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Scaffold characteristics and their fabrication techniques for tissue engineering applications

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Abstract

Tissue engineering (TE) involves using cells, engineering materials, and biochemical factors from natural sciences to enhance biological tissue through replacement and repair. Despite being utilized for many years, the use of traditional medical and surgical treatments has been restricted due to their side effects. The scaffolds that play a supporting role promote the formation of new tissue-like structures by cells. Scaffolds have also regulated the delivery of growth factors and medications within the damaged area. The strengths and weaknesses distinguish these two separate categories of biomaterials. Synthetic polymers possess excellent mechanical durability and can be readily adjusted in terms of their form and rate of degradation. Using engineered tissues has offered a fresh approach to regenerate missing tissues. Creating a scaffold is a crucial part of the TE procedure. This review provided an overview and evaluation of the features of a scaffold such as biocompatibility, biodegradability, mechanical properties, structure, and production methods. Furthermore, this research offered a thorough examination of the various scaffolds and how they are created.

Introduction

According to Nyame *et al*. (2015), the skin serves as the primary defense system against external threats and helps regulate body temperature. The structure consists of upper epidermis, lower dermis, and subcutaneous hypodermis layers. Every layer in the body has its own unique functions such as preventing dehydration, serving as a barrier against injury, providing sensory perception, facilitating vitamin D synthesis, and carrying out immune surveillance, all crucial for body biology systems (Brohem *et al*., 2011; Mogoşanu and Grumezescu, 2014; Chaudhari *et al*., 2016). Any harm to a layer caused by mechanical, physical, or metabolic (especially from diabetes

mellitus) factors can lead to permanent harm and endanger the patient's health. In cases of injury or healing, the main focus is on stopping bleeding and avoiding microbial infection through the arrival of inflammatory cells such as neutrophils, macrophages, and lymphocytes. It is crucial to quickly heal to restore the function of damaged tissue or cells. The process of wound healing involves various stages: an initial inflammatory phase with immune cell infiltration and cytokine production, a proliferative phase for tissue repair, and a final remodeling phase with scar formation. Wound healing progresses through several stages. The first stage involves an inflammatory or exudative phase characterized by the infiltration of neutrophils, macrophages, and lymphocytes, as well as cytokine production (Mogoşanu and Grumezescu, 2014). Next is the proliferative or fibroplasia phase, which includes the removal of damaged tissue and the formation of granulation tissue in the wound bed (Shevchenko *et al*., 2009). Following this is the maturation or remodeling phase, during which fibroblasts and fibrocytes produce the extracellular matrix, and the formation of scar tissue marks the completion of the wound healing process (Shevchenko *et al*., 2009; Mogoşanu and Grumezescu, 2014; Chaudhari *et al*., 2016; Oryan *et al*., 2019b).

Wounds can be categorized as epidermal, dermal, or dermo-epidermal, depending on their extent and seriousness (Shevchenko *et al*., 2009). The molecular process of skin wound healing primarily entails the production of different growth factors like epidermal growth factors (EGF) and tissue growth factors alpha and beta (TGF-α, TGF-β) (Mogoşanu and Grumezescu, 2014; Chaudhari *et al*., 2016). The healing of wounds and regeneration of skin rely on various factors such as the type of wound (epidermal, deep dermal, full thickness) as well as tissue damage caused by burns or physical trauma, inflammation, secondary infections, etc (Mogoşanu and Grumezescu, 2014; Chaudhari *et al*., 2016). The stages of skin wound healing involve fibroblasts and fibrocytes producing extracellular matrix (ECM), keratinocyte formation and proliferation in the epidermal layers, and the differentiation of keratinocytes to create the outermost epidermal layers (Mogoşanu and Grumezescu, 2014; Chaudhari *et al*., 2016; Oryan *et al*., 2019b). Factors such as disease conditions (e.g., diabetes, renal infections), presence of foreign bodies, malnutrition, and age can impact the normal wound healing process and tissue restoration. These factors need to be taken into consideration when developing various grafts for skin tissue regeneration (Mogoşanu and Grumezescu, 2014; Nyame *et al*., 2015; Alaribe *et al*., 2016; Oryan *et al*., 2017a).

The development of organ regeneration has long been regarded as the gold standard in the field of tissue engineering (Chaudhari *et al*., 2016). Tissue engineering is a multidisciplinary field involving medicine, biological science, and engineering, aiming to create medical implants that can aid in tissue regeneration. In the last three decades, tissue engineering has gained attention as a viable medical treatment (Oryan *et al*., 2017b; Costantini and Barbetta, 2018). Tissue engineering primarily focuses on restoring and enhancing tissue function by generating new biocompatible alternatives or reconstructing the tissues (Dhandayuthapani *et al*., 2011; Alaribe *et al*., 2016). The use of cell implants and different matrices, like scaffolds, can accelerate the healing and regeneration processes by creating three-dimensional (3D) tissue structures (Alaribe *et al*., 2016; Chaudhari *et al*., 2016). Researchers have developed various scaffold matrices to enhance cell growth in 3D structures. These scaffolds are highly compatible with skin tissue, biodegradable, and serve as effective dressing materials for wound healing. A recent advancement in skin tissue engineering involves the use of scaffolds with cell populations, such as keratinocytes and fibroblasts (Norouzi *et al*., 2015).

In this overview, we talked about skin tissue engineering and different types of scaffolds, focusing on the ones commonly used in skin tissue regeneration. The text also covers the discussion on the materials, benefits, and drawbacks of scaffolds used in skin tissue engineering, emphasizing their role as appropriate matrices for supporting the growth and differentiation of skin cells. In addition, the important characteristics of suitable scaffolds, including biocompatibility, biodegradability, structure, and mechanical properties, have been thoroughly examined.

