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Review

Biomimetic Scaffolds—A Novel Approach to Three Dimensional Cell Culture Techniques for Potential Implementation in Tissue Engineering

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Abstract: Biomimetic scaffolds imitate native tissue and can take a multidimensional form. They are biocompatible and can influence cellular metabolism, making them attractive bioengineering platforms. The use of biomimetic scaffolds adds complexity to traditional cell cultivation methods. The most commonly used technique involves cultivating cells on a flat surface in a two-dimensional format due to its simplicity. A three-dimensional (3D) format can provide a microenvironment for surrounding cells. There are two main techniques for obtaining 3D structures based on the presence of scaffolding. Scaffold-free techniques consist of spheroid technologies. Meanwhile, scaffold techniques contain organoids and all constructs that use various types of scaffolds, ranging from decellularized extracellular matrix (dECM) through hydrogels that are one of the most extensively studied forms of potential scaffolds for 3D culture up to 4D bioprinted biomaterials. 3D bioprinting is one of the most important techniques used to create biomimetic scaffolds. The versatility of this technique allows the use of many different types of inks, mainly hydrogels, as well as cells and inorganic substances. Increasing amounts of data provide evidence of vast potential of biomimetic scaffolds usage in tissue engineering and personalized medicine, with the main area of potential application being the regeneration of skin and musculoskeletal systems. Recent papers also indicate increasing amounts of in vivo tests of products based on biomimetic scaffolds, which further strengthen the importance of this branch of tissue engineering and emphasize the need for extensive research to provide safe for humansbiomimetic tissues and organs. In this review article, we provide a review of the recent advancements in the field of biomimetic scaffolds preceded by an overview of cell culture technologies that led to the development of biomimetic scaffold techniques as the most complex type of cell culture.

Keywords: biomimetic scaffolds; 3D bioprinting; biologically derived materials; 4D materials

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1. Introduction

Tissue damage resulting from cancer, congenital defects, and trauma requires new and effective treatments that facilitate tissue regeneration. Tissue engineering presents significant potential in this regard, as it enables the restoration of native tissue architecture and functions through the fusion of cells to specific scaffolds [1]. The goal of tissue engineering (TE) is to restore, preserve, or enhance the structure and function of damaged tissues or organs by integrating biological signals and biological scaffolding strategies [2].

Cell cultures have traditionally been studied on 2D platforms, consisting of cells interacting with a culture dish in a medium environment. However, this method has limitations as the growth of cells on a flat surface does not accurately reproduce their actual functioning in the 3D environment of other cells. The 3D model overcomes these limitations by recreating the in vivo environment. Scientists have provided evidence that scaffolding animal cells can induce the formation of 3D colonies that resemble the natural environment, both molecularly and phenotypically [3]. The extracellular matrix (ECM) plays a crucial role in the transport properties, cell communication, mechanotransduction, and growth factor signaling of the 3D hierarchical microstructure due to its electromechanical nature. This is achieved through the interaction of the ECM with receptors on the cell surface, as well as the binding of growth factors and other signaling molecules [4]. Zhang et al. suggest that the ECM scaffold is a highly promising candidate for tissue engineering applications. Among biomimetic ECM scaffold materials, decellularized ECM scaffolds (dECMs) derived from natural ECM are particularly noteworthy due to their natural components and microenvironment [5].

The goal of regenerative medicine is to repair and replace damaged tissue, and the use of three-dimensional scaffolds is one of the most promising techniques for tissue repair. Hydrogels are one of the most extensively researched types of scaffolds. They have demonstrated positive results in preclinical studies by mimicking the fundamental signals that promote local tissue regeneration [6]. Recent advancements in computer-aided design and 3D printing have helped the production of macroporous hydrogels, enabling the creation of more intricate structures. This approach may lead to the development of fully reconstructed organs. The field of hydrogels is a promising area of research in regenerative medicine, with applications in most tissues of the human body [7].

Biocompatible scaffolds promote cell adhesion, proliferation, and differentiation to facilitate tissue regeneration. These scaffolds provide strength, mechanical stability, flexibility, and an ideal environment for cell growth. Scaffolds can be divided into natural and synthetic categories [5]. Bioscaffolds provide a niche for cell growth, while synthetic scaffolds offer greater control over the size and morphology of regenerated tissues. In this review, we present a comprehensive overview of the current state of knowledge regarding biomimetic scaffolds. We begin by examining recent advancements in scaffold technology and their pivotal role in tissue engineering. Subsequently, we delve into the cell culture techniques that have contributed to the conceptualization and development of biomimetic scaffolds, as well as those evolving in tandem with this technology. In the final section, we highlight recent advancements in biomimetic scaffolds and explore their potential applications in biotechnology and tissue engineering.

2. Scaffolds in Tissue Engineering—Recent Findings and Current Research

In recent years, there has been an observable increase in the diversity of areas studied in the field of tissue engineering. Scientists are seeking more complex opportunities to apply recent technological advancements beyond the classic tissue engineering of the skeletal system [8]. An increasing number of studies provide data indicating usage of scaffolds in tissue engineering of different systems, including endocrine, muscle, genitourinary, digestive and pulmonary systems.

Recent advancements in regenerative endocrinology include the development of novel methods of protecting pancreatic β-cells from destruction by immune cells in hybrid devices consisting of scaffolds made out of polycaprolactone scaffolds and pancreatic

β-cells encapsulated in alginate microcapsules. This method prolongs the functionality of the device and potentially eliminates the need for immunosuppressants [9]. Also, the development of prevascularized thyroid organoids opens new possibilities for potential new ways of hypothyroidism treatment [10]. Another advancement in tissue engineering of endocrine glands is development of a 3D-bioprinted functional model of the pancreas using pancreatic and endothelial cell lines with an ability to moderate insulin secretion answering to changes in glucose levels in medium [11]. Also, in the field of human reproductive hormones, there are some significant changes with the introduction of decellularized extracellular matrix providing optimal microenvironment for in vitro spermatogenesis [12].

In the treatment of volumetric muscle loss, scientists have developed a photoreactive hydrogel with the ability to change its stiffness. This allows the determination of the optimal level of hydrogel stiffness, which significantly increases the regeneration of muscle tissue [13].

In the field of muscle tissue regeneration, a recent study has also provided a description of complex artificial muscle tissue consisting of a layer of myofibroblasts connected with motor neurons derived from induced pluripotent stem cells. The motor neurons contracted the layer of myofibroblasts by creating neuromuscular junctions [14].

