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*MINIREVIEWS*

# Bioengineering breakthroughs: The impact of stem cell models on advanced therapy medicinal product development

José Mauro Granjeiro, Priscila Grion de Miranda Borchio, Icaro Paschoal Brito Ribeiro, Katiucia Batista Silva Paiva

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## Abstract

The burgeoning field of bioengineering has witnessed significant strides due to the advent of stem cell models, particularly in their application in advanced therapy medicinal products (ATMPs). In this review, we examine the multifaceted impact of these developments, emphasizing the potential of stem cell models to enhance the sophistication of ATMPs and to offer alternatives to animal testing. Stem cell-derived tissues are particularly promising because they can reshape the preclinical landscape by providing more physiologically relevant and ethically sound platforms for drug screening and disease modelling. We also discuss the critical challenges of reproducibility and accuracy in measurements to ensure the integrity and utility of stem cell models in research and application. Moreover, this review highlights the imperative of stem cell models to align with regulatory standards, ensuring using stem cells in ATMPs translates into safe and effective clinical therapies. With regulatory approval serving as a gateway to clinical adoption, the collaborative efforts between scientists and regulators are vital for the progression of stem cell applications from bench to bedside. We advocate for a balanced approach that nurtures innovation within the framework of rigorous validation and regulatory compliance, ensuring that stem cell-base solutions are maximized to promote public trust and patient health in ATMPs.

**Key Words:** Stem cells; Advanced therapy medicinal products; Tissue-engineered products; Health; Three-dimensional cell culture



**Core Tip:** Stem cells play a crucial role in tissue engineering by offering the potential for regenerating of damaged tissues, which is critical for developing advanced therapy medicinal products. Stem cells can differentiate into specific cell types and promote tissue repair through various mechanisms. When combined with tissue engineering techniques, stem cell therapy enhances cell viability, differentiation, and therapeutic efficacy, overcoming disease treatment limitations. However, translating stem cell research into approved clinical therapies has been challenging. Regulatory bodies have provided guidelines to ensure the safety and efficacy of advanced therapy medicinal products utilizing stem cells before the approval for clinical use.

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## INTRODUCTION

Advanced therapy medicinal product (ATMP) was defined by European Union (Directive 2001/83/EC) and amended by the ATMP Regulation (EC No 1394/2007), whereas, in the United States, the term used is cellular and gene therapy products. In this review, we will adopt ATMP. These products represent a significant advancement in medical treatment, focusing on innovative therapies for new regenerative approaches for well-know or rare diseases/conditions (orphan and unmet needs)[1]. These biological products include three main categories: Gene therapy, somatic cell therapy, and tissue-engineered products (TEPs), all of which aim to treat the root causes of diseases rather than just their symptoms. Stem cells can differentiate into multiple cell types and secrete trophic factors, making them an attractive tool for ATMPs, revolutionizing the field of regenerative medicine. New three-dimensional (3D) cell culture techniques have enhanced cell properties relevant for tissue regeneration, such as cell viability, differentiation, and secretion of pro-regenerative factors, overcoming the limitations of stem cell therapy alone for organ replacement in the tissue engineering concept. In this review, we discuss the current use of stem cells in ATMP development, specifically, for TEP and the regulatory landscape worldwide.

### *ATMPs*

ATMPs represent a diverse category of medicinal products based on, or the combination of them and the addition of medical devices. ATMPs are medicines that are based on the manipulation of biological materials (genes, cells, and/or tissues) and combined with medical devices to achieve therapeutic effects (Figure 1). The three main categories include: (1) Gene cell therapy medicine (GCTM)- which involves the introduction, removal, or alteration of genetic material within a patient's cells to treat or prevent disease; (2) Somatic cell therapy medicine (SCTM) - this therapy transfers genetic material into somatic (non-reproductive) or stem cells to treat diseases, ensuring that future generations do not inherit the changes; and (3) TEP: This therapy contain engineered cells or tissues designed to regenerate, repair, or replace damaged human tissues. Clinical use of SCTM and TEP is referred as regenerative medicine.

Recent reports show numerous ongoing clinical trials for ATMPs, with a significant proportion in the early development phases. These trials predominantly focus on oncology, genetic disorders, cardiovascular and musculoskeletal diseases. The complexity of ATMPs often necessitates innovative trial designs, including small sample sizes and adaptive methodologies to accommodate the unique characteristics of these therapies[2].

A recent study assessed the efficacy of ATMPs in healing long bone delayed unions and non-unions through clinical and radiological consolidation at 3, 6, and 12 months of the initial fracture[3]. Clinical consolidation occurred earlier, while radiological consolidation reached 92.8% at 12 months. Bone biopsies confirmed bone formation around bioceramic granules, with better consolidation in non-smokers and slight delays in tibial non-unions. The study showed effective bone healing using expanded human bone marrow mesenchymal stem cells (MSCs) with biomaterials, though consolidation rates were lower in smokers.

However, ATMPs come with a number of known and unknown risks, many of which are unique to this product class. Some of the main risks associated with ATMPs include: (1) Related to the novel mechanisms of action (may cause new risks to patients due to their novel mechanisms of action); (2) Related to manufacturing complexity (ATMPs present a high degree of technical complexity and substantial challenges to their manufacture and risks are related to improper handling, post-release of the product and prior to its use, have the potential to impair the quality and safety of the product as well as increase risks associated with the production process); (3) Related to extensive manipulation (products subjected to substantial manipulation in the laboratory or that perform a function in the recipient that is different from

