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Engineered living materials for *in situ* production of therapeutics

EU-IN Horizon Scanning Report

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Executive summary

Engineered Living Materials (ELMs) are biomaterials composed entirely or partly by living cells capable of self-replication and adaptation. Among other applications, ELMs can be leveraged for their potential of producing therapeutic molecules *in situ*. This horizon scanning report describes current state of art and emerging trends of ELMs development and explores challenges and opportunities related to their ability to deliver, produce and release therapeutic substances within the body to treat the designated area.

Engineered materials and synthetic biology are recognized by global bodies like the World Health Organisation, the Organisation for Economic Co-operation and Development and the World Economic Forum as transformative emerging technologies. Moreover, research and development of ELMs for therapeutic purposes is supported by ongoing calls for action and grants such as the Pathfinder challenge launched by the European Innovation Council.

Examples of potential applications include engineered bacteria embedded in hydrogels for localised anti-cancer drug delivery, ELMs producing antimicrobial agents, ELMs that protect and promote skin healing and ELMs releasing growth factors to stimulate tissue regeneration and repair.

Applications of ELMs for *in situ* production of therapeutics offer potential advantages such as continuous production of molecules within the body, better stability of produced therapeutics, potential increased efficacy and possible reduction of side effects.

Despite growing interest and research effort in this field, several challenges in the development of ELMs are faced like ensuring batch consistency and scalability of manufacturing, maintaining viability and functionality of cells over time, and addressing the biosafety of these products. From the regulatory point of view, there is no official harmonised definition of engineered living materials nor a dedicated framework.

The main recommendations to tackle these challenges include increasing clarity regarding terminology and definitions of ELMs, increasing information on specifications for standardised testing procedures to enhance reproducibility, reliability and stability as well as discussing specific requirements for clinical trials.

More experience and exposure of the regulators to ELMs development will determine the need for a dedicated regulatory framework and further guidance.

1. Introduction

1.1. Rationale and objectives of the report

Horizon scanning is the systematic analysis of information to identify early indicators of scientific and technological advancements that may present new regulatory challenges or public health opportunities. The European Medicines Agency (EMA) conducts horizon scanning in collaboration with experts and groups like the EU Innovation Network (EU-IN) (1). The main objective of horizon scanning activities is to proactively anticipate and address upcoming challenges and opportunities by analysing and forecasting the most relevant upcoming topics and reporting their estimated impact on the European Medicines Regulatory Network (EMRN) over the next three to 10 years. Reports contain recommendations for the EMRN, supporting developments and facilitating innovation reaching patients. Horizon scanning is a key activity to achieve the strategic goals of the European Medicines Agencies Network Strategy (EMANS) to 2028 (2).

Engineered living materials (ELMs) was identified as a relevant topic based on literature screening, the result of a survey conducted among stakeholders and interactions with scientific groups of EMA, the EMRN and the European Innovation Council (EIC).

This horizon scanning report describes current state of art and emerging trends of ELMs development and explores challenges and opportunities related to their ability to deliver, produce and release therapeutic substances within the body to treat the designated area.

1.2. Defining Engineered Living Materials (ELMs)

The definition of ELMs used in this report is engineered materials composed, either entirely or partly, by living cells. The term "engineered" is used when biomaterials (e.g., cells, tissues, materials that integrate living cells, etc.) do not retain their original function or if they have been subject to substantial manipulation. ELMs can have a broad application in health (tissue engineering, wound healing, targeted production of molecules, medical implants, diagnostic devices) and even beyond (e.g., sustainable architecture, environmental remediation…) (3). Moreover, engineered living materials can be leveraged to develop New Approach Methodologies (NAMs). Nevertheless, this report focuses on one specific application of ELMs for health, the *in situ* production of therapeutics. The term *in situ* refers only to a localised area within the body where the therapeutics are initially delivered, produced and released and does not include bedside manufacturing. This definition is also used by other European initiatives like the EIC Pathfinder challenge on engineered living materials (4).

Despite this definition, it should be noted that ELM is not a standardised term in most EU languages and varies vastly from country to country. Currently, there is no official position of the European Directorate for the Quality of Medicines & HealthCare (EDQM) on the matter. Moreover, the constant evolution of the ELM field may change the most common definition of the term in the future.

ELMs use their components, living cells and scaffolding, to actively replicate and, possibly adapt to the environment. ELMs utilize the energy and chemical cues provided by their environment to assemble themselves into systems capable of responding and adapting to internal and external stimuli, and also present several unique properties like regeneration, self-replication, self-organisation, self-repair and sustainability (3).

Modifying living materials and delivering them at various body sites holds significant potential for managing a range of diseases, either directly or indirectly. ELMs can persist in different body regions for extended periods and offer a promising platform for the targeted production of molecules (e.g. biomolecules or chemicals), specifically *in situ*. These molecules could be leveraged for therapeutic and diagnostic applications.

For example, currently, biological medicines in clinical settings are limited to the administration of externally produced biomolecules from engineered cells. However, engineered living materials that can be implanted and can produce or induce molecules directly within the body offer vast opportunities, including better stability of biomolecules, increased efficacy, and possible reduction of side effects (5). This report describes different types and applications of ELMs for *in situ* production of therapeutic molecules and highlights challenges and opportunities related to their development.

1.3. Classification of ELMs

ELMs can be divided into two different main types, based on their composition. The first type is called biological ELM (bio-ELMs) and includes all the materials that are entirely composed by living cells or by living cells and material produced by cells (e.g. biomineralization). Bio-ELMs also retain the ability to completely self-assemble without the need for other additional components (6,7). The second type, hybrid engineered living materials (h-ELMs), are not entirely composed by living cells. They also contain abiotic components, like scaffolds, and rely on top-down building processes, such as bio casting, embedment in artificial matrices (8) and bioprinting in 2D, 3D and even 4D (9).

While the two categories of bio-ELMs and h-ELMs are the most prevalent in the literature, the classification of ELMs is evolving towards a more granular classification like taxa inspired by Woese's system, a popular taxonomy for life, which uses three domains (Archaea, Bacteria and Eukaryotic living materials), complemented with an additional domain for ELMs containing also synthetic cells (10,11).

2. Applications of ELMs for *in situ* production of therapeutics

This section provides a non-exhaustive overview of fabrication techniques and highlights applications of ELMs that produce therapeutics *in situ*. Furthermore, relevant examples from the ongoing Pathfinder challenge portfolio launched by the European Innovation Council on engineered living materials are discussed.

2.1. ELMs fabrication

Living organisms can use their own secreted substances, and fungal hyphal structures, to create ELMs (12–14). Examples of secreted substances include amyloid protein and extracellular polysaccharides. Amyloid protein is a key part of the microbial extracellular matrix, and structural framework of biofilms (15). Amyloid-secreting microbes can be leveraged for designing living functional materials through bioengineering. The engineered biofilms produced can behave like hydrogels and can be precisely tuned using 3D printing and microencapsulation techniques (16).