A study conducted on rabbits demonstrated another potential application of scaffolds in tissue engineering of different systems. It presents the potential usage of adipose tissue engineering as a tool in the treatment of postoperative complications. In order to reduce epidural fibrosis after the procedure of laminectomy, the researchers reconstructed local adipose tissue using a scaffold made from ECM imbued with mesenchymal stem cells [15].

Another area of tissue engineering development is tissue engineering of bile ducts using hydrogels as the scaffold [16].

Scientists have also recently used tissue engineering technologies in the regeneration of the genitourinary system. They created a device consisting of a scaffold and mesenchymal stem cells overexpressing basic fibroblast growth factor. They have provided evidence of the device's potential usage in regenerating full-thickness injuries of the uterus [17].

Tissue engineering is a developing field that has generated interest in creating scaffold-based artificial organs with higher functionality. For instance, a recent study focused on contractile vascular grafts that retain their contractility. The graft is composed of decellularized pulmonary artery and progenitor cardiovascular cells obtained in a bioreactor under physiological flow conditions [18].

3. 2D Cell Cultures—Limitation of Most Common Type of Cell Cultures

Culture vessels with a single surface are used for 2D cell culture. The proliferating cells cover the surface of the culture environment, forming a monolayer cell culture. The crucial factor affecting cell proliferation is confluence, which refers to the degree of surface coverage by cells. As confluence approaches 100%, the metabolism of cultured cells changes and the expression of proliferation markers decreases [19]. The most commonly used technique for culturing cells is on a flat surface due to its simple processing and cost-effectiveness. A monolayer of cells also enables straightforward observation and measurement. There are two types of 2D cultivations: simple cultures and co-cultures. Simple culture involves the interaction of cells with a dish in a culture medium environment. In co-cultures, cells from different tissues are cultured together in a single medium, allowing for direct or indirect interactions, as illustrated in Figure 1. Direct co-culture involves growing different types of cells on the surface of a shared dish, facilitating plenty of interactions. Indirect co-culture, on the other hand, is based on separate cultivation surfaces but with interaction occurring through a common culture medium. One disadvantage of this 2D cultivation model is the lack of representation of real cell surroundings. Although growing cells on a flat, plastic surface covered with dry plasma can enhance cell adhesion, it is not an appropriate method for studying cell metabolism in a natural environment where cells are surrounded by other cells in three dimensions. However, this model is useful for analyzing simple interactions under controlled conditions.

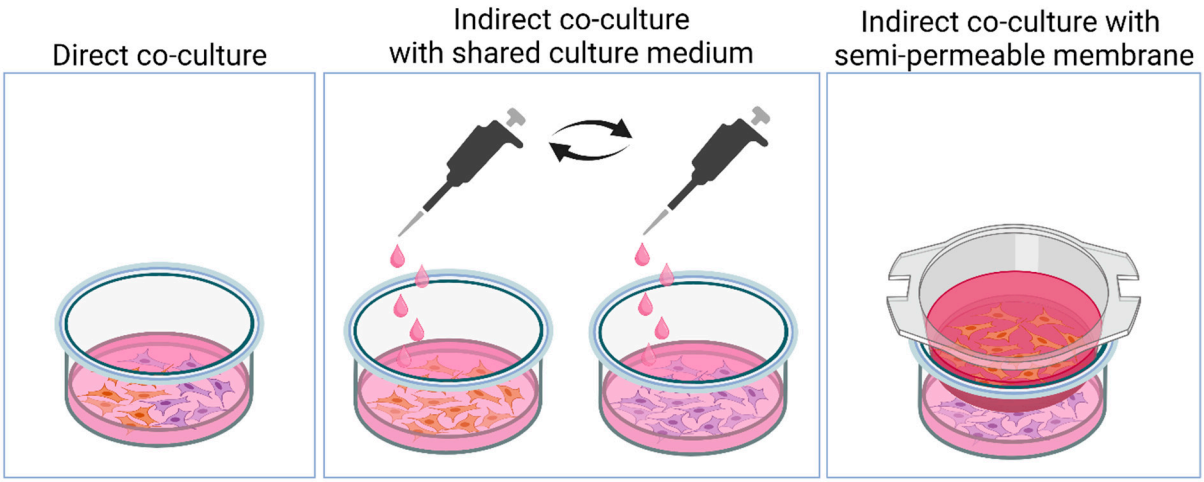

Figure 1. Types of direct and indirect co-culture systems. Created with BioRender.com.

4. 3D Cell Cultures

The next stage in comprehending physiological and pathological processes involving cell cultures is through the use of three-dimensional cell cultures. The interest of researchers in 3D human cell culture has grown rapidly since Hamburger and Salmon published one of the first papers on the subject in 1977 [20,21]. Although 2D cell cultures are the most commonly used method of studying living cells in vitro, there are still some significant drawbacks to this approach. According to research, cells cultured in two-dimensional conditions exhibit less similarity to those found in natural tissues [22]. This is due to changes in cell morphology, reduced cell–cell and cell–ECM interactions, and altered gene and protein expression resulting from the lack of a complex microenvironment [23]. Researchers were compelled to explore 3D culture techniques due to impaired cell polarity, ample access to oxygen and nutrition, and the absence of an external matrix, among other characteristics [24]. In the early 2000s, it became evident that 3D cultures were an emerging technology [25]. While the prediction that 3D culture would replace 2D culture was somewhat misguided, there is a growing trend towards more studies utilizing 3D culture. Three-dimensional cell cultures offer several advantages, the most significant of which is a closer representation of natural tissues [26]. However, 3D cultures also have some disadvantages, including higher costs, lower reproducibility, and greater difficulty in interpretation [23].

In fact, three-dimensional cell culture is a more complex method than two-dimensional culture. 3D cultures often involve co-culturing, where more than one type of cell is cultured simultaneously. In addition, there are various methods for replicating living 3D structures. 3D culture can be categorized as either scaffold-free or scaffold-based [26–29]. In scaffold-free 3D cultures, cells interact with each other and form structures such as spheroids [30]. Scaffold-based 3D cultures are capable of creating more intricate structures by utilizing cell–cell and cell–EMC interactions, which can closely resemble natural tissue [31]. There are two main types of 3D culture methods: those that use scaffolding in the cell culture (scaffold techniques) and those that do not (scaffold-free techniques).