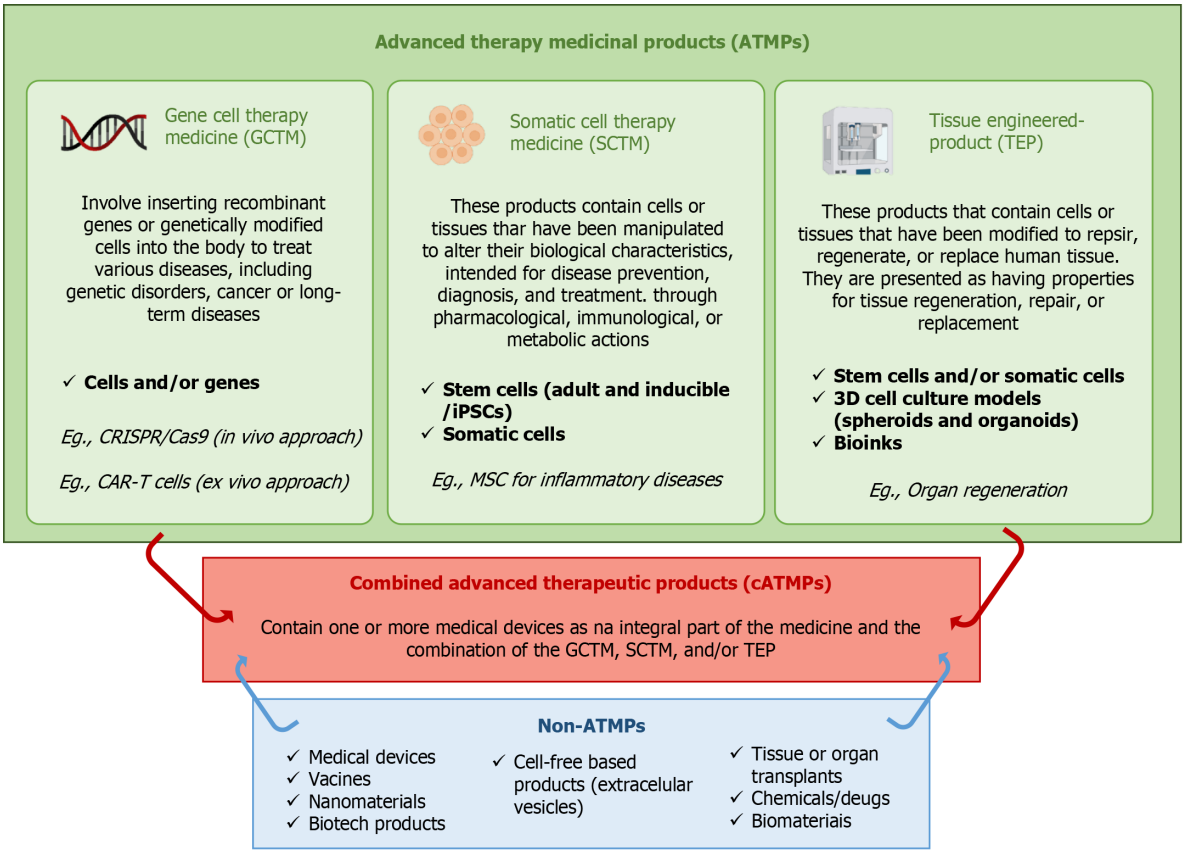


**Figure 1 Three main types of advanced therapy medicinal products.** Non-advanced therapeutic products are regulated separately from advanced therapeutic products and may be used in combination with advanced therapeutic products. ATPs: Advanced therapeutic products; cATMP: Combined advanced therapy medicinal product; SCTM: Somatic cell therapy medicine; GTMP: Gene therapy medicinal product; TEP: Tissue-engineered products; iPSC: Induced pluripotent stem cell; MSC: Mesenchymal stem cell; CRISPR/Cas9: Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9; CAR-T: Chimeric antigen receptor T; 3D: Three-dimensional.

the function performed in the donor pose a high intrinsic risk to health and the degree of manipulation to which the cells have been subjected has more impact on risk assessment than the origin of the cells - autologous or allogenic); and (4) Related to improper administration (the detailed description of the conditions of use of the ATMP must be carefully elaborated and informed by the manufacturer to the person in charge of the use/application of the product and should be carried out only in authorized specialized centers). To mitigate these risks, a flexible approach to risk identification, evaluation and mitigation is needed, considering all areas of development including the biological activity, quality attributes, manufacturing process steps and therapeutic administration procedures. Appropriate risk minimization measures, such as specialized trainings for physicians and targeted educational materials, may also be necessary[2,4,5].

### *Stem cells in ATMPs*

Over time, advancements in stem cell research have led to the development of cell-based therapies to address diseases resistant to conventional treatments[6]. A significant milestone leading to these advancements was the breakthrough in stem cell cultivation, which led to the discovery of human embryonic stem cells (ESCs) and the development of xeno-free culture systems[7]. These foundational advancements laid the groundwork for harnessing the therapeutic potential of stem cells, shaping the trajectory of regenerative medicine. By harnessing the regenerative capacity of stem cells, researchers can overcome limitations in current treatment modalities, paving the way for personalized therapies. ATMPs utilize a variety of cells and tissues, and their specificity directly affects their effectiveness and safety. In Table 1, the main cells for each category of ATMPs are summarized.

Among adult stem cells, MSCs also known as mesenchymal stromal cells or medicinal stem cells, are multipotent stem cells that can differentiate into various cell types. They are primarily found in the bone marrow but can also be isolated from other tissues. MSCs have garnered significant attention for their therapeutic potential, particularly in regenerative medicine and tissue engineering. They are being investigated in over 1000 clinical trials for various applications, including treating of inflammatory diseases, tissue repair, and immune modulation. Their ability to respond to inflammation and promote tissue regeneration makes them a focal point in current biomedical research. Despite the promising potential of MSCs, challenges remain regarding their mechanisms of action, optimal isolation methods, and the intricacies of their differentiation pathways. Ongoing research aims to unlock their full therapeutic potential through an improved understanding of their biology and the development of effective clinical applications[8,9].

**Table 1 Type of somatic cells employed in specific advanced therapy medicinal products (ATMPs)**

| ATMP | Intended use | Cells | Applications | Action in the tissue |
|---|---|---|---|---|
| **GTMPs** | The focus is primarily on the genetic material that is delivered into the patient's cells to correct or replace defective genes | Terminally differentiated: T-cells | Often modified for cancer immunotherapy (*e.g.*, CAR-T cells) | They can be performed *in vivo* (directly in the patient) or *ex vivo* (cells are modified outside the body and then reintroduced), which are leading to therapeutic, prophylactic, or diagnostic effect |
| | | Hematopoietic stem cells | Used for genetic disorders affecting blood cells | |
| **SCTMPs** | Can involve cells that have been manipulated to alter their biological characteristics for therapeutic purposes, not intended to be used for the same essential function(s) in the recipient as in the donor | Terminally differentiated: Like fibroblasts and chondrocytes | Which are used for repairing specific tissues | They can repair or replace damaged tissues or to treat diseases, which are leading to therapeutic, prophylactic, or diagnostic effect. Unlike GTMPs, somatic cell therapy does not necessarily involve genetic modification but rather the application of cells to restore function |
| | | Mesenchymal stem cells: Isolated adult tissues | These cells are known for their ability to differentiate into various cell types and are used in regenerative medicine | |
| | | Induced pluripotent stem cells: These are reprogrammed adult cells | Can differentiate into any cell type, providing a versatile option for therapy | |
| **TEPs** | Cells that are used in combination with scaffolds to create functional tissues, not intended to be used for the same essential function(s) in the recipient as in the donor | Progenitor: Such as those derived from stem cells | Can differentiate into specific tissue types | They are designed to repair, regenerate, or replace damaged tissues or organs |
| | | Engineered: Cells that have undergone substantial manipulation to achieve desired characteristics | For tissue repair or regeneration | |

