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Breakthroughs in Tissue Engineering Techniques

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Abstract

Tissue engineering techniques represent a cutting-edge approach in biomedical science, aiming to regenerate, repair, or replace damaged or malfunctioning tissues and organs. This abstract provides an overview of tissue engineering techniques, highlighting their importance, methodologies, and applications in regenerative medicine. Tissue engineering involves the integration of cells, biomaterials, and biochemical factors to create functional tissue substitutes that mimic the structure and function of native tissues. Key components of tissue engineering include the selection of appropriate cell sources, biomaterial scaffolds, and growth factors, as well as the optimization of culture conditions and bioreactor systems to support tissue development. Various tissue engineering strategies have been developed to address specific clinical needs, including scaffold-based approaches, cell-based therapies, and bioprinting technologies. Scaffold-based approaches utilize biocompatible materials to provide structural support and promote cell attachment, proliferation, and differentiation. Cell-based therapies involve the transplantation of cultured cells or stem cells into damaged tissues to facilitate regeneration and repair. Bioprinting technologies enable the precise deposition of cells and biomaterials in predefined spatial patterns to create complex three-dimensional tissue constructs. Tissue engineering techniques hold great promise for the treatment of a wide range of medical conditions, including cardiovascular diseases, musculoskeletal disorders, and neurodegenerative diseases. Continued research and development in tissue engineering are essential for advancing regenerative medicine and improving patient outcomes in the future.

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Introduction

Patients suffering from diseased and injured organs are often treated with transplanted organs, a treatment that has been in use for over 50 years. However, as modern medicine increases the human lifespan and the aging population grows, the need for donor organs increases as well, leading to a critical shortage. These problems have led physicians and scientists to explore the interdisciplinary field of tissue engineering, which applies engineering and life sciences principles to develop biological substitutes that aim to restore or improve tissue function.[1]

Tissue engineering and regenerative medicine involve combining scaffolds, cells like stem cells, biologically active molecules, and tools like bioreactors to create functional 3D tissue models mimicking the extracellular matrix environment. The goal is to assemble constructs that can recreate cells, rebuild tissues and whole organs, and ultimately provide cures for complex diseases instead of just treatments.[2]

A. Overview of Tissue Engineering

Tissue engineering evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. Artificial skin and cartilage are examples of engineered tissues that have been approved by the FDA; however, currently they have limited use in human patients.

B. What are tissue engineering and regenerative medicine?

Tissue engineering and regenerative medicine are closely related fields that aim to develop biological substitutes to restore or improve tissue function. Regenerative medicine is a broad field that includes tissue engineering but also incorporates research on self-healing – where the body uses its own systems,

sometimes with help from foreign biological material, to recreate cells and rebuild tissues and organs. The terms “tissue engineering” and “regenerative medicine” have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex, often chronic, diseases.

C. How do tissue engineering and regenerative medicine work?

Cells are the building blocks of tissue, and tissues are the basic unit of function in the body. Groups of cells make and secrete their own support structures, called extracellular matrix. This matrix, or scaffold, does more than just support the cells; it also acts as a relay station for various signaling molecules. Cells receive messages from many sources in their local environment, and each signal can start a chain of responses that determine what happens to the cell. By understanding how individual cells respond to signals, interact with their environment, and organize into tissues and organisms, researchers have been able to manipulate these processes to mend damaged tissues or even create new ones.

D. Scaffolds, Cells, and Stimuli for *In Vitro* Modeling

Scaffolds represent important components for tissue engineering. However, researchers often encounter an enormous variety of choices when selecting scaffolds for tissue engineering. Scaffolds, typically made of polymeric biomaterials, provide the structural support for cell attachment and subsequent tissue development.[3-6]

Apart from blood cells, most, if not all other, normal cells in human tissues are anchorage-dependent residing in a solid matrix called extracellular matrix (ECM) as shown in Fig. 1. Firstly, ECM provides structural support and physical environment for cells residing in that tissue to attach, grow, migrate and respond to signals. Secondly, ECM gives the tissue its structural and therefore mechanical properties, such as rigidity and elasticity that is associated with the tissue functions. Thirdly, ECM may actively provide bioactive cues to the residing cells for regulation of their activities. Fourthly, ECM may act as reservoir of growth factors and potentiate their bioactivities. Fifthly, ECM provides a degradable physical environment so as to allow neovascularization and remodeling in response to developmental, physiological and pathological challenges during tissue dynamic processes namely morphogenesis, homeostasis and wound healing, respectively. Intuitively, the best scaffold for an engineered tissue should be the ECM of the target tissue in its native state. Nevertheless, the multiple functions, the complex composition and the dynamic nature of ECM in native tissues make it difficult to mimic exactly. Therefore, contemporary concept of scaffolding in tissue engineering is to mimic the functions of native ECM, at least partially.

E. Key Requirements for Scaffolds

1. **Architecture:** Scaffolds should provide void volume for vascularization, new tissue formation and remodeling so as to facilitate host tissue integration upon implantation.