4.1. Scaffold Free Techniques

Spheroids

The term ‘spheroid’ describes the spherical shape of cell colonies, whether they are single-cell or multicellular. In traditional monolayer cultures, cell–flask interactions are the primary type of interaction that contributes to the formation of the culture. In traditional monolayer cultures, cell–flask interactions are the primary type of interaction that contributes to the formation of the culture. However, spheroids emerge due to cell–cell and cell–ECM interactions. The process of forming a spheroid involves three main steps

presented on Figure 2. Firstly, the extracellular matrix (ECM) fibers, which are rich in RGD motifs, allow for the binding of integrins expressed on the cell membrane surface, resulting in upregulated cadherin expression. Secondly, cadherins localize on the cell surface. Finally, cadherin-cadherin homophilic bindings between nearby cells cause stronger cell-to-cell adhesion and spheroid formation [24,32,33].

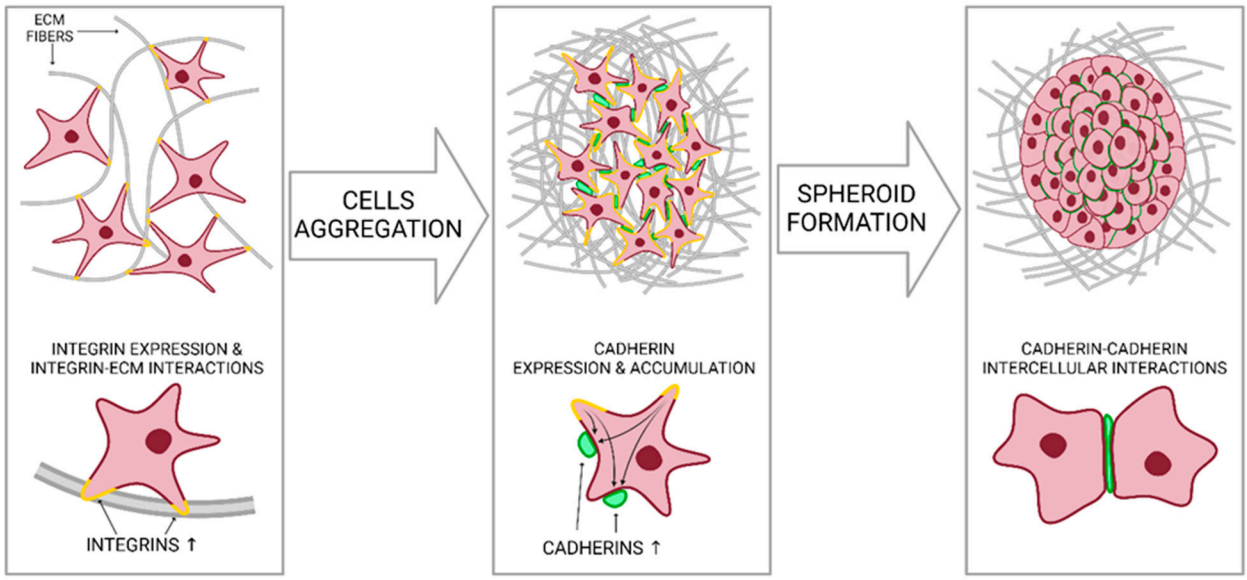

Figure 2. Steps of cell aggregation and spheroid formation (ECM, extracellular matrix). Created with BioRender.com.

There are various methods for manufacturing spheroids. This goal can be achieved using various technologies, such as hanging drop, low-adherence substrate, bioreactor, microwells, magnetic manipulation, and microfluidics [33–36]. The properties of spheroids are more important than the technologies used to manufacture them. Due to their three-dimensional nature, spheroids closely resemble in vivo conditions, including oxygen gradients, nutrition, and metabolic products [35,37].

However, spheroids have some limitations despite their similarities to in vivo processes. This method of cell culture is less well-known than 2D monolayer cultures, and there are also major problems with standardizing spheroid size [33]. Nonetheless, spheroids are an important tool for understanding physiological and pathological processes. For instance, the proliferation rate of spheroid surface cells is significantly higher than that of the inner zone. In fact, due to the oxygen gradient, the inner zone may even consist of a necrotic core instead of living cells [35]. Therefore, spheroids are a superior model to 2D cultures for anti-cancer drug testing [38].

Currently, researchers are exploring the use of spheroids in various fields of bioengineering and medicine. This type of 3D culture is widely studied as an example of cells that mimic cancer tumors. In the case of co-cultures, which are spheroids made up of cancer cells and other cells such as cancer-associated fibroblasts, the similarities to in vivo cancer tumors are even greater [37]. The number of recently published papers on the use of spheroids in cancer research is still increasing. One of the main advantages of using spheroids in cancer research is the ability to measure drug response. Multiple studies have been conducted involving spheroids derived from various types of cancer, including colorectal cancer [39–42], breast cancer [41,43,44], lung cancer [45,46], ovarian cancer [1,47,48] and even Ewing sarcoma cells [49]. It is important to note that 3D cultures, particularly spheroids, are a valuable and effective tool for studying cancer biology. They can be used to assess potential drug resistance, identify new therapeutic targets, and investigate molecular mechanisms [50].

4.2. Scaffold Techniques

4.2.1. Organoids

Organoids are more complex structures than spheroids and are named as such because they represent the complexity of organs, unlike spheroids, which generally represent only one type of tissue [30]. Organoids can be derived from various types of stem cells, including embryonic, induced pluripotent, or tissue-specific cells [24]. Due to their complexity, organoids are valuable models for studying and simulating physiological processes that occur in living organisms' organs. Organoids are self-organising and self-renewing structures, meaning that they have some level of freedom in their organisation. While 2D cultures are easier to control, 3D cultures, especially more complex ones, are less predictable in terms of deterministic processes. Therefore, organoids can closely mimic living organs. Similarly, 3D cultures can be scaffold-based or scaffold-free [51]. However, scaffolds are more commonly used in organoid manufacturing to recreate a natural environment [52].

Organoids are often referred to as mini-organs, and current research is focused on creating fully functional organs on a mini-scale. Examples include 'mini-guts' [53], 'mini-hearts' [54], and 'mini-livers' [55]. The name 'organoid' accurately describes a miniaturised model of an entire organ. Organoids are commonly used in cancer research, with patient-derived organoids (PDOs) proving to be a valuable tool in drug testing and the study of cancer biology [56–58]. Additionally, organoids are utilised as models for a range of other diseases, including lung diseases [59], liver cirrhosis [60], inflammatory bowel disease [61], and brain disorders [62].