Extracellular polysaccharides can stabilise biofilms by interacting with each other. They can retain water, are highly permeable, and biocompatible, and they can be tuned and leveraged to create living functional materials through metabolic engineering (17,18). In addition to tuning proteins and polysaccharides, novel assembly methods have been developed that modify the surface of microbial cells with ligands and receptors capable of binding to facilitate self-assembly among microbes (19).

Extracellular polymeric substances have limitations in their three-dimensional structure and mechanical performance like low density, weak bonding, defect caused by unchecked internal microstructures and long-term growth limitations (20). These limitations can be mitigated by adding external scaffolds which can support long-term microbial growth and the co-cultivation of different microbial zones. Examples of fabrication methods using exogenous scaffolds or inorganic components include the sol-gel process (8) that allows the creation of hybrid ELMs for use as injectable materials or implant coatings (21), 3D printing, encapsulation, and spinning.

3D printing allows the flexible assembly of living materials with complex geometric designs. Examples of the mostly used techniques are inkjet, micro-extrusion, and laser-assisted printing (22). 3D bioprinting ELMs allows precise spatial control over the positioning of biomaterials, essential for mimicking natural tissue architectures (23), enables the incorporation of multiple cell types and biomaterials (24), and provides a scalable and automatised method for producing ELMs. Bioprinting can also improve reproducibility of ELMs production, which can ensure greater consistency in batch manufacturing. The combined use of smart materials, biomaterials that can respond to stimuli, and bioprinting has resulted in the development of 4D printing, i.e. the incorporation of stimuli-responsive biomaterials in the printing process. This results in the creation of dynamic structures that can develop and adapt over time (9,25).

The development of capsules made of polymeric shells that contain a liquid core can serve as containers capable of regulating the diffusion of molecules, such as nutrients, metabolic products, and oxygen. They also provide a suitable microenvironment for sustained microbial growth and co-cultivation of diverse cell types. Moreover, their robust shell and the ability to tune their size facilitate seamless integration with raw materials and bioprinting techniques, such as micro-extrusion (26,27).

Spinning is another technique used for ELM fabrication but requires the material to withstand harsh conditions, which has limited its use with living organisms. Only wet spinning and electrospinning have been used to create living materials (28).

2.2. ELM applications

ELMs for the production of therapeutics *in situ* have an increasing area of applications with current focus on oncology, wound dressing, infectious diseases and regenerative medicine.

2.2.1. Oncology

There are several cancer treatments under development that leverage genetic engineering for the *in situ* production of anti-cancer drugs, often based on bacterial strains of Salmonella (29,30) or Clostridium (31,32). Suitable bacterial chassis plays a major role in the development of ELMs, or more broadly, bacterial-based cancer therapies.

Some bacterial species are more suitable for engineering due to characteristics and features such as well-characterised genetic tools, ease of genetic modification, innate anti-tumour activity, and the ability to invade and colonise specific tumours. These attributes are exemplified by Gram-negative bacteria like the Salmonella chassis and *E. coli*. Progress with Gram-positive bacteria has been slower due to less established cloning protocols, incompletely annotated genomes, uncharacterised biological components, and limited options for gene expression (33).

Many engineered bacteria-based therapeutic strategies for cancer consists of single bacterial strains genetically modified to perform a therapeutic function such as secrete or metabolise a substance (33–35). However, ELM systems with a defined extracellular structure and scaffold (a biomaterial) have also been explored as therapeutical strategy in oncology. One example is the ELM consisting of a

genetically modified *E. coli* strain, embedded in a hydrogel matrix, that can secrete the anticancer molecule deoxyviolacein in a light-regulated manner for up to 42 days (36). The hydrogel matrix not only supports the bacteria growth and containment in the desired site but also allows the regulated diffusion of the secreted molecule in the microenvironment upon blue light stimulation (36).

Novel ELMs have the potential to improve treatment delivery. Activation and production of treating agents directly into the target area, for example a malignant tumour, or its near proximity not only increases the local concentration of the therapeutic but will also likely reduce the dosage of treatment needed, improve pharmacokinetics, delay degradation of the payload thus potentially decreasing side effects that usually appear following systemic exposure. This would result in a greater safety and cost-effectiveness of therapeutics and could also enable the reintroduction and further development of treatments that had been discontinued due to their side effects (34,35).

2.2.2. Infectious diseases

ELMs applications related to infectious diseases include physical protection, virus detection, drug discovery, medical equipment, and instruments. This section will cover the elements related to *in situ* production of therapeutics. ELMs can interact with the viral replication cycle and can be modified to recognise and target specific antigens on the cell surface. Depending on their composition, ELMs can also be susceptible to environmental conditions or stimuli such as light (36,37) or pH, enabling control over *in situ* treatment delivery and possibly preventing side-effects like immune cytokine storm often caused by rapid payload release (38). Furthermore, the possibility to integrate dyes in the bacteria not only increases the chances of monitoring ELMs *in vivo*, but also allows the delivery of antigens and adjuvants using second near-infrared (NIR-II) technology and bioluminescence (39).

One example of an ELM system for the treatment of infections is a genetically modified *E. coli* anchored in a dextran-based hydrogel with large pores that reduces the risk of an unwanted bacteria release in the body (40). *E. coli* is genetically modified to produce and secrete lysostaphin, a bacteriocin that inhibits the growth of pathogens such as *Staphylococcus aureus in vitro* (40).

2.2.3. Wound dressing

Multiple studies have shown that ELMs can be a very effective and sturdy barrier to protect wounds and absorb the excessive exudations that might cause infections, and maintain a level of moisture ideal for healing (41,42). This is especially relevant for some partial deep dermal and full thickness burns (>25%) that represent an unmet medical need. Products for treatment of such burns have been granted EMA orphan designation. An example is the bilayer engineered collagen hydrogel-based skin graft composed of autologous keratinocytes and fibroblasts that has been granted orphan designation in April 2015 for the treatment of partial deep dermal and full thickness burns (43).

2.2.4. Regenerative medicine

Major advances have been made in regenerative medicine, especially growth factor engineering, the development of robust peptidomimetics, and controlled release matrices. Injectable and implantable ELMs that produce and release growth factors (e.g., pro-angiogenic proteins) have been developed and have shown to be able to bind collagen and promote angiogenic network formation among vascular endothelial cells, indicating their regenerative potential (44). These ELMs allow diffusion of nutrients, gases, growth factors, cytokines and the metabolites of cellular activity.

Additionally, the bio inductive qualities of ELMs not only allow the adhesion and differentiation of the implanted cells and the production of extracellular matrix, but also favours the migration of endogenous cells, thus favouring the regeneration of damaged tissue. Lastly, bacteria like genetically modified *E. coli* have been leveraged to create living glues, stimuli-responsive ELMs capable of autonomous mechanical work and damage repair. A relevant example includes an ELM system that can sense blood and respond by repairing blood-leakage sites in a microfluidic device that mimics an internal bleeding (45).

