ISSN 1990-519X, Cell and Tissue Biology, 2023, Vol. 17, No. 5, pp. 454–464. © Pleiades Publishing, Ltd., 2023.

Application of 3D Scaffolds in Tissue Engineering

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Received December 11, 2022; revised January 17, 2023; accepted January 27, 2023

Abstract—Three-dimensional (3D) scaffolds are often used in tissue engineering applications to produce an environment that is conducive to the integration of cells or growth factors to repair or replace damaged tissues or organs. These scaffolds are utilized to mimic the microenvironment seen in vivo, where cells interact and respond to mechanical cues from their three-dimensional surroundings. Consequently, cellular response and fate depend greatly on the material properties of scaffolds. These three-dimensional scaffolds' porous, networked pore structures enable the movement of nutrients, oxygen, and waste. This article looks at the many manufacturing procedures (such as conventional and rapid prototyping techniques) used to create 3D scaffolds with variable pore sizes and porosities. The various methods for determining pore size and porosity will also be covered. It has also been investigated if scaffolds with graded porosity may more accurately mimic the *in vivo* situation in which cells are exposed to layers of various tissues with changing characteristics. Following a look at the extracellular matrix, nature's own scaffold, the ability of scaffold pore size and porosity to affect biological responses and mechanical qualities will also be investigated. We will talk about the problems with the current ways of building scaffolds for tissue engineering applications and offer some new and exciting alternatives.

Keywords: scaffold, repair, regenerative medicine, regeneration

DOI: 10.1134/S1990519X23050061

INTRODUCTION

In the discipline of biomedical engineering known as tissue engineering, biology and engineering are combined to produce tissues or biological products outside of the body. Cell and molecular biology, physiology and systems integration, stem cell proliferation and differentiation with lineage traits, extracellular matrix chemistry and chemicals, and endocrinology are significant components of this subject. By incorporating other scientific areas into the translation of tissue engineering structures to therapeutic applications, it is possible to guarantee that cutting-edge generated tissues are accepted and employed by doctors (Ikada, 2006; Abdolmaleki et al., 2020). The development of extracorporeal organs or ex vivo tissue for use as grafts or replacements for failing in vitro organs has given rise to the area of regenerative medicine. Clinical trials are now being conducted for tissues like cartilage, bone, skin, brain, and hepatic tissue, and it is anticipated that in the next few years, many more tissues will also be used as healing mechanisms (Khil, 2003; Abdolmaleki et al., 2020). The utilization of porous three-dimensional scaffolds to provide physical support and a local environment for cells to enable and facilitate tissue development is a key idea in tissue engineering (Vacanti and Langer, 1999). To stimulate tissue creation in vitro and in vivo, scaffolds may be seeded with stem cells, progenitor cells, fully differentiated cells, or cell co-cultures. The capacity to transmit biomolecules and temporal/spatial signals to promote functional regeneration in damaged tissues and organs allows them to be directly implanted in vivo as well (Griffith, 2002). To produce tissue engineering scaffolds, it is necessary to understand the connections between the characteristics of the scaffold and biological processes. Throughout tissue development, morphogenetic elements are dynamically present in the extracellular environment. Biological processes are driven by cell-specific and intricately dynamic ECM structures. Each scaffold feature has to be assessed not just in a reasonably controlled in vitro setting but also in light of the physiological function of the tissue (Hollister, 2005; Asadi et al., 2020). The outcome of the tissue development process may be significantly influenced by a variety of scaffold qualities