Characteristics of scaffolds

Scaffolds are considered the most suitable materials for rejuvenating, upholding, and enhancing tissue function in tissue engineering as mentioned in a study funded by the national science foundation (O'brien, 2011; Chaudhari *et al*., 2016). They have a crucial function in tissue repair and regeneration as they create a proper environment for the supply of necessary factors for cell proliferation and differentiation, while also degrading and being replaced by new tissues over time (Dhandayuthapani *et al*., 2011; O'brien, 2011; Norouzi *et al*., 2015; Nyame

et al., 2015; Chaudhari *et al*., 2016). Indeed, scaffolds with supportive functions promote the formation of new tissue-like structures by cells. Another important function of scaffolds is regulating the gradual release of growth factors and medications within the damaged region. Scaffolds need specific qualities like biocompatibility, biodegradability, and mechanical properties for their intended use

Biocompatibility

Biocompatibility is the scaffold's capacity to aid in cellular growth and proliferation, as well as to support molecular and mechanical signaling systems, cell differentiation, and ECM secretion without triggering an immune response in the host (Velasco *et al*., 2015; Li *et al*., 2016; Bhardwaj *et al*., 2018). The biocompatibility or cytocompatibility of a scaffold is frequently determined by its structural characteristics like topography, porosity, surface charge, and exposed chemical groups (Balint *et al*., 2014; Gupta *et al*., 2016; Bhardwaj *et al*., 2018). The behavior of cells may be influenced by the interaction between the cells and the surface of the material (Gupta *et al*., 2016; Bhardwaj *et al*., 2018).

Biodegradability

Materials possessing biodegradability can break down into safe and non-toxic substances in both *in vitro* and *in vivo* environments, as stated by Bhardwaj *et al*. (2018) and Velasco *et al*. (2015). If the rate of degradation aligns with the rate of restoration, the host system typically absorbs the degraded products, classifying the scaffold as biodegradable. Biodegradability is connected to biocompatibility by ensuring that the broken-down substances do not cause an immune reaction in the host tissues; they should be safe, not provoke an immune response, and can be metabolized easily (Armentano *et al*., 2013; Bhardwaj *et al*., 2018). Various biodegradable natural and synthetic materials have been utilized in the production of scaffolds that can be broken down by enzymes or hydrolyzed without generating harmful byproducts. A biodegradable scaffold should not degrade too quickly or too slowly because this could impact its mechanical properties and ability to regenerate tissue (Hussein *et al*., 2016).

Architecture

The field of tissue engineering assists in the healing and restoration of injured tissues by creating synthetic grafts that replicate the structure and function of a natural organ (Bhardwaj and Kundu, 2010; Mandal *et al*., 2012; Gupta *et al*., 2016). Therefore, it is important to attain a structure that is alike, has a chemical composition that is compatible, exhibits mechanical properties that are similar, and has adequate porosity for applications in skin regeneration (Bhardwaj *et al*., 2014). Traditional techniques for creating 3D scaffolds include freeze-drying, phase separation self-assembly, particle leaching, decellularized matrix, radiation crosslinking, and electrospinning (Pereira *et al*., 2013; Bhardwaj *et al*., 2014; Chouhan *et al*., 2017). Advanced techniques consist of photopolymerization processes, microfluidic assembly, 3D bioprinting, and extrusion-based methods like 3D fiber deposition (Ng *et al*., 2016). Scaffolds have been built to preserve the interaction between cells and ECM for optimal skin regeneration, emphasizing the "dynamic confluence between cells and ECM" (Macri and Clark, 2009). The final phase of wound healing is tissue healing, during which the characteristics of ECM continue to evolve. Tissue healing involves MMPs, TIMPs, and ECM component secretion happening simultaneously (Macri and Clark, 2009; Bygd *et al*., 2015).

The advancement of state-of-the-art 3D printing technology has enabled accurate and automated cell deposition and scaffold construction through computer-aided design and manufacturing (Pereira *et al*., 2013; Murphy and Atala, 2014; Bhardwaj *et al*., 2018). Various bioprinting technologies, including laser-assisted direct writing, inkjet printers, and micro-dispensing methods, have been used recently to print various biomaterials, cells, and bioactive molecules (Pereira *et al*., 2013; Bhardwaj *et al*., 2018). Hassanajili *et al*. (2019) employed an indirect 3D printing method in the creation of bone scaffolds utilizing PLA/PCL/HA composites. The scaffolds were assessed for cytotoxicity, and it was found that the cells thrived and multiplied effectively on them. The findings also suggested that the composite scaffold with a 70/30 weight ratio of PLA/PCL demonstrated superior characteristics in terms of biocompatibility, viability, and osteoinduction properties (Hassanajili *et al*., 2019).

Mechanical properties

Tissue's functional performance is closely tied to its mechanical strength and is crucial for the development of skin substitutes. The skin's dermal layer is made up of a complicated structure of enzymatically connected ECM, as well as collagen and elastin fibers, which offer biomechanical properties and flexibility (Ushiki, 2002). The subpar skin substitutes cause skin contraction,

fibrosis, and scarring resulting in unsuccessful skin repair (Vogel, 1994). The cellular behaviors influenced by scaffolds' mechanical properties include cell viability, cell-matrix interactions, cell proliferation and differentiation, and size of focal adhesions. Scaffolds also offer structural support for skin tissue in both *in vitro* and *in vivo* settings until integration and ECM regeneration (Lutolf and Hubbell, 2005; Levy-Mishali *et al*., 2009; Collins *et al*., 2010). Researchers previously analyzed the mechanical characteristics of wound healing by utilizing collagen scaffolds (with and without FGF-1) in various studies (Pandit *et al*., 1998; Pandit *et al*., 1999; Pandit *et al*., 2000). Enhanced mechanical properties in regenerated skin led to improved wound healing and reduced contraction rate after treatment.

