Recent Advances in Tissue Engineering Scaffolds and Commercial Applications

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Abstract: *Scaffolds for tissue engineering are support structures that help cells grow and multiply after being implanted into a patient. To allow cellular adhesion, proliferation, and differentiation, the optimal scaffolds should have the right surface chemistry and microstructures. Furthermore, the scaffolds must have sufficient mechanical strength and a low rate of biodegradation with no unwanted by-products. Regenerative medicine efforts currently rely on the transplantation of cells in combination with supporting scaffolds and macromolecules to restore pathologically damaged tissue architectures. Biologically active scaffolds, which are based on analogues of the extracellular matrix that have spurred tissue and organ creation, have attracted a lot of attention in recent years. A scaffold is required to restore function or regenerate tissue, as it will serve as a temporary matrix for cell proliferation and extracellular matrix deposition, with further ingrowth until the tissues are completely restored or regenerated. Different technologies have been employed for fabrication of scaffolds for regeneration of different organs and tissues like skin, cartilage, bone, heart, lungs, liver and kidney. This review focuses on the different strategies used to construct the scaffold for the above-mentioned tissues and organs along with their commercial applications.*

Keywords: *Tissue Engineering, Scaffold, Electrospinning, Nanofiber, Heart valve, Kidney Decellularization*

Introduction:

Controlling the interplay between materials (scaffolds), cells, and growth factors to create environments that promote the regeneration of functional tissues and organs is one goal of tissue engineering [1]. Approximately 230 million of people suffer tissue loss or end-stage organ failure every year, and the treatments include transplantation, surgical reconstruction, or medical device implantation [2]. Tissue engineering, in combination with additive manufacturing, has emerged as a promising method for regenerating damaged tissues and organs by creating patient-specific substitutes that restore, improve, or maintain tissue function [3]. The creation of functional tissues or organs necessitates the use of a scaffold that serves as a template for tissue regeneration. The extracellular matrix (ECM), a main regulatory and structural component of tissues made up of fibrous proteins, proteoglycans, and glycoproteins, responds to various stimuli, and mimicking these cues with synthetic analogues of the ECM (scaffolds) has been a major research topic in the tissue-engineering field [4]. Tissue engineering's main paradigm employs a method in which

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biomaterials are designed and engineered to encourage living cells to repair and restore damaged tissues and organs while maintaining normal function. The first step is to create a tissue construct in vitro by seeding cells on a biodegradable scaffold that provides a metabolically and mechanically supportive environment for the cells to attach to and proliferate. The cell–scaffold construct is implanted in the appropriate anatomical location in the second phase, with the goal of in vivo remodeling to restore normal tissue structure and lead to viable organ function [5]. Biomaterials play an important role as "scaffolds" in this tissue engineering approach. The main function of the biomaterial is to maintain the mechanical integrity of the scaffold while promoting cell attachment and proliferation within the porous structure. Scaffold materials used in tissue engineering are generally thought to be biocompatible, easy to handle and process during manufacture, and biodegrade into nontoxic products. Furthermore, the scaffold should have a highly porous macrostructure that is mechanically stable at first [6].

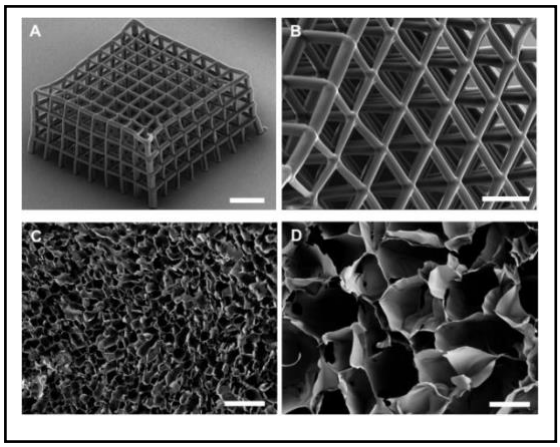

Fig.1: Scaffolds used in Tissue Engineering. Reference: http://bioxriv.org/

This review focuses mainly on the recent advances in tissue engineering scaffolds and the commercial application of each of the type of it. We will look at the types of materials (more specifically, polymeric materials) that can modulate tissue regeneration via direct and indirect chemical control over transplanted or host cells and also over the continuous challenges this field of science brings for the scientists to work on it.

1. Scaffolds in Skin Tissue Engineering:

To heal damaged tissues, polymeric nanofibrous scaffolds have the potential to interact and regulate specific regenerative mechanisms at the molecular level [7]. The most extensively used technology for fabricating nanofibers for tissue engineering purposes is electrospinning [8]. Because of their structural similarities to the native extracellular matrix, electrospun ultrafine fibres can be tailored to exhibit appropriate pore distribution, high surface area–to–volume ratio, cell adhesion, and proliferation [8]. Electrospun polymeric nanofibers have several advantages as skin substitutes, including the ability to limit fluid and protein loss from wounds, aid in exudate drainage, suppress microbial infection, have strong anti–adhesion capabilities, and direct endogenous cells to proliferate and remodel [7-8]. Nanofibrous scaffolds are now being developed in conjunction with growth factors and/or cells to speed up wound healing. There are several