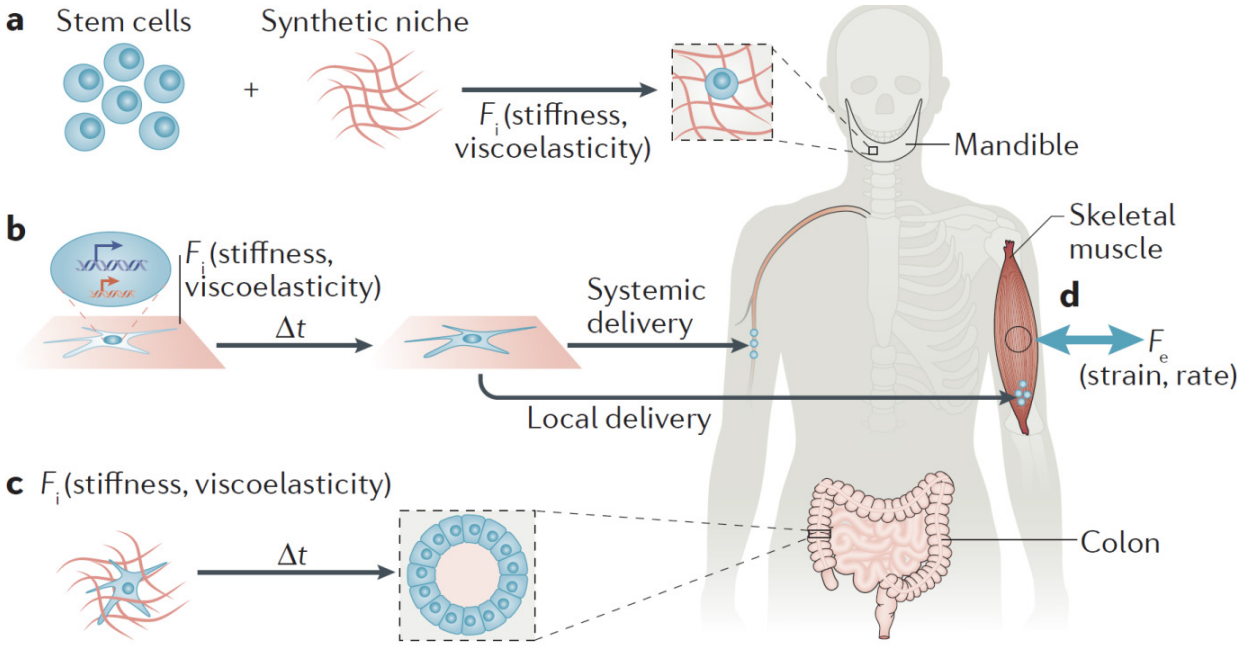

Fig. 1: **Tissue Engineering and Regenerative Medicine**

2. **Cyto- and tissue compatibility:** Scaffolds should provide support for either extraneously applied or endogenous cells to attach, grow and differentiate during both *in vitro* culture and *in vivo* implantation.
3. **Bioactivity:** Scaffolds may interact with the cellular components of the engineered tissues actively to facilitate and regulate their activities.
4. **Mechanical property:** Scaffolds provide mechanical and shape stability to the tissue defect. The intrinsic mechanical properties of the biomaterials used for scaffolding or their post-processing properties should match that of the host tissue.

F. Stem Cell Guidance and Stimuli

One major issue is the need to determine how much guidance or instruction stem cells require in order to regenerate tissues, and in what form these instructions should be provided. Many clues can be drawn from developmental and regenerative biology, where endogenous stem and progenitor cells are recruited to form new tissue in response to environmental stimuli. These stimuli can be provided through various means, including secreted or matrix-embedded signaling molecules, matrix chemistry and physical forces.Classical tissue engineering strategies aim to recreate this ECM environment to direct cell behavior on a scaffold of choice, with the eventual goal of implantation at the site of injury or disease to restore tissue function. Ideally, a microenvironment would be formed in which the ECM induces certain cell behavior, and cells would respond in turn by remodeling the substrate, establishing a dynamic feedback cycle that fashions the ECM according to the changing needs of the cell, allowing the cells and ECM to dictate the repair process.[7-11]

G. Scaffold Requirements for Bone Tissue Engineering

One basic requirement for a scaffold is that the material should support necessary cell activity leading to bone regrowth, including cell attachment, proliferation, and differentiation, as outlined in Section 3.1. Several studies have demonstrated the ability of bone marrow-derived mesenchymal cells to adhere, proliferate, and undergo osteogenic differentiation on two-dimensional silk fibroin films.[56-58] These films establish the suitability of silk fibroin as a stem cell-supporting biomaterial; however, due to their two-dimensional (2D) format, application of these films for wound healing is limited to use as coatings for other three-dimensional (3D) scaffolds to alter surface properties.[12]

Because silk fibroin is a flexible material that can be processed in several different ways, it is not limited to 2D monolayer cell culture. Silk substrates can also be formed three-dimensional (3D) materials suitable for *in vivo* implantation into the site of bone or cartilage damage. For example, silk fibroin solution can undergo a sol-gel transition to form 3D hydrogels, which can be used as tissue culture substrates.[13] Hydrogels can also be further processed by lyophilization to generate porous sponges. Silk fibroin sponges and hydrogels have supported chondrocyte-based cartilage tissue engineering *in vitro* [14] and guided repair of critical-sized cancellous bone defects *in vivo*,[15] respectively. Another promising processing option is the formation of porous scaffolds from silk fibroin solutions by salt leaching, gas foaming, and freeze drying. Scaffold topography and geometry play a critical role in tissue formation by dictating cell adhesion, proliferation, and distribution, as well as nutrient and oxygen availability. Thus, ideal scaffolds should be capable of forming various geometries for tissue-specific needs. The architecture and morphology of silk scaffolds can be controlled by processing options such as fibroin solution concentration, salt particle size, and solvent (aqueous or organic).As shown in Fig. 2, adjustment of these properties can result in favorable conditions for cartilage and bone engineering.