Different methods like blending, chemical crosslinking, and co-polymerization have been utilized to enhance the mechanical properties of scaffolds (Shevchenko *et al*., 2009; Bhardwaj *et al*., 2014; Oryan *et al*., 2014; Oryan *et al*., 2018b). In a previous study, scientists demonstrated enhanced mechanical characteristics in collagen scaffolds by utilizing crosslinking with EDC. The mechanically stable matrices with cross-linking displayed decreased contraction rates during both culture and wound healing processes (Park *et al*., 2003). In a different research, Garcia *et al*. (2008) utilized an enzyme-focused method to create cross-linked collagen matrices. In a different research, the use of glutamyl-lysine based cross-linking was found to enhance the mechanical stability of the scaffold and decrease wound contraction in an animal model (Garcia *et al*., 2008).

Chaudhry *et al*. (2009) also employed nano-indentation to study how collagen matrices behave mechanically at the nanoscale level. Moreover, various studies have demonstrated the advantageous effects of combining synthetic polymers with natural polymer matrices to enhance the mechanical properties of tissue engineered skin substitutes (Bhardwaj *et al*., 2018). Kumara *et al*. (2019) studied how copper nanoparticles impact the physical and chemical characteristics of a scaffold made from chitosan and gelatin for use in skin tissue engineering. According to Kumari *et al*. (2019), it was demonstrated that copper nanoparticles have the capability to enhance the physico-chemical characteristics of the scaffold.

Manufacturing technologies

Cost plays a significant role in the commercialization of medical products like implants or tissue-engineered constructs, as high prices often prevent products from being produced and discourage payment from both the NHS and private sectors. However, it is extremely important to establish scalable manufacturing processes to meet good manufacturing practice (GMP) standards. Packaging, storage, sterilization, and transportation are additional crucial factors that must be taken into account (Mozafari *et al*., 2019).

Fabrication techniques

Porous scaffolds

In the 1980s, Joseph Vacanti and Robert Langer introduced the concept in the development of tissue engineering that a porous biocompatible and resorbable material could serve as a scaffold to direct tissue regeneration (Rey and St-Pierre, 2019). Various types of porous scaffolds are available, including sponge, foam, mesh, and nanomicroscale biodegradable fibers (Liu *et al*., 2010b; Sundaramurthi *et al*., 2014). The creation of porous scaffolds is commonly done through the utilization of porogens for controlling pore size and shape in biomaterials, prototyping, electrospinning nanofibers in a layer-by-layer fashion, and the latest method of 3D printing (Chan and Leong, 2008; Chaudhry *et al*., 2009; Norouzi *et al*., 2015). These structures contain interconnected pores to promote the creation of extracellular matrix for efficient cell interaction in their surroundings. A porous scaffold allows cells to adhere and create their own ECM while also enhancing nutrient delivery to the device's center, decreasing the risk of necrosis (Dhandayuthapani *et al*., 2011). A suitable porous structure has a defined pore size and a suitable surface-to-volume ratio to allow for the diffusion of nutrients, medication, and other substances (Ouriemchi and Vergnaud, 2000; Wei and Ma, 2004; Chaudhry *et al*., 2009). Poly-ethylene glycol (PEG), PLA, PGA, PLGA, and PCL are among the synthetic biodegradable polymers utilized as porous scaffolding materials (Zhang and Ma, 1999) (Fig. 1).

Solvent casting and porogen leaching

The technique of solvent casting and porogen leaching (SCPL) was first introduced by Mikos *et al*. in 1994 and is a straightforward process with two steps. Usually, a mixture is made by combining a polymer solution dissolved in an organic solvent with particles (known as the porogen) that do not dissolve in the solvent to form slurry.

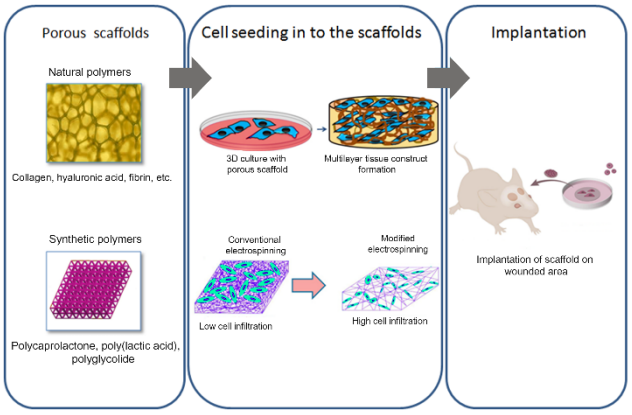

Fig. 1. Porous scaffolding using various biomaterials. Various natural, synthetic and biodegradable materials are used for generation of highly porous scaffolds. These scaffolds provide a suitable environment for cell growth and proliferation. The porous nature of such scaffolds facilitates the regular supply of nutrients and oxygen for the skin cells, such as keratinocytes and fibroblasts. The full thickness skin grown on such scaffolds is used for wound transplant.

This blend is poured into a membrane, and the liquid is removed through evaporation to create a solid blend of the polymer and porogen. The particles are then removed from the structure by floating them in a water solution to create interconnected pores in the membrane. A depiction of this process can be seen in Fig. 2. Salt particles have been widely utilized as porogen, but other substances like sugar, gelatin, and even paraffin particles (requiring an aliphatic solvent for extraction) have also been employed. The selection of porogen determines the size, shape, and consistency of pores in the structure, while the amount of porogen in the slurry controls the porosity and, to some extent, the connectivity between the pores. The development of a compact polymer layer on the external surfaces of the material, known as the skin layer, may restrict the entry of cells into the internal porous structure and affect the usability of the scaffolds created using this method.