4.2.2. Hydrogels

Modern 3D cell culture scaffold techniques often rely on the use of various biomaterials, including hydrogels. Hydrogels are hydrophilic, cross-linked polymer chains [63,64] that can be pre-prepared to facilitate 3D cell culture due to their similarity to the extracellular matrix [65]. Although hydrogels can vary greatly in water content, chain composition, and ionic charge [66], their use in 3D cell culture is well-established. The potential applications of hydrogels are vast, as demonstrated by the numerous classifications available. Hydrogels can be classified based on their source (natural or synthetic), size (nano-, macro-, or bulk hydrogels), chain composition, ionic charge, method of crosslinking, response to various stimuli, or biodegradability [67]. Matrigel® is one of the most well-known hydrogels in scientific research [68]. The substance is a natural biomaterial derived from secretions of Engelbreth–Holm–Swarm (EHS) mouse sarcoma cells. Hydrogels can also be obtained naturally by crosslinking collagen [69], gelatin [70], hyaluronic acid [71], or alginate [72]. Synthetic hydrogels, in contrast to natural ones, can be manufactured with greater precision, allowing for better control of desired properties such as water absorption and ionic charge.

4.2.3. Advanced 3D Bioprinting

3D bioprinting is a promising and increasingly popular tool for scaffold production, presenting many advantages. These include high levels of structure customization, which can imitate the structure of desired tissue, as well as the freedom to mix components and impregnate them with additional substances [73]. It allows for the creation of complex tissue structures that scientists around the world are looking to apply in medicine [74]. Current research primarily focuses on using 3D bioprinting to produce advanced scaffolds for skin bioengineering [75], as well as for musculoskeletal, cardiovascular, and neural systems [73].

The recent development of 3D bioprinting technologies has led to the creation of 4D bioprinting, which represents the next generation of biomaterials. These materials are capable of changing their shape, properties, and functions in response to external stimuli such as heat, changes in pH, light, and humidity [76]. A wide range of intelligent polymers and materials are used in this process. For example, iron-sensitive hydrogels, polyethylene glycol that responds to temperature fluctuations, or special ink containing poly(lactic acid) polymer that assembles into tubular shapes under the influence of a magnetic field [77].

This new generation of biomaterials is proposed to have applications in various areas of medicine, such as drug delivery systems (encapsulation devices) and biosensors [78–80]. However, the most significant potential of 4D-printed biomaterials is in tissue engineering [76]. Multi-material 4D-printed implants with heterogeneous morphology can provide an environment that changes over time that promotes cell activity and differentiation, and increases the regenerative capabilities of nearby tissues [81]. Currently, scientists are conducting research on 4D-printed biomaterials in a few applications. This includes the production of functional meniscal implants [82], regeneration of cartilage with chitosan derivatives [83], bone replacement implants for treating losses caused by trauma or genetic diseases [84–87], and tooth implants [88]. Additionally, there is evidence of the potential usage of 4D-printed biomaterials in the engineering of the cardiovascular system. Scientists are currently developing 4D-printed patches to aid heart function after myocardial infarction [89], as well as a model of an artery to study the pathomechanism of cardiovascular diseases [90]. Additionally, there are projects focused on creating 4D-printed heart valve implants [91]. 4D-printed biomaterials may also have applications in otolaryngology, such as craniofacial, tracheal, nasal, and aural implants [77]. Studies have shown that 4D-printed biomaterials can successfully guide stem cell differentiation and fate [92]. The potential applications of 4D-printed biomaterials include regeneration in craniofacial skeletal muscle [93], wound healing [94], implants for bladder disorders [95], and in vitro models of fibroblast remodeling [96].

Bioprinting techniques can be divided into two categories based on the place of synthesis. The most common technique is ex vivo, where the scaffold is combined with cells and other biomolecules outside of the body [76]. The newer approach, in situ, aims to print tissue or organs directly in the human body at the required site of trauma [97]. This technology has a wide variety of potential applications. There are various materials that are considered for use in in situ bioprinting, such as hydrogels based on Gallol-Functionalized Hyaluronic Acid [98], methacrylate-based gelatin [99], and bioinks based on alginate–chitosan and kaolin [100]. Current research focuses on in situ bioprinting applications for direct cartilage repair [101,102] or bioprinting human mesenchymal cells that will transdifferentiate into chondrogenic tissue [103]. In situ bioprinting may also be used for skin wound regeneration, providing accurate coverage of the affected area [104]. Scientists have developed a 3D bioprinter called the 'SkinPen' for skin regeneration, which uses a complex hydrogel controlled by ultrasound and ultraviolet light to enhance adhesive and morphological properties [105]. Currently, studies are being conducted to combine these two goals and create a robot-assisted in situ bioprinter for skin and hair follicle regeneration [106]. The study investigates the use of gelatin methacryloyl (GelMA) with zinc and silicon ions for hair follicle regeneration in a mouse model [107]. Additionally, researchers are exploring the use of robotic technologies for cranial bone regeneration through in situ bioprinting [108]. Robots are also being utilized to develop minimally invasive bioprinting systems for liver tissue bioprinting and regeneration [109]. In endoscopic surgery, in situ bioprinting is seen as a potential new technology for intestine regeneration [110]. Scientists have presented evidence of the potential use of in situ bioprinting for printing neurons, vascular and muscle tissues [111–113]. Intraoperative in situ bioprinting can also be used in orthopaedics for bone tissue regeneration [114]. Additionally, researchers are attempting to bioprint bone tissue with complete vascularisation, adding an additional level of complexity to the constructs created in situ [115]. Scientists see another potential application of in situ bioprinting in functional segmental tracheal reconstruction, where this technique can be used to regenerate destroyed parts of the trachea [116]. In addition, in situ bioprinting may have possible applications in dentistry as a means of regenerating dental pulp [117].

4.2.4. Alternative Materials Employed in the Context of 3D Bioprinting

The three most commonly used techniques in 3D printing are Fused Deposition Modeling (FDM), Stereolithography (SLA), and Selective Laser Sintering (SLS). The crucial difference between these methods is the form of the delivered material and the way the

printing material hardens, as shown in Figure 3. FDM is the most commonly used technique, where a solid filament is heated, melted, and extruded through a nozzle. The base of the SLA technique is a container with liquid resin that is hardened with a laser beam. Printing with SLS technique also involves a laser beam, but the printing material used is polymeric powder. The materials can be modified and supplemented in various ways due to the printing technique.