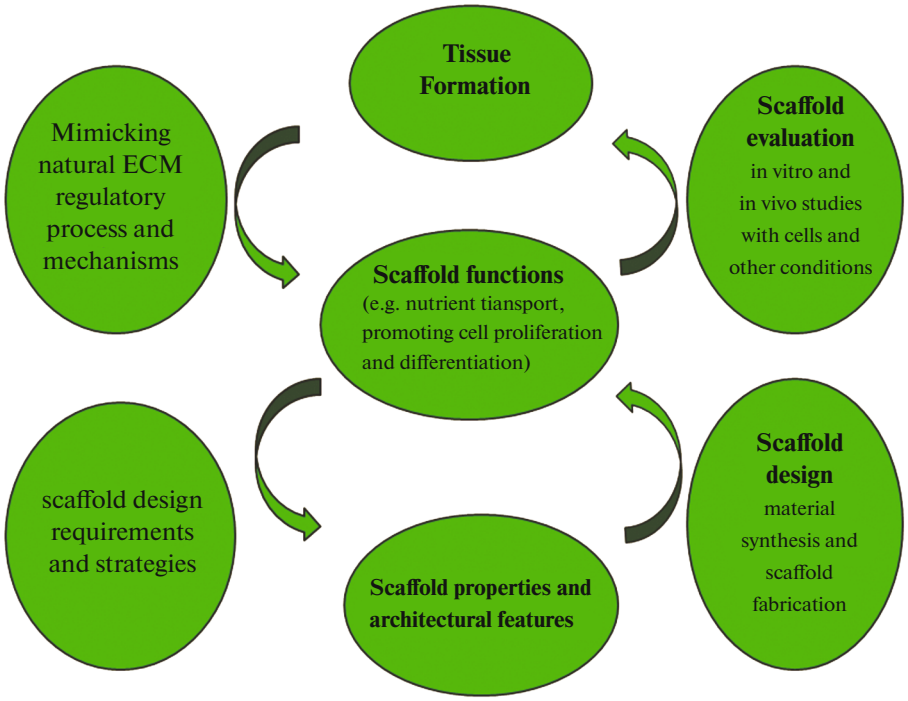

Fig. 1. Scaffold synthesis is a set of procedures for determining design concepts and constructing supporting techniques.

and environmental factors. The scaffold designs have been improved by using these connections (Lutolf and Hubbell, 2005). So that tissue growth may be started and controlled in vitro and/or at the site of implantation in vivo, scaffold features and their regulatory functions should be reproducibly included in a scaffold design (Saska et al., 2021).

The intrinsic complexity of biological systems makes it challenging to mimic natural processes and build tissues using scaffolds. Many factors might cause complications, including the fact that we don't fully comprehend natural cell-ECM interactions (Stevens and George, 2005). It's possible that as scaffold manufacturing technology advances, so will our knowledge of how to best use it. There may be other issues, such as the appropriate level of sophistication for a tissue-engineering scaffold (Malda et al., 2004) (Fig. 1).

In this work, we investigate the various scaffold design methodologies currently in use as well as key ideas that might facilitate the rational multifunctional design of tissue engineering scaffolds. The management of design factors through the use of different materials and manufacturing methods, as well as their effects on controlling tissue development, are identified and studied. The primary goal of the present study is to concentrate on the methods used in the Tissue engineering sector for scaffold production. This study focuses in particular on examining the two most popular methods, namely electrospinning and 3D printing. The study briefly examines the applicability, limitations, and anticipated needs of these cutting-edge Tissue engineering methods, including how they may be used to create full body organs.

TISSUE ENGINEERING

The primary objective of tissue engineering is a regeneration or replacement of tissues or organs damaged by disease, injury, or congenital anomalies. At present, tissue engineering repairs damaged tissues and organs with artificial supporting structures called scaffolds. These are used for attachment and subsequent growth of appropriate cells. During the cell growth gradual biodegradation of the scaffold occurs and the final product is a new tissue with the desired shape and properties. In the sixteenth century, the Italian surgeon Tagliacozzi described his forearm flap-based nose repair. Modern surgery arose in the nineteenth century as a result of the development of sterile technology and an understanding of the germ theory of illness. In the evolution of human treatment, techniques that result in the restoration of function via structural replacement have become more essential (Atala et al., 2002). Transplantation is, in some respects, the most invasive kind of reconstructive surgery. Biological changes have happened because of the ways that tissue is moved from one place to another. For instance, redirecting urine into the colon may result in deadly colon cancer 20 to 30 years later. Using the stomach as a urinary system replacement might result in permanent scarring and blockage. The largest challenge is collecting sufficient tissue and organs for

everyone in need. In the United States, 115 940 people are on transplant waiting lists, and many will pass away while waiting for available organs. Over time, immune system dysfunction results in continuous rejection and destruction. The restrictions have necessitated the development of innovative tissue-supply systems. Using live cells to build new and functioning living tissues, which are often connected to a matrix or scaffolding to assist tissue creation, in this setting, tissue engineering has emerged as a new field. In 2006, the authors stated that new cell sources including several types of stem cells, have been discovered, revitalizing interest in the issue (Vacanti, 2006). Scaffolds may be made from natural or synthetic materials, or a combination of the two. The Nobel Prize was granted to Drs. Gurdon and Yamanaka for their contributions to the area of cell reprogramming. In 2006, they revealed the modest decellularization of vital organs while maintaining the circulatory supply's architecture (Nerem, 2006).