Phase separation

Phase separation is a method for fabricating scaffolds where a uniform polymer solution is divided into polymer-rich and polymer-poor phases due to thermodynamic instability (Martínez-Pérez *et al*., 2011). This can be achieved by lowering the temperature of the solution below the freezing point of the solvent to start crystal formation, causing phase separation through a process known as thermally induced phase separation. After the solution is fully frozen, the solid material is then sublimated to eliminate the solvent, leaving behind the polymer-rich areas to create the walls of the scaffold, while the polymer-poor sections create pores within the structure. Adjusting fabrication variables like polymer concentration, temperature, and surfactant utilization can provide a level of influence on pore size and distribution, aiming to regulate crystal formation (Akbarzadeh and Yousefi, 2014; Rey and St-Pierre, 2019). It is important to note that this technique usually results in pores that are generally at the smaller end of the appropriate size range for tissue engineering applications (frequently less than 200 μm).

Scaffolds made using this method can only reach a thickness of 2–3 mm due to difficulties in the leaching process with thicker structures, as noted in studies by Prasad *et al*. (2017), Sumayya and Muraleedhara Kurup (2018), and Rey and St-Pierre (2019).

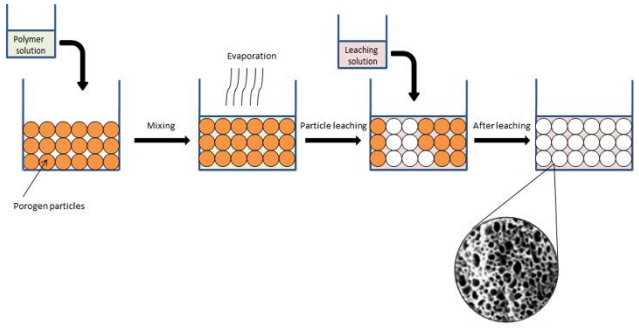

Fig. 2. Schematic illustration of SCPL fabrication process.

Gas foaming

Gas foaming is also widely employed to create scaffolds for tissue engineering purposes. This category of methods depends on adding a blowing agent to create gas bubbles in a solid polymer sample, resulting in the formation of pores (Rey and St-Pierre, 2019). In their initial use of this method for tissue engineering, Mooney and colleagues found that they created poly(lactic-co-glycolic) acid discs through compression molding in high-pressure CO2 for long durations. Afterward, they quickly returned the samples to regular atmospheric pressure to enable the trapped CO2 to expand within the polymer matrix (Mooney *et al*., 1996). One main benefit of this method compared to other traditional scaffold fabrication methods is that it does not require the use of solvents

or porogens, therefore eliminating the potential risks of cytotoxic effects. Yet, achieving control over both the size of pores and their connectivity has been difficult. The even porosity and connection of pores are two drawbacks of this method (Dehghani and Annabi, 2011).

Electrospinning

Electrospinning is the most widely used method in tissue engineering for textiles. This technique depends on creating an electric field between a polymer solution dispensed at a regulated rate (often using a needle) and a collector in order to pull the solution into a fiber (Braghirolli *et al*., 2014; Rey and St-Pierre, 2019). This happens when a polymer solution is exposed to a high enough voltage, causing it to acquire a charge that allows electrostatic repulsion to overcome surface tension and result in thread elongation. This fiber hardens as it moves towards the collector because the quick evaporation of the solvent is affected by its high surface area-to-volume ratio. The result is a layer of nonwoven fibers that have been placed on the collector by Rey and St-Pierre in 2019.

The interconnected nature of porosity results in pore sizes that are typically smaller than those produced by previously mentioned scaffold production techniques. This may be seen as a benefit for various uses, such as creating blood vessels or membranes in tissue engineering to keep transplanted cells in a tissue gap (Chia *et al*., 2006; Ercolani *et al*., 2015). Manipulating various process parameters such as polymer concentration, solvent choice, flow rate, humidity, voltage difference, needle size, and the distance between the needle and collector can influence the diameter of the fibers. Precise adjustment of these factors enables the creation of fiber sizes that vary from a couple of micrometers to the smaller nanometer ranges. These factors also affect the level of leftover solvent in fibers when they reach the collector, thus regulating the bonding strength at fiber contact points (Saha *et al*., 2011; Garg *et al*., 2013; Rey and St-Pierre, 2019).

The inclusion of bioactive materials in electrospun PCL scaffolds is important in tissue engineering because they mimic ECM, are biocompatible, and biodegradable. Investigations have recently been conducted on utilizing electrospun PCL and its blends/composites for skin reconstruction. The possibility of enhancing cell attachment and antimicrobial properties of the scaffolds by including active agents like growth factors, medications, and nanomaterials has been studied. Electrospun wound dressings/skin substitutes have the capability to speed up wound healing and boost cell proliferation, according to Joseph *et al*. (2019).

Hybrid scaffolds

Several studies have demonstrated the advantages of mixing or altering the fabrication methods mentioned earlier in order to create anisotropic structures. This method first emerged in cartilage tissue engineering, where hybrid scaffolds were created to guide ECM deposition in a way that mirrors the organization of natural tissue. An investigation recently incorporated SCPL with electrospinning to create scaffolds with anisotropic mechanical characteristics, surpassing those made solely through porogen leaching (Steele *et al.*, 2014; Cao *et al.*, 2022). These scaffolds also offer greater porosity for tissue deposition compared to anisotropic scaffolds produced by electrospinning each layer with different fabrication parameters (McCullen *et al.*, 2012).

