An Overview of Tissue Engineering Scaffolds- Recent Advances and Limitations to practical Applications

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ABSTRACT:

Geriatrics and population with multiple co-morbidities across the world live an unhealthy life owing to improperly functional/dysfunctional tissues/organs. The remedy of tissue/organ transplantation incurs the limitations of either tissue rejection or timely unavailability of a suitable donor. Thus tissue/organ engineering in a 3D artificial substrate i.e. scaffold mimicking host's native ECM offers solution to the challenge. In this review the so far work done in this domain including all the attempts and advances made in the horizon of biomaterials, fabrication techniques, challenges and practical applications have been comprehensively summarized from both theoretical and practical point of view. The till date advances in each domain have been summarized in tabulated form which is the novelty of this review. Despite much work done little applications have been seen in practical domain. In this respect an exclusive analysis of the recent advancement in the zone of practical applications has been done. Challenges/limitations to their practical applications have been crucially evaluated and future direction towards their solution is carved to extend and utilize their benefits practically.

1 INTRODUCTION:

In United States and other developed countries, elderly populations in union with multiple co-morbidities i.e. diabetes, cardiovascular problems and obesity have led to enhanced prevalence of compromised tissue/organ function. Chronic wounds, periodontal diseases, orthopedic problems and compromised functioning of bio valves are being an obstruction to quality life [1]. Organ transplantation has been a promising approach towards Sir Harold Gillies' idea of "replacing like with like". It refers to the replacement of a damaged or diseased tissue/organ with a healthy one, donated by a healthy donor and resumption of the quality of life [2]. But approximately less than a half of these patients expire awaiting a suitable donor [3]. Also that tissue rejection of the transplanted organ by the host immune system is not very unlikely because transplanted organs have genomes that are distinct from the recipient's genome [4]. Thus, there is a great need for biocompatible tissue replacement materials with excellent native microenvironment and ECM mimicking properties. Tissue Engineering is a multidisciplinary field that utilizes the principles of engineering and life sciences for the development of novel constructs as biological substitutes with natural, synthetic, or semisynthetic materials that restore, maintain, or improve tissue function or a whole organ [5, 6]. The pivot objective of tissue engineering is to generate or regenerate tissues and eventually organs [7]. This field utilizes three approaches; 1) the replacement of the cells providing the required function by the isolated cells or cell substitutes; 2) the delivery of tissue engineering substances, i.e. growth and differentiation factors, to targeted sites; (3) growing cells in an artificial three dimensional porous biomaterial matrix structure i.e. scaffolds. The first two approaches are exercised when the deformities or flaws are small and well constrained but limited when it comes to engineering actual size scale

and predestined shapes. Therefore, the third approach, i.e. use of scaffolds has become pivotal for the engineering of practical size tissues [8].

1.1 SCAFFOLDS:

A scaffold is an artificially produced template matrix or a substrate structure to aid three-dimensional tissue growth. These structures are constructed for the engineering of new functional tissues via bringing about desired cellular interactions. The cells impact their own microenvironment because scaffolds mimic native extra cellular environment. These substrate structures mimic the native extracellular environment, allowing seeded cells to adhere, proliferate, grow and differentiate. The regeneration of new functional tissues and their differentiation into different organs is facilitated by provision of natural extracellular substrate and other utilities i.e., growth factors and other biological molecules in the scaffolds. The stem cells have the potential to differentiate which makes them a pivotal aid for their applications in tissue engineering. These cells possess two unique characteristics – the potential to produce new stem cells and the ability to become multiple cell types [9]. Stem cells attach, multiply, migrate and function onto it to replace damaged organs [7].

1.1.1 TYPES OF SCAFFOLDS:

There are different types of scaffolds.