natural and synthetic polymers that have been electrospun in nanofibrous form and used in skin tissue engineering. Collagen is the most biomimetic skin substitute for full thickness burns due its biological origin and have been proved to reduce the size of wound [9-10]. Plant-derived human collagen type I promoted cell proliferation at a level equal to or better than human tissue-derived collagen in previous experiments, implying that plant-derived collagen is a viable alternative to human tissue-derived collagen. Plant-derived Human Collagen Scaffolds for Skin Tissue Engineering are a promising raw material for the fabrication of tissue engineering scaffolds with great repeatability and no risk of disease transmission [11]. Gelatin, on the other hand, acts as a skin substitute with a dermal-epidermal component and suitable for dermal-epidermal skin substitution with high cell infiltration [10,12]. In addition to this, Chitosan scaffolds have been fabricated using cross linkers like dimethyl 3-3, dithio bis propionimidate (DTBP) [13]. Human keratinocytes and fibroblasts fabricated from Silk Fibroin are cytocompatible and this biomaterial is good for wound dressing [14]. Research shows that Myoglobin and Hemoglobin can help heal wounds by preventing hypoxia [15]. Promotion of cell proliferation and enhancement of angiogenesis PHBV (Poly3–hydroxybutyrate– co–3–hydroxy valerate) an excellent biomaterial for use as a scaffold skin tissue engineering [16] while properties like in-vitro compatibility and possession of anti-adhesiveness is attributable to the use of PLGA (Polylactide–co– glycolide) in scaffold fabrication [17]. The above mentioned natural or synthetic polymers can be utilized for making polymeric hydrogels. These hydrogels are nowadays preferred as scaffolds in skin tissue engineering because of their remarkable hydrophilic properties and lack of cytotoxicity in cells [18].

Moreover, when skin is wounded, bone marrow–derived mesenchymal stem cells (BM–MSCs) are targeted to the lesion sites by chemotactic signals and contribute to epidermal cells for skin regeneration in vivo, according to numerous studies [19]. Nanofibers with biofunctional properties encapsulating Human mesenchymal stem cells (hMSC) with a polyelectrolyte complex had a well-organized cytoskeleton and gene expression. This biotechnological breakthrough construct could be utilised as a framework for tissue engineering [20]. In addition to these, Bm-MSCs also express vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF–β1), keratinocyte growth factor (KGF), platelet–derived growth factor (PDGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), and fibroblast growth factor (FGF) which stimulate wound repair. Besides, BM–MSCs can also mediate skin tissue repair or regeneration by promoting collagen synthesis and employing inflammatory cells and stem cells [21-24]. In acute full-thickness skin wounds, tissue-engineered skin grafts made of biodegradable nanofiber scaffolds (NFS) enriched with bone marrow–derived mesenchymal stem cells (BM–MSCs) can speed wound healing and skin regeneration [25]. As a result, trapping BM–MSCs on a nanofibrous scaffold ensures that these cells are available on the wound bed to facilitate healing, acting as a bioreactor. In acute full thickness wound healing, BM–MSC implanted on a nanofibrous collagen–PLGA scaffold has showed encouraging outcomes [25]. Ligands (for specific cell receptors), growth factors, and stem cells could be integrated into nanofibers to provide a possible scaffold with improved tissue regeneration efficiency. Electrospinning in combination with layer-by-layer construction of nanofibers and cells can be used for making scaffold that can be seeded fibroblasts or other cell types, which can blend in nicely with the host tissue and help in tissue regeneration (Fig 2).