Before transplantation, living cells may migrate inside the implant or be connected to a matrix in culture. These cells may be retrieved as fully formed cells from the tissue being recreated. The application of this new discipline to human health care may be seen as a refinement of previously stated medical concepts (Langer et al., 2000). The ability to create three-dimensionally organized, functional tissue is a benefit of tissue engineering over cell transplantation. This chapter describes some of the challenges that must be overcome before tissue engineering may be included in physicians' and surgeons' therapeutic toolkits. The bulk of the challenges are social and scientific in nature (Okita et al., 2007). Tissue engineering may make use of what we have learned about cell and stem cell biology, biochemistry, and molecular biology to create new tissues. The practical application of engineering principles to biological systems has been made possible by developments in materials science, chemical engineering, and bioengineering. The practice of human treatment by surgeons and physicians is another related area of study (Ott et al., 2008). When it comes to therapeutic stem cells, iPS cells have the potential to be the holy grail since they can be produced in endless quantities, are patient-specific, and are immune-neutral. In terms of ethics and legislation, there is still much to discover about these cells, but the study is still going well. As we go closer to human applications, the fundamental biophysical limitation of mass transfer of live tissue must be addressed individually (Codrea et al., 2021) (Fig. 2).

The scope of this study is immense since tissue engineering has such a wide range of possible applications. In order to create tissue-engineered structures, several research teams are analyzing the ideal chemical and physical assemblages of innovative biomaterials. Growth in the industry will need a generation of materials scientists and chemical engineers (Simmons et al., 2020). A vascularized mesodermal component, such as smooth muscle, cartilage, or fibrous tissue, makes up every tube in the human body. The development of new skin, which includes the keratinocyte-filled epithelial layer and the dermis and its accompanying fibroblasts, is now the focus of research (Jonathan, 2020). Future research must concentrate on neural regeneration, neural ingrowth, and neural function in end-organ tissues like skeletal or smooth muscle. Some tissues may be designed as universal tissues that may be exploited by anybody, while others may be modified to meet the requirements of a specific patient. Apart from their architectural complexity, the cells therein have a very high metabolic demand. Consequently, isolating a significant number of living cells is quite challenging (Sharma et al., 2019). 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 (Lysaght and Reyes, 2001).

3D SCAFFOLDS

Scaffolds are medically designed materials that help in the development of new, functioning tissues. Scaffolds mimic the original tissue's extracellular matrix, reproducing the in vivo environment and allowing cells to exercise control over their microenvironments (Mertsching et al., 2009). High porosity and proper pore size are required to permit cell seeding and diffusion of cells and nutrients throughout the whole structure. Given that scaffolds should ideally be absorbed by the surrounding tissues without the need for surgical removal, biodegradability is often a crucial concern (Widmer and Mikos, 1998). Material selection is an integral part of the scaffolding process (Stock and Vacanti, 2001). Natural or synthetic biodegradable materials may be employed. They must be biocompatible, causing no damage to cells, and simple to clean and discard (Kajihara et al., 2003). Natural polymers consist of long chains, including nucleotides, amino acids, or monosaccharides made of repeating covalently bonded groups. Biofunctional molecules which ensure bioactivity, biomimetic nature, and natural restructuring are typically found in such polymers. Bioactivity, biocompatibility, 3D geometry, antigenicity, non-toxic byproducts of biodegradation, and intrinsic structural resemblance are the most important properties of natural polymers (Heidary and Mahdavi, 2015; Singh et al., 2016). Even long noncoding RNAs (lncRNAs), which include pseudogenes and circRNAs, are required for construction of a molecular scaffold that can support cellular activity (Roshancheshm et al., 2022).

Synthetic polymers are advantageous in a few characteristics such as tunable properties, endless forms, and established structures over natural polymers.