1.1.1.1 POROUS SCAFFOLDS:

Porous scaffolds have three dimensional, well interconnected porous network structure with good porosity that facilitates the transport of materials. They are either Sponge porous or Foam porous (Fig.1). They are good tissue engineering templates being providing well interconnected porous network for better material and nutrient transport and hindering the growth of contact-

inhibited cells. They have found applications in the reconstruction of bones and organ vessels. Open spaces present throughout the structure pose a limit to their use and require improvement in the pore interconnectivity. Porous scaffolds with better controlled pore size, diameter, surface area to volume ratio and crystallinity are prepared using the synthetic materials i.e. PLGA, PCL, PLLA, PGA, PDLLA, PEE by solvent casting, particulate leaching and electro spinning fabrication techniques [6].

Figure 1: Physical appearance of collagen: chitosan porous scaffold prepared by freeze drying method for wound healing (reproduced in cropped form under http://creativecommons.org/licenses/by/3.0/) [10]

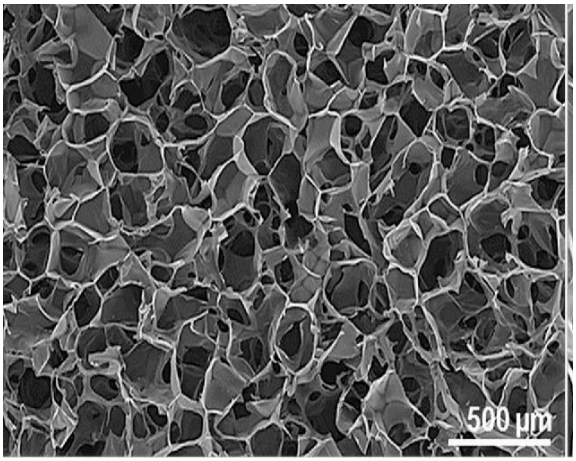

Figure 2: SEM image of Porous PEDOT:PSS scaffolds prepared by freeze drying method for bone tissue engineering (reproduced in cropped form under https://creativecommons.org/licenses/by/4.0/) [11]