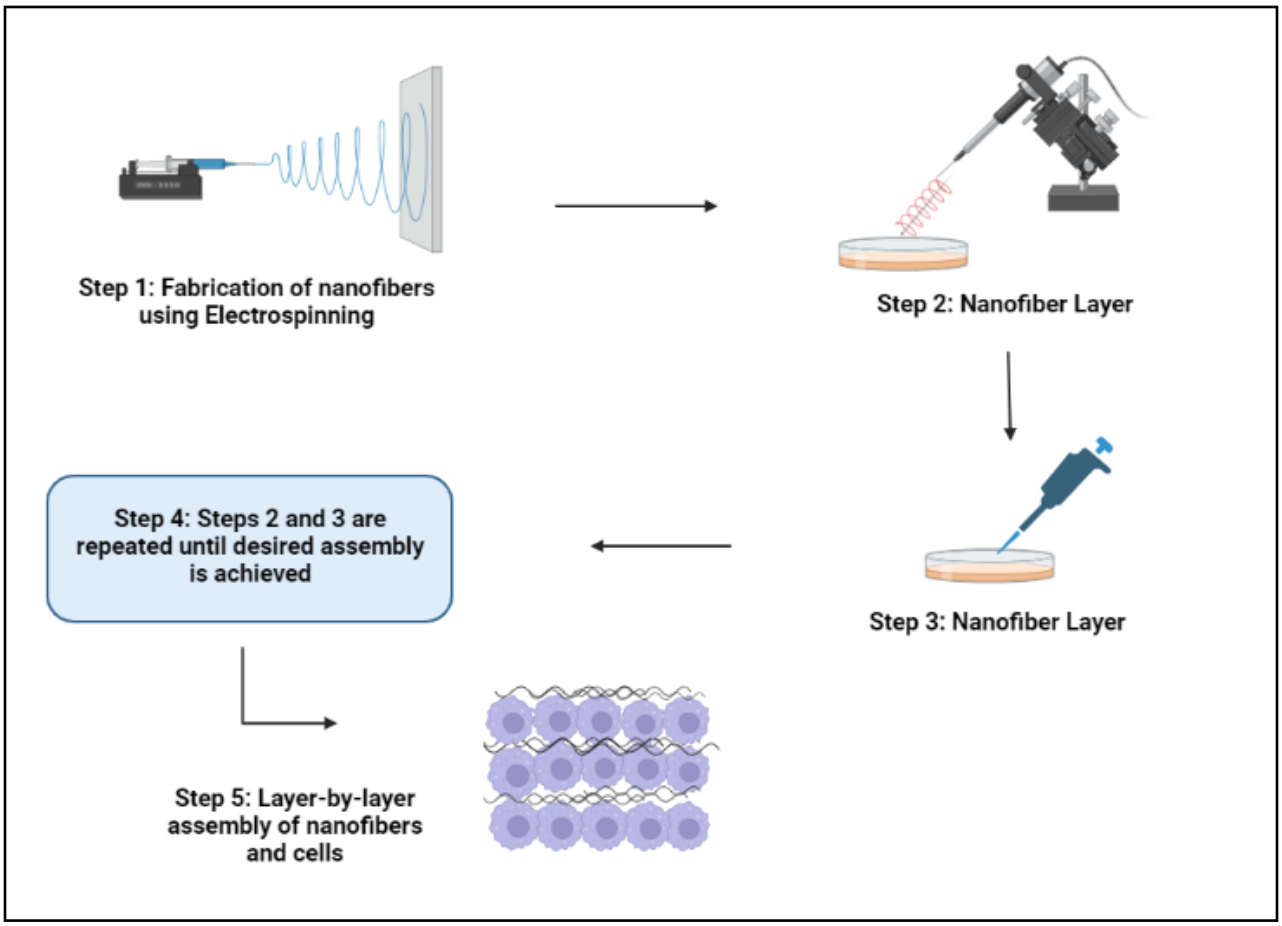

Fig.2: Procedure of making Nanofiber Scaffolds by Electrospinning and layer-by-layer assembly for Skin Tissue Engineering

2. Scaffolds in Lung Tissue Engineering:

Pulmonary illness is a global public health issue that has a detrimental effect on people's quality of life. It increases the requirement for hospitalization as well as the chance of dying prematurely. Lung transplantation is the primary treatment for severely injured lungs due to adult lung tissue's low regenerative capability. But the scarcity of lungs for transplantation is a prevalent problem, and patients must take immunosuppressive medicines for the remainder of their lives to prevent immune rejection of the transplanted organs. Recent developing technologies of lung tissue engineering have been found helpful in curing many pulmonary diseases.

The selection of scaffold material, design, and fabrication are very important in the development of the human lung.

From all attempted procedures, a pioneering approach is decellularized scaffold. ECM scaffolds are complex biostructures made up of a variety of structural and functional components that have been specially organized and suited to the desired tissue [26]. These compositions contain molecules like collagen, laminin, elastin, and fibronectin [27]. Peterson and his colleagues used a decellularization process to create a lung extracellular matrix that protected hierarchical branching

structures of airways and vessels while leaving the basement membrane, which contains collagen IV, laminin, and fibronectin, intact [28].

Biological scaffolds are also promising for constructing biomimetic extracellular matrices. Douglas et al. first reported a 3D cell culture of rat fetal lung cells on a collagen matrix to develop a model for studying lung epithelial cell biology [29]. In another study, a vascular gelatin-based sponge (Gelfoam) was used as a model matrix in which germinal lung cells were grafted into the lung parenchyma of adult rats [30].

Synthetic scaffolds are of increasing interest in tissue engineering, but their low biocompatibility has limited their application. Shigemura et al. reported that polyglycolic acid (PGA) has worked well as a patch grafted onto an incised lung in a rat model. The PGA seeded with adipose-derived stem cells (ASCs) was successful in regenerating alveolar and vascular tissues [31].

3. Scaffolds in Cartilage Tissue Engineering:

Articular cartilage is a load-bearing tissue that lines the surface of bones in diarthrodial joints. But intrinsic repair capacity of this avascular tissue is very low. Microfracture and arthroplasty are some of the treatment options for articular cartilage defects, though, these strategies fail in many cases. The limitations of the present treatment options have escalated the development in the field of cartilage tissue engineering.

To date, a diverse set of scaffolds have emerged to induce the formation of cartilaginous constructs.

Collagen, gelatin, polysaccharides (alginate, agarose, chitosan, hyaluronic acid), and fibrin are the most common biomaterials that have been widely used in cartilage tissue engineering [32]. A study showed that within poly (L-lactic acid) (PLLA) scaffolds, collagen matrix or collagen gel prompted chondrocytes to regenerate cartilage expressing type II collagen [33,34]. Alginate beads stimulated chondrogenesis of ingrowing cells while preserving the original scaffold shape, according to Marijnissen et al. [35]. Gelatin has recently been widely used as a carrier for bioactive molecules such as TGF-β and FGF-2 in cartilage tissue engineering [36,37]. Fibrin is also an appealing biomaterial because it is biocompatible and biodegradable, and it can be infused with growth factors [38].