Additive manufacturing (AM)

The techniques mentioned restrict the design of scaffolds, giving a limited amount of control over pore shape, size, and connectivity. Recent advances in AM technologies have allowed for better control over the shape, size, and connectivity of pores in scaffolds, enabling a more scientific approach to their fabrication. AM techniques enable the creation of scaffolds, or more generally structures, by following a layer-by-layer approach using information from a computer-aided design (CAD) drawing. These techniques also make it easier to create the scaffolds. Blending AM with CAD drawings created from tissue imaging data aids in the implementation of personalized medicine in tissue engineering. The next part focused on several AM techniques that have become prominent methods in tissue engineering (Rey and St-Pierre, 2019; González-Henríquez *et al*., 2022).

Powder-bed three-dimensional printing

Powder-bed 3D printing is an additive manufacturing method which involves selectively depositing a binder onto a powder bed using an inkjet printer (Sachs *et al*., 1992). Using powder layers mixed with binder and drying step creates 3D structures during the fabrication process. The scaffold design CAD drawing is utilized to create the necessary cross-sectional data to manage the binder's spatial application. The uncovered powder bed not coated by

the binder helps to maintain the structure during construction. After removing the loose powder, a post-processing sintering step is carried out to mix the powder particles and create a strong mechanical structure. The effectiveness of this method relies heavily on factors such as the characteristics of the powder (e.g., size, application onto the powder bed) and binder used for creating the green material, along with the specific printer technology employed (Bose *et al*., 2018). Shrinkage, deformations, and cracks that occur during sintering are constraints of this method. Therefore, the volume loss should be anticipated and considered in the initial CAD design (Shanjani *et al*., 2010; Rey and St-Pierre, 2019; Islam, 2022).

Selective laser sintering

Selective laser sintering (SLS) is another additive manufacturing technique that employs powder for creating tissue engineering scaffolds. This method involves using the layer-by-layer technique like in powder-bed 3D printing, but instead of binding the powder particles with a binder and sintering them later, they are sintered directly in the powder bed according to the CAD drawing data. In SLS, sintering occurs by using a high-power laser to heat the powder bed locally (Mazzoli, 2013). In addition to the influence of powder traits on this method, the size of the laser spot is also a crucial factor. Hence, the minimum pore sizes achievable with this method, along with the pros and cons of the process, closely resemble those achieved through powder-bed 3D printing (Rey and St-Pierre, 2019; Schappo *et al*., 2022).

Fused deposition modeling

Fused deposition modeling (FDM) is a type of additive manufacturing (AM) technique that utilizes melt extrusion, in which a polymer is melted in a heated nozzle and then extruded based on spatial coordinates from a CAD model (Crump, 1992). Using several extrusion nozzles can be utilized to distribute the polymer responsible for the scaffold structure and also serving as a temporary support for the structure. This method is generally a cost-effective substitute for other AM technologies, but it is limited to creating basic and uniform porous structures (Rey and St-Pierre, 2019; Song *et al*., 2021).

Stereolithography

Stereolithography (SLA) utilizes UV or laser light to solidify a photosensitive liquid monomer film selectively in space. The 3D configuration is created by alternating between applying thin liquid layers and spatially controlled photopolymerization stages (Hull, 1986). The unprocessed polymer can be extracted to uncover the desired design, then can be further polymerized to enhance the mechanical strength between layers. SLA-based techniques generally provide better resolution than other AM methods and are easier to remove unreacted material compared to powder-based techniques. Nonetheless, the limited availability of noncytotoxic reactive resins poses a significant challenge for using this method in tissue engineering (Melchels *et al.*, 2010; Alkaissy *et al.*, 2022). Moreover, altering the composition of the construct in SLA is challenging due to the material being initially in liquid form.

Different types of scaffolds in skin tissue engineering

Scaffolds may consist of either synthetic or naturally occurring polymeric materials that can be degradable or non-degradable (Ma, 2004; Ciardelli *et al*., 2005; Gunatillake *et al*., 2006; Nair and Laurencin, 2007).

Polymeric scaffolds: synthetic and natural

Various types of biodegradable polymeric materials have already been utilized in tissue engineering applications for scaffold fabrication, making them the primary materials for this purpose. They can be categorized as: (1) natural-based materials like polysaccharides (starch, alginate, chitin/chitosan, hyaluronic acid derivatives) or proteins (soy, collagen, fibrin gels, silk); (2) synthetic polymers like poly(lactic acid) (PLA), poly(glycolic acid) (PGA), poly(3-caprolactone) (PCL), and poly (hydroxyl butyrate) (PHB) (Shin *et al*., 2003; Koegler and Griffith, 2004; Wen and Tresco, 2006; Alkaissy *et al*., 2022).

Polysaccharide natural biomaterials

Collagen provides strength for the growth of tissues. It is secreted by fibroblasts to promote faster healing of wounds and is the most prevalent natural protein biomaterial made in the human body. Collagen protein consists of a helical polypeptide containing glycine, proline, and hydroxyproline in repetitive sequences (Holmgren *et al*., 1999; Rho *et al*., 2006; Chaudhry *et al*., 2009; Norouzi *et al*., 2015; Reddy *et al*., 2021). The collagen-based skin substitutes, both acellular (Integra® and Brisbane®) and cellular (Apligraf® and Transcyte®), help speed up wound healing by creating an optimal environment for