ATMP: Advanced therapy medicinal product; GTMP: Gene therapy medicinal product; SCTMP: Somatic cell therapy medicinal product; TEP: Tissue-engineered product; CAR-T: Chimeric antigen receptor T.

Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cell that can be generated directly by reprogramming adult somatic cells back into an embryonic-like pluripotent state through the forced expression of specific genes and factors important for maintaining the properties of ESCs. iPSCs are similar to ESCs in many aspects, including the expression of ESC markers, chromatin methylation patterns, ability to form embryoid bodies and teratomas, and potential to differentiate into various cell types. The breakthrough discovery of iPSCs allows researchers to obtain pluripotent stem cells without using embryos, providing a novel method to "de-differentiate" cells whose developmental fates were traditionally assumed to be determined. Patient-specific iPSCs carrying disease-relevant genetic backgrounds can be used to study disease mechanisms, evaluate drug activity and toxicity, and develop next-generation cell therapies. Tissues derived from iPSCs will be a nearly identical match to the cell donor, an important factor in disease modeling and regenerative medicine applications. Besides their advantages, challenges remain in ensuring the safety and efficacy of iPSC-based therapies, such as the potential for genetic and epigenetic abnormalities during reprogramming and differentiation[10].

Using stem cells for ATMPs presents several challenges that can impact their development, safety, and efficacy. Key challenges include: (1) Safety and efficacy concerns; (2) Regulatory inconsistencies; (3) Manufacturing challenges; and (4) Public perception and misuse[4].

## SAFETY AND EFFICACY CONCERNS

Measurement plays a crucial role in developing therapeutic products and mimetic models as alternatives to animal testing. Accurate and precise measurement techniques are essential to assess the efficacy, safety, and quality of TEPs and models. However, challenges in measurement can jeopardize the advancement of therapeutic products and mimetic models in several ways, as depicted in Figure 2[11-15].

Metrology, the science of measurement, is increasingly recognized as pivotal in life sciences, particularly in the development of ATMPs. As discussed by Plant *et al*[16], the application of metrological principles involves meticulous planning and thorough documentation to diminish uncertainties and biases, thereby enhancing the reliability of experimental results in varied research contexts. Investigating the technical aspects of metrology in greater depth by focusing on precision and specificity is crucial for addressing the inherent variability present in biomedical research[17]. The importance of metrology in biological research has been highlighted[18], as it plays a crucial role in creating reference materials and defining measurement uncertainty. Due to the inherent variability of this field, standardization is essential for advancing biological research. Establishing precise, reliable and transparent measurement techniques, calibration, standards, and quality control is critical for achieving robustness and reproducibility in scientific research, particularly in developing therapeutic products. The Bureau International des Poids et Mesures (BIPM) is instrumental in this context.

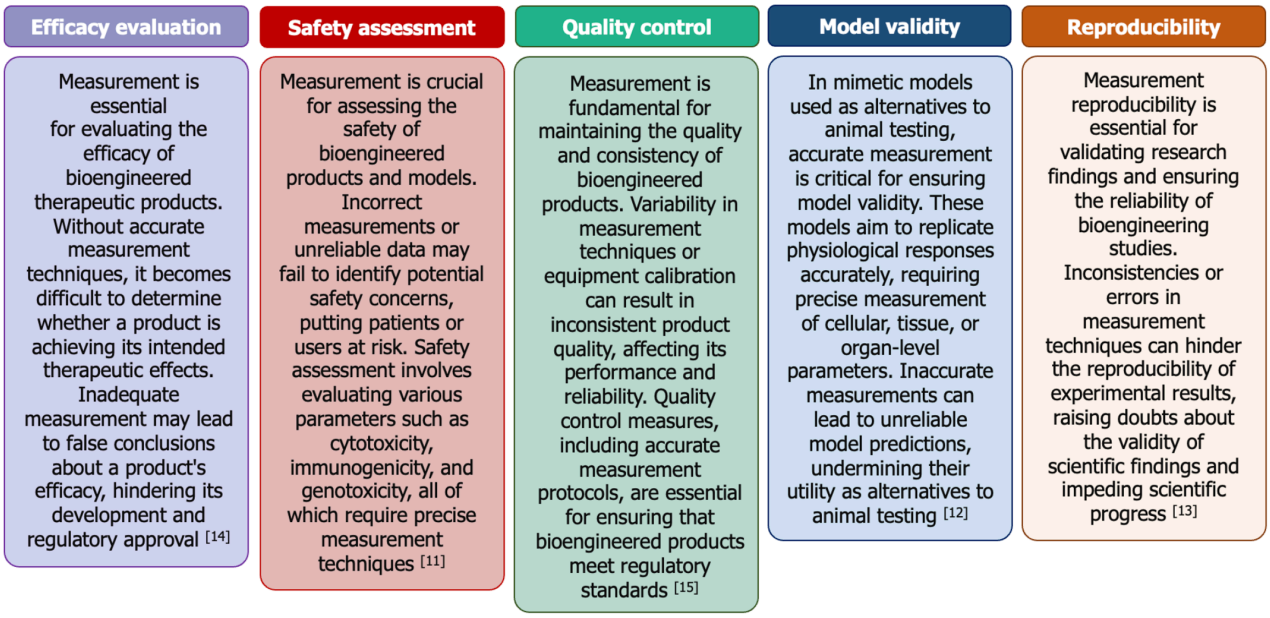


**Figure 2 Measurement challenges that can hinder the progress of advanced therapeutic product development.**

BIPM strives to improve measurement standards and methodologies in cellular analysis and product quality assurance. Collaborations with organizations like the European Association of National Metrology Institutes and initiatives like Quality by Design reflect the commitment of BIPM to enhance measurement accuracy and reliability, which is essential for the efficacy and safety of ATMPs applications and therapeutic interventions[19]. The use of MSCs and iPSCs in ATMPs raises important safety and efficacy concerns that need to be addressed.