Synthetic biomaterials are of great interest as they can be readily tailored to the demands of clinical applications. Because of their biocompatibility and FDA approval for clinical application since 1990, poly (α-hydroxy esters) such as PGA, PLA, and their copolymers are the most widely investigated synthetic biodegradable polymers for cartilage tissue engineering [32]. PCL and PPF are two other polyesters that can be used to achieve a variety of mechanical and degradable properties. When PCL is copolymerized with PGA or PLA, it acquires elastic properties that are beneficial for cartilage regeneration [39]. PPF has recently been investigated as a thermo-reversible hydrogel scaffold for articular cartilage engineering [40].

4. Scaffolds in Bone Tissue Engineering

Large bone defects or injuries, caused by old age, traffic accident, autoimmune diseases, injuries, obesity, are serious problems in orthopedics, and they bring great harms to health and the quality of life. Synthetic bone void filler, allografts, autografts, distraction osteogenesis, vascular bundle

insertion, and cement casting are the most common therapies for bone deficiencies today. Autografting is considered as the "gold standard" among treatment techniques; it entails extracting bone from one side of the patient and transferring it into the wounded portion of the same patient for bone healing [41]. The autograft approach, however, has numerous drawbacks, including a limited supply of grafts, persistent discomfort, high donor site morbidity, secondary injury, and infections.

Bone tissue engineering has become a promising strategy for mending bone abnormalities in recent years, because to the fast growth of tissue engineering technologies. The three main component of bone tissue engineering are- scaffolds, stem cells and growth factors (Fig 3).

Scaffolds are extremely important in bone tissue engineering. Their goal is to replicate the structure and function of natural bone. Biomaterials are used for the evaluation, treatment, augmentation, repair or replacement of tissues or organs of the body [42]. Some of scaffolds include- collagen, chitosan, fibrin, PLA, PGA etc. Stem cells are the foundation of bone tissue engineering since they can self-renew and differentiate into at least one kind of tissues. Ideal applicants of stem cells should satisfy the following requirements: many sources and easy sampling, robust in vitro cell passage with an immobile phenotype, great adaptation to the receiving zone's environment, capability to replace lost cells and restore tissue function, safe clinical application [43]. Growth factors (GFs) are important in BTE because they promote cell growth and differentiation, which is necessary for the proper fracture healing response. GFs are often deposited in the extracellular matrix and released after damage to influence metabolic processes. Bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs), transforming growth factor- (TGF-), and fibroblast growth factor (FGF) are some of the GFs that are used during bone healing [44].