2.3. EIC projects

Over the past decade, national and international stakeholders have shown significant interest in developing ELMs and their potential for public health has also been highlighted by the World Health Organization report on emerging technologies and scientific innovation in 2023 (46–50), by the Organisation for Economic Co-operation and Development with a dedicated report on synthetic biology (51) and by the World Economic Forum that included engineered living therapeutics as one of the top 10 emerging technologies in 2025 (52). The main drivers of progress in this field are ongoing calls for action and grants, with several EU-funded projects focusing on ELMs development (4,53). The most relevant initiative is the Pathfinder challenge by EIC (HORIZON-EIC-2021-PATHFINDERCHALLENGES-01-05) (4), aiming to develop breakthrough ELM technologies and make EU companies leaders in ELMs production via coordinated actions. The EIC ELMs portfolio of projects are funded under this call and the Pathfinder Open call (53). This initiative encourages collaboration among researchers in synthetic biology, materials engineering, and artificial intelligence.

Key projects under the Pathfinder challenge include:

- Engineering a living human mini-heart and a swimming bio-robot (BioRobot MiniHeart) (54)
- Producing multi-cellular mycelium-based ELMs with computational capability (Fungateria) (55)
- Supervised morphogenesis in gastruloids (SUMO) (56)
- Closed-loop control of fungal materials (LoopOfFun) (57)
- PRInted Symbiotic Materials for living tissues production (PRISM-LT) (58)
- Living therapeutic and regenerative materials with specialized layers (NextSkins) (59)

These projects aim to develop at least two ELMs with different compositions or uses, focusing on multi-cellular ELMs and adaptable technologies for various cell types, and for application in two different sectors.

Among them, **NextSkins** and **PRISM-LT** aim at developing ELMs capable of *in situ* therapeutics production.

NextSkins focuses on mimicking skin layers to create two engineered living materials:

- Living therapeutic skin: a wearable patch for treating skin disorders, made of a bacterial cellulose hydrogel matrix with sense-and-respond cells.
- Living regenerative material: a tough, impact-resistant material for protective garments, capable of self-reinforcement and regeneration (59).

The **PRISM-LT** project aims to create a flexible platform for manufacturing living tissues using a hybrid living materials concept. This involves bio-ink with stem cells and engineered helper bacteria or yeast cells to form structured assemblies. The goal is to develop two symbiotic materials for biomedical and food applications, demonstrating the platform's versatility (58).

Five additional projects, not initially part of the call, were presented at subsequent events like the first EIC ELMs Annual Meeting and an EIC-EMA meeting on the regulatory framework of ELMs, such as:

- **Bio-Hybrid Hierarchical Organoid-Synthetic Tissues** (Bio-HhOST) (60): developing bio-hybrid materials with living and artificial cells that communicate dynamically to regulate living cells.
- **Implantable Ecosystems of Genetically Modified Bacteria for the Personalized Treatment of Patients with Chronic Diseases** (ISOS) (61): developing the first biomedical product for the *in situ* fabrication and auto-renewed delivery of therapeutic compounds employing complex ecosystems of probiotic genetically engineered bacteria integrated in a biomaterial-based bioreactor.
- **Bacteria Biofilm as bio-factory for tissue regeneration** (BIOACTION) (62): developing innovative bio-hydrogels by engineering the peri-implant orthopaedic and dental biofilm *in situ* and transforming it into a producer of pro-regenerative factors to treat implant-associated infections.
- **Archibiome tattoo for resistant, responsive, and resilient cities** (Remedy) (63): developing compatible bio fabrication processes that allow personalised design in the architectural context.
- **ENable LIGHT- and synthetic biology-driven volumetric bioprinting of functional human tissues** (ENLIGHT) (64): combining synthetic biology, 3D printing, and photonics to create optogenetic volumetric bioprinting, allowing ultra-fast production of high-resolution living tissues and organoids, most notably bioprinted engineered pancreatic cells capable of secreting insulin.

3. Challenges during ELMs development

This section provides an overview of current challenges related to ELMs development. The main challenges have been summarised based on literature but also on regulatory interactions between ELMs developers and European regulators.

3.1. Quality and manufacturing

The development of ELMs for the targeted delivery of therapeutics can present challenges to manufacturing and quality control methods. The following subparagraphs highlight challenges related to batch-to-batch consistency and quality standards, bioprinting, processing, and storage.

3.1.1. Batch-to-batch consistency and quality standards

The living components of ELMs may lead to a lack of batch-to-batch consistency. The fluctuating purity and potency of organisms in ELM products may result in a lack of uniformity and standardisation, even more when the ELM assembly takes place *in situ* (e.g., directly inside the body). These factors make it difficult to define detailed specifications for each batch to ensure quality and safety. The quality and safety of ELMs for therapeutic purposes, especially those with minimal processing, heavily depend on donor screening and sample testing for pathogens.

Topics discussed with regulators include:

- Suitability of the proposed potency assays
- Batch release:

- the proposed drug product release process
- the possibility of replacing routine batch tests for impurities
- potency assay for batch release testing

3.2. 3D printing

The process of 3D printing to produce ELMs can be challenging, especially if different facilities handle raw materials and printing (65,66). Different approaches are being explored to overcome the challenges of 3D bioprinting used during development and clinical translation of ELMs:

- Selecting suppliers with a strict quality control process towards all the bioprinting components, including bioinks, printers, consumables and living organisms.
- Adopting a centralised management software. Using a robust management software can streamline operations by allowing remote control and monitoring of multiple printers from a single interface to manage print queues, reducing downtime, and ensure efficient use of resources.
- Standardizing the types of 3D printers and materials used across facilities can simplify maintenance and training. It also ensures consistency in the quality of printed parts (66).
- Regular maintenance and calibration to improve operational efficiency. Furthermore, implementing a regular maintenance schedule for all printers can prevent unexpected breakdowns and ensure consistent print quality.
- Standardised calibration routines to ensure that each printer operates at optimal performance levels, reducing errors and rework (23).
- Introducing comprehensive training for staff on the use of 3D printers and management software that includes troubleshooting common issues and understanding the workflow from design to print (24).
- Establishing a centralised and regularly updated repository for design files and print settings to maintain consistency and reducing errors that is accessible to all facilities (67).
- Planning for scalability (e.g., investing in scalable software solutions and ensuring that infrastructure can support increased demand) (68).

During ELMs manufacturing, protocols may be considered to minimise the risk of contamination, including the minimisation of reusable equipment while handling the raw materials and screening of any potential printing cartridge produced by a third party.

3.3. Process and storage

The impact of processing and storage on ELMs also needs to be considered. Given their smart properties, processing may significantly affect the living component of ELMs.