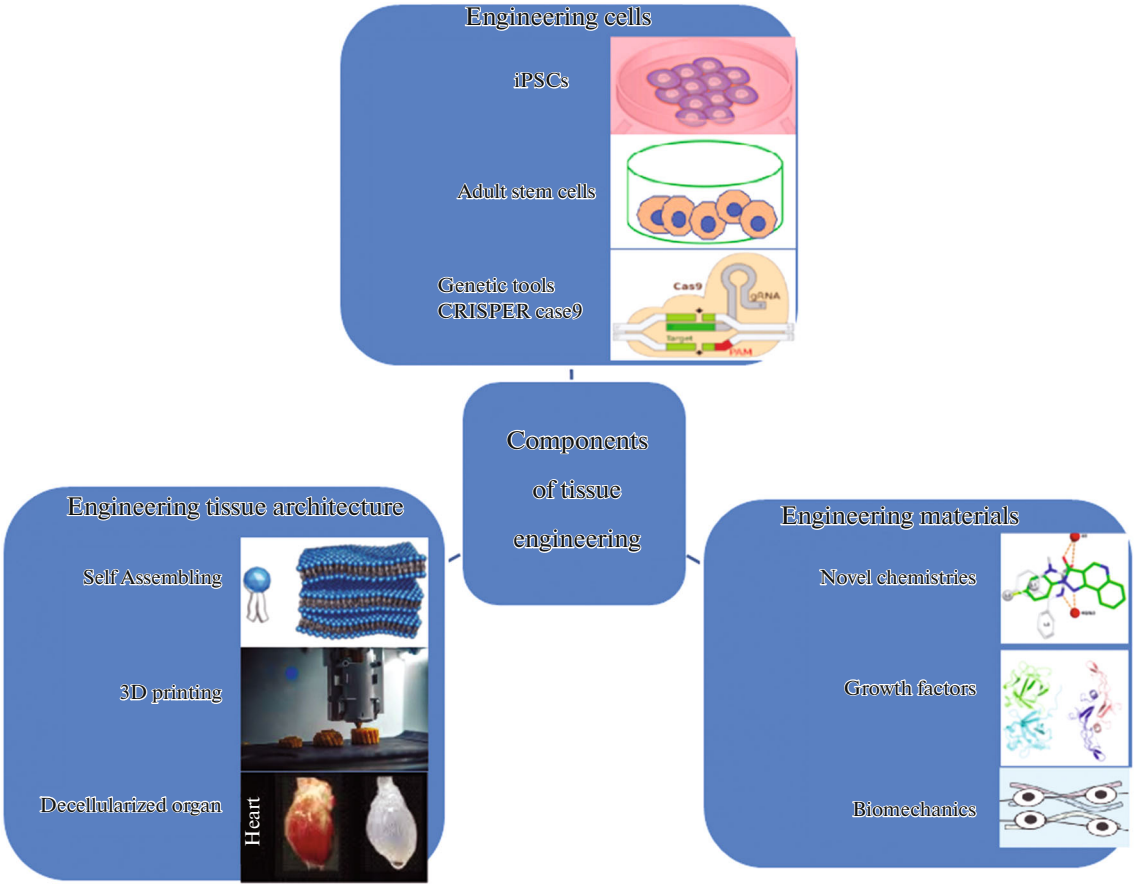

Fig. 2. The different facets of tissue engineering are shown. Materials, cells, and tissue topologies may all be changed individually or in combination to achieve therapeutic goals.

Polymerization, interlinkage, and functionality (changed by block structures, by combining them, by copolymerization) of their molecular weight, molecular structure, physical and chemical features make them easily synthesized as compared to naturally occurring polymers (Geckil et al., 2010; Kluge and Mauck, 2011). In biodegradable polymers, synthetic polymers are a major category and can be produced under controlled conditions. In a broad spectrum, the mechanical and physical characteristics are predictable and reproducible, such as strength, Young's modulus, and degradation rate. Poly(α-hydroxy esters) including PCL, PGA, PLA, and their copolymer PLGA and poly(ethers) including PEO and PEG, PVA, and PU are the most widely studied degradable synthetic materials (Cascone et al., 2001; Jayakumar and Nair, 2012). These are probably the most popular examples, although there are currently many other synthetic materials being sought. These polymers have various levels of biodegradability, biocompatibility, and mechanical properties, but no single polymer holds all three of these critical properties at the optimum level (Ciardelli et al., 2005).

Each application requires a certain material, which is characterized by its mechanical qualities. Bone tissue engineering, for instance, will need a far more rigid scaffold than skin regeneration (Takagi et al., 2017). The synthetic polymer polylactic acid (PLA) is among these materials. In the human body, this polyester dissolves into lactic acid, a naturally occurring and easily excreted molecule. As a result of its adaptability and biocompatibility, it is an excellent scaffolding material. The consequence of combining PLA with PGA is poly-lactic-co-glycolic acid (PLGA) (Gentile et al., 2014).

Natural materials may also be used to create scaffolds; for instance, several extracellular matrix derivatives have been examined to see how well they stimulate cell development. Polysaccharides such as chitosan and proteins like collagen and fibrin are examples of biomaterials (Park et al., 2011) Glycosaminoglycans (GAGs) have been shown to be compatible with cells. Hyaluronic acid is one of the GAGs that may be used as scaffold material; it can be mixed with cross-linking agents (e.g., glutaraldehyde, water-soluble carbodiimide, etc.). A non-bioactive molecule may be linked to an extracellular matrix protein frag-

ment, such as the RGD peptide (Pomeroy et al., 2020).