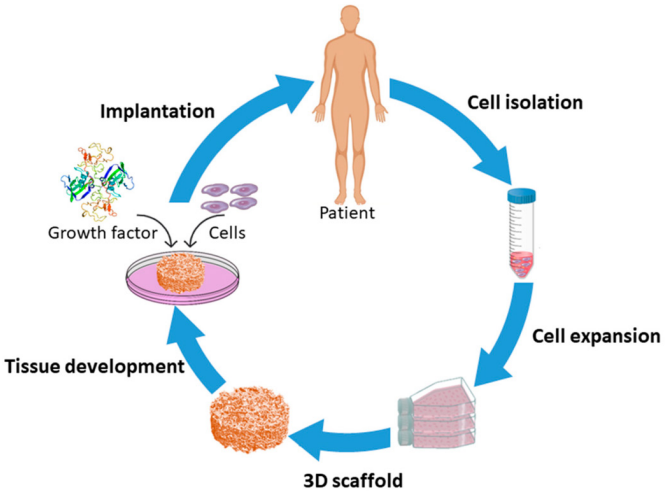

Fig. 2: **Fabrication and Plasma Modification of Nanofibrous**

H. Biological Signaling for Bone Healing

Tailoring of the physical properties of biomaterials is necessary for developing an environment that promotes bone healing. Equally important are the biological signaling requirements of the healing tissue. The cellular events underlying *in vivo* skeletogenesis are regulated by an array of signaling factors, many of which have similar

functions in both bone development and bone repair. Three major categories of signals have been identified: pro-inflammatory cytokines; growth and differentiation factors; and metalloproteinases and angiogenic factors .[16]

From the large pool of biochemical factors known to stimulate developmental and regenerative bone formation, tissue engineers can select the most promising target molecules for incorporation into scaffolds to stimulate tissue regeneration. In addition to the strategic choice of signaling molecules, scaffold design is an important component of efficient delivery of biochemical stimuli. There are several strategies for incorporating biological stimuli in a bioengineered scaffold in order to enhance tissue functionality when cultured *in vitro* and, more importantly, when implanted *in vivo*. Many of these approaches have been taken with silk fibroin materials to improve osteochondral tissue formation, taking into account the growth factors, cytokines, and other factors that are known to play a critical role in endogenous skeletogenesis.[17]

Bone *In Vitro* Models

The engineering of extracellular matrix (ECM) that mimics native tissue matrix begins with the identification of a biomaterial that is critical in the formation of a scaffold. The choice of biomaterial is dependent on the tissue being modeled. Biomaterials are based on three categories: (a) polymers, (b) metallics, and (c) ceramics. Factors that influence the choice of materials are the type of tissue being mimicked, structural integrity, adequate mechanical environment, bioactivity, biocompatibility, and biodegradability. The biomaterial should provide structural support for cellular attachment, growth, proliferation, and migration while consisting of adequate mechanical properties and an environment native tissue matrix provide to cells. Materials should be bioactive and biocompatible to provide bioactive cues and growth factors while reducing the risk of immunological response in the presence of an artificial scaffold. Additionally, the scaffold or matrix should act as a support structure facilitating correct localization and retention at the site of tissue damage. While biodegradability is key for the formation of the vascular network and allows for the patient's own ECM to replace the scaffold and degrade over time without any cytotoxic effects, this factor is organ-specific. For example, in regenerative medicine for hard tissues such as bone or teeth, materials are engineered from metallic or ceramic biomaterials to reduce the rate of biodegradability.

Table 1: Summary of the various biomaterials and their pros and cons.

Biomaterial	Properties	Limitations	References
Hydroxyapatite	Natural/ Synthetic Synthesis, Bioactive, biocompatible, hydrophilic	Brittleness, low tensile strength and fracture toughness	[98] [99]

In Vitro Bone Remodeling Models

Even the most minimal *in vitro* models of bone remodeling fundamentally require the co-culture of osteoblasts and osteoclasts. However, these can be performed either indirectly or directly. Indirect methods include conditioned media and the use of transwell inserts. The former takes media from one cell type and adds it to another, whereas the latter uses a permeable insert to provide two culture surfaces in the same well, allowing exchange of soluble factors but no cell-cell contact between the two types. Direct methods co-culture both cell types on the same surface, be it a planar, two-dimensional(2D) tissue culture well or a three-dimensional scaffold (Figure 4). A recent review suggested that when testing bone implant and repair materials *in vitro*, overlooking bone remodeling is a key limitation of the approaches currently taken (Kohli et al., 2018). That paper makes a strong case for improving our ability to replicate this process for *in vitro* biomaterial testing; therefore, biomaterials testing is not discussed in this review. Rather, the current review summarizes the key research that has been done thus far in creating an *in vitro* model of bone remodeling, focusing on fundamentals, 3D models of the process, and disease-oriented models.[18]

B. Cell Models for *In Vitro* Bone Modeling

Advancement in the development of *in vitro* bone models requires the selection of suitable cell models which can behave similarly to the ones *in vivo*.Immortalized cell lines such as MC3T3-E1, MLO-A5, and MG-63 have been used extensively in bone tissue engineering due to their ease of access, high expansion capacity, and reproducibility of outcomes. However, these cell models do not always behave similarly to primary bone cells.Primary osteoblasts and osteocytes can both be directly isolated from bone tissue and provide an alternative to cell lines for bone-related studies. Several protocols and methods are available for the isolation of human osteoblasts, including enzymatic digestion and spontaneous outgrowth cultures from bone biopsie. Isolation of primary osteocytes is more challenging due to

their location within the mineralized bone matrix, which requires multiple digestion and decalcification steps.