In terms of safety concerns, we need to consider for MSCs: (1) Tumorigenicity (there is a risk of tumor formation associated with MSC therapies, particularly due to their ability to proliferate and differentiate and is heightened in cases where MSCs are derived from pluripotent sources or manipulated extensively, which may lead to uncontrolled growth *in vivo*; (2) Thromboembolic events (MSCs can express tissue factor - TF/CD142, which is procoagulant and can trigger coagulation cascades, linking to thromboembolic complications during the infusion of MSC products, necessitating careful monitoring and potential use of anticoagulants in clinical protocols[20]; (3) Immunogenicity (although MSCs are generally considered to have low immunogenicity, there is still a possibility of immune reactions, especially when using allogeneic/donor-derived cells); (4) Heterogeneity (MSCs are a heterogeneous population and inter-donor variations in their characteristics can impact their safety and efficacy as well as differences in isolation methods, culture conditions, and donor sources can lead to inconsistencies in product quality, which poses challenges for regulatory approval and clinical application[12]; (5) Quality control issues (variability in manufacturing processes can affect cell potency and safety, making it essential to establish stringent quality control measures throughout the production and handling of MSCs for ATMPs)[8].

In terms of safety concerns, we need to consider for iPSCs: (1) Genetic and epigenetic abnormalities (the reprogramming process to generate them can introduce genetic mutations and epigenetic aberrations that may compromise the safety and functionality of the cells); (2) Teratoma formation (the pluripotency and proliferative capacity of iPSCs and their derivatives increases the risk of uncontrolled growth and teratoma formation upon transplantation); and (3) Immunogenicity (even autologous iPSC-derived cells may trigger immune responses and rejection upon transplantation due to genetic and epigenetic changes acquired during reprogramming and culture). To address these concerns, researchers are exploring non-integrative methods for iPSC generation, such as episomal vectors and mRNA transfection, which offer higher safety while maintaining reprogramming efficiency. Genome editing tools like clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 are being used to correct genetic defects in patient-derived iPSCs before differentiation and transplantation. Ongoing research aims to further optimize the differentiation protocols, enhance the safety and efficacy of iPSC-derived cells, and develop robust quality control measures to ensure these cells' safe and effective use of these cells in ATMP applications[10].

In terms of efficacy concerns, we need to consider for MSCs: (1) Variability in cell characteristics (since MSCs are a heterogeneous population, and their properties can vary significantly based on the source (*e.g.*, bone marrow, adipose tissue, umbilical cord) and the individual donor and this variability can lead to inconsistent therapeutic outcomes, making it challenging to predict the efficacy of MSC-based therapies across different patients and conditions; (2) Differentiation potential (the ability of MSCs to differentiate into specific cell types is crucial for their therapeutic effectiveness; however, factors such as donor age, health status, and culture conditions can affect their differentiation capacity, leading to the limited or inconsistent differentiation that can result in suboptimal therapeutic effects, particularly in regenerative applications); (3) Quality control issues (inadequate quality assurance during manufacturing can lead to variations in cell potency and viability, ultimately affecting the efficacy of the final product; the lack of standardized protocols further complicates efforts to ensure consistent quality); (4) Immunogenicity and rejection (although MSCs are generally considered immunoprivileged, there is still a risk of immune responses, especially with allogeneic MSCs, and variability

in the immunogenic profile can lead to reduced efficacy or failure of the treatment due to immune rejection); (5) Long-term efficacy (many studies focus on short-term outcomes, and there is limited data on the long-term efficacy of MSC therapies and understanding how MSCs behave and maintain their effects in the long term is crucial for evaluating their overall therapeutic potential); and (6) Transport and handling (MSCs are living cells that require specific conditions to maintain their viability and functionality during transport and storage; any deviations from optimal conditions can compromise the efficacy of the product before administration, leading to questions about the reliability of the treatment) [21-23].

In terms of efficacy concerns, we need to consider for iPSCs: (1) Variability in differentiation efficiency (the differentiation efficiency of iPSCs can vary significantly between cell lines and may be lower compared to ESCs for certain lineages; (2) Epigenetic memory (iPSCs may retain an epigenetic memory of their somatic cell of origin, which can restrict their differentiation potential and skew their lineage commitment); and (3) Integration of transgenes (the use of integrative methods for iPSC generation, such as retroviral vectors, can disrupt tumor suppressor genes and increase the risk of tumorigenicity)[24].

## REGULATORY CONSIDERATIONS

Bringing ATMPs to market involves navigating complex regulatory frameworks to ensure safety and efficacy. Challenges include defining appropriate regulatory pathways, addressing unique characteristics of ATMPs (such as the personalized nature and novel mechanisms), and establishing robust manufacturing processes[25,26].

Regulatory bodies play a crucial role in safeguarding public health by establishing and enforcing standards for new therapies. They assess preclinical and clinical data, oversee manufacturing practices, and conduct inspections to ensure compliance with regulations. Through rigorous evaluation, these bodies mitigate risks and promote the development of safe and effective treatments. However, the regulatory approach varies among agencies worldwide[27-29] (Figure 3). Each country's regulatory body has established comprehensive guidelines and regulations to manage the complex challenges presented by ATPs. They focus on robust scientific evaluation to ensure the safe integration of these innovative products into healthcare systems to provide advanced treatment options for patients.

Regulatory guidelines require ATMP manufactures to conduct cell differentiation assays[30], quantify impurities and metabolites, detect mycoplasma[31], perform sterility tests, analyze endotoxins, and use specific methods for quantifying dimethyl sulfoxide, penicillin, and streptomycin. The accuracy of these methodologies is essential to ensure the safety and efficacy of ATMPs[32,33]. This list is not exhaustive, and it is crucial to consult the specific guidelines of each country to identify the regulatory tests required for registering each type of ATMP. Each regulatory body also defines strict procedures for clinical trials to test ATMPs[34].