Conventional sterilization methods may be incompatible with ELMs. Standard techniques such as autoclave, gamma irradiation, or ethylene oxide exposure could kill, or damage engineered living cells and degrade bio-hybrid matrices. Alternative approaches are limited and even less destructive techniques like filtration are able to reduce contaminants but often fail to provide long-term sterility without affecting ELM performance.

While some injectable liquid ELMs can be frozen without losing effectiveness, other, more sensible materials may face issues around moisture, light and oxygen levels that do not fit their design. These strict environmental requirements could also be a challenge for the transport and storage of ELMs. Managing cold chain logistics, storage, and temperature monitoring throughout the supply chain is crucial for temperature-sensitive ELMs.

3.4. Pre-clinical

Testing biocompatibility and tumorigenicity of the ELMs components are a common topic for discussion during regulatory interactions, with a focus on:

- Validity of the tumorigenicity tests proposed
- Design of *in vivo* tumorigenicity study
- The use of the second development batch for toxicology studies
- Whether the 3-month toxicity data are sufficient to support the initiation of a clinical trial
- The ideal waiting period between the first and the second patient receiving ELMs containing allogeneic cells as well as the safety period between two cohorts proposed for a clinical trial.

3.5. Clinical

For early phase clinical trials, conducting risk analysis for the ELM under development is well aligned with the guidelines provided by applicable good practices, particularly chapters 2 and 9.7 of GMP part IV, which emphasize a risk-based approach (69).

Additional risks may arise during subsequent stages of product development and the manufacturing process. These emerging risks should be documented in an accompanying report that outlines the Quality Risk Management strategy for innovative product development. Each identified risk should be linked to a risk management plan and mitigation measures to address or reduce the identified risks.

3.6. Other challenges

3.6.1. Ethics

Key questions, such as the ethical implications of ELMs that display autonomous functionalities, potential health risks, and issues related to the rightful ownership and liability of such materials derived from donor cells, need attention. They mainly involve ensuring equitable access to the technology and preserving individual autonomy. While protecting intellectual property can stimulate innovation, it should be balanced with equitable access to ELMs benefits. Several applications of ELMs may be linked to healthcare technologies, such as monitoring parameters in the human body or serving as neural interfaces, functioning as active implantable devices with potential biofeedback capabilities (70).

3.6.2. Design

Despite the growing interest in ELMs, there are hurdles that need to be addressed to maximise their integration in the biomedical field. The following paragraph highlights challenges related to the design and characterisation of ELMs and their functionalities.

One of the inherent challenges of ELMs implanted *in situ* for the production of therapeutics is related to the interactions they have with the microenvironment and how they can influence each other. This is especially true for rheological properties and can lead to great variability in the results (71,72). ELMs

intended for delivery and production of therapeutics *in situ* need to adapt to a particular environment and a variety of new techniques have been introduced to expand the experimental scope and reliability of future products (73).

Engineered cells can be developed and tested through polymer physics models, organoids, and organ-on-a-chip technologies to investigate their behaviour when they are exposed to biochemical agents and physical stress for a certain period of time, mimicking the *in vivo* environment (74–76). The characterisation of other more complex elements like the embedded genetic circuits, which can be used to tune gene expression and biomolecular interactions, are still not fully explored (77,78).

Another challenge of ELMs is to introduce more complex or varied engineered behaviour in ELMs to transition to a higher level of smart material, for example to tailor ELMs responses to multiple or more subtle stimuli. Challenges have been encountered in first generation stimuli-responsive ELMs. For example, the use of temperature-responsive, physically cross-linked injectable hydrogels in tissue engineering can be impaired by the temperature increase during room temperature subcutaneous injection that causes clogging of needles and results in failure of injection (79).

Lastly, a current pressing challenge of ELMs development relates to mechanistic mathematical modelling to quantify reaction kinetics (74). The scarcity of tools to efficiently measure dynamic conditions in cells and hydrogels, combined with the increase in complexity, prevents the quantification of functional parameters (80). Machine learning, data-driven modelling, transfer learning, and autonomous experimentation for the discovery, design, and optimisation of soft and biological materials offer opportunities to simplify this part of the product design (81).

3.6.3. Biosafety

A key factor for developing ELMs, especially those based on microbes, are the genetic tools available to modify such organisms. This is already seen with engineered gut microbes, like probiotics, which have natural elements such as curli fibers that help them reach and colonise specific body areas, like the intestine, or the tumor microenvironment (33,82). However, using ELMs in clinical settings faces challenges, including biosafety and meeting regulatory requirements, similar to other genetically modified organisms (GMOs).

Biosafety concerns associated with the use of ELMs are mainly related to engineered cells. Most notably the risk of immune rejection, tumorigenicity, and the potential for abnormal proliferation or escape from the desired site. Imaging technologies like second near-infrared (NIR-II) fluorescence can be used to monitor ELMs *in vivo* (39). Additional precautional measures include introducing biocontainment circuit designs which typically involve an input that is specific to the permissive environment and repressive to the killing circuit, but upon exit from the permissive environment, causes the expression of lethal components (83). The most prominent examples of said circuits are auxotrophic cells and genetic kill switches. Auxotrophic cells need constant external supplements of specific molecules to survive and in case of escape, they would be extremely unlikely to survive in another environment where the molecule is not supplied (84). However, the need for a continuous supply of essential factors to their probiotic environment relies on external intervention which can limit the applicability of this strategy.

The use of genetic kill switches induces lysis or the expression of toxins in case cells leave the desired site. One example is a two-fold toxin/antitoxin kill switch system to limit the growth of a genetically engineered strain of *E. coli* (85). This is achieved using a cold-inducible promoter to induce the expression of toxin protein CcdB if the temperature falls below 37°C, resulting in microbial cell death (86,87). However, appropriate modifications of intrinsic cellular DNA-repair and mutagenesis pathways should be considered as a complementary measure of biocontainment approaches since innate

mutagenesis can interfere with the expression of such kill switches (88). Therefore, genetically stable kill switches could be very valuable for any future ELM application, as these materials may need to be stored for long periods of time without compromising their safety.

Hence why the development of broad-host kill-switch systems able to adhere to these requirements will be vital to large scale implementation of ELMs (17).

In addition to biological approaches, the physical encapsulation of bacteria in hydrogels can also act as a biocontainment tool capable of both greatly reducing the chance of inadvertent escape and ensuring the sustained viability of microbial cultures. Examples include the encapsulation of microbial cells in a nonporous, highly crosslinked hydrogel matrix or the use of a bilayer thin film hydrogel (40,44,89). Lastly, capsules with polymeric shells and liquid cores have been also explored as an alternative approach for compartmentalising microbial cells for biosafety purposes (90).

3.7. Regulatory framework applicable to ELMs

ELMs often involve one or more complex manufacturing processes, a great variety of components, including manipulated living cells, and different characteristic features. As a result, ELMs may require defined quality standards and regulations for development, evaluation and approval processes.