Primary cells have greatly enhanced the knowledge of bone biology; for instance, a recent study has shown the development of an *in vitro* model to investigate the interaction of primary human osteoblasts and osteocytes. But due to their need for a bone biopsy, slow proliferation rate, short life-span, decreased doubling time after two or three passages, long isolation procedures, limited accessibility, restricted pool of potential donors (they are usually acquired during orthopedic surgery), their use for developing personalized human *in vitro* models is restricted. The use of progenitor cells of the bone-specific cell types could be more promising to develop human *in vitro* bone models. Mesenchymal stem cells (MSCs) are osteoblast/osteocyte progenitor cells which were primarily extracted from bone marrow and later from other tissues such as adipose tissue, muscle, peripheral blood, dental pulp as adult tissue sources and umbilical cord, umbilical cord blood, placenta, amniotic fluid as fetal and perinatal tissue sources. Hematopoietic stem cells (HSCs) are multipotent and self-renewing cells that can give rise to immune and blood cells. HSCs are primarily located in the bone marrow and can be mobilized into the bloodstream, which makes bone marrow and peripheral blood the common tissue sources for HSC extraction.Taken together, the most promising cell models for the generation of personalized human *in vitro* bone models are progenitor cells. To develop these models, the patient's own progenitor cells should ideally be extracted from one source, which makes the procedure more convenient for the patient, as well as results in less demanding clinical procedure. Among all adult tissue sources, due to the possibility to extract both MSCs and HSCs from bone marrow and peripheral blood, they can be considered the most suitable sources for the isolation of osteoblast/osteocyte and osteoclast progenitor cells.[18]

In Vitro Cardiac Tissue Models

Recent studies on microfluidic devices employing stem cells under laboratory set-up have revealed meticulous events pertaining to the pathophysiology of myocardial infarction (MI) occurring at the infarcted site. This discovery also underpins the appropriate conditions in the niche for differentiating stem cells into mature cardiomyocyte-like cells and leads to engineering of the scaffold via mimicking of native cardiac physiological conditions. The most advanced technology, using iPSC-derived cardiomyocyte (CM)-loaded microfluidic devices, has now been providing unprecedented opportunities to understand the mechanisms of MI development. This technology can also be employed to study the effects of drugs in the preclinical drug screening phase.

Tissue engineering combines the principles of engineering and life sciences to better understand the relationship between the structure and function of normal and pathological tissues, and allows for the production of biological tissues for drug testing, disease modeling, and further research for regenerative medicine. Currently, there are various approaches to cardiac tissue engineering, such as an injectable in situ delivery of cells, and *in vitro* engineering of contractile tissue constructs for transplantation. Another approach includes manipulated patch constructs, which are often associated with higher engraftment rates and appear to support damaged myocardium more effectively than transplanted cells.

Advances in stem cell biology have made it possible to use various cells such as adult stem cells and induced pluripotent stem cells (iPSCs), and advances in bio-3D printing have made it possible to create large tissue structures for transplantation. An approach utilizing scaffolds involves seeding a vesicle into a three-dimensional and porous scaffold using a biodegradable polymer material, mixing and pouring cells and gel-like scaffolds (e.g., collagen) into a mold, then arranging

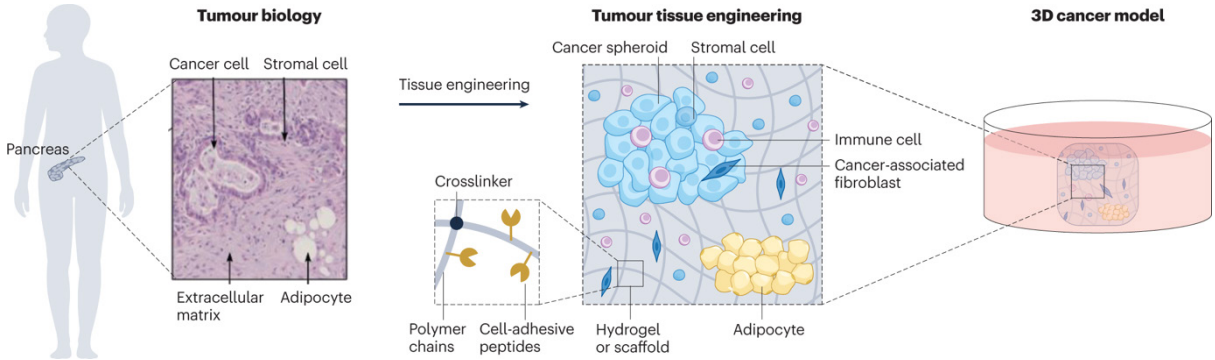

Fig. 3: Biomaterial-based platforms for tumour tissue engineering

the cells using a 3D printer and a small amount of gel. Another method, bioprinting, has been pursued as well where fiber-like cell-containing gels are bundled together.[19]

A. 3D Cardiac Tissue Models

Spheroids and organoids (clumps of cells) have also attracted attention. By seeding these cell masses in metal needles or metal devices, organizing then extracting them, one can create a three-dimensional structure that does not contain scaffolds (Figure 2a). Early research used tissue printing techniques, where the printed constructs worked well with the cardiac system and demonstrated high survival rates after 7 days of culture. 3D printing stacks up the previously mentioned spheroids, a method that leads to a higher function expression compared to two-dimensional cultures. Spheroids also promote the differentiation of the myocardium, promoting maturation.