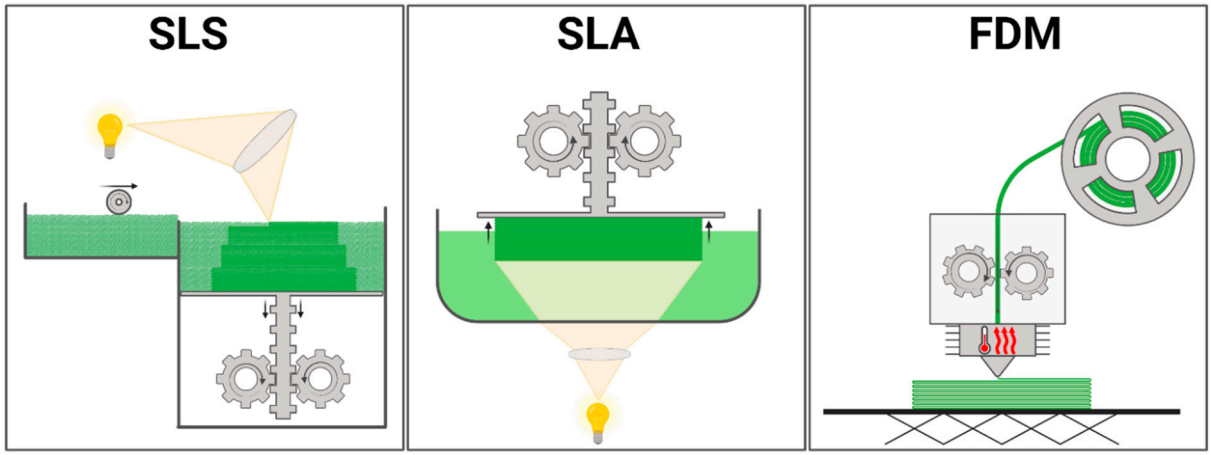

Figure 3. Main 3D printing technologies (abb. SLS, stereolitography; SLA, selective laser sintering; FDM, fused deposition modeling). Created with BioRender.com.

Fused Deposition Modeling (FDM): FDM is a method commonly used for 3D printing with several thermoplastic polymers, including poly (L-lactic acid) (PLA), poly-l-lactide-co-ε-caprolactone (PLCL), and gelatin methacrylate (GelMA), along with various supplements. PLA, which has stiff and cytocompatible mechanical properties, is most often used for bone tissue scaffolds [118]. PLA is obtained through the condensation of lactic acid or the polymerization of lactide. Commercially available polylactic acid (PLA) is typically derived from fermented plant starch, such as corn, cassava, sugarcane, or sugar beet pulp. However, PLA has limitations, including poor mechanical properties, insufficient surface wettability, and a low degradation rate, which restricts its use in biomedical applications [119]. Therefore, there are multiple proposals to enhance the PLA composition or indirectly use PLA for forming bioscaffolds. The use of a PLA printed shape as a frame for low-viscosity bioink can limit the ink from spreading beyond the established shape [120]. This strategy involves using PLA material as a scaffold to form the proper bioscaffold. To mimic tissues, particularly bone tissue, PLA is supplemented to improve biomineralization and physical properties. The addition of hydroxyapatite (HAp) significantly increases the strength and stiffness of PLA. Simultaneously, hydroxyapatite (HAp) contains sites for the deposition of salts and apatite species in the scaffold, which increases the rate of material mineralization in an in vitro study [121]. Bioscaffolds formed from HAp-polylactic acid (PLA) composites can improve bone regeneration in in vivo grafting. As demonstrated in the rabbit model, HAp-PLA scaffolds are biocompatible, degrade over time, and form bone trabeculae and marrow cavities on the surface of the scaffolds. Moreover, the safety and efficacy of HPa-PLA in repairing cranial defects in rabbits are comparable to that of autologous bone transplantation [122]. Bioglass (BG) can also be used to enrich the scaffold and mimic the bone tissue environment. The PLA-BG composite exhibits greater durability than PLA alone [123]. Furthermore, the addition of BG enhances cell viability and the expression of endothelial marker genes in vitro, as demonstrated on human umbilical vein endothelial cells (HUVECs), indicating a positive effect on angiogenesis [124]. Pearl powder [125], graphene oxide [126], and cold argon plasma treatment [119] are all valuable supplements that can influence bone cell differentiation, proliferation, and scaffold strength.

PLCL is a copolymer composed of L-lactic and ε-caprolactone. It is widely used in the production of scaffolds for soft tissue engineering due to its flexibility and rubber-like elasticity. With the adjustment of 3D-printing parameters, it is possible to obtain scaffolds with extensibility comparable to native human tissues, such as vessels, cartilage, and ligaments, while maintaining full cytocompatibility and cell adhesion [127]. A combination of PLA and PLCL has been found to be an effective mimic for cartilage tissue. The inclusion of PLA in the material enhances its mechanical resistance and stiffness, as well as the processability of PLCL for 3D printing. In line with this, the PLCL-PLA scaffold has been shown to improve the proliferation and chondrogenesis of in vitro-seeded chondrocytes more effectively than the PLCL scaffold alone [128]. The hybrid scaffold containing PLCL and decellularized extracellular matrix (dECM), specifically adipose tissue dECM, is a promising technique with potential clinical applications. These adipose-mimicking scaffolds possess mechanical properties comparable to native tissue and have the potential to enhance tissue regeneration. The in vitro test demonstrated that the dECM-PLCL scaffold promotes adipogenesis and angiogenesis, as well as adipose tissue formation, while suppressing apoptosis of human adipose-derived stem cells (hADSC) in vivo [129,130].

Gelatin methacryloyl (GelMA) is a hydrogel made from proteins. It has a porosity of almost 90% and a disordered pore arrangement, which imitates the structure of the extracellular matrix (ECM) due to its high collagen content. This porosity promotes cell viability and is crucial for proper development and metabolism [131]. To cross-link GelMA, it must be exposed to UV light in the presence of photo-initiators. The advantage of this process is its simplicity in modulating mechanical properties by adjusting the time and intensity of UV light and the concentration of photo-initiators. Commonly used photo-initiators include Irgacure 2959 (I2959), lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), and Eosin-Y [132]. However, a disadvantage of photo-crosslinking is the accelerated enzymatic degradation of the scaffold with collagenase, which makes the material less suitable for the regeneration process. The combination of GelMA and tyramine-conjugated 8-arm poly(ethylene glycol) (8PEGTA) reduces degradation while maintaining ECM-mimicking conditions during early tissue regeneration [133]. GelMA can also act as a carrier and support nervous tissue development. A 3D-printed canal filled with 7,8-dihydroxyflavone (7,8-DHF) successfully reconstructed and reconnected a 12-mm nerve defect [134]. The properties of elastic GelMA include the potential to mimic vessel structures. When supplemented with chitin nanocrystals, the material exhibits improved mechanical resistance, cell adhesion, proliferation, and vascularization [135]. GelMA can be used to mimic bone tissue. Supplementing with BG improves mechanical properties, cell adhesion, proliferation, and, most importantly, osteoblast differentiation. This also enhances the release of osteocalcin (OCN), a factor in bone mineralization that orchestrates the osteogenesis process [136].