The EU-Innovation Network (EU-IN) Borderline Classification Group (BLCG) works as a multidisciplinary forum for informal discussions on innovative product classification between European competent authorities (91).

ELM borderline classifications discussed by the BLCG include a keratinocyte-based product. The main points discussed focussed on what constitutes substantial manipulation of cells and tissues. Cells or tissues shall be considered 'engineered' only if they are not used for the same essential function as in the donor or if they have been subject to substantial manipulation (92). The effects of mechanical and enzymatic dissociation onto the cells have been debated and whether they should be considered a non-substantial manipulation.

Other cases discussed including local sustained drug delivery systems, a wound dressing for the prevention of microbial colonialisation, and hydrogels, focussed on defining and establishing the principal mode of action, intended action and which of the component of a composite medical item is ancillary. Based on their nature and mode of action, most ELMs would fall within the Advanced Therapy Medicinal Products (ATMPs) regulatory framework (92). Currently, there is no agreed EU nor national approach to classify ELMs.

Furthermore, there is currently no regulatory guidance specifically addressing ELMs-based products. However, ATMP specific guidance can provide support to ELMs developers. These include:

- Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials (EMA/CAT/852602/2018) (93)
- Guideline for human cell-based medicinal products (EMEA/CHMP/410869/2006) (94)
- Reflection paper on clinical aspects related to tissue engineered products (EMA/CAT/573420/2009) (95)

EMA early discussion forum like the Innovation Task Force (ITF) (96,97) provide opportunities to discuss early developments with European regulators. It is currently not certain whether a dedicated regulatory framework and guidance for ELMs are needed. More experience and exposure of the regulators to ELMs development will determine the need for further guidance.

4. Recommendations

The following recommendations related to the development of ELMs for *in situ* production of therapeutics are based on current regulatory requirements, experience and interactions between regulators and ELMs developers, including a joint EIC-EMA meeting on ELMs that took place in February 2025.

4.1. Regulatory classification frameworks

To support the development and authorisation of ELMs for *in situ* therapeutic production, increased information on the applicable classification framework of different types of ELMs and the related regulatory requirements would provide clarity and predictability for developers and alignment between regulatory authorities. This could be achieved through the development of joint guidance documents of regulatory authorities in charge of medicinal products and medical devices. Approaching EDQM to propose a list of standardised terms to clarify ELMs-related vocabulary should also be considered.

4.2. Guidance on pharmaceutical requirements

Acknowledging that variability of ELMs presents pharmaceutical development challenges, guidance on quality control and batch-to-batch consistency would be of relevance to developers and regulatory authorities. This would include information on specifications for standardised testing procedures to enhance reproducibility, reliability and stability.

4.3. Guidance on non-clinical and clinical requirements

To address the unique characteristics of ELMs, their development and use, specific guidance for the non-clinical and clinical development should be considered. This would include setting out ELMs specific requirements for clinical trials and genetically-modified organisms (GMO), including environmental release.

5. Reference List

1. EU Innovation Network (EU-IN) | European Medicines Agency (EMA) [Internet]. 2017 [cited 2025 Apr 14]. Available from: https://www.ema.europa.eu/en/committees/working-parties-other-groups/eu-innovation-network-eu

2. European medicines agencies network strategy | European Medicines Agency (EMA) [Internet]. 2025 [cited 2025 Apr 15]. Available from: https://www.ema.europa.eu/en/about-us/how-we-work/european-medicines-regulatory-network/european-medicines-agencies-network-strategy

3. Chayaamor-Heil N, Perricone V, Gruber P, Guéna F. Bioinspired, biobased and living material designs: a review of recent research in architecture and construction. Bioinspir Biomim. 2023 Jun;18(4):041001.

4. EIC Pathfinder challenge: Engineered living materials [Internet]. 2021 [cited 2023 Jun 1]. Available from: https://eic.ec.europa.eu/eic-funding-opportunities/calls-proposals/eic-pathfinder-challenge-engineered-living-materials_en

5. Flores Bueso Y, Lehouritis P, Tangney M. In situ biomolecule production by bacteria; a synthetic biology approach to medicine. Journal of Controlled Release. 2018 Apr;275:217–28.

6. Stephanopoulos N, Ortony JH, Stupp SI. Self-Assembly for the Synthesis of Functional Biomaterials. Acta Mater. 2013 Feb 1;61(3):912–30.

7. Teague BP, Guye P, Weiss R. Synthetic Morphogenesis. Cold Spring Harb Perspect Biol. 2016 Sep;8(9):a023929.

8. Kamanina OA, Saverina EA, Rybochkin PV, Arlyapov VA, Vereshchagin AN, Ananikov VP. Preparation of Hybrid Sol-Gel Materials Based on Living Cells of Microorganisms and Their Application in Nanotechnology. Nanomaterials. 2022 Jan;12(7):1086.

9. Rivera-Tarazona LK, Shukla T, Singh KA, Gaharwar AK, Campbell ZT, Ware TH. 4D Printing of Engineered Living Materials. Advanced Functional Materials. 2022;32(4):2106843.

10. Lantada AD, Korvink JG, Islam M. Taxonomy for engineered living materials. Cell Reports Physical Science. 2022 Apr 20;3(4):100807.

11. Srubar WV. Engineered Living Materials: Taxonomies and Emerging Trends. Trends in Biotechnology. 2021 Jun 1;39(6):574–83.

12. Wang Y, Liu Y, Li J, Chen Y, Liu S, Zhong C. Engineered living materials (ELMs) design: From function allocation to dynamic behavior modulation. Curr Opin Chem Biol. 2022 Oct;70:102188.

13. Orozco-Hidalgo MT, Charrier M, Tjahjono N, Tesoriero RF, Li D, Molinari S, et al. Engineering High-Yield Biopolymer Secretion Creates an Extracellular Protein Matrix for Living Materials. mSystems. 2021 Mar 23;6(2):10.1128/msystems.00903-20.

14. Elsacker E, Zhang M, Dade-Robertson M. Fungal Engineered Living Materials: The Viability of Pure Mycelium Materials with Self-Healing Functionalities. Advanced Functional Materials. 2023;33(29):2301875.

15. Olubajo S, Tükel Ç. Functional Amyloids as Key Biofilm Matrix Components. In: Reichhardt C, editor. Biofilm Matrix [Internet]. Cham: Springer Nature Switzerland; 2024 [cited 2025 Apr 16]. p. 131–52. Available from: https://doi.org/10.1007/978-3-031-70476-5_4

16. Jimenez EM, Nguyen C, Shakeel A, Tesoriero RJr, Charrier M, Stull A, et al. Genetically Modifying the Protein Matrix of Macroscopic Living Materials to Control Their Structure and Rheological Properties. ACS Synth Biol. 2024 Dec 20;13(12):3936–47.