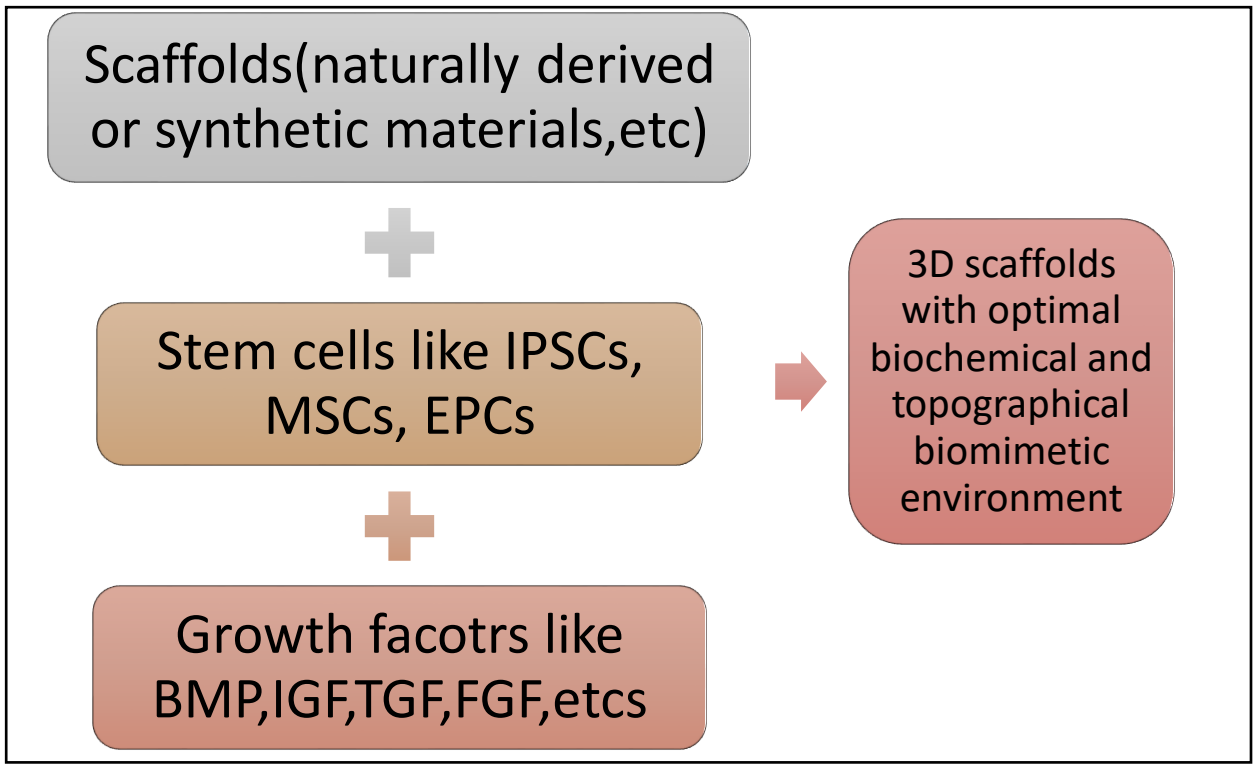

Figure 3: Components of bone tissue engineering

Scaffold fabrication technique for Bone Tissue Engineering-

4.1 Nano-Featured Scaffolds: Scaffolds acts as temporary and synthetic Extra-cellular matrix replica that supports cell attachment and guides three-dimensional bone tissue formations. The main constituents of bone ECM are in nanoparticles range, and it has been well established that native bone cells interacts well with nano size proteins and minerals [45-47]. Scientists were able to drastically enhance the surface area, surface roughness, and surface-area-to-volume ratios of the scaffold by shrinking the material size to the nanoscale, resulting in superior physiochemical characteristics. The BTE scaffold's osteoinductivity and osseointegration are strongly influenced by nanotopography. Electrospinning [48], molecular self-assembly [49], and phase separation [50] are some of the scaffold production techniques that enable the construction of nano-featured scaffolds. Nano-featured scaffolds can also be made from self-assembled into nanotubes/nanofibers that can even more accurately simulate the dimensions of natural entities, such as collagen fibers.

4.2 Scaffold-induced cell homing: Stem cell homing refers to stem cell attraction to wounded tissues or their capacity to travel to various niches/locations after mobilization [51]. Scaffold-based homing relies on biodegradable scaffolds put in the defect location to release the chemokines responsible for MSC homing. Several critical molecules have been identified as crucial elements in the mobilisation of major cellular players, despite the fact that the processes of mobilisation have yet to be fully explained [52]. Various mimetic peptide sequences (e.g., arginine-glycine-aspartic acid (RGD), glycine-phenylalanine-hydroxyproline-glycine-glutamate-arganine (GFOGER), Tyr-Ile-Gly-Ser-Arg (YIGSR), Arg-Glu-Asp-Val (REDV), and Ile-Lys-ValAlaVal (IKVAV) may be employed to facilitate cell attachment and dissemination of cells drawn to the defect location, and such scaffolds have been shown to improve osteoblast functioning and osseointegration in vivo [53].

4.3 Engineering Scaffolds for Orthopaedic Tissue Interfaces: Several variables contribute to the complexity of rebuilding hard tissue–soft tissue orthopaedic interfaces (i.e., bone to soft tissues such as ligament, tendon, or cartilage). The structure of orthopaedic tissue interactions is varied and complex. The natural regulated distribution of non-mineralized and mineralized interface areas, as well as collagen fibre organisation, should be taken into consideration when engineering the mechanical characteristics of soft tissue to bone. A variety of multi-phased scaffolds have been designed to structurally and functionally mimic native soft tissue-to-bone to support the formation of integrated multi-tissue systems [54]. To imitate the three interface zones, a tri-phasic layered scaffold was created (ligament, fibrocartilage, and bone). Phase 1 is made up of PLGA (10:90) mesh for soft tissue (i.e. ligament) formation, phase 2 is made up of PLGA (85:15) microspheres for the interface fibrocartilage region, and phase 3 is made up of sintered PLGA (85:15) and 45S5 bioactive glass composite microspheres for bone formation. The multi-tissue areas were formed in three separate but continuous phases thanks to this novel scaffold architecture [55-56].

4.4 Other scaffold fabrication techniques: Tissue engineers may create custom-made and customised complicated scaffold designs for the treatment of complex bone defects that are commonly seen in craniomaxillofacial operations by combining computer-assisted design (CAD) and computer-assisted manufacturing (CAM). Grayson et al. successfully utilized the CAD/CAM systems to engineer personalized, clinically sized anatomically shaped bone grafts for the repair of human temporomandibular joint (TMJ) (Fig 4) [57].

Furthermore, as an osteoinductive scaffold system, a unique hybrid technique combining the combination of mechanically robust, porous scaffolds and nano-featured self-assembling peptide hydrogels is being examined. The mechanically robust scaffold component of this technique will allow for loadbearing defect site mechanical stability, while the hydrogel phase will allow for effective cell distribution into the defect implantation site, cell niche creation, and mineralization promotion [58].

Despite the fact that Bone Tissue Engineering methods are presently not the gold standard in clinical practice due to expensive prices and a lack of universal manufacturing processes, new research has found viable ways for rapid bone regeneration, opening the way for the implementation of Bone Tissue Engineering methods in the clinic.