Several ways of generating porous materials that may be used as tissue engineering scaffolds have been discussed in the literature. Each strategy has its own merits, but none of them is without disadvantages. Molecular self-assembly is one of the few techniques for creating biomaterials with comparable qualities to the natural in vitro extracellular matrix (ECM) (Cassidy, 2014). For tissue engineering applications, nonwoven polyglycolide structures have been studied; these fibrous structures may be advantageous for culturing diverse cell types. Solvent casting and particle leaching may produce structures with consistent porosity and restricted thickness (SCPL). Compression-molded disc-shaped items may now be produced utilizing a process that employs gas as a porogen (Nam and Park, 1999).

The TIPS approach uses a solvent with a low melting point that is easy to evaporate. In SCPL, the emulsion is immediately frozen in liquid nitrogen after being poured into a mold. The polymeric structure is subsequently removed from the mold and cured, resulting in a porous, stiff structure. A porous scaffold is created after cooling below the solvent's melting point and vacuum drying (Anstey et al., 2021).

Electrospinning is a versatile technology for producing fibers with diameters ranging between a few microns and a few nanometers. The suitable scaffold material is dissolved in a solvent and placed in a syringe in a typical electrospinning setup. A high voltage is applied to the needle's tip and a conducting collecting surface as the solution travels through the needle (Anstey et al., 2021). Tissue engineering has adopted computer-assisted design and production techniques since the bulk of older technologies lacked the ability to control porosity and pore size. Initially, CAD software is used to build a three-dimensional structure, and algorithms may be employed to adjust the porosity (Melchels et al., 2011).

The scaffold is then built using polymer powder ink-jet printing or polymer melt Fused Deposition Modeling (Nam and Park, 1999). The cell survival rate for possible therapeutic applications increases as the pore size of a bioreactor and the mechanical stress (to reproduce in vitro conditions) decrease. Replacement of articular cartilage may act as an "alternative to standard tissue healing" by shortening recovery time and increasing transplant efficacy (Ma and Elisseeff, 2005).

In a 2012 study, intended to determine whether multicellular 3D patterns could be created in a natural matrix using laser-assisted bioprinting (LaBP), and if the resultant structures were functional and capable of creating tissue. LaBP is used to organize small amounts of live cell cultures into high-resolution patterns (Koch et al., 2012). A group of scientists claims to have created a 3D-printed cell construct that might be useful for drug research and toxicity testing. Other cell types (such as melanocytes, Schwann cells, and hair follicle cells) may be included in the 3D cell construct, according to the researches. It is feasible to evaluate the activities of these cells in an in vitro environment analogous to their natural environment (Lai et al., 2011).

Gustafsson and his associates (Gustafsson et al., 2020) found that spider silk self-assembled at the aqueous solution interface to form freestanding, bioactive membranes with a cm^2 area but only a 250 nm thickness. Uniquely, the membranes combine nanoscale thickness, biodegradability, ultrahigh strain and strength, protein permeability, and cell adhesion and proliferation into a single package (Qasim et al., 2019) (Fig. 3).

APPLICATION OF 3D SCAFFOLDING IN TISSUE ENGINEERING

Application in Bone Tissue Engineering

Researchers have combined scaffolds for 3D printing bone tissue with stem cells. These scaffolds may perform similarly to bone, cartilage, and the extracellular matrix (ECM) in that they facilitate cellular attachment, development, and differentiation. The optimum scaffolds for bone tissue engineering should have the right form, with the right porosity, surface area ratio, mechanical support, and surface activity (Bose et al., 2013). Stem cells are self-renewing and developing cells that may multiply endlessly and specialize into many germ layers. Stem cells are classified as embryonic stem cells or adult stem cells based on their developmental stage. Historically, tissue engineering and regenerative medicine have concentrated on blastocyst-stage inner cell mass-derived stem cells (Whiting et al., 2015). The study of iPS cells has improved organoid creation, drug development, illness mechanism research, and disease treatment (Samsonraj et al., 2017). ASCs may differentiate into a variety of tissues and cell types and offer significant promise for tissue repair and the treatment of illness (Han et al., 2019). In 2006, Yamanaka and Takahashi successfully created iPS cells from cultured mouse fibroblasts by mixing four components (Takahashi and Yamanaka, 2006). The use of additive manufacturing (AM), especially 3D printing, is expanding in the production of bioactive implantable devices. A structure's ability to support intracellular mobility, nutrient delivery, and ECM formation depends on its high porosity, high interconnectivity, and specified pore size and shape (Do et al., 2015). Pore geometry, pore size distribution, and interconnectivity are all changed when routine procedures like gas foaming, pore-forming agent leaching, or phase separation methods are utilized (Prasopthum et al., 2018). Furthermore, 3D printing has a greater therapeutic effect and improved material properties (Pantea et al., 2021). Within a