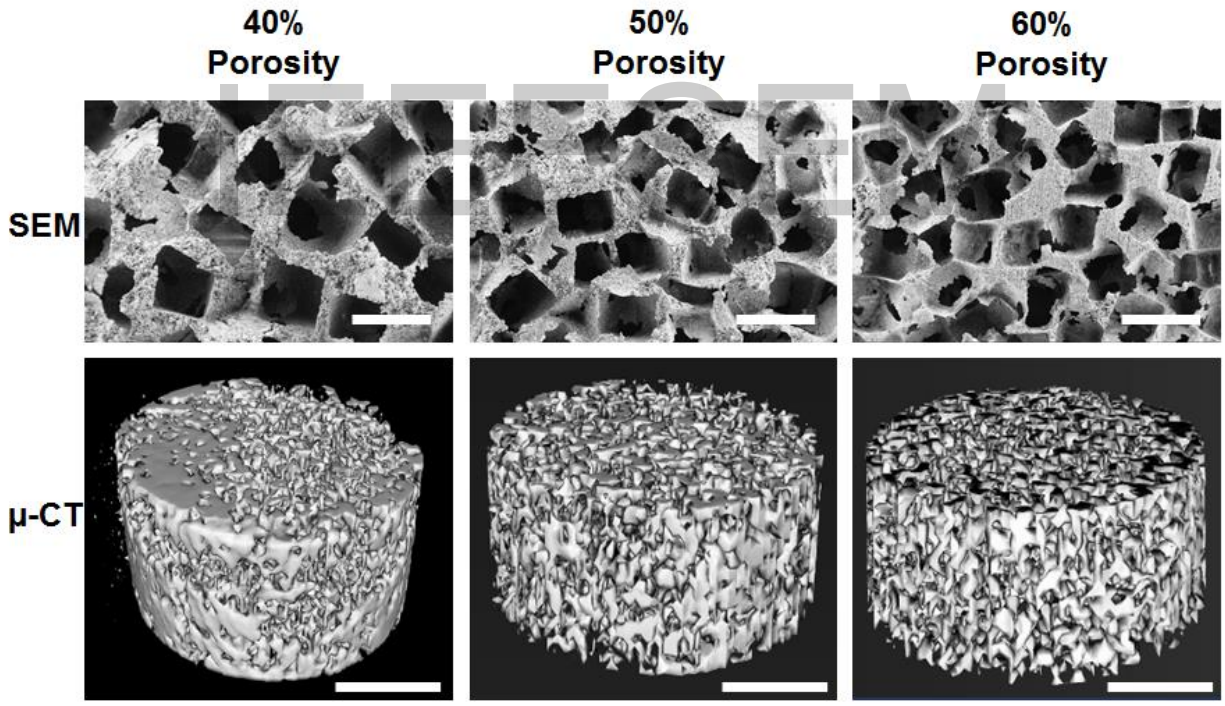

Figure 3: SEM and µ-CT images of porous PCL:Borophosphosilicate composite scaffolds with various pore sizes prepared by solvent casting and particulate leaching method for bone tissue engineering [12]

1.1.1.2 HYDROGEL SCAFFOLDS:

Hydrogel scaffolds are the gel network structures prepared by either natural or synthetic polymers that form gels by covalent or non-covalent cross-linking of the network. The natural polymers invoke biocompatibility and well synchronized biodegradability with intrinsic cellular processes. The synthetic macromolecules overcome the limits of mechanical strength and batch to batch variation imposed by natural polymers. The natural polymers used to prepare hydrogels include collagen, fibrin, gelatin, chitosan, alginate and HA while synthetic macromolecules involve PLA derived copolymers, derivatives of PEG and PVA. The advantages of these tissue engineering templates are their high water content, good physical structure for cellular as well as nutrient transport and angiogenesis. They found applications in wound dressing and healing, cartilage and bone reconstruction as well as in drug delivery [6].

Figure 4: Physical appearance of collagen hydrogel scaffold for periodontal healing (reproduced in cropped form under https://creativecommons.org/licenses/by-nc/4.0/legalcode) [13]

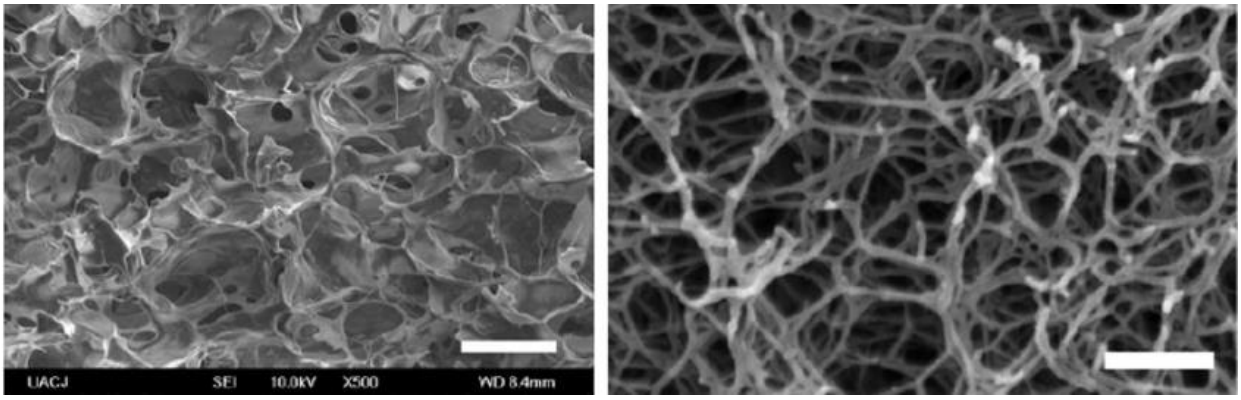

Figure 5: FESEM image of agarose hydrogel and agarose hydrogel for cancer drug delivery (reproduced in cropped form under http://creativecommons.org/licenses/by/3.0) [14]