In terms of regulatory concerns are significant and multifaceted and we need to consider for both MSCs and iPSCs: (1) Compliance with good manufacturing practice (GMP) principles (stem cells must be produced under strict GMP conditions to ensure their safety, quality, and efficacy, including comprehensive documentation, quality control, and validation of manufacturing processes; compliance can be challenging, especially for academic institutions with limited experience in regulatory protocols; also, upgrading existing manufacturing processes to meet GMP standards can be costly and complex, particularly for large-scale production necessary for clinical trials; this often requires collaboration with industrial partners to achieve the necessary scale and compliance)[25]; (2) Inter-donor variability (can vary significantly between donors, leading to inconsistencies in product quality and therapeutic outcomes; establishing standardized protocols for cell isolation, expansion, and characterization is crucial but challenging); (3) Manufacturing standardization (there is a need for standardized procedures across different sources of MSCs to ensure consistent product quality and variability in manufacturing processes can complicate regulatory approval and clinical application); (4) Regulatory framework differences (varies significantly between regions and cells could be authorized under a hospital exemption clause, for example, in European Union); (4) Risk-based controls (the production of stem cells involves inherent risks, necessitating rigorous risk assessment and control measures throughout the manufacturing process, including ensure the safety, identity, purity, and potency of the final product); and (5) Quality consistency validation (ATMPs must undergo validation for quality consistency and successful demonstration of manufacturing processes; investigational ATMPs may not require full verification of analytical procedures, but authorized products must meet stringent validation standards). Addressing these regulatory concerns is essential for successfully developing and commercializing of stem cell-based ATMPs. In summary, Table 2 shows degree of cell manipulation, regulatory considerations, clinical trial design, and surgical considerations[8,35,36].

We focused on nine main regulatory frameworks. The United States Food and Drug Administration oversees ATMPs through various frameworks, including the Center for Biologics Evaluation and Research for biological products and the Center for Drug Evaluation and Research for drugs. Regulatory pathways such as investigational new drug applications, biologics license applications, and device premarket approvals are utilized[27].

ATMPs in the European Union fall under the European Medicines Agency Regulation 1394/2007 (Advanced Therapy Medicinal Products Regulation). This regulation encompasses gene cell therapy medicine, SCTM, and TEP. The European Medicines Agency provides centralized marketing authorization to ensure efficacy, safety, and compliance with quality standards[27]. Health Canada adopts a flexible regulatory framework tailored to the unique characteristics of ATMPs. Regulatory requirements prioritize safety and efficacy while fostering innovation in ATP development. The Medicines and Healthcare Products Regulatory Agency in the United Kingdom implements its regulations for ATMPs, focusing on stringent assessment and inspection processes to uphold patient safety and product effectiveness[37].

**Table 2 Comparison between many aspects surrounding stem cells applied in advanced therapy medicinal products (ATMPs)**

| ATMPs | Degree of cell manipulation | Regulatory considerations | Clinical trial design | Surgical considerations |
|---|---|---|---|---|
| **SCTMPs** | Substantial manipulation | Often involves rigorous scrutiny of the manipulation processes and the resultant biological characteristics of the cells. Regulatory bodies require extensive data on safety and efficacy, particularly because these therapies may involve significant changes to the cells' original functions. Clinical trials must demonstrate not only the safety of the therapy but also its therapeutic benefits in the intended patient population | Often include endpoints that assess both the manufacturing process and the therapeutic outcomes. This may involve feasibility studies to ensure that the cells can be successfully harvested, manipulated, and reintroduced to the patient. The complexity of these therapies necessitates close coordination between clinical teams and manufacturing facilities | The administration may require less invasive procedures, depending on the therapy. For instance, T-cell therapies can often be administered through infusion after manipulation outside the body |
| **TEPs** | Substantial manipulation | The focus is more on the engineering processes and the ability of the product to integrate and function in the body. The regulatory framework may emphasize the physical and biological properties of the engineered tissues, requiring evidence that they can effectively repair or replace damaged tissues | May be more focused on demonstrating the functional integration of the engineered tissues and their ability to restore tissue function. The design of these trials often involves assessing the physical and biological properties of the implanted tissues and their long-term performance in the body | Typically involves more complex surgical procedures for implantation, which can introduce additional risks associated with surgery, such as infection or complications from the surgical site. The success of these products is closely tied to the surgical technique and the patient's ability to heal and integrate the new tissue |

ATMP: Advanced therapy medicinal product; SCTMP: Somatic cell therapy medicinal product; TEP: Tissue-engineered product.