Bioprinted human cardiac muscle patches (hCMPs) without scaffolding are produced by loading spheroids one by one into an array of needles, fusing them, then removing the hCMP and culturing it until the holes in the needle are filled with the surrounding tissue. Breeckwoldt et al. developed hCMPs by differentiating human embryonic stem cells (hESCs) into hiPSC-CMs, a design that allows one to analyze human heart diseases by checking the engineered heart tissue (EHT) force strength. A system for evaluating the 3D contractile force of the heart's structure is important for new drug development. Another study without the use of a scaffold consists of a tubular EHT (T-EHT), created using bio-3D printers with hiPSC-CMs and needle arrays, which produced beating conduits for patients suffering from monoventricular disease.To better recapitulate the 3D complexity of cardiac mechanobiology, there is considerable research interest in 3D *in vitro* model systems that mimic the complex 3D architecture and mechanics of the myocardium. By means of biomaterials, external loading, or tissue processing techniques, it is possible to include the mechanical cues from the anisotropic and continually-beating cardiac tissue in 3D *in vitro* models.Over the past decades, many scaffold materials have been explored to mimic the stiffness and 3D architecture of myocardial extracellular matrix (ECM). Hydrogels composed of crosslinked polymer networks such as gelatin methacryloyl, polyethylene glycol (PEG), and alginate are regularly used, of which the elastic modulus can be tuned in the range of physiological and pathological myocardium. To take advantage of the interplay between crosslinked polymer networks and natural polymers, hybrid biomaterials have been developed, such as poly(lactic-co-glycolic acid)/gelatin/elastin and poly(caprolactone) (PCL)/gelatin. Moreover, material processing techniques, such as melt-based electrohydrodynamic printing and electrospinning, are used to mimic the hierarchical microarchitecture of the myocardium in 3D scaffold materials.

To incorporate the mechanics of preload and afterload in 3D *in vitro* models, Eschenhagen et al. pioneered the design of engineered heart tissue (EHT). This cardiac microtissue was composed of a cell-laden, ECM mimicking hydrogel molded between two stretching posts. The posts were used to maintain mechanical stretch of the tissue, and hence the initial prestretch of the sarcomeres, and simultaneously functioned to quantify contractile forces exerted by the tissue. By changing the static prestretch of the EHT constructs preload can be varied. By incorporating their EHT system in a Flexcell device to vary the preload of engineered tissues, van Kelle et al. found that tissue contractile force increased with increasing preload.Overall, the EHT approach enables independent control over mechanical cues applied to (e.g., stretch) and inside (e.g., hydrogel properties) the microtissues. Classical EHTs, however, are confined in one direction to mimic the myocardium's anisotropy, which is not representative of most pathological tissues. To mimic the pathological, chaotic tissue organization and mechanical force distribution of the myocardium, *in vitro*, Van Spreeuwel et al. demonstrated the fabrication of cardiac microtissues consisting of cardiomyocytes and fibroblasts that could be constrained either uniaxially and biaxially, thereby manipulating the organization and mechanical forces within the microtissue while simultaneously quantifying the contraction force exerted by the tissue.[20]

More recently, researchers have put effort into better recapitulating ventricle anatomy and function *in vitro*. By means of advanced 3D bioprinting and electrospinning techniques the characteristic ECM fiber anisotropy has been mimicked while building a left ventricle chamber of few millimeters. The scaffolds were seeded with iPSC derived cardiomyocytes and showed functional electrophysiological activity leading to contraction and, even, ejection fraction.An ideal *in vitro* cardiac model should accurately recapitulate the physiological or pathological conditions of the human heart, including three-dimensional (3D) anisotropic tissue structure, orientation of the extracellular matrix (ECM) network, vascularization, and circulation (Figure 1). Traditional 2D *in vitro* systems, although informative, cannot accurately mimic the complex 3D conditions due to their inability to recapitulate the dynamics of the biological and mechanical properties of the *in vivo*

microenvironment. The 3D models are characterized by establishment of adhesion complexes and tissue polarity, and by changes in cytoskeletal structure and cell volume that are significantly different from those found in cells cultured as monolayers. As a result, the translational results in 2D conditions are fundamentally different from those in 3D.

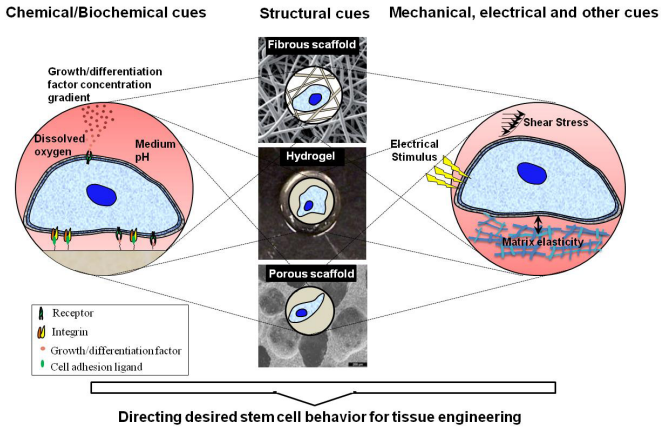

Fig. 4: Stem Cells in Tissue Engineering

Given the key role of ECM in heart development and mechanical functions, development of an *in vitro* cardiac model requires biomaterials, methods, and systems to host the cells, control the cell-cell and cell-ECM interactions, and regulate the cell fate and functions. From Fig. 4, an optimal *in vitro* model would incorporate the aforementioned hiPSCs into an *in vivo*-like tissue structure while providing researchers with precise control over cell types, ECM composition, cell-cell interactions, and microenvironment geometry.Biomaterials have played a major role in creating 3D tissue models, since they not only support cell attachment and alignment, but also transmit load, provide physiologically relevant stiffness, and ideally can be degraded and replaced over time by cell-secreted ECM proteins. Synthetic biomaterials provide an attractive alternative to natural materials, as researchers can control the entire synthesis process as well as the materials' mechanical properties, topography, and structure. Key requirements for synthetic scaffolds are that they recapitulate the native 3D hierarchical fibrillar structure, possess biomimetic surface properties, and demonstrate mechanical integrity.Microengineered *in vitro* models with multiple readouts have a great potential to better mimic the *in vivo* physiology and provide a deeper understanding of the physiological events that characterize cardiac development and function. An ideal *in vitro* cardiac tissue model should be physiologically relevant with multiple biological, mechanical, and electrical readouts, ensuring different functional endpoints for a particular application.[21-23]

Pancreas and Liver *In Vitro* Models

The fundamental goal of biomedical research is to decipher the molecular mechanisms of human disease in order to develop more effective or even new models for diagnosis, prevention, and therapeutic approaches. Due to ethical and other concerns, basic research on human (patho)physiology requires *in vitro* approaches.