fibroblast and keratinocyte growth and maturation, as shown in studies by Bürck *et al*. (2013) and Norouzi *et al*. (2015). Multiple studies have documented various forms of collagen dressings used for healing wounds and burns such as collagen sponges for deep skin wounds, Glycagen®, collagen absorbable membrane, collagen composite films, a blend of type III collagen with polysaccharides, microfiber collagen scaffolds, and electrospun collagen nano-fibrous scaffolds. Oryan *et al*. (2019) recently studied how collagen hydrogel-scaffold dressings, with or without topical *Saccharomyces cerevisiae*, affect healing of cutaneous burn wounds in rats (Lee *et al*., 2022). They demonstrated that a combination of collagen scaffold and hydrogel could effectively regulate inflammation, particularly when used together. Nevertheless, optimal outcomes were achieved when the mix of collagen scaffold and hydrogel was combined with probiotic (Oryan *et al*., 2018a). Different electrospinning methods have been used to create collagen nanofibrous scaffolds for skin replacement, including spinning collagen type I and type III, aligning collagen nanofibers, solvent spinning collagen, coating scaffolds with collagen, and cross-linking collagen (Matthews *et al.*, 2002; Zeugolis *et al.*, 2008; Liu *et al.*, 2012; Norouzi *et al.*, 2015).

However, gelatin is not as immune-reactive as collagen and promotes greater cell attachment because of the existence of arginine-glycine aspartic acid (RGD) sequences (Lee and Mooney, 2001; Mota *et al*., 2014; Lam *et al*., 2022). Scaffolds composed of gelatin nanofibers created through electrospinning technique have displayed promise for use in wound healing procedures (Norouzi *et al*., 2015). Various types of gelatin dressings like gelatin-alginate sponges, gelatin with EGF, and gelatin films have demonstrated promising uses for treating wounded and burned skin tissue.

Greater focus has been given to silk fibroin (SF) based scaffolds made through electrospinning as wound dressing materials, as they enhance consistent collagen spreading and cell adhesion on their surface in comparison to a basic SF film (Min *et al*., 2004). Because of its high biocompatibility and flexibility, SF is highly beneficial for use in wound dressings and skin grafts (Mogoşanu and Grumezescu, 2014). The scaffolds, nanofibers, sponges, and membranes based in San Francisco, along with cytocompatible porous films made from silk fibroin, have displayed encouraging outcomes in the healing of wounds (Liu *et al*., 2010a; Mogoşanu and Grumezescu, 2014; Hussain *et al*., 2022). Fibrinogen, another natural polymer, has been commonly used for skin regeneration purposes as well. Fibrinogen, a non-globular protein with a fibrous structure, has the potential to serve as a scaffold based on matrix for wound treatment (McManus *et al*., 2006; Sell *et al*., 2008). Various animal-derived biomaterials like keratin, bovine serum albumin, egg shell membrane proteins, etc., have shown efficacy in the field of skin regeneration products. Keratin and its related substances have been utilized in different dressing products to deliver antibiotics or growth factors, making them beneficial in the process of wound healing (Vasconcelos and Cavaco-Paulo, 2011). Besides the protein-based biomaterials mentioned, certain plant proteins along with polysaccharides like soy protein linked with glutaraldehyde and sago starch also work well for wound and burn dressing purposes (Silva *et al*., 2003; Mogoşanu and Grumezescu, 2014).

Biological materials made from polysaccharides such as hydrogels play a key role in treating skin injuries like wounds and burns. These materials fall into categories like neutral (glucans, dextrans, cellulose), acidic (alginic acid, hyaluronic acid), basic (chitosan), or sulfated (heparin, chondroitin) polysaccharides (Mogoşanu and Grumezescu, 2014; Cui *et al*., 2021; Torkaman *et al*., 2021). Different forms of D-glucans sourced from yeast, grains, and fungi have been utilized in creating gel formations to facilitate successful wound healing (Lehtovaara and Gu, 2011). Bacterial cellulose (*Acetobacter xylinum*) is a distinctive nanostructured biomaterial with significant potential for wound dressings and tissue engineered skin according to studies by Czaja *et al*. (2006), Fu *et al*. (2013), Radu *et al*. (2021), and Torkaman *et al*. (2021).

Chitosan creates and deposits collagen and hyaluronic acid at the wound site, which are crucial biological substances in ECM. Chitosan not just aids in wound healing but also accelerates scar-free healing by stimulating the production of other biological substances (Norouzi *et al*., 2015; Oryan and Sahvieh, 2017). Alginate helps with absorbing wound fluid and enables the moist wound environment to remain (Shalumon *et al*., 2011). Norouzi *et al*. (2015) and Shalumon *et al*. (2011) have both reported on the utilization of alginate-based electrospun scaffolds in the regeneration of skin tissue. HA, heparin, and chondroitin sulfate are crucial for skin regeneration since they constitute the primary elements of the ECM (Salbach *et al*., 2012; Masri and Fauzi, 2021). The HA hydrogel scaffolds are famous for promoting tissue regeneration by aiding in angiogenesis and neurite growth, leading to improved wound healing (Kogan *et*

al., 2007; Masri and Fauzi, 2021).

These two distinct categories of biomaterials are characterized by numerous benefits and drawbacks. Synthetic polymers exhibit strong mechanical properties and can be easily adjusted in terms of shape and degradation rate. However, they have hydrophobic surfaces and do not have cell-recognition signals. Naturally derived polymers may have the benefit of biological recognition to promote cell adhesion and function, however, their mechanical properties are subpar.