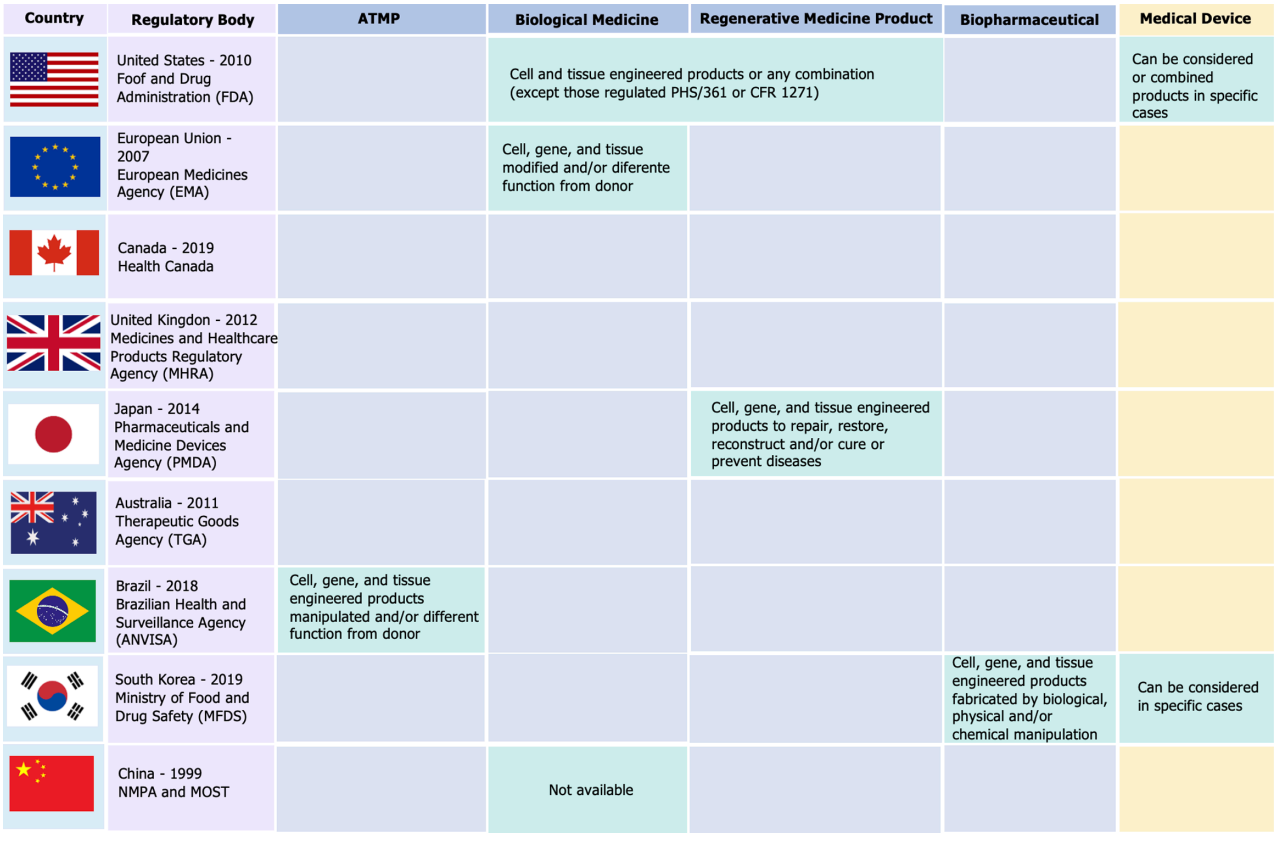


**Figure 3 Regulatory landscape for advanced therapeutic products worldwide.** ATMP: Advanced therapeutic product; MOST: National Health Commission of the People's Republic of China; NMPA: State Administration for Market Regulation.

Japan's Pharmaceuticals and Medicine Devices Agency has ensured adherence to quality, safety, and efficacy standards before market approval[38]. The Therapeutic Goods Administration in Australia regulates ATMPs under the Therapeutic Goods Act 1989 and relevant regulations, assessing them for safety, quality, and efficacy throughout their lifecycle, including post-market surveillance. The Brazilian Health and Surveillance Agency in Brazil has established specific regulatory frameworks for ATMPs, including Instruction Normative 270/2023, RDC 506/2021, and RDC 505/2021. These regulations govern good manufacturing practices, clinical trials, and product registration while ensuring compliance with rigorous standards for quality and safety[5]. The Ministry of Food and Drug Safety in South Korea is the

central regulatory authority. It has established a comprehensive regulatory framework to oversee the development, clinical testing, and commercialization of ATMPs. South Korea aims to harmonize its regulations with international standards[39].

The State Administration for Market Regulation and National Health Commission of the People's Republic of China regulates ATMPs as innovative biologics under the same framework as other pharmaceutical products, encompassing laws, regulations, departmental rules, and technical guidelines. China has seen a surge in policies promoting drug innovation, with the Center for Drug Evaluation significantly enhancing its capacity and efficiency in evaluating ATMPs. The ATMP industry in China is expanding rapidly. Although it lags in innovative target and indication coverage, there has been growth in diversity of product types, targets, and indications in recent years. This regulatory system encourages risk-based regulation and cross-discipline collaborations to advance more ATMPs towards market authorization in China, emphasizing expedited regulatory programs for efficient review processes, especially for highly innovative products from small companies. The National Drug Regulation Science Program of China has initiated the issuance of ATMP regulatory guidelines, supporting high-quality regulation of stem cell and gene therapies to achieve more targets in the coming years. Strengthening the regulatory framework involves updating guidelines, communicating effectively with stakeholders, and fostering partnerships with international regulatory agencies for convergence[40,41].

## ETHICAL CONSIDERATIONS

Stem cell research brings critical ethical and societal issues to the forefront. Ethical concerns are primarily focused on the use of human embryos in research. The research ultimately involves their destruction, which raises questions about the embryo's moral status. This concern extends to issues of consent for the donation of tissues, particularly when it comes to reproductive cells or embryos that could potentially develop into a person[42]. Despite these challenges there is a growing societal consensus that the potential benefits of stem cell research may outweigh the ethical costs due to the promise of treating or even curing debilitating diseases[43]. In 2021, the International Society of Stem Cells Research released the "Guidelines for Stem Cell Research and Clinical Translation". The most crucial topics related to ethical and social considerations of stem cell use include: (1) Genetic material and confidential personal information; (2) Informed consent; (3) Genetic manipulation of the cells; and (4) Intellectual property and patents[44].

Moreover, the evolution of this field has led to a reduction in animal testing, aligning with societal values prioritizing compassion towards animals and ethical research practices[45] as reinforced by the Food and Drug Administration Modernization Act 2.0 which "allows for alternatives to animal testing for purposes of drug and biological product applications". Adhering to the principles of the "Replacement, Reduction, and Refinement" in animal research, stem cell studies contribute to more ethical scientific protocols and heighten the integrity and public perception of scientific research[46,47].

For instance, 3D bioprinting has been used to create complex tissue models that closely mimic human physiology, such as liver and cardiac tissues, allowing for precise replication of the human disease environment and drug responses[48]. High-throughput methods for creating multicellular spheroids, which more accurately represent the *in vivo* tumor microenvironment, have been developed to enhance cancer research without relying on animal models[49]. Human liver spheroids and advanced 3D bioprinting techniques are emerging as effective alternatives to animal testing for evaluating hepatotoxicity and drug efficacy in treating liver diseases, such as non-alcoholic steatohepatitis[50]. These practices foster trust in scientific research and stimulate the development of innovative methods that are both humane and potentially more indicative of human biological responses[51,52].