A. Pancreatic *In Vitro* Models

In the case of the pancreas, particularly the islets of Langerhans, basic research into the development and evolution of diabetes largely relies on a variety of animal models (mainly rodents, particularly mice) at present. Despite their many similarities, there are many structural and physiological differences between humans and rodents in the islets of Langerhans, leading to differences in functional coupling between cells and, ultimately, differences in the complex dynamics of insulin secretion. This means that not all aspects of the results obtained in animal models can always be reliably extrapolated to humans.Moreover, current two-dimensional (2D) pancreatic culture models are unable to mimic the dynamics of insulin secretion in response to glucose or maintain beta cell viability over extended periods of time. In view of this, there is a great need for the development of increasingly sophisticated human *in vitro* models of the pancreas that more closely approximate pathophysiology at the tissue and organ levels, so that they recapitulate features that are essential to disease etiology and progression.The research focusing on pancreatic *in vitro* models has already been described and assembled in some excellent reviews, especially those focusing on pancreatic cancerand pancreatitis. In view of this, this review provides an overview of *in vitro* tissue and disease models with a focus on the endocrine pancreas.

B. Overview of Pancreatic Models

1. An overview of the anatomy, physiology, and pathophysiology of the human pancreas is provided, focusing on the islets of Langerhans and beta cell dysfunction.
2. The basic elements that should be considered when developing an *in vitro* disease model, such as the type and source of cells, are discussed.
3. This is followed by an overview of the current types of *in vitro* models, ranging from simple monolayer monoculture and co-culture models to self-assembled organoids and complex scaffold-based 3D microphysiological systems.

4. As an integral part of 3D *in vitro* models, the importance of material selection and 3D bioprinting as an emerging technology for fabricating biomimetic scaffolds is addressed.
5. Finally, breakthroughs in the field of *in vitro* models, focusing on bioreactors and organs-on-a-chip, are reviewed, and a future outlook is provided.

C. Liver *In Vitro* Models

The current inability to expand human hepatocytes *in vitro* is an obstacle not only for cell therapy, but also for pharmaceutical drug development, because of the cells' importance in assessing the metabolism of xenobiotics. Thus, the generation of hepatocytes from expandable precursors is of considerable interest.It is noteworthy that cells with properties virtually identical to hepatic oval cells can also emerge in the pancreas, especially after ablation of acinar cells. Upon transplantation, these pancreas-derived "oval cells" can differentiate into functional hepatocytes and bile ducts.Several reports have suggested that the reciprocal trans-differentiation is also possible, i.e. the conversion of liver cells toward the pancreatic endocrine fate. Forced expression of pancreatic transcription factors elicit insulin expression in the liver and corrects experimental diabetes.

Together these findings suggest that both the adult liver and pancreas contain cells with epigenetic memory of their common embryonic origin. The existence of potential ß-cell precursors in the adult liver is of obvious medical interest.Since pancreatic exocrine cells greatly outnumber ß-cells, it is also exciting that they can be reprogrammed to make functional ß-cells *in vivo* by viral delivery of the developmental transcription factors Pdx1, Ngn3, and Mafa.Pluripotent stem cells, including embryonic stem cells (ESC) and induced pluripotent stem cells (iPS), are a potentially abundant source of hepatocytes and ß-cells. Numerous groups have been developing ESC differentiation protocols that attempt to mimic normal embryonic development.Despite remarkable progress, the resulting cells often fail to achieve complete function sufficient for regenerative therapy, remaining only "hepatocyte- or ß-cell-like". It is not yet clear how precisely the known developmental signals must be orchestrated to properly program hepatic and pancreatic cells at will, but detailed studies of the activated signaling pathways and their cross-regulatory interactions during embryogenesis will be informative.

D. Advantages of *In Vitro* 3D Models

In the near future, 3D tissue-engineered models are expected to become useful tools in the preliminary testing and screening of drugs and therapies and in the investigation of the molecular mechanisms underpinning disease onset and progression. Besides their high scientific potential, these models also bring some advantages in terms of ethical and economic issues. Economic aspects should be also considered: the actual costs for successfully transforming a drug candidate from a new molecular entity (NME) to a clinical product are between $800 million and $2.2 billion, with development timelines spanning 8–12 years (DiMasi et al., 2016). Moreover, there is a high failure rate for NMEs in lead development, especially those in expensive late-stage clinical trials.

In order to overcome the limitations of current drug-screening methodologies and reduce the use of animals, 3D models have been investigated and a number of studies are in progress, with the aim of making them more and more reliable and sophisticated. Unlike animal models, 3D *in vitro* models give the possibility to independently identify and modulate cellular and molecular factors responsible for disease onset and progression, allowing the investigation of the contribution of each of them on the development of a specific disease and thus changing the way to study tissue physiology and pathophysiology. The introduction of these models in the biomedical research practice may lead to numerous advantages, such as the reduction of animal use as well as the overcoming of the limits associated with traditionally employed models (i.e., animal and 2D cell culture models), and the achievement of more reproducible data, thanks to the possibility to tightly control the experimental parameters, with lower cost and less time.