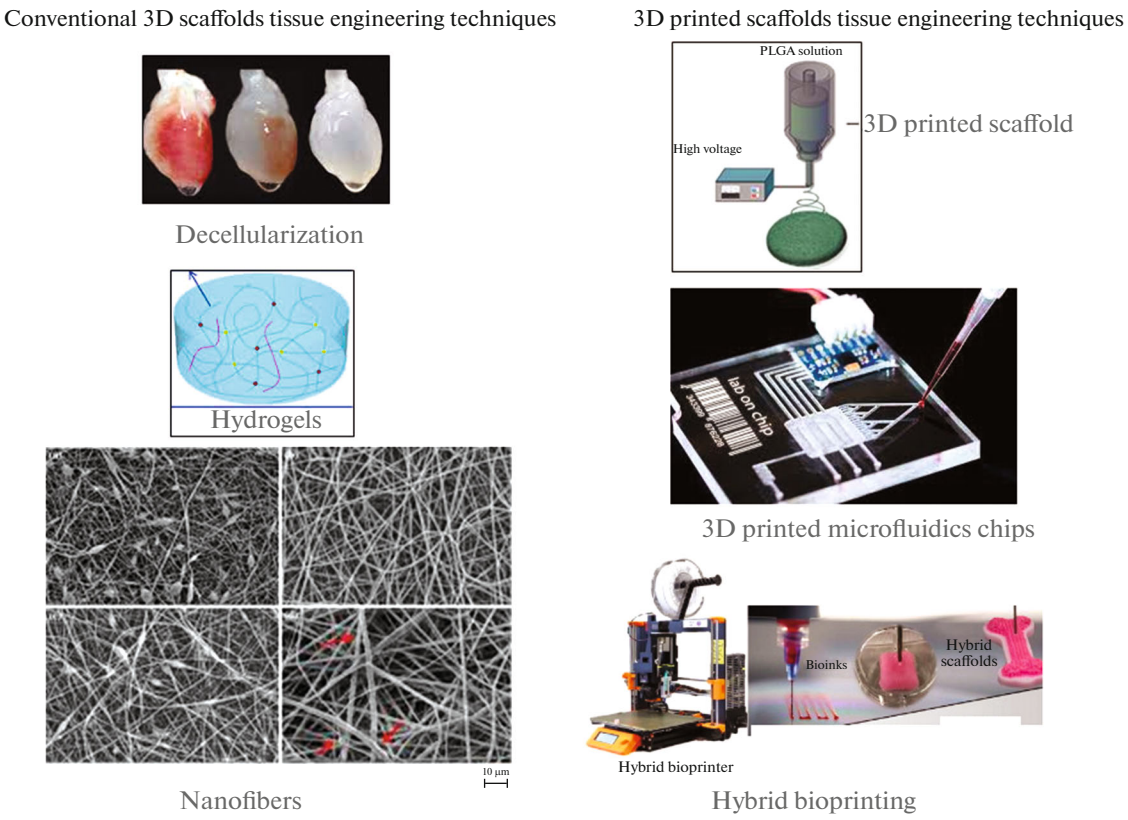

Fig. 3. Traditional and contemporary 3D printed scaffold-based tissue engineering techniques include decellularization, hydrogels, nanofibers and hybrid bioprinting; 3D scaffold printing; and 3D printed microfluidics chips. The acronym for three-dimensional is dimensional.

therapeutic setting researchers found that compared to solid or hexagonal porous scaffolds, square-shaped scaffolds promoted greater human mesenchymal stem cell (hMSC) proliferation and differentiation (Aliabouzar et al., 2018). This enables the construction of structures for bone tissue regeneration via 3D printing, as well as promoting stem cell-directed differentiation and proliferation (Song et al., 2018). The creation of well-specified porosity structures is possible via 3D printing. For those with tissue impairments, it may also help create a tailored implant structure when used in conjunction with 3D computer imaging. It is possible to rapidly create CAD models from CT scan data (STL) (Marro et al., 2016). A hydrogel is a three-dimensional network of natural or synthetic polymers that is insoluble in water. Due to their porosity, hydrogels are utilised as matrices in tissue engineering (Zhu, 2010). Various building processes combine COL and minerals to generate MCS with a similar structure to bone tissue. MCSs produced by 3D printing were employed as a replacement for full or even partial skeletal abnormalities (Chocholata et al., 2019; Mabrouk et al., 2020; Li et al., 2021; Yahya et al., 2021) . One of the future challenges in bone tissue engineering is to design and to manufacture biodegradable scaffolds with a homogeneous growth rate over their entire volume, using pore size gradients or specific distributions of embedded growth factors. This requires manufacturing processes with higher resolution and bio-fabrication capabilities. Haider et al. fabricated various nHA/PLGA nanofibers scaffolds with the purpose of bone tissue regeneration. From the obtained results, they concluded that their nHA/PLGA composite scaffolds had enhanced osteogenesis (bone formation) (Haider et al., 2020).