1.1.1.3 NANO FIBROUS SCAFFOLDS*:*

Nano-fibers scaffolds are prepared by electro-spinning, phase separation or self-assembly technique by either natural or synthetic polymers. High-surface-to-volume-ratio in junction with micro porous structure is the specialty of this structured type. These two properties of nano-fibers induce all the ideal properties desired in a tissue engineering template i.e. cell anchorage, growth, movement and differentiation. The natural polymers used to produce nano-fibers are collagen, chitosan, gelatin and silk fibroin while the synthetic polymers used for this purpose include PLA, PCL, PU, and copolymers thereof i.e. PLGA PEVA, PLLA-CL. In order to have successful applications of nano-fibers, the functional group of the polymers is to be functionalized through physical mixing, coating or surface grafting techniques. Specific drugs, growth factors or genetic material can be blended into the polymers in order to achieve their controlled release. Nano-fibers scaffolds have found their applications in the regeneration of skin, bones, ligament, cartilage, cardiac and neural tissues [6].

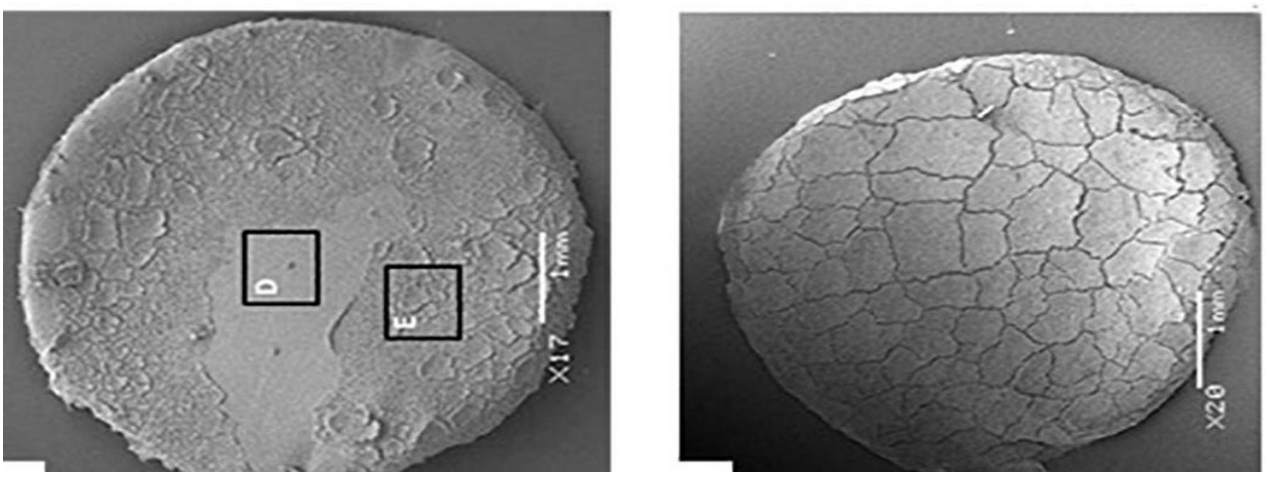

Figure 6: Physical appearance of nanofibrous PET scaffolds for human intestinal epithelium (reproduced under http://www.frontiersin.org/AuthorConditions.aspx) [15]