17. Molinari S, Tesoriero RF, Ajo-Franklin CM. Bottom-up approaches to engineered living materials: Challenges and future directions. Matter. 2021 Oct 6;4(10):3095–120.

18. Lu C, Huang Y, Cui J, Wu J, Jiang C, Gu X, et al. Toward Practical Applications of Engineered Living Materials with Advanced Fabrication Techniques. ACS Synth Biol. 2024 Aug 16;13(8):2295–312.

19. Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China., Kiprono SJ, National Engineering Research Centre for Nano-Medicine, Huazhong University of Science and Technology, Wuhan 430074, China, Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, 190-50100, Kakamega, Kenya, Ullah MW, Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, PR China., et al. Surface engineering of microbial cells: Strategies and applications. Eng Sci [Internet]. 2018 [cited 2025 Sep 8]; Available from: http://www.espublisher.com/journals/articledetails/127/

20. Geng Y, Jia Z, Li L. Structural engineered living materials. Nano Res. 2024 Feb 1;17(2):715–33.

21. Catauro M, Ciprioti SV. Characterization of Hybrid Materials Prepared by Sol-Gel Method for Biomedical Implementations. A Critical Review. Materials. 2021 Jan;14(7):1788.

22. Dell AC, Wagner G, Own J, Geibel JP. 3D Bioprinting Using Hydrogels: Cell Inks and Tissue Engineering Applications. Pharmaceutics. 2022 Dec;14(12):2596.

23. Santoni S, Gugliandolo SG, Sponchioni M, Moscatelli D, Colosimo BM. 3D bioprinting: current status and trends—a guide to the literature and industrial practice. Bio-des Manuf. 2022 Jan 1;5(1):14–42.

24. Dababneh AB, Ozbolat IT. Bioprinting Technology: A Current State-of-the-Art Review. Journal of Manufacturing Science and Engineering [Internet]. 2014 Oct 24 [cited 2025 Mar 3];136(061016). Available from: https://doi.org/10.1115/1.4028512

25. Ramezani M, Mohd Ripin Z. 4D Printing in Biomedical Engineering: Advancements, Challenges, and Future Directions. Journal of Functional Biomaterials. 2023 Jul;14(7):347.

26. Ladeira BM, Custódio CA, Mano JF. Core–shell microcapsules: biofabrication and potential applications in tissue engineering and regenerative medicine. Biomater Sci. 2022 May 4;10(9):2122–53.

27. Nadine S, Patrício SG, Barrias CC, Choi IS, Matsusaki M, Correia CR, et al. Geometrically Controlled Liquefied Capsules for Modular Tissue Engineering Strategies. Advanced Biosystems. 2020;4(11):2000127.

28. Rodrigo-Navarro A, Sankaran S, Dalby M, del Campo A, Salmerón-Sánchez M. Engineered living biomaterials. Nature Reviews Materials. 2021 Aug 31;6.

29. A Phase 2 Study of Saltikva (Attenuated Salmonella Typhimurium Containing the Human Gene for Interleukin-2) in Patients With Metastatic Pancreatic Cancer - AdisInsight [Internet]. [cited 2023 Jun 27]. Available from: https://adisinsight.springer.com/trials/700329057

30. MDB 401 B - AdisInsight [Internet]. [cited 2023 Jun 27]. Available from: https://adisinsight.springer.com/drugs/800060923

31. Pilot Study to Evaluate the Biologic Effect of CBM588 in Combination With Cabozantinib/Nivolumab for Patients With Metastatic Renal Cell Carcinoma - AdisInsight [Internet]. [cited 2023 Jun 27]. Available from: https://adisinsight.springer.com/trials/700340105

32. Clostridium butyricum M588- Miyarisan Pharmaceutical - AdisInsight [Internet]. [cited 2023 Jun 27]. Available from: https://adisinsight.springer.com/drugs/800023453

33. Sieow BFL, Wun KS, Yong WP, Hwang IY, Chang MW. Tweak to Treat: Reprograming Bacteria for Cancer Treatment. Trends in Cancer. 2021 May 1;7(5):447–64.

34. Taniguchi S. In Situ Delivery and Production System (iDPS) of Anti-Cancer Molecules with Gene-Engineered Bifidobacterium. Journal of Personalized Medicine. 2021 Jun;11(6):566.

35. Fan JX, Niu MT, Qin YT, Sun YX, Zhang XZ. Progress of engineered bacteria for tumor therapy. Advanced Drug Delivery Reviews. 2022 Jun 1;185:114296.

36. Sankaran S, Becker J, Wittmann C, del Campo A. Optoregulated Drug Release from an Engineered Living Material: Self-Replenishing Drug Depots for Long-Term, Light-Regulated Delivery. Small [Internet]. 2019 Feb [cited 2025 Sep 5];15(5):1804717. Available from: https://onlinelibrary.wiley.com/doi/10.1002/smll.201804717

37. Xie L, Pang X, Yan X, Dai Q, Lin H, Ye J, et al. Photoacoustic Imaging-Trackable Magnetic Microswimmers for Pathogenic Bacterial Infection Treatment. ACS Nano [Internet]. 2020 Mar 24 [cited 2025 Sep 5];14(3):2880–93. Available from: https://pubs.acs.org/doi/10.1021/acsnano.9b06731

38. Tang Z, Kong N, Zhang X, Liu Y, Hu P, Mou S, et al. A materials-science perspective on tackling COVID-19. Nat Rev Mater. 2020 Nov;5(11):847–60.

39. Wang W, Yang Q, Du Y, Zhou X, Du X, Wu Q, et al. Metabolic Labeling of Peptidoglycan with NIR-II Dye Enables In Vivo Imaging of Gut Microbiota. Angew Chem Int Ed. 2020 Feb 10;59(7):2628–33.

40. Guo S, Dubuc E, Rave Y, Verhagen M, Twisk SAE, Van Der Hek T, et al. Engineered Living Materials Based on Adhesin-Mediated Trapping of Programmable Cells. ACS Synth Biol. 2020 Mar 20;9(3):475–85.

41. Liu X, Gao L, Fu S, Zhao W, Wang F, Gao J, et al. Polycaprolactone nanofiber-alginate hydrogel interpenetrated skin substitute for regulation of wound-substitute interface. Materials & Design. 2023 Mar 1;227:111706.

42. Elangwe CN, Morozkina SN, Olekhnovich RO, Polyakova VO, Krasichkov A, Yablonskiy PK, et al. Pullulan-Based Hydrogels in Wound Healing and Skin Tissue Engineering Applications: A Review. International Journal of Molecular Sciences. 2023 Jan;24(5):4962.