Challenges and Future Perspectives

One of the most significant challenges in tissue engineering is the replication of complex tissue structures and functions, particularly for organs with high cellular heterogeneity and intricate architectures, such as the liver or kidneys. While scaffold technology and stem cell therapy have shown promise, achieving the level of precision necessary for these organs remains a daunting task. Vascularization, or the process of forming blood vessels within engineered tissues, is another hurdle. Without proper blood flow, even the most meticulously engineered tissues cannot survive, let alone function, after transplantation. The regulatory landscape adds another layer of complexity. Ensuring that new tissue engineering products are safe and effective requires navigating a maze of approval processes, which can be time-consuming and costly. This is a necessary challenge, however, as it ensures patient safety and the efficacy of treatments.

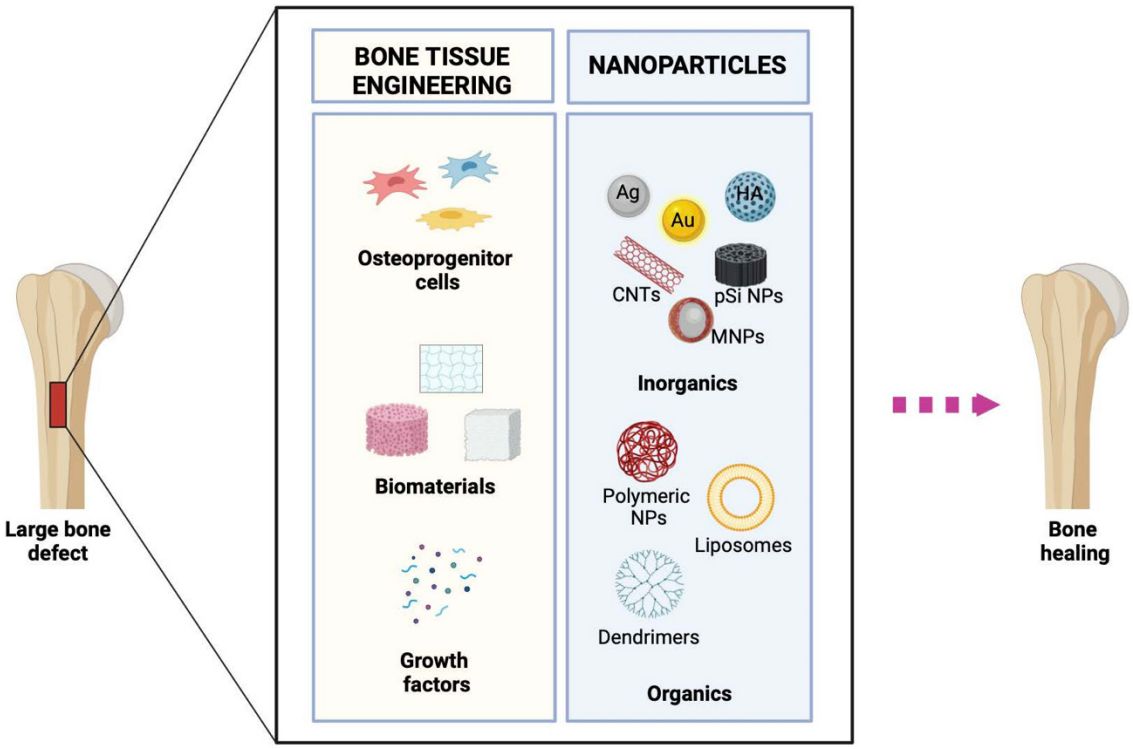

Fig. 5: Bone Tissue Engineering and Nanotechnology

Despite these challenges, the future of tissue engineering is brimming with potential. The convergence of bioengineering, materials science, and digital technology is paving the way for novel approaches. For instance, advancements in bioprinting technologies promise the ability to construct tissues layer-by-layer, potentially overcoming current vascularization issues.Another exciting frontier is the development of smart biomaterials that can respond to their biological environment, providing cues for tissue healing or regeneration as needed. The integration of such materials into emerging technologies such as nano- and micro-technology could lead to the development of next-generation implants that can adaptively respond to a patient's needs. With the growing emphasis on personalized medicine, tissue engineering is poised to offer more customized solutions. The use of patient-specific genetic information could allow for the engineering of tissues that are more compatible with an individual's biology, reducing the risk of rejection and enhancing therapeutic outcomes.In the broader scope of the field, interdisciplinary collaboration will be vital to overcoming current limitations. Combining insights from different scientific disciplines will not only accelerate the pace of innovation but also enhance the translatability of bench research into clinical applications. As researchers continue to untangle the complexities of human tissues, the day when organ shortages and irreparable tissue damage are problems of the past edges closer to reality.

Applications of *In Vitro* Tissue Models

The combination of bioprinting and organ-on-a-chip techniques would promote the engineering of suitable microenvironments to better represent the complexities of human tissues, thus mimicking their true functions, which would increase the reliability of pre-clinical tests. Moreover, bioprinting technologies can be used to devise *in vitro* models allowing for better characterization and understanding of the mechanisms of viral infections (Saygili et al., 2020). Additionally, the merging of bioprinted tissue models with chip devices could provide an important combination taking the two separate concepts into synergistic fruitful outcomes (Zhang and Khademhosseini, 2020). This combined strategy could perhaps lead to better representative models that not only feature volumetric structures but also compartmentalization and dynamic flows as in Fig. 6.