Stereolitography: Compared to FDM printing, SLA printing is more efficient in printing smaller and more precise models with higher resolution. Stereolithography is a 3D printing process that uses a light source to harden the material. Even when using the same materials, such as GelMA, which is also used in SLA printing, the liquid state of the materials allows for the use of resins such as poly(D,L-lactide) (PDLLA) or poly(propylene fumarate) (PPF). Even when using the same materials, such as GelMA, which is also used in SLA printing, the liquid state of the materials allows for the use of resins such as poly(D,L-lactide) (PDLLA) or poly(propylene fumarate) (PPF).

Factory tests have demonstrated the possibility of utilising GelMA and a low-cost, commercially available printer to conduct three-dimensional in vitro culture. The scaffold produced in this manner is characterised by high printing accuracy and good biocompatible properties [137]. Similar to the FDM method, the enrichment of GelMA with other materials significantly expands and diversifies its potential uses, allowing it to be adapted to mimic various tissues. The combination of PLCL and GelMA has been shown to replicate the properties of small intestine tissue when cultured in vitro [138]. It is important to maintain a clear and logical structure, use precise language, and avoid bias and grammatical errors. Additionally, the supplementation of HAp has successfully aided in the regeneration of

bone tissue in vivo [139]. Modifications using the FDM technique may also allow for the replication of other tissue types and material properties.

PDLLA is a flexible polymer with numerous medical applications, including controlled drug delivery [140]. It has a high capacity to mimic bone tissue and has been successfully used for bone regeneration in vivo. The best results in the treatment of bone defects were obtained using a hybrid of PDLLA and PPF. This hybrid also supported the expression of key markers for osteogenesis, such as osteocalcin, collagen, and runt-related transcription factor 2 (RUNX2) [141].

Photo-crosslinkable PPF is a popular choice for bone-mimicking scaffolds due to its superior mechanical resistance, stiffness, and biodegradability. To apply PPF, SLA is necessary using a solvent. Diethyl fumarate (DEF) is a commonly used solvent for PPF, but studies have shown that this combination can have a cytotoxic effect on seeded cells. Ethyl acetate (EA) is a potentially safer alternative. It has been found not to reduce scaffold biocompatibility on preosteoblasts cell-line in vitro [142]. By properly optimizing the PPF material composition, it is possible to create a drug-releasing bioscaffold with the strength and stiffness of native bone [143].

Selective Laser Sintering: Selective laser sintering is advantageous due to its ability to quickly and precisely print complex structures without the need for additional support. The printed elements are supported by powder, which also serves as a printing material. While there is limited research on the use of SLS as a source of scaffolds in medical applications, some materials have been described in this field. Borate-based bioactive glass was used to create bone-mimicking scaffolds that efficiently regenerate bone defects. A powder polymeric binder was used to merge the components, and after the fabrication process, the parts were heated to remove the binder [144]. One type of polymeric binder that can be used is thermoplastic polyurethane (TPU), which is also a suitable scaffold material. TPU is an elastic material with cytocompatible properties that could be used to mimic soft tissues. This enhances biological properties, maintains cell viability, promotes cell proliferation, and differentiates neural stem cells [145].

5. Biomimetic Scaffolds—From Advanced Engineering to Biological Application

Biocomposite structures consist of macromolecules, such as proteins, lipids, polysaccharides, minerals, and polynucleotides, that are naturally present in tissues. The extracellular matrix (ECM) contains various types of proteins, including collagen, elastin, gelatin, and other glycoproteins. Collagen, which is abundant in connective tissues, forms a fibrillary structure that provides strength and structural support. The collagen fibers in the bone tissue are saturated with minerals, which increases their stiffness and mechanical strength. Bioactive domains in collagen are involved in interacting with cell membrane receptors, such as integrins (i.e., $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$ and $\alpha11\beta1$). Collagen-binding integrins influence fibroblast activity, regulating differentiation and synthesis of ECM components, which is crucial in tissue wound healing [146]. The presence of elastin in tissues provides elasticity to various organs, such as bladder and artery tissues. The mechanical properties of blood vessels, fibrous connective tissue, and skin are mainly determined by the cooperation of collagen and elastin percentage composition, which provides strength and structural support while also providing elasticity and resilience. Another crucial protein is fibronectin, which has domains that bind to other ECM proteins, such as collagen, heparin, and integrins. Interactions between cells and the extracellular matrix (ECM) play a crucial role in ECM development, homeostasis, and wound healing [147]. As extracting ECM proteins can be expensive, gelatin has been proposed as a cost-effective alternative scaffold base [148]. Polysaccharides, such as chitosan, alginate, dextran, and hyaluronic acid, have advanced scaffold development due to their low cost, ease of commercialization, biocompatibility, and biodegradability. They are similar to the extracellular matrix (ECM), which is rich in glycosaminoglycans, glycoproteins, and glycolipids [149–152]. Tissue engineering scaffolds are generally produced as pre-fabricated or in situ cross-linked hydrogels, with many using 3D printing technology. Pre-fabricated scaffolds primarily focus on the presence of pores

and interconnected channels to enhance the viability of seeded cells. Figure 4 present 2 possible concepts of biomimetic scaffolds fabrication that employ various techniques, including traditional methods like decellularization but also electrospinning, rapid prototyping-based microfabrication, and modular hierarchical assembling. Each method provides different characteristics to porous scaffolds. These include degradability to nontoxic materials, production of ultrafine fibers with varying diameters, production of final 2D structures, and production of large and complex 3D structures based on 3D programmed images. In situ gelled hydrogel involves creating biomimetic hydrogel scaffolds using peptide-based biomaterials to repair tissue, as well as controlled drug delivery. Proteins possess molecular properties that enable them to interact with other macromolecules and regulate the hard and soft tissue of an organism.