43. Union Register of medicinal products [Internet]. [cited 2025 Jan 22]. COMMISSION IMPLEMENTING DECISION of 14.12.2015 relating to the designation of "Bilayer engineered collagen hydrogel-based skin graft composed of autologous keratinocytes and fibroblasts" as an orphan medicinal product under Regulation (EC) No 141/2000 of the European Parliament and of the Council. Available from: https://ec.europa.eu/health/documents/community-register/html/reg_od_act.htm?sort=a

44. Dhakane P, Tadimarri VS, Sankaran S. Light-Regulated Pro-Angiogenic Engineered Living Materials. Advanced Functional Materials. 2023;33(31):2212695.

45. An B, Wang Y, Jiang X, Ma C, Mimee M, Moser F, et al. Programming Living Glue Systems to Perform Autonomous Mechanical Repairs. Matter [Internet]. 2020 Dec [cited 2025 Sep 5];3(6):2080–92. Available from: https://linkinghub.elsevier.com/retrieve/pii/S259023852030504X

46. European Innovation Council to award up to €145 million to achieve breakthroughs in emerging, strategic areas of technology [Internet]. [cited 2023 Jun 22]. Available from: https://eic.ec.europa.eu/news/european-innovation-council-award-eu145-million-achieve-breakthroughs-emerging-strategic-areas-2022-04-07_en

47. GOV.UK [Internet]. [cited 2023 Jun 22]. Government to look at ways new high-tech materials could advance UK industry. Available from: https://www.gov.uk/government/news/government-to-look-at-ways-new-high-tech-materials-could-advance-uk-industry

48. Shrestha P. UK announces £95m for advanced materials' research and development [Internet]. Energy Live News. 2022 [cited 2023 Jun 22]. Available from: https://www.energylivenews.com/2022/11/07/uk-announces-95m-for-advanced-materials-research-and-development/

49. Albert JME. Bold Goals for U.S. Biotechnology and Biomanufacturing: Harnessing Research and Development to Further Societal Goals.

50. 2023 emerging technologies and scientific innovations: a global public health perspective — preview of horizon scan results [Internet]. [cited 2023 Jun 1]. Available from: https://www.who.int/publications-detail-redirect/WHO-SCI-RFH-2023.05

51. Robinson DKR, Nadal D. Synthetic biology in focus: Policy issues and opportunities in engineering life [Internet]. 2025 Feb [cited 2025 Sep 5]. (OECD Science, Technology and Industry Working Papers). Available from: https://www.oecd.org/en/publications/synthetic-biology-in-focus_3e6510cf-en.html

52. Top 10 Emerging Technologies of 2025 - Flagship report June 2025 [Internet]. World Economic Forum; Available from: https://reports.weforum.org/docs/WEF_Top_10_Emerging_Technologies_of_2025.pdf?_gl=1*1v581fg*_up*MQ..*_gs*MQ..&gclid=EAIaIQobChMIj7bC0J_BjwMVPKaDBx304ACyEAAYASAAEgJQlPD_BwE

53. EIC Pathfinder Open 2022 [Internet]. Available from: https://cordis.europa.eu/programme/id/HORIZON_HORIZON-EIC-2022-PATHFINDEROPEN-01-01

54. Engineering a living human Mini-heart and a swimming Bio-robot [Internet]. Available from: https://cordis.europa.eu/project/id/101070953

55. Enlisting synthetic fungal-bacterial consortia to produce multi-cellular mycelium-based ELMs with computational capability [Internet]. Available from: https://cordis.europa.eu/project/id/101071145

56. Supervised morphogenesis in gastruloids [Internet]. Available from: https://cordis.europa.eu/project/id/101071203

57. Closed-loop control of fungal material [Internet]. Available from: https://cordis.europa.eu/project/id/101070817

58. CORDIS | European Commission [Internet]. [cited 2023 Jun 23]. PRInted Symbiotic Materials as a dynamic platform for Living Tissues production | PRISM-LT Project | Fact Sheet | HORIZON. Available from: https://cordis.europa.eu/project/id/101070913

59. CORDIS | European Commission [Internet]. [cited 2023 Jun 23]. Living Therapeutic and Regenerative Materials with Specialised Advanced Layers | NextSkins Project | Fact Sheet | HORIZON. Available from: https://cordis.europa.eu/project/id/101071159

60. CORDIS | European Commission [Internet]. [cited 2024 Jun 30]. Next Generation 3D Tissue Models: Bio-Hybrid Hierarchical Organoid-Synthetic Tissues (Bio-HhOST) Comprised of Live and Artificial Cells. | Bio-HhOST Project | Fact Sheet | HORIZON. Available from: https://cordis.europa.eu/project/id/101130747

61. ISOS-Implantable Ecosystems of Genetically Modified Bacteria for the Personalized Treatment of Patients with Chronic Diseases [Internet]. Available from: https://cordis.europa.eu/project/id/101130454

62. Bacteria Biofilm as bio-factory for tissue regeneration [Internet]. Available from: https://cordis.europa.eu/project/id/101098972

63. Archibiome tattoo for resistant, responsive, and resilient cities [Internet]. Available from: https://cordis.europa.eu/project/id/101185862

64. CORDIS | European Commission [Internet]. [cited 2024 Jun 30]. ENable LIGHT- and synthetic biology-driven volumetric bioprinting of functional human tissues | ENLIGHT Project | Fact Sheet | H2020. Available from: https://cordis.europa.eu/project/id/964497

65. Yu Y, Wang Q, Wang C, Shang L. Living Materials for Regenerative Medicine. Engineered Regeneration. 2021 Jan 1;2:96–104.

66. Kesti M, Fisch P, Pensalfini M, Mazza E, Zenobi-Wong M. Guidelines for standardization of bioprinting: a systematic study of process parameters and their effect on bioprinted structures. BioNanoMaterials. 2016 Sep 1;17(3–4):193–204.

67. Schmieg B, Brandt N, Schnepp VJ, Radosevic L, Gretzinger S, Selzer M, et al. Structured Data Storage for Data-Driven Process Optimisation in Bioprinting. Applied Sciences. 2022 Jan;12(15):7728.

68. Eghosasere E, Osasumwen E, Emmanuella O. 3D Bioprinting in Tissue Engineering: Advancements, Challenges, and Pathways to Clinical Translation. JSM Regenerative Medicine and Bioengineering. 2025 Jan 10;7(1):1–15.

69. Guidelines on Good Manufacturing Practice specific to Advanced Therapy Medicinal Products [Internet]. Available from: https://health.ec.europa.eu/document/download/ad33d9dd-03f0-4bef-af53-21308ce2187d_en?filename=2017_11_22_guidelines_gmp_for_atmps.pdf

70. Ebbesen M, Korvink JG, Islam M, Lantada AD. The ethics of engineered living materials. Trends in Biotechnology. 2024 Jan 1;42(1):5–9.

71. Lavrador P, Gaspar VM, Mano JF. Engineering mammalian living materials towards clinically relevant therapeutics. eBioMedicine. 2021 Dec 1;74:103717.