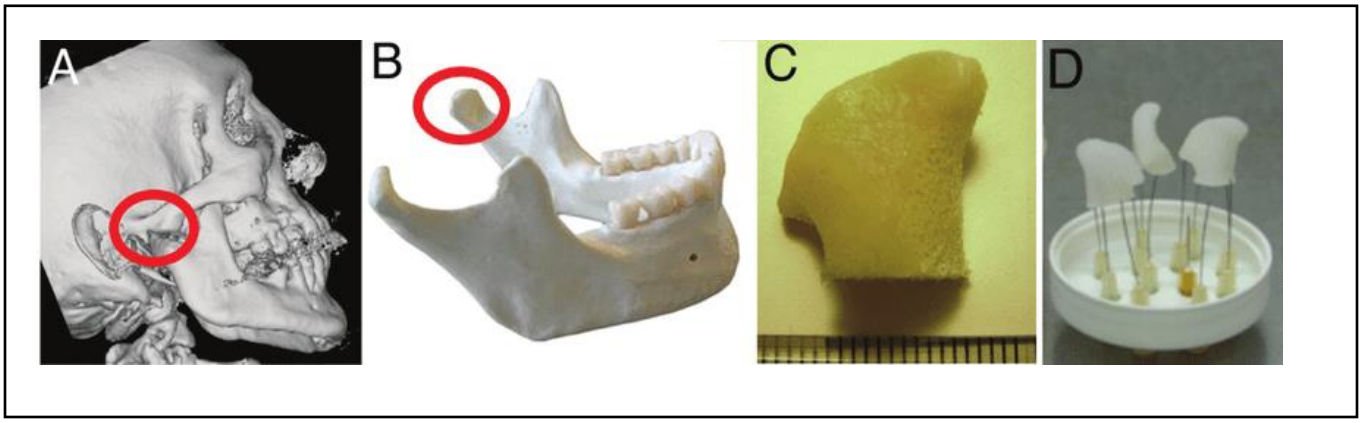

Figure 4: Anatomically shaped bone transplant tissue engineering. (A–C) Preparation of the scaffold. (A, B) High-resolution digital data was obtained from clinical CT scans in order to rebuild the precise geometry of human TMJ condyles. (C) These data were used to create TMJ-shaped scaffolds from entirely decellularized trabecular bone using MasterCAM software. (D) A shot depicting the complicated geometry of the final scaffolds, which varies significantly in each projection [57].

5. Tissue Engineering Scaffolds For Heart

Heart diseases contribute to a staggering number of deaths all around the world, especially in the United States. Meanwhile, heart valve disease (particularly pulmonary and aortic valve stenosis) is a major public health concern being the cause of significant morbidity and mortality globally. This dysfunction occurs when there is narrowing of one or more of the heart valves, restricting the blood flow, or when one or more of the valves work inadequately, failing to make a tight seal during diastole [58]. The pulmonary and aortic valves consist of three semicircular leaflets attached to a fibrous annulus called the root [59]. These leaflets, also called cusps, consist of three distinct layers, namely, the fibrosa, spongiosa and ventricularis. These layers are composed of valvular interstitial cells within an extracellular matrix (ECM) of collagen, elastin and glycosaminoglycans (GAGs) [63]. To mimic this intricate organization, an adequate scaffold structure is needed. The scaffold, besides having the basic requirements (like biocompatibility, biodegradability, and mechanical integrity), should have certain additional properties like resistance to calcification and thrombosis and being able to oppose the particular hemodynamic pressures and flows of the environment of the heart from the moment of implantation [60]. The concept of heart valve tissue engineering was introduced by Shinoka et al. in the mid to late 1990s [61].

Primarily two types of scaffolds have been developed: decellularized native heart valve scaffolds from allogeneic/xenogeneic sources; and fully artificial scaffolds fabricated from synthetic or natural polymers [64] which can be further classified as porous, fibrous, and hydrogel scaffolds [58,60]. Decellularized scaffolds maintain the original valve structure as well as many of the ECM molecules, providing potential advantages over the latter. The cells are removed using one of many tested detergent or enzymatic methods of decellularization involving various combinations and concentrations of reagents such as EDTA, trypsin, SDS, RNase, DNase, and Trito X-100, thereby minimizing damage to the original structure to avoid problems during subsequent recellularization or implantation [60,65,68]. However, cell removal may compromise the physical and biomechanical properties of the valve leaflet. Matrix/polymer hybrid scaffolds heart valve leaflet engineering with enhanced biomechanical characteristics may be advantageous and provide superior replacement valves [66,67]. After decellularization, acellular scaffolds are sometimes treated with cross-linkers such as pentagalloyl glucose (PGG) before implantation to stabilize the scaffold matrix, reduce immunogenicity and avoid undesirable consequences such as calcification [69].

On the other hand, fabricated polymeric scaffolds offer certain benefits compared to acellular scaffolds including better control over mechanical characteristics, degradation rates, fabrication, repeatability and lower immunogenic responses. Synthetic materials like polyglycolic acid (PGA) and polylactic acid (PLA) were initially studied for tissue engineering heart valve cusps but the cusps were found to be thicker and less flexible compared to natural cusps [61,70]. Sodian et al. [73,74] then used porous polyhydroxyalkanoate (PHA) as material for designing a biodegradable and biocompatible tri-leaflet heart valve scaffold (Fig. 1) via both in vivo and in vitro experiments which demonstrated the presence of viable cells and formation of ECM. Additionally, the mechanical properties of PHA, such as elasticity and mechanical strength, outperform those of the previously used material. Furthermore, the possibility of dip coating the non-woven PGA mesh in P4HB (poly-4-hydroxybutyrate), a PHA-based polymer, was examined to overcome the flexibility issue and increase the mechanical performance of PGA [71,72]. P4HB [75], PCL [76], and PEG [77, 78] among others are some of the synthetic polymers that have been studied for scaffold formation. In past few years, certain studies [79] have indicated the possibility of using polyglycerol sebacate (PGS) as a scaffold material for heart valve engineering. The investigations revealed that the prepared scaffold had good biodegradability, rigidity and cell adhesion properties.