Several studies have already demonstrated the successful engineering of *in vitro* models for investigating coronaviruses by combining human bronchial tracheal mesenchymal cells (HBTCs) with adenovirus-12 SV40 hybrid virus transformed bronchial epithelial (BEAS-2B) cells (Albright et al., 1990) or by using pulmonary Oct-4+ stem/progenitor cells (Ling et al., 2006; Suderman

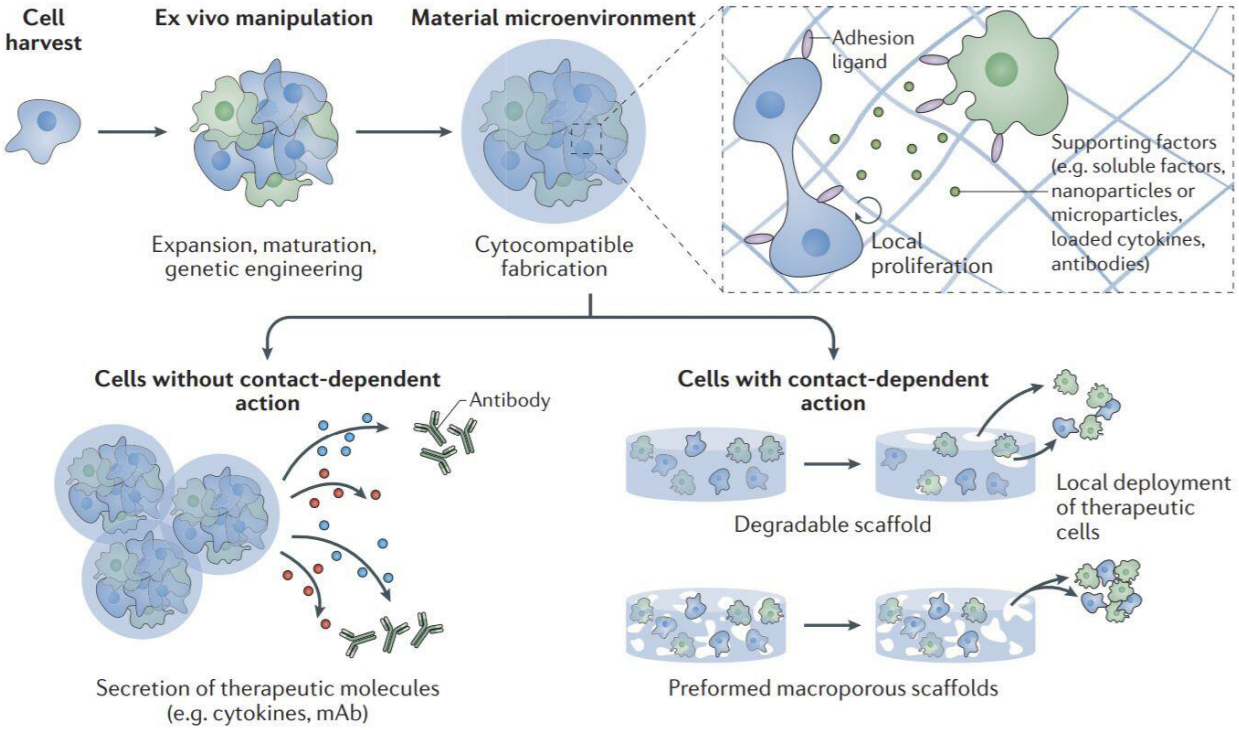

Fig. 6: Tissue Engineering and Regenerative Medicine

McCarthy et al., 2006), influenza A by using A549 human alveolar epithelial cells and a hydrogel comprising of Matrigel, alginate, and gelatin or by employing primary human small airway epithelial cells (HSAEpCs) in combination with a chitosan-collagen scaffold (Berg et al., 2018; Bhowmick et al., 2018), and other respiratory system-related viruses (Gardner and Herbst-Kralovetz, 2016; Huh et al., 2012a; Miller and Spence, 2017; Nichols et al., 2013). Taking the lead from these studies, using the proper tissue types and by making additional efforts, 3D models suitable for the study of the SARS-CoV-2 virus represent an excellent opportunity to quickly understand the disease, rather than waiting for animal models – in fact, mature animal models for SARS-CoV-2 infection are rarely available.

A. Reliable *In Vitro* Disease Models

Reliable *in vitro* human disease models that capture the complexity of *in vivo* tissue behaviors are crucial to gain mechanistic insights into human disease and enable the development of treatments that are effective across broad patient populations. Injury to cells and tissues sets in motion a series of events that contain the damage and initiate the healing process by means of regeneration and repair. Inadequate tissue repair following trauma or surgery and misregulated tissue regeneration and repair responses, such as diabetes mellitus, aging, cancer, osteoarthritis and fibrosis, affect millions of patients worldwide each year. The molecular mechanisms underlying tissue repair or its failure are not completely understood and current therapeutic options are limited. Thus, tissue regeneration technology has emerged as a useful platform for the development of reliable *in vitro* systems, with applications in drug development and disease modeling.

Conclusion

In conclusion, tissue engineering techniques represent a promising frontier in regenerative medicine, offering innovative solutions for the treatment of various medical conditions. The integration of cells, biomaterial scaffolds, and growth factors enables the creation of functional tissue substitutes that closely mimic the properties of native tissues. Through scaffold-based approaches, cell-based therapies, and bioprinting technologies, tissue engineers strive to develop tailored solutions for tissue regeneration and repair. Despite significant advancements, challenges remain in optimizing the efficacy, safety, and scalability of tissue engineering techniques for clinical applications. Continued research and collaboration across multidisciplinary fields are essential to overcome these challenges and translate tissue engineering innovations into clinical practice. With ongoing advancements in biomaterials, cell biology, and biofabrication methods, tissue engineering holds immense potential to revolutionize healthcare by providing personalized therapies and improving patient outcomes in the future.

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