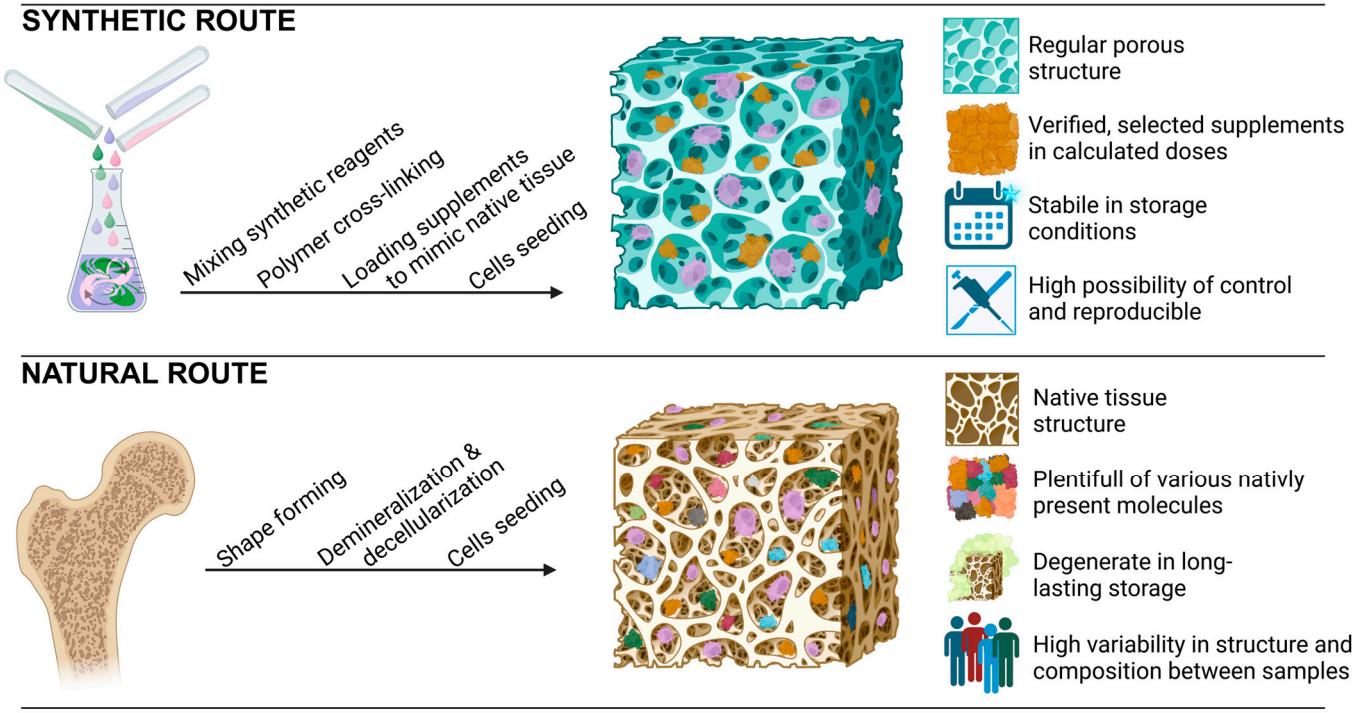

Figure 4. Synthetic and natural route of proceeding biomimetic scaffolds. Created with BioRender.com.

The scaffold is a tool that creates space for tissue formation de novo. It mimics the natural environment to provide optimal conditions for cell growth. The properties of the culturing environments vary depending on the cultured tissue, not only with the material and composition of supplements but also with the mechanical aspects. Tissue engineering employs engineering and life science to generate biological structures to restore, repair, and maintain organ tissue and improve its function. Biological scaffolds can be made from native tissues or synthetically synthesized and can be either degradable or nondegradable [153]. The optimization of scaffold production relies on adjusting the material's biological and chemical properties, such as biocompatibility, immunogenicity, and impact on cell metabolism [154]. The mechanical properties of the bioscaffold are equally important. The adjustment of material cross-linking density enables the regulation of porosity and resistance. A material with a denser cross-linking exhibits greater strength, tear resistance, and stiffness, which may be crucial for supporting connective tissue but can reduce cell viability [155]. Biomechanical resistance testing involves determining ultimate strain and stress and the energy required for failure, particularly under tensile forces [156]. Additionally, scaffolds have been utilized as controlled release tools to maintain therapeutic concentrations of diffusible tissue inductive factors. Loading a bioscaffold with the long-term released proangiogenic factor, leonurine hydrochloride

(LH), can increase the osteogenic differentiation of bone marrow stem cells (BMSC) and promote vessel formation in vivo [157]. The long-term release of the anti-bacterial polyhexamethylenebiguanide (PHMB) efficiently reduces bacterial content in regenerating skin wounds [158]. Cell culture is a primary in vitro biological tool and a crucial component of transgenesis, bioengineering, and regenerative medicine. The extracellular matrix (ECM) plays a critical role in tissue structure and function. For example, tendons contain thick bundles of collagen type 1, which are responsible for their high tensile strength. Collagen and elastin fibrils are responsible for the elasticity of the skin. In terms of tissue function and activity, there are examples such as the Arg-Gly-Asp (RGD) sequence on fibronectin that triggers a binding event or heparin sulfate proteoglycans that facilitate basic fibroblast growth factor (bFGF) activities. ECM provides a degradable environment that is crucial for angiogenic sprouting and remodeling during tissue dynamics, including morphogenesis, homeostasis, and wound healing [159,160]. Embryonic ECM is replaced during morphogenesis to accommodate tissue growth, with an ECM half-life of 7 to 10 h [161]. After tissue development is complete, the physiological degradation of the ECM shifts to maintaining ECM homeostasis. Both morphogenesis and homeostasis strictly involve the p53/laminin pathway [162]. The p53 protein responds specifically to laminin, a key component of the ECM, and regulates the expression of molecules required to establish homeostatic form and dynamics [163]. The production of nitric oxide (NO), an important intercellular signaling molecule [164], also supports another link in the pathway.

Biomimetic scaffolds of the extracellular matrix (ECM) are designed to provide a framework for cell culture to develop tissue with appropriate signal cross-talk. Intercellular communication in 3D co-cultures is more efficient with the involvement of paracrine signaling [165]. An ideal biomimetic scaffold should imitate natural ECM properties and create conditions for positive interaction with cells to increase cell adhesion, growth, migration, and differentiation [166]. Decellularized extracellular matrix (dCEM) scaffolds contain extracellular macromolecules, including collagen, elastin, fibronectin, laminin, and matricellular proteins [158]. These scaffolds are prepared through a decellularization process, which preserves signals and biological performance, providing a 3D biological support structure for subsequent cell seeding on damaged organs. In the model of mice myocardial infarction, a bioengineered cardiac patch was created using decellularized tissue with seeded cells. This approach demonstrated more efficient tissue regeneration compared to using only a scaffold or cell-seeding therapy [167]. Additionally, decellularized grafts were found to provide proper reconstruction of rats' small intestine, with a well-organized structure and intact nervous system in vivo [168]. Studies have shown that dECM scaffolds can create a favourable microenvironment that promotes tissue regeneration. They provide a tissue-specific template for the healing and functional regeneration of various tissues, such as skin [169], cartilage [160], or dense regular connective tissue [170]. During tissue regeneration, fibroblasts and other surrounding cells recreate the tissue environment. It would be advantageous to degrade the used material, making space for newly formed tissue and avoiding the need for surgical removal.