### *TEP*

Recent advancements include in TE the integration of biomaterials, cellular components, and engineering principles to fabricate functional tissues and organs[13,53,54] (Figure 4). This multidisciplinary approach has revolutionized regenerative medicine, offering novel tissue repair and organ replacement solutions. Recent breakthroughs in TE have revolutionized the field, with significant advancements in CRISPR technology, bioinformatics, and nanotechnology[55]. 3D bioprinting, organ-on-a-chip, and stem cell technologies have seen remarkable progress[52,56]. Nanoengineering has significantly enhanced the performance and functionalities of biomaterials with potential applications in developing biomedical treatments and techniques[57]. Further developments involve the utilization of immunoengineering and regenerative immunotherapies to guide tissue reconstruction[58]. Notably, incorporating techniques like electrical stimulation and nanoparticle synthesis to promote cell proliferation and differentiation have emerged[59]. These breakthroughs collectively represent the cutting edge of TE, which can potentially transform various industries and improve human health. Stem cell technology emerged as a disruptive force in TE by challenging conventional paradigms and offering unprecedented therapeutic potential. The 3D stem cell culture systems in TEPs offers numerous benefits over traditional 2D cultures. Here are the key advantages: Enhanced physiological relevance: (1) Mimicking *in vivo* conditions: 3D cell cultures better replicate the tissue's natural architecture and microenvironment of tissues compared than 2D cultures. This allows for more accurate modeling of cellular behavior, interactions, and responses to stimuli, which is crucial for studying tissue development and disease processes[60]; and (2) Improved cell-cell and cell-extracellular matrix (ECM) interactions: In 3D cultures, cells can interact with each other and with the ECM in a manner that closely resembles their behavior *in vivo*. This promotes more natural cell proliferation, differentiation, and function, leading to more relevant results in tissue engineering applications[60]. Scalability and versatility: (1) Scalable production: 3D culture systems can be designed to produce large quantities of tissue constructs, making them suitable for various applications in regenerative medicine and tissue engineering. This scalability is essential for developing clinically relevant

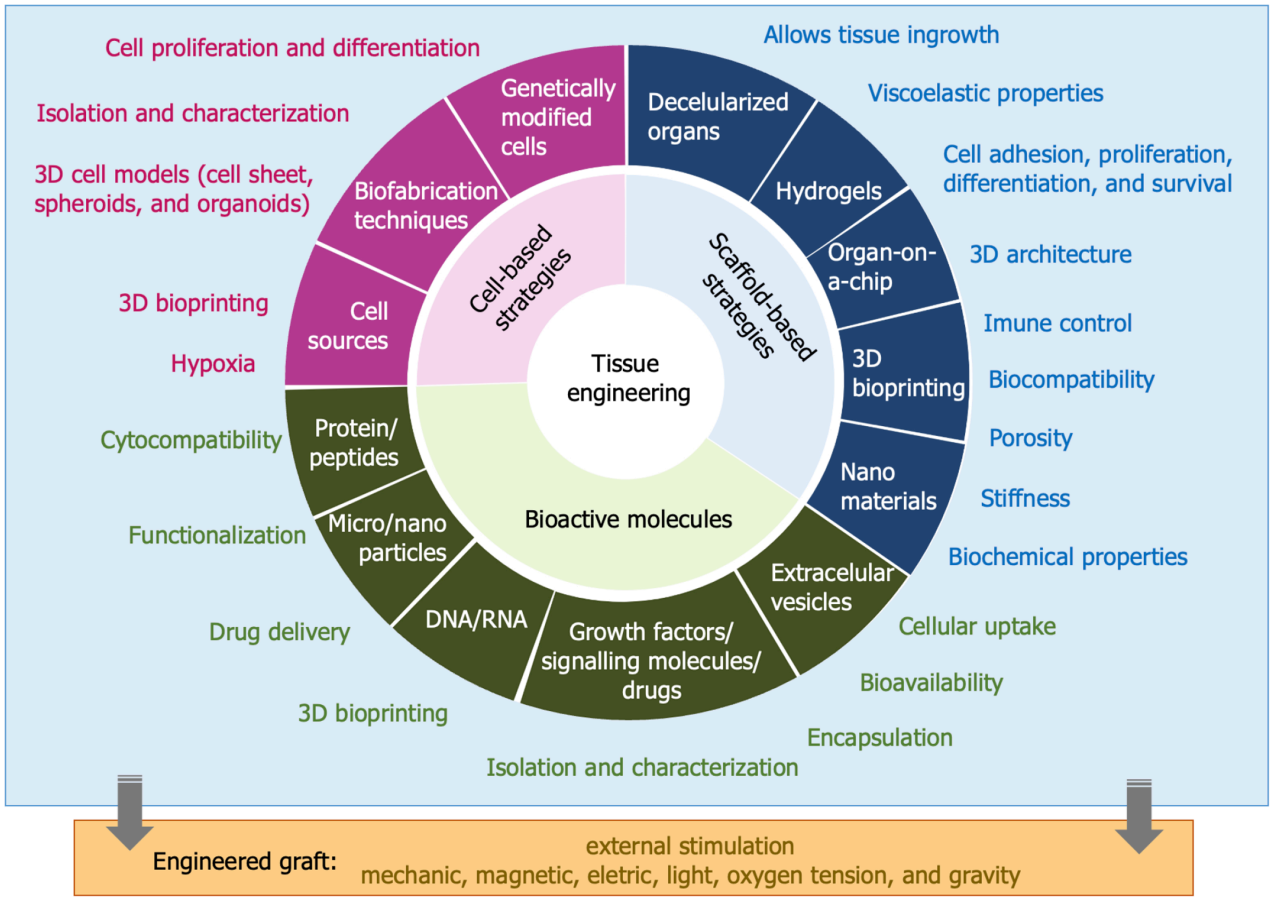


**Figure 4 Tissue engineering basics.** Three essential components required for tissue regeneration are: (1) Living cells that can proliferate and differentiate to form new tissue; (2) Scaffolds that provide structural support for cell attachment and tissue formation; and (3) Bioactive molecules that promote cell proliferation, differentiation, and tissue development. 3D: Three-dimensional.

products[61]; and (2) Diverse applications: 3D cell cultures can be applied for various 3D microenvironments, such as in many scaffolds (hydrogels, decellularized matrix) and microfluidic systems. Enhanced biocompatibility and integration: (1) Better biocompatibility: 3D cell cultures often utilize biomaterials that mimic the natural ECM, improving cell adhesion and promoting tissue integration when implanted *in vivo*. This is critical for the success of tissue-engineered products[61]; and (2) Facilitated nutrient exchange: Unlike 2D cultures, where nutrient access is uniform, 3D cultures create gradients of nutrients and oxygen, which can influence cell behavior and viability. This feature is vital for maintaining the health and functionality of engineered tissues over time[60].

