytokine release, metabolic activity, or pathogen burden for clinician monitoring or automatic adjustment of therapeutic gene circuit activity. In closed-loop systems, biosensor outputs inform implantable or wearable controllers regulating gene circuits, hydrogel properties, or dosing regimens, establishing an integrated digital-biological therapeutic ecosystem that enhances safety, adherence, and personalization [68]. Microfluidic and nano plasmonic biosensors enable real-time, label-free monitoring of live cell protein secretion, such as VEGF [69]. Conceptual frameworks for "living programmable materials" emphasize integrating sensing and computation within materials for responsive digital-biological therapeutic systems [64].

Results and Discussion

Consolidated Therapeutic Outcomes

In vitro cytotoxicity testing supports both biological interpretation and regulatory decision-making and has evolved from basic viability assays toward multifaceted, mechanistically informed systems. Modern *in vitro* toxicology uses complementary endpoints to generate coherent, biologically plausible toxicity profiles and to support regulatory decision-making [70]. LTMs show promising efficacy in preclinical studies; greater emphasis on safety profiling, mechanism-of-action studies, manufacturing robustness, and standardized evaluation frameworks are essential to facilitate their translation into clinical applications [48].

There exists a stepwise framework for the preclinical evaluation of nanocarrier-based drug delivery systems that encompasses *in-vitro, ex-vivo*, and *in-vivo* models, each contributing distinct insights into safety, efficacy, and translational potential. *In vitro* studies help in rapid screening of cytotoxicity, cellular uptake, and mechanistic interactions; on the other hand, *ex vivo* models provide an intermediate platform between cell culture and animal studies. *In vivo* investigations remain essential for assessing pharmacokinetics (ADME), biodistribution, immunogenicity, and therapeutic efficacy [37].

Despite significant progress, the preclinical models show an inability to fully replicate human physiological complexity, often resulting in discrepancies between preclinical and clinical outcomes. Emerging technologies, including organ-on-chip platforms and computational modeling, are being developed to address these limitations. Advanced nanoimmunosafety assessment, such as 3D cultures, organoids, and organ-on-chip systems, better

mimics tissue-specific immune microenvironments [71-72]. Consistent with findings from preclinical evaluation studies, LTMs demonstrate improved therapeutic efficacy over conventional systems, as shown in Fig. 5.

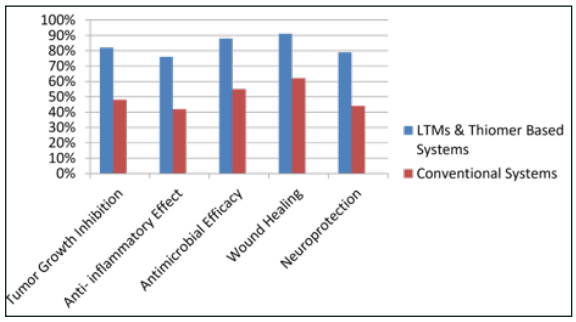

Figure 5: Comparative therapeutic efficacy of LTMs vs. conventional systems

Future Perspectives

The emerging personalized treatment strategy, which was an unmet need, seems to be a realizable dream of hope with living cell-based biohybrid systems. The *in-situ* delivery of patient-specific therapeutic molecules through biocompatible natural polymers [3]. Personalized microbial scaffolds thus represent a shift from "one-size-fits-all" pharmacotherapy toward precision biotherapeutics, where treatment is dynamically modulated by patient-specific cues such as pH, cytokine levels, or metabolic state.

The pivotal role of the human microbiome in maintaining metabolic homeostasis and immune regulation through the direct delivery of bioactives and probiotics into dysbiotic environments [2]. Hence, localized modulation of gut, skin, or mucosal microbiota and further treatment of novel strategies for inflammatory bowel disease, acne, and metabolic syndrome to reshape microbial ecosystems will be a dream come true situation. LTMs not only hold promise for the above-mentioned conditions but can also be used for diabetes and autoimmune disorders.

Summary & Conclusion

Living Therapeutic Materials (LTMs) represent a paradigm shift by offering dynamic responsiveness, sustained activity, and localized therapeutic effects. They are not only reducing the dosing frequency but also enhancing the patient's compliance (1). The versatile behavior of these hybrid systems is addressing the unmet clinical needs of oncology, wound management, and infectious and metabolic conditions [3].

Alongside their potential, there exist critical translational barriers, which include interactions of microbes with hosts that might trigger immune responses, unstructured approval pathways, and undue delay, manufacturing hurdles due to preclinical assessment challenges and ethical concerns, etc [73].

The demanding collaborative efforts needed for LTMs to make them successful visionary contributors to health care and biomedical technology are not only limited to biotechnology and pharmacy, but also to regulatory science to ensure frameworks are in place for ethically valid, scientifically non-compromised clinical potential validation [8]. Collaborative efforts involving academia, industry, and regulatory agencies are essential to establish standardized protocols and accelerate clinical trials. Digital integration and AI-driven monitoring improve the innovative potential and personalization for long-term adaptive therapeutic interventions.

Conflict of Interest

Authors declare no conflict of interest.

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