The future of biomimetic scaffolds involves the development of nanobiotechnology or nanotechnology to assemble and control the function of proteins [171]. Molecular biomimetics provides solutions for the control and fabrication of large-scale nanostructures to assemble materials in two and three dimensions. The concept of forming 4D scaffolds relies on adding the dimension of time in 3D biomaterial processing. Under the influence of external factors, such as heat or moisture, 4D biomaterials change their properties and shape in accordance with the controlled treatment process. Bioinspired 4D objects with multiple activities have been introduced as a new approach to producing highly complex smart materials [172]. In drug delivery, small-sized lipid nanoparticles (LNPs) have potential, but their poor stability and intracellular trafficking weaken their effectiveness. The potential of creating hydrophobic scaffolds can be increased by using transformed, self-assembled LNP containing lipids and proteins that have been previously used for gene or drug delivery [173]. The choice of scaffold depends on the specific biological application

and intended usage, which can range from biotechnology to regenerative medicine for organ regeneration.

6. Application of Biomimetic Scaffolds in Biotechnology

Stem cells are a fundamental tool for tissue engineering and regenerative medicine due to their pluripotency and differentiation ability. The potential of stem cells is limited by their source, with the fertilized oocyte being the cell with the widest potency, giving rise to cells from all germ layers. Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) are used in tissue engineering and are influenced by their environment [153]. ESCs are more effective than adult stem cells (ASCs) and can generate any organ through tissue-specific differentiation conditions in vitro. However, ASCs and tissue-specific cells are limited in number, making it difficult to grow a complete organ. The primary sources of stem cells for tissue engineering are bone marrow, umbilical cord blood, and circulating blood [174,175]. Recent stem cell research has shown the potential for nuclear transfer of somatic cells and reprogramming, which can convert fully or partially differentiated cells back to their embryonic state, restoring their stemness [176]. This technology has the advantage of using autologous cells, which helps to overcome issues with immune rejection. The extracellular matrix (ECM) plays a crucial role in determining stem cell fate. The chemical composition, surface chemistry, porosity, degradation behavior, and mechanical strength of a scaffold can all influence the fate of stem cells [177]. Both natural and synthetic biomaterials can create bioactive scaffolds that regulate stem cell differentiation [178]. Another biotool used in biotechnology is gene transfer, which involves managing RNA, DNA, proteins, and other macromolecules, such as lipids, for various purposes. There are different techniques of gene delivery for different biotechnological purposes, including viral and nonviral techniques. Nonviral techniques, such as transfection, can be performed through methods such as injection of naked DNA, electroporation, particle bombardment, and cationic liposomes. Contemporary viral gene transfer occurs in vivo using retroviruses, adenoviruses, or adeno-associated viruses. An alternative strategy involves incorporating DNA directly into a polymeric scaffold or genetically manipulated cells. This technique can protect DNA from degradation, offer better control of transgene and protein levels, and potentially reduce inflammation. In tissue engineering, transfections are utilized to deliver drugs or produce growth factors that can reduce the need for recombinant proteins, thus supporting tissue regeneration [179]. Scaffolds provide a more efficient method for gene delivery to specific cell populations while protecting vectors against extracellular barriers that may reduce their therapeutic efficacy and immune responses. Scaffolds can enhance cell adhesion, maintain cell–cell interaction, and increase protein expression levels [180]. They also offer the potential to alter cell function and fate. Hydrophobic scaffolds have been utilized for drug and gene delivery to treat diseases and develop DNA vaccines due to their increased adsorption capacity for proteins and other supplements [181]. Biopolymers can be used as a carrier for DNA vaccines at the organism scale. Plasmid DNA can be incorporated or surface-adsorbed onto carriers to create a complex that can be encapsulated in layers of other polymers or incorporated into multistructured polymer forms or matrices [182].

7. Application of Biomimetic Scaffolds in Advanced Regenerative and Reconstructive Medicine

For decades, scientists have recognized that the human body provides biological scaffolds and biopolymers that are highly attractive for tissue engineering and regenerative medicine. The human body has the ability to regenerate most organs, so damage to major organs can cause failure and serious disorders. Regenerative medicine combines tissue engineering with cell-based therapy, gene therapy, and immunomodulation. One potential use of dCEM scaffolds is for organ transplantation and tissue repair to regenerate and restore organ function [158]. Primary or genetically engineered cells can be transplanted by seeding them onto tissue-like three-dimensional structures to repair tissue damage. The traditional method of injecting cell solutions into tissues by hand encounters many

difficulties, including high mortality rates for implanted cells. Biomimetic scaffolds consist of extracellular matrix (ECM) proteins and growth factors [148], which facilitate the transportation of high-density cells to affected tissues. Tissue engineering, a subfield of regenerative medicine, has the potential to regenerate almost all tissues and organs in the human body. Tissue engineering strategies generally involve implanting a construct into an organism, and delivering growth factors or supplements to enhance cell viability, proliferation, and adhesion to surrounding tissues [160]. Biomimetic materials are often used to replace unhealthy tissue in the body, while sutures are necessary for certain types of wounds. Fabrication methods can improve the production of customizable defect-fillable scaffolds for tissue regeneration in regenerative medicine [130]. Decellularized tissue can effectively replace native tissues as a biomimicking wound dressing [166]. Additionally, sutures for wound sewing can be manufactured with lyophilized decellularized ligament to biomimic the surrounding tissues. The spinning of fibers enabled the creation of thread that is as durable as silk fibers, which has potential use in wound sewing [105]. Biomimetic scaffolds have numerous applications in medicine, including artificial skin, arteries, and joint replacements in the body [183,184].

8. Conclusions and Future Perspectives

Considering the current state of knowledge regarding biomimetic scaffolds, it is difficult to overestimate their potential future applications. They have enhanced traditional 3D cell culture techniques by adding another dimension of modifiable parameters to already complex structures. Further advancement in the field of biomimetic scaffolds may be the branch of tissue engineering that will bring to light concepts like tailor-made organ implants that eliminate the risk of transplant rejection complications, laboratory modelling of patient-specific tumors, and the cultivation of healthy cells in an environment that closely mimics natural conditions. The potential applications of biomimetic scaffolds in regenerative, translational, and personalized medicine are potentially very vast, but due to high complexity of the constructs further extensive studies are still required.

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