Synthetic biomaterials

PLA, PGA, copolymers, and PLGA are commonly utilized in tissue engineering as linear aliphatic polyesters (Chu *et al*., 1999; Zhang and Ma, 2001; Bolland *et al*., 2008; Patel *et al*., 2021). They have been proven to be safe for living organisms and break down into harmless substances at a manageable pace within the body. Synthetic biomaterials have been used for a long time as biodegradable surgical sutures and have received approval from the FDA for clinical use. These polymers break down by the hydrolysis of the ester bonds, and the resulting breakdown products are eventually expelled from the body as carbon dioxide and water (Lin *et al*., 2003). PGA's hydrophilic properties make it a popular choice for scaffold production, as stated by Li in 1999. It breaks down quickly in water and loses strength within two to four weeks PGA has been made into non-woven fiber fabrics and is commonly used in tissue engineering scaffolds (Li, 1999). PLA undergoes de-esterification through hydrolysis, resulting in lactic acid formation. The structure and quality of the molecules impact how quickly PLA breaks down and its strength, making the scaffold last longer in both lab and living conditions. PLA, PGA, and PLGA are some of the synthetic polymers approved by the FDA for specific human clinical uses. Other linear aliphatic polyesters like poly(3-caprolactone) (PCL) and poly (hydroxyl butyrate) (PHB) have been utilized in tissue engineering research by a small number of researchers, according to Bendix (1998), Lepoittevin *et al*. (2002), and Li *et al*. (2002). PCL breaks down much more slowly than PLA, PGA, and PLGA (Kang *et al*., 2007). Gradual deterioration decreases the appeal of PCL in biomedical uses, yet enhances its desirability in extended implants and controlled release applications. Griffith (2000) demonstrated that PCL can serve as both a suture material and a drug delivery system for extended periods of time.

Polymer materials can also serve as scaffolds for seeding cells in order to create cell sheets that release ECM. Cell sheets that secrete extracellular matrix can be utilized for transplants (Chan and Leong, 2008). The majority of synthetic polymers containing nanomaterials for skin wound dressings are created through electrospinning (Khang *et al*., 2010; Norouzi *et al*., 2015). Dai *et al*. (2012) demonstrated that nanofibrous membranes made of a blend of polyvinyl pyrrolidone through electrospinning are efficient in drug delivery and show improved potential for healing wounds. Likewise, movies created from fatty acid polyurethane and biodegradable poly-3-hydroxybutyrate-poly-"-caprolactone have demonstrated encouraging outcomes in wound care (Gultekin *et al*., 2009). Moreover, poly-"-caprolactone homopolymers and poly-L-lactide-"-caprolactone matrices could be used in tissue repair (Mogoşanu and Grumezescu, 2014; Sowmya *et al*., 2021). In a model of sheep dermal wound healing, the dressing material comprising silicone-coated non-woven polyester improved re-epithelialization at the wound site according to Losi *et al*. in 2012. The majority of artificial materials have been widely utilized in skin tissue regeneration along with natural polymers and are examined in the next section as composite biomaterials.

Composite Biomaterials

Composite biomaterials are commonly utilized in skin tissue engineering to promote wound healing and tissue regrowth. The mixed composite could consist of various natural or man-made polymers, or a blend of both types. A blend of plant proteins mixed with a polysaccharide, like cross-linked soy protein with sago starch, has shown effectiveness in dressing wounds and burns according to Mogoşanu and Grumezescu's study in 2014. Various composite mixtures suitable for skin or dermal tissue regeneration are chitosan nanoparticles with fibrin gels, thrombin receptor agonist peptide (TRAP) in poly(N-vinyl caprolactam)-calcium alginate hydrogel films, biopolymeric matrices with angiogenic growth factors, and micro- and nanoparticulates delivering epidermal growth factor (EGF) (Strukova *et al.*, 2001; Değim, 2008; Zhou *et al.*, 2011a; De Luca *et al.*, 2021; Bayer, 2022).

In recent years, microbial cellulose has been utilized to create various composite sheets in combination with montmorillonite (MMT). The mixture of microbial cellulose and MMT in composite films enhances their antibacterial and therapeutic effects in tissue regeneration and wound healing

(Mogoşanu and Grumezescu, 2014). Many chitosan-based composite biomaterials exist, including films containing mixtures of chitosan, cellulose, and silver nanoparticles, spongy mixtures of chitosan and gelatin, and mixtures of chitosan, gelatin, and antibiotics, with potential uses in wound healing and tissue regeneration (Mogoşanu and Grumezescu, 2014). Hydrogel composites containing PVP, KC, potassium chloride, polyethylene glycol, and PVP-KC have been studied for their use in tissue engineering (Şen and Avcı, 2005; Kesharwani *et al*., 2021). Cho *et al*. (2002) demonstrated that incorporating hyaluronic acid (HA) and silver sulfadiazine into composite polyurethane foams leads to a significant reduction in wound size in experimental rat models. A different composite hydrogel made from HA is created for tissue repair with crosslinking of HA with glycidyl methacrylate groups and DNA (Chen, 2012; Dubey and Sharon, 2022). Sponges composed of gelatin crosslinked with different materials have been utilized successfully as well (Ulubayram *et al*., 2002). Combining polyvinyl alcohol with gelatin or carboxymethyl cellulose, polyacrylic acid, and polyethylene glycol shows potential for wound healing (Smith *et al*., 2009; Akila and Janani, 2021; Tabriz and Douroumis, 2022).