Application in Cartilage Tissue Engineering

As it provides the articulating bones with a practically frictionless and low-wear bearing surface and aids in mechanical stress absorption, articular cartilage is crucial for smoothly moving joints (Johnstone et al., 1999). Its avascularity and relative acellularity limit its ability to repair and renew following injury or disease. Current treatment methods for cartilage healing include microfracture, mosaicplasty, and implantation of autologous chondrocytes (Abdolmaleki et al., 2020). Therefore, to restore the injured cartilage in the chondral defect, innovative cartilage repair techniques are required. In order to restore tissue functioning and achieve a positive therapeutic result, polymeric scaffolds are used in cartilage tissue engineering as an

alternative to traditional healing methods (Temenoff and Mikos, 2000). The synthetic materials used most often in the production of tissue engineering scaffolds are poly L-and D-lactic acid (PLLA and PDLA), polyglycolic acid (PGA), and their copolymers. Cell adhesion, survival, and matrix growth are all determined by the way these constructs are designed and built. Pore dimensions, shape, total porosity, and the substance employed all have an impact on chondrocyte biology (Taboas et al., 2003).

Scaffolds have to be made to mimic the strength and stiffness of healthy tissues. The laydown pattern, which comprises the angle of deposition, the breadth of the material deposited, and the spacing between the strands, may be changed to change the pore morphology of various structures. It has been suggested that mechanical stress may stimulate the in vitro development of tissue-engineered cartilage constructions (Wu et al., 1999).

As a result, it may be necessary to employ a dynamic bioreactor to apply mechanical stress in order to create tissue-engineered cartilage structures in vitro. Implanting a scaffold with biomechanical characteristics similar to actual cartilage has enormous potential. Its rigidity would be sufficient to support weight quickly. Early force transmission over the healing site may cause cartilage to develop that has similar biomechanical properties to the articular cartilage around it. Additionally, patients would heal more quickly (Landers et al., 2002).

Application in Cardiac Tissue Engineering

In a number of approaches, scientists have created 3D bioengineered alternatives that duplicate natural body structures at macro, micro, and nanoscale levels (Mathur et al., 2016). When biological tissues or organs are injured, scaffolds are short-term, three-dimensional supports for cell growth and proliferation. Many natural tissues, including the heart, kidneys, pancreas, lungs, nerves, and cardiac valves, may be imitated by a scaffold. After being implanted into a patient's body, scaffolds make it easier for new tissues to grow, leaving behind a biologically sound system. These scaffolds could provide cutting-edge treatment options for cardiovascular diseases instead of organ donation (Ng et al., 2016).

The best scaffold biomaterial for a cardiac patch is an elastic material that can stretch and relax hundreds of times without affecting the heart's ability to contract and relax. Due to its high hydrophilicity, permeability, biodegradability, flexibility, and capacity to bond with other biopolymers, poly (vinyl alcohol) (PVA) holds a lot of potential for the development of cardiac scaffolds. Without dissolving, PVA may hold onto a significant volume of water or biological fluids (Mathur et al., 2016). A 3D PVA fibrous scaffold was produced by Roy et al. (Ng et al., 2016) using an electrospinning technique and glutaraldehyde crosslinking. Polydopamine was used to coat the surface of the scaffold to help human mesenchymal stem cells (hMSCs) stick to it.

We show how to create biocompatible and biodegradable 3D porous scaffolds for cardiac tissue engineering (CTE) applications using a hybrid approach. The goal was to differentiate human induced pluripotent stem cells into cardiomyocytes in order to provide a useful template for manufacturing cardiac cells. The proposed scaffold has mechanical properties similar to the extracellular matrix (ECM), which enables the contraction of heart cells (Qasim et al., 2019) (Fig. 4).

Application in Vascular Tissue Engineering

Tissue engineering is a multidisciplinary method for improving or replacing the function of biological tissue. Researchers can thoroughly examine vascular network formation and vessel interactions with the environment thanks to vascular tissue architecture. Treatment for cardiovascular illness, ischemia, and burn wounds may benefit from an understanding of and control over the development and differentiation of human vasculature (Kalogeris et al., 2012). The physiology and operation of human organs depend on a healthy circulatory system. Because of a shortage of oxygen, nutrients, and the inability to eliminate metabolic waste, tissue malfunction and necrosis may arise from ischemia or a momentary or long-term decrease in blood flow to a target organ (Auger et al., 2013). Although 3D blood vessel creation has advanced significantly, creating a functional vascular multiscale system has remained difficult (Song et al., 2018). Numerous techniques have been used to mimic the intricacy, distinctive architecture, and function of human blood veins in vascular networks. Vascular tubes have traditionally been produced through sheet rolling, tube molding, and direct scaffolding of biomaterials, with or without cells. Due to improved control over vascular development and repeatability of the fabrication process, 3D bioprinting has emerged as a critical method for the production of vascularized bioconstructs (Cui et al., 2012).