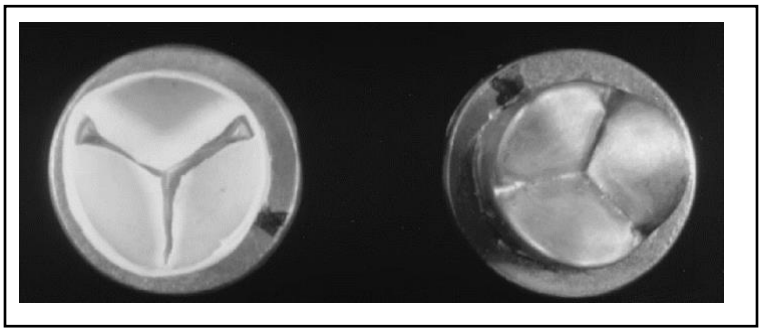

Fig 5. Porous heart valve scaffold and aluminum cast for fabrication of scaffold (seen from the top) (adapted from [73]).

Synthetic scaffolds might offer many advantages, however, their ability to generate a toxic response when degraded is a significant drawback, and their structure may not entirely mirror the complex structure and function of genuine tissue. Collagen, chitosan [82], small intestinal

submucosa (SIS), hyaluronic acid (HA) [77,85], and fibrin [83, 84] are among the biological materials being experimented on for heart valve scaffold construction. The major ECM protein of natural cardiac valves is collagen, and the majority of its mechanical and tensile strength is provided by type I collagen which is predominantly present. Additionally, collagens are relatively mildly immunogenic and are biodegradable due to their proteinaceous nature [58,62,63,]. But, collagen based scaffolds have low biodegradability that can be overcome by combining them with other biomaterials and cross-linking between polypeptide chains [80,81]. A carbodiimide-based sequential crosslinking approach was used by Nazir et al. [81] to prepare an ECM resembling hybrid scaffold from collagen type I and HA with tailorable cross-linking densities that displayed enhanced bending moduli up to 1660 kPa. Fibrin gel is another type of natural scaffold with tunable biodegradability and polymerization characteristics. They also have the benefit of being easily manufactured from the blood of the patient, resulting in an autologous scaffold with no harmful degradation products or inflammatory reactions. Furthermore, fibrin interacts biologically with cells and can promote cell growth or migration [63]. Ye et al. [82] demonstrated cell proliferation and collagen formation in fibrin gels implanted with aortic-derived myofibroblasts using aprotonin to suppress fibrin degradation rates. These composites, however, exhibited poor mechanical characteristics as well as gel shrinkage. Jockenhoevel et al. [83] reported on the fixation of fibrin gels with poly-l-lysine and were somewhat successful in avoiding shrinking and increasing collagen formation by creating inner stress, resulting in enhanced mechanical characteristics. Hyaluronic acid (HA) is the primary GAG of the original valve leaflet and is required for heart morphogenesis. It is naturally non-immunogenic and non-thrombogenic. These properties make it a promising scaffold material for tissue engineering cardiac valves [58,62]. It can be crosslinked using various methods to form hydrogels. However, hydrogels have weak mechanical properties, and hence, for augmenting these properties, HA is used in composite scaffolds [84]. Modified PEG hydrogel — polyethylene glycol diacrylate (PEGDA) — is an appealing material for heart valve tissue engineering because of its controllable mechanical and biological properties [77].

6. Tissue Engineering Scaffolds in Liver

Liver is one of the vital organs of our body. It plays an important role in detoxification, glycogen storage, production of bile, and controls chemical levels in the blood. Liver diseases often lead to liver failure which becomes fatal to patients. Liver transplantation is the only cure for liver failure. Here, arises the problem of scarcity in the number of donors. The number of recipients most often outnumbers the number of donors. So, liver tissue engineering has become a promising field in cases of production of Bioartificial Liver.

The choice of scaffold material is an important criterion that determines the success rate of liver tissue engineering.

One of the most promising scaffolds in liver tissue engineering is the decellularized whole liver scaffold. The texture of the decellularized liver is identical to that of the original organ. This natural structure can act as a 3D poral matrix for cell proliferation, differentiation, and function in cultivated cells. Perfusion with EDTA, SDS, Triton-X, or other detergent and enzymatic agents mixed in phosphate buffer solution (PBS) into the liver portal vein is used to decellularize the liver. All hepatocytes are removed from the liver after a time of perfusion, while the extracellular matrix is left intact with blood and bile arteries [85]. Decellularized Human Liver is used as a natural scaffold. It was produced by tissue decellularization, and the remaining ECM was used as a

scaffold for culture. It perfectly represents the structural and biochemical components of the original human liver matrix. It has certain limitations like an elaborate production process and limited availability of donor tissue [86].