Scaffolds based on hydrogels

Hydrogel structures made from natural macromolecules or artificial polymers show great promise because of their biocompatibility, biodegradability, and capacity for cellular interaction. Recent progress in the development and utilization of biodegradable hydrogels has resulted in significant improvement in tissue engineering (Cabodi *et al*., 2005; Alemzadeh *et al*., 2019). Hydrogels are created by either covalently or non-covalently linking polymers together (Hoffman, 2012). Maintaining a proper balance between cell adhesion to the scaffold and the degradation rates of hydrogel scaffolds is crucial for facilitating the development of new tissues (Hubbell, 1999; Lee and Mooney, 2001). Hydrogels like hyaluronan–fibronectin and chitosan–gelatin combined with PLGA nanofibrous scaffolds are beneficial for wound healing in skin tissue engineering (Shevchenko *et al*., 2009; Franco *et al*., 2011; Gugerell *et al*., 2015). Additionally, dextran-derived hydrogels have resulted in the full restoration of skin in the process of wound healing by promoting effective formation of blood vessels (Sun *et al*., 2011). In recent years, the potential of hydrogel-based scaffolds for regenerating dermal and epidermal tissues has been shown in studies by Hartwell *et al*. (2015), Li *et al*. (2003), Lin *et al*. (2014), Tsao *et al*. (2014), and Zhao *et al*. (2016). A gelatin hydrogel containing keratinocytes that can be crosslinked with a photo has been found to be useful as a replacement for skin, wound coverings, and for research purposes in the lab (Zhao *et al*., 2016; Sharma *et al*., 2021). A biohybrid hydrogel-collagen-glycosaminoglycan (GAG) that quickly transitions from a viscous liquid to a solid scaffold has been shown to promote healing in rabbit wounds according to Hartwell *et al*. (2015). A 3D porous chitosan-alginate scaffold was created by incorporating Poly(ethylene glycol)-g-chitosan (C-PEG) hydrogel to provide a bi-layered micro-environment for fibroblasts and keratinocytes support (Tsao *et al*., 2014). Moreover, bilayer hydrogel scaffolds (either with a fibrous mat or porous scaffold) have been mentioned for successful drug delivery to boost wound healing and enhance skin regeneration by seeding stem cells from debrided human burn skin (Jaiswal *et al*., 2013; Natesan *et al*., 2013). In more recent times, there have been reports of self-assembling peptide-based hydrogel scaffolds speeding up the healing of burn wounds and the multiplication of skin cells with optimistic potential for regenerating dermal tissue (Bradshaw *et al*., 2014; Loo *et al*., 2014; Binaymotlagh *et al*., 2022).

Acellular Scaffolds

Collagenous matrices devoid of cells are widely used for producing acellular scaffolds due to their popularity in various studies (Dahms *et al*., 1998; Yoo *et al*., 1998; Chen *et al*., 1999; Alemzadeh *et al*., 2019; Oryan *et al*., 2019a; Chen *et al*., 2022). When placed on injuries, these structures break down gradually and are mainly substituted by ECM proteins made by the growing and changing fibroblasts. As depicted in Fig. 3, cells are extracted from tissues like kidneys, skin, etc., and the resulting de cellularized material can be utilized as a porous scaffold for seeding different types of cells to enable their growth, facilitating the use of such implants for multiple purposes. Because of the proximity of the decellularized material to the natural components of tissues and organs, these scaffolds could be beneficial for effective transplantation. Acellular scaffolds have an advantage over other scaffolds in maintaining the ECM structure and cell adhesion ligands. This promotes the growth of tissue resembling the natural tissue, while also decreasing immune reactions to the grafts, leading to their extended functionality in the long run (Dhandayuthapani *et al*., 2011).

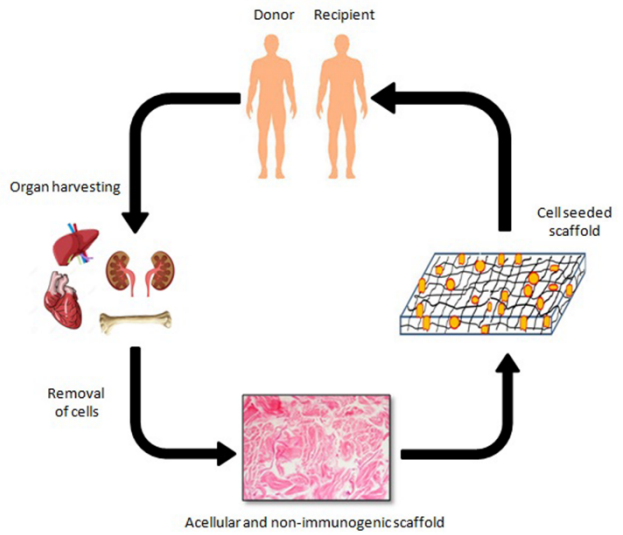

Fig. 3. Acellular scaffolding approach. In this approach, complete de-cellularization of the organs performed to create extracellular (ECM) based matrices. The cells of interest such as skin cells, liver cells or any other organ specific cells can be then effectively grown on such scaffolds.

Approved acellular scaffolds derived from different extracellular matrices are used for human tissue regeneration, including heart, intestines, and urinary bladder regeneration (Gilbert *et al*., 2006). Several acellular scaffolds based on dermal and epidermal tissue have been documented in the field of skin regeneration (Debels *et al*., 2015). The acellular dermal allografts are de-epithelialized by eliminating cells, infectious materials, and antigenic components (Shores *et al*., 2007; Debels *et al*., 2015). Acellular dermal substitutes are created using either natural or synthetic polymers, or a blend of both, according to Chaudhari *et al*. (2016). A recent review article by Debels *et al*. (2015) examined various kinds of acellular scaffolds utilized in skin tissue engineering. Multiple acellular scaffolds sold on the market are considered successful skin replacements for healing wounds (Debels *et al*., 2015; Nyame *et al*., 2015). Acellular allografts like Alloderm®, DermaCELL®, DermaMatrix®, FlexHD®, Graftjacket®, Graftjacket Xpress®, and Integra® as well as Aplicaf® (both bovine) are used in wound healing applications, while xenografts such as EZdermMediskin®, OASIS Ultra®, MatriStem®, and MicroMatrix® (all porcine) are also utilized (Debels *et al*., 2015; Nyame *et al*., 2015; Depani and Thornton, 2022; Tang *et al*., 2022).

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Conflict of Interest

All authors declare that they have no conflicts of interest.

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