Despite the advantages, several challenges remain in 3D stem cell culture: (1) Standardization (there is a lack of standardized protocols for 3D culture systems, leading to variability in results and complicating comparisons across studies); (2) Cost and complexity (the materials and technologies required for 3D cultures can be expensive, and the complexity of these systems may require specialized expertise for effective management and analysis); (3) Assessment and analysis (current assays for analyzing 3D cultures are less developed compared to those for 2D cultures, making it difficult to quantify outcomes and assess cellular responses consistently.

Several emerging sources of MSCs are being explored for their potential use in TEPs: (1) Menstrual blood-derived MSCs (can be obtained non-invasively from healthy women; have shown good proliferative capacity, multi-lineage differentiation potential, and immunomodulatory properties; however, challenges remain in standardizing the manufacturing process due to potential variability based on the day of the menstrual cycle when the cells are obtained); (2) Dental pulp-derived MSCs (obtained from extracted teeth; exhibit characteristics similar to bone marrow-derived MSCs and have been explored for regenerative therapies in dentistry and orthopedics; while preclinical and early clinical studies are promising, more translational research is needed to consolidate the results and establish standardized manufacturing protocols for ATMP development); and (3) iPSC-derived MSCs (can be reprogrammed to generate MSCs, providing an unlimited and consistent cell source for ATMP manufacturing; have shown comparable characteristics to MSCs from other sources and may offer advantages in terms of scalability, consistency, and potential for genetic modification; however, challenges remain in ensuring the complete elimination of residual undifferentiated iPSCs in the final product and establishing robust quality control measures to mitigate the risk of tumorigenicity)[62].

While specific numbers of approved TEPs can vary, it is noted that TEPs constitute less than 5% of all ATMPs currently in clinical trials. The approval landscape is evolving, with ongoing clinical trials and regulatory adaptations to facilitate introducing more TEPs into the market. Currently, the total number of TEPs that have received regulatory approval is limited, highlighting the challenges associated with their development and commercialization in regenerative medicine

and tissue engineering[63].

Until now, the number of approved TEPs remains relatively low compared to the overall number of ATMPs. As of recent reports, the first TEP to receive approval was ChondroCelect, an autologous cartilage cell-based product in association with collagen membrane for patellar and trochlear articular cartilage repair approved by European Medicines Agency in 2009, however was withdrawn in 2016. Since then, the development of TEPs has been slow, with only a few products reaching the market: (1) MACI (Genzyme): Matrix-applied characterized autologous cultured chondrocytes for repair of symptomatic cartilage defects of the knee in combination with porcine collagen scaffold and a first combined ATMP approved by European Medicines Agency in 2013 and Food and Drug Administration in 2016, however the authorization has expired; (2) Holoclar (considered an orfan medicine): *Ex vivo* expanded autologous human corneal epithelial cells (limbic biopsy) containing stem cells on a fibrin membrane (cell sheet) for causes of physical or chemical ocular burns, causing limbal stem cell deficiency, approved by European Medicines Agency in 2015; and (3) Spherox (CO.DON): Spheroids (10-70 spheroids/cm$^2$ suspension for implantation) of human autologous matrix-associated chondrocytes for repair of symptomatic articular cartilage defects of the femoral condyle and the patella of the knee, approved by European Medicines Agency in 2017[63].

## CONCLUSION

ATMPs are at the forefront of medical innovation, offering new hope for patients with previously untreatable conditions. As the field continues to evolve, the focus remains on ensuring safety, efficacy, and accessibility while navigating the complexities of regulatory frameworks, market dynamics, and pricing and reimbursement[64-66]. The ongoing development and implementation of ATMPs could revolutionize treatment paradigms across various medical disciplines, significantly impacting patient care and outcomes.

Required safety-related changes can inadvertently reduce the safety or efficacy of the ATMP. For example, modifications such as removing serum or feeder layers in culture can significantly decrease the yield of desired stem cells, necessitating alternative approaches to ensure safety and efficacy. There is a critical need to formally demonstrate the efficacy of stem cell therapies, as invasive procedures are often involved. The challenge lies in providing robust clinical evidence that meets regulatory standards, especially in life-threatening diseases where traditional therapies may not be effective[67].

There are inconsistencies between regulatory authorities regarding the advice and requirements for stem cell-based ATMPs. This can complicate the process of preserving the drug's potency during manufacturing scale-up and validation, leading to potential product rejection. Also, regulatory bodies often require changes to clinical protocols for generating regulatory-grade data without fully understanding the biological mechanisms involved. This can result in protocols that are not suitable for specific treatments, complicating the development process[67].

The production of stem cell-based ATMPs involves complex and variable processes that must comply with GMP standards. Ensuring consistency and reproducibility in the manufacturing process is essential but can be difficult due to the inherent variability of biological materials. Maintaining high-quality standards throughout the manufacturing process is critical. This includes ensuring that the final product is safe and effective, which can be complicated by the unique characteristics of stem cells.

The proliferation of unregulated stem cell therapies can lead to public skepticism and fear regarding legitimate stem cell treatments. Patients may be exposed to ineffective or harmful therapies, undermining trust in scientifically validated treatments. The subjective nature of assessing outcomes in some stem cell therapies can lead to inflated reports of efficacy due to placebo effects, complicating the evaluation of true therapeutic benefits[4,67]. Addressing these challenges requires a collaborative approach involving researchers, regulators, and healthcare providers to ensure that stem cell-based ATMPs are developed safely and effectively, with a strong emphasis on education and public awareness.

### *Emerging trends*

3D bioprinting: Advances in bioprinting technology enable the creation of complex tissue-like structures with precise spatial organization of cells and hydrogels, enhancing the potential for functional tissue engineering[68]; and dynamic culture systems: Innovations in perfusion (microfluidics) and bioreactor systems provide dynamic environments that mimic physiological conditions, improving nutrient and oxygen delivery to 3D cultures and supporting cell viability and function[69].

## FOOTNOTES

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