Poly PHBVHHx, a member of the polyhydroxyalkanoates (PHA) family, was originally employed in tissue engineering. PHA is made in nature by bacteria fermenting sugar or fat, giving it good biocompatibility and biodegradability. They have varying ductile and more or less elastic properties according to their composition, which provides the material with the necessary mechanical strength [87]. PHBVHHx under the microscope shows a honeycomb-like structure with varying pore sizes. It was implanted with MSC into a rat model of carbon tetrachloride-induced liver failure. The rat's liver functions substantially better than the control group's liver 28 days after implantation. Poly PHBVHHx has a high capability of forming 3D scaffolds which can be used for liver tissue engineering [88].

7. Tissue Engineering Scaffolds in Kidney

Chronic kidney disease (CKD) is becoming more common around the world at an alarming rate [89]. For patients with end-stage renal illness, kidney transplantation is the most effective therapeutic option. Kidney transplantation is a much better and more effective option than long-term dialysis [90]. Despite an increase in the number of patients on the transplant waiting list, the number of available kidneys has remained stable.

Decellularization of xenogeneic or allogeneic donor kidneys is a possible alternative strategy for creating scaffolds for complete kidney engineering. To eliminate the antigenic parenchyma from the entire renal matrix, detergents, enzymes, or other cell-lysing solutions are perfused antegrade through the renal vasculature [91,92]. Few cases of decellularized whole-kidney ECM scaffold transplantation have been recorded to date, owing to the nonendothelialized vasculature's inherent thrombogenicity.

The renal artery and vein of decellularized swine kidneys were anastomosed to the aorta and vena cava of recipient pigs, respectively, by Orlando et al. [93]. During 60 minutes of intraoperative monitoring, the scientists saw adequate blood flow through the scaffold without bleeding, but 2 weeks later, they discovered significant thrombosis throughout the non-endothelialized kidney scaffolds. Despite a nonspecific inflammatory response, the authors found no evidence of immunological rejection, implying that the decellularized allogeneic scaffolds were compatible with the host animals. Although the duration of in-vivo perfusion was not specified, the authors reported no thrombosis or bleeding during the implantation time. The vasculature must be pre endothelialized in vitro to prevent thrombotic blockage of the vessels after implantation in order to efficiently transplant decellularized kidney scaffolds over lengthy periods of time [92].

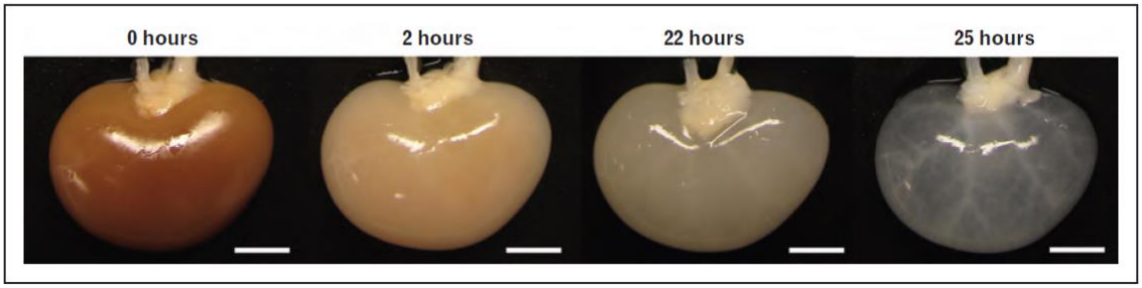

Fig 6: Whole Kidney Decellularization. Shown in this figure is a representative macroscopic view of a Sprague-Dawley rat kidney undergoing a detergent-based decellularization protocol. As cellular material is removed from the scaffold, the kidney gradually fades in colour. At the end of the decellularization process, the kidney is transparent in appearance, and the vasculature can easily be visualized. Scale bar: 5 mm. [94]

Electrospinning and other polymer fabrication techniques have the potential to provide a non-woven path for renal tissue engineering. Primary rat kidney cells were extracted and planted on electrospun poly (lactic acid) scaffolds. The ability of electrospun polymer scaffolds to act as a conveyor for kidney cells makes them an ideal candidate for kidney tissue engineering; the non-woven path offers morphological control as well as superior mechanical properties with degradation over a tuneable time frame, making them a better option than decellularised tissue [95].

Conclusion:

Bioengineered scaffolds for any sort of wound healing have made significant progress from repairing to regenerative processes, but the quest for the perfect therapy continues. Wound healing is aided by biomaterial scaffolds, which have been studied extensively. Scaffolds are based on the characteristics and structures of biomaterials. Understanding the idea behind biomaterial scaffold design allowed us to create scaffolds that range from basic cross-linking, electro-spinning, and 3D bio-printing to cell-matrix interactive and immune-modulating scaffolds that encapsulate cells or bio-molecules. Tissue formation in the body is a complicated process in which cell populations self-assemble into functional units as part of the development or repair process. Finding ways to replicate these processes outside the body has picked academic, medical, and commercial interest. With the demonstration of skin and cartilage tissue engineering in the laboratory, there is currently international activity in the in vitro regeneration of tissues such as nerves, liver, bone, heart valves, blood vessels, bladder, and kidney using tissue engineering scaffolds as a future of therapeutics. Following this research, we were able to identify several domains in which contemporary biological technology has a bright future ahead of it and can progress even farther provided proper monitoring and community culture are maintained.

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