72. Zhang J, Miao Y, Li H. 7 - Dynamic behaviors of the hydrogel. In: Li H, Silberschmidt V, editors. The Mechanics of Hydrogels [Internet]. Woodhead Publishing; 2022 [cited 2023 Aug 2]. p. 139–57. (Elsevier Series in Mechanics of Advanced Materials). Available from: https://www.sciencedirect.com/science/article/pii/B9780081028629000026

73. Ahearne M. 4 - Mechanical testing of hydrogels. In: Li H, Silberschmidt V, editors. The Mechanics of Hydrogels [Internet]. Woodhead Publishing; 2022 [cited 2023 Aug 2]. p. 73–90. (Elsevier Series in Mechanics of Advanced Materials). Available from: https://www.sciencedirect.com/science/article/pii/B9780081028629000038

74. Liu Z, Zheng S, Li Z, Xu S, Jincheng Lei J, Toh W. 9 - Multiscale modeling of hydrogels. In: Li H, Silberschmidt V, editors. The Mechanics of Hydrogels [Internet]. Woodhead Publishing; 2022 [cited 2023 Aug 2]. p. 187–222. (Elsevier Series in Mechanics of Advanced Materials). Available from: https://www.sciencedirect.com/science/article/pii/B9780081028629000129

75. Wu T. 11 - Mechanically driven phase transition of physical hydrogels. In: Li H, Silberschmidt V, editors. The Mechanics of Hydrogels [Internet]. Woodhead Publishing; 2022 [cited 2023 Aug 2]. p. 269–88. (Elsevier Series in Mechanics of Advanced Materials). Available from: https://www.sciencedirect.com/science/article/pii/B9780081028629000130

76. Goh KB. 13 - Enzyme functionalized hydrogels: relationship between stimuli and mechanical response. In: Li H, Silberschmidt V, editors. The Mechanics of Hydrogels [Internet]. Woodhead Publishing; 2022 [cited 2023 Aug 2]. p. 307–19. (Elsevier Series in Mechanics of Advanced Materials). Available from: https://www.sciencedirect.com/science/article/pii/B9780081028629000087

77. Liu X, Inda ME, Lai Y, Lu TK, Zhao X. Engineered Living Hydrogels. Advanced Materials. 2022 Jul;34(26):2201326.

78. Tang TC, An B, Huang Y, Vasikaran S, Wang Y, Jiang X, et al. Materials design by synthetic biology. Nat Rev Mater. 2021 Apr;6(4):332–50.

79. Li Y, Yang HY, Lee DS. Biodegradable and Injectable Hydrogels in Biomedical Applications. Biomacromolecules. 2022 Mar 14;23(3):609–18.

80. Sohail A. Genetic Algorithms in the Fields of Artificial Intelligence and Data Sciences. Ann Data Sci. 2023 Aug 1;10(4):1007–18.

81. Ferguson AL, Brown KA. Data-Driven Design and Autonomous Experimentation in Soft and Biological Materials Engineering. Annual Review of Chemical and Biomolecular Engineering. 2022;13(1):25–44.

82. Duraj-Thatte AM, Praveschotinunt P, Nash TR, Ward FR, Nguyen PQ, Joshi NS. Modulating bacterial and gut mucosal interactions with engineered biofilm matrix proteins. Sci Rep. 2018 Feb 22;8(1):3475.

83. Stirling F, Naydich A, Bramante J, Barocio R, Certo M, Wellington H, et al. Synthetic Cassettes for pH-Mediated Sensing, Counting, and Containment. Cell Reports. 2020 Mar;30(9):3139-3148.e4.

84. Wiebking V, Patterson JO, Martin R, Chanda MK, Lee CM, Srifa W, et al. Metabolic engineering generates a transgene-free safety switch for cell therapy. Nat Biotechnol. 2020 Dec;38(12):1441–50.

85. Rottinghaus AG, Ferreiro A, Fishbein SRS, Dantas G, Moon TS. Genetically stable CRISPR-based kill switches for engineered microbes. Nat Commun. 2022 Feb 3;13(1):672.

86. Omer R, Mohsin MZ, Mohsin A, Mushtaq BS, Huang X, Guo M, et al. Engineered Bacteria-Based Living Materials for Biotherapeutic Applications. Front Bioeng Biotechnol. 2022;10:870675.

87. Stirling F, Bitzan L, O'Keefe S, Redfield E, Oliver JWK, Way J, et al. Rational Design of Evolutionarily Stable Microbial Kill Switches. Molecular Cell. 2017 Nov 16;68(4):686-697.e3.

88. Lee JW, Chan CTY, Slomovic S, Collins JJ. Next-generation biocontainment systems for engineered organisms. Nat Chem Biol. 2018 Jun;14(6):530–7.

89. Bhusari S, Kim J, Polizzi K, Sankaran S, del Campo A. Encapsulation of bacteria in bilayer Pluronic thin film hydrogels: A safe format for engineered living materials. Biomaterials Advances. 2023 Feb 1;145:213240.

90. Vilabril S, Nadine S, Neves CMSS, Correia CR, Freire MG, Coutinho JAP, et al. One-Step All-Aqueous Interfacial Assembly of Robust Membranes for Long-Term Encapsulation and Culture of Adherent Stem/Stromal Cells. Advanced Healthcare Materials. 2021;10(10):2100266.

91. Regulatory Science and Innovation | EMA Annual Report 2021 [Internet]. [cited 2023 Sep 1]. Available from: https://www.ema.europa.eu/en/annual-report/2021/regulatory-science-and-innovation.html

92. Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 (Text with EEA relevance) [Internet]. OJ L Nov 13, 2007. Available from: http://data.europa.eu/eli/reg/2007/1394/oj/eng

93. Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials [Internet]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-clinical-requirements-investigational-advanced-therapy-medicinal-products-clinical-trials_en.pdf

94. GUIDELINE ON HUMAN CELL-BASED MEDICINAL PRODUCTS [Internet]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-human-cell-based-medicinal-products_en.pdf

95. Reflection paper on clinical aspects related to tissue engineered products [Internet]. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/reflection-paper-clinical-aspects-related-tissue-engineered-products_en.pdf

96. Supporting innovation | European Medicines Agency (EMA) [Internet]. 2020 [cited 2025 Apr 28]. Available from: https://www.ema.europa.eu/en/human-regulatory-overview/research-development/supporting-innovation

97. Uster DW, Cordo' V, Cormier E, Ehmann F. Insights into Early Interactions on Innovative Developments with European Regulators. Ther Innov Regul Sci. 2024 Nov;58(6):1108–19.

6. Appendix

6.1. Methodology

The current state of art and key emerging trends have been identified via a systematic literature search using Web of science, PubMed, Embase, Cochrane and CINAHL.

Regulatory challenges and opportunities related to ELMs have been informed using relevant EMA internal documents, mostly produced during Scientific Advice, Innovation Task Force, and Business Pipeline Meetings, now Portfolio and Technology Meetings.