CONCLUSIONS

Tissue engineering may provide a way to deal with recurring issues with tissue regeneration. Scaffolds, a fundamental ingredient that supports mechanical properties, cell adhesion, proliferation, and specialisation, may be produced by modifying essential factors including material selection and the technology utilised to generate 3D structures. Certain older procedures, such freeze drying, are still helpful for this when compared to modern approaches, but cutting-edge techniques hold enormous promise for the more challenging subject of tissue engineering. Both the industrial and healthcare sectors have quickly boosted

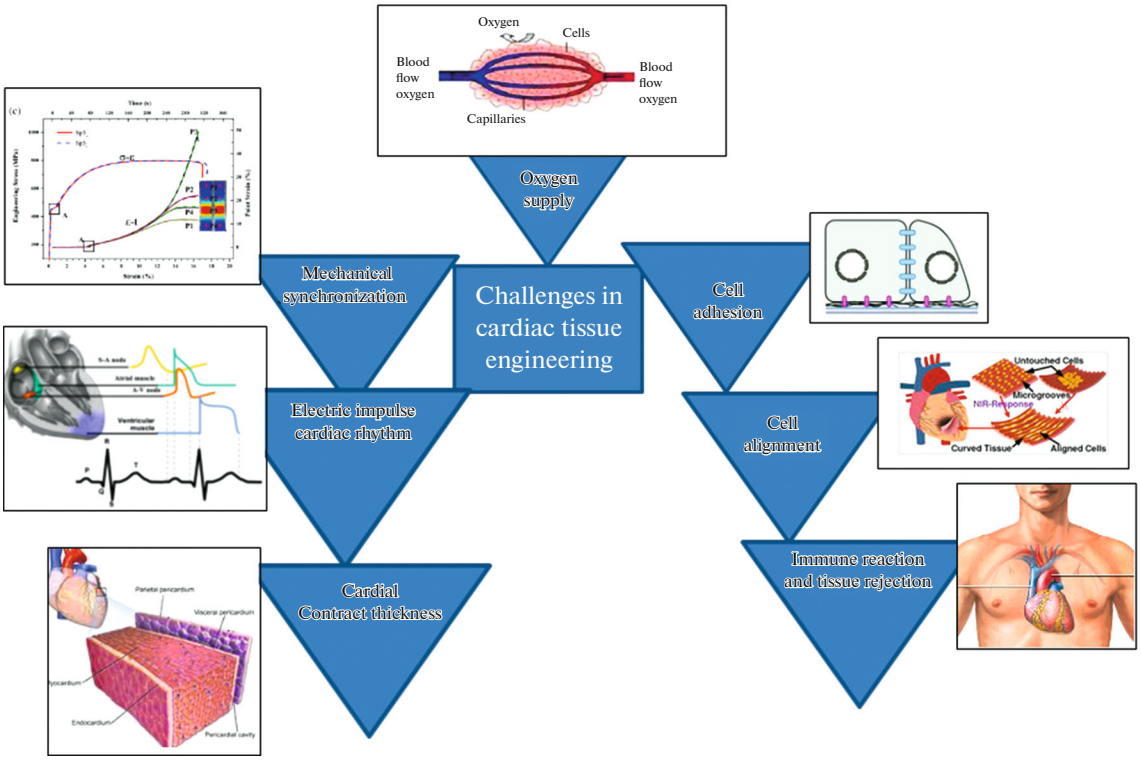

Fig. 4. The picture highlights the challenges of cardiac tissue engineering.

their usage of electrospinning. Compared to electrospinning, 3D printing advances more slowly since it is a complex process needing several technological components that ignore biological processes. With the aid of cutting-edge techniques and biomaterials, researchers studying tissue engineering are now able to construct 3D structures.

ACKNOWLEDGMENTS

The authors would like to thank Mohaghegh Ardabili University for concerning this manuscript.

FUNDING

The research was funded by University of Mohaghegh Ardabili.

COMPLIANCE WITH ETHICAL STANDARDS

All authors of this manuscript say that they have no conflicts of interest to disclose. The work did not involve animals or human beings as experimental subjects.

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