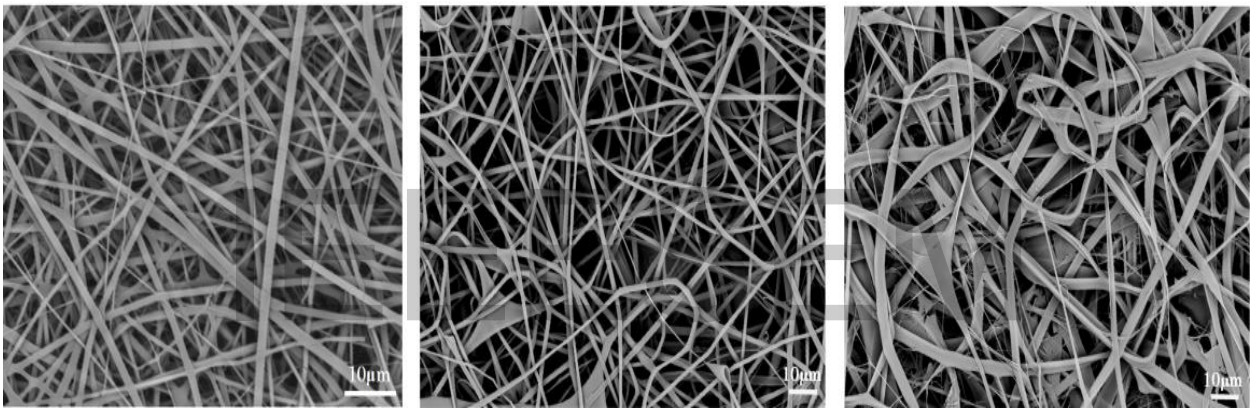

Figure 7: SEM images of silk:TMOS fibrous scaffolds prepared by electrospinning for bone tissue engineering (reproduced under http://creativecommons.org/licenses/by/3.0/) [16]

1.1.1.4 MICROSPHERE SCAFFOLDS:

Microsphere scaffolds are microencapsulated polymer matrix for controlled drug release pattern i.e. slow drug release over extended time duration. They are prepared by heat sintering, solvent vapor treatment method and particle aggregation technique. Microsphere produced by different techniques have different properties and hence applications. Bilayered scaffolds produced by particle aggregation method have osteochondrial tissue engineering applications. PLAGA sintered microsphere scaffolds are used for the reconstruction of load bearing bone. Injectable microspheres are also prepared for controlled drug delivery applications. These scaffolds have

also found applications in gene therapy. The high molecular polymers invoke rapid drug release properties while low molecular weight polymers induce slow drug release pattern into these structures. These scaffolds are characterized by good porosity i.e. required three dimensional pore size and good pore interconnectivity [6].

1.1.1.5 POLYMER-BIOCERAMIC COMPOSITE SCAFFOLDS:

Polymer-bioceramic composite scaffolds fulfill the demands of the host tissue by adding rather good mechanical, biocompatible and degradation properties which either one cannot induce separately. Ceramic materials possess certain valuable properties i.e. good biocompatibility, resistance to compression and corrosion, degradability ranging from relatively inert, semi inert to non-inert. Polymers are flexible in nature. The composite of the two pose ideal properties to the scaffolds structures i.e. uniform cell adhesion, proliferation and differentiation. Complications in their achieving optimum results are lack of synchronization in the stability and strength during cellular process i.e. degradation and tissue replacement. The bioceramic material used for preparing bone tissue engineering constructs includes CP, HAP and TCP. The best polymer bioceramic composite scaffold is PLGA/HAP which possess excellent biocompatibility and bioactivity properties and used for osteoconductive applications [6].

Figure 8: Physical appearance of Galss:GO composite scaffold prepared by high temperature calcination technique for bone tissue engineering (reproduced in cropped form under https://creativecommons.org/licenses/by/4.0/) [17]

1.1.1.6 ACELLULAR SCAFFOLDS:

Acellular scaffolds are prepared by removing cellular components of natural tissues by chemical or mechanical means without altering the remaining composition, mechanical integrity and function of the matrix. They possess the native ECM, intact natural mechanical strength and minimum immunological inflammation due to decellularisation. Polymer coating can further amount to mechanical stability and haemocompatibility properties and make them more desirable for tissue engineering applications. Some applications include heart valves, small intestine and urinary bladder which have been decellularized. The mechanism involves utilization of biologic/polymer composite as starter matrix for tissue reconstruction. One example is tissue engineering of heart valve with decellularized porcine aortic valve along with dip coated biodegradable polymer [6].

1.1.2 PROPERTIES OF IDEAL SCAFFOLDS:

Since scaffolds are meant to be colonized by foreign cells trigger of an immune or inflammatory response is likely [18]. Therefore, an ideal tissue engineering scaffolds must possess a few

fundamental requirements in order to minimize the provocation of an immune reaction but optimize the cell growth [18] [6, 19].

1.1.2.1 BIODEGRADABILITY:

The material used to design polymer scaffold must be biodegradable having non-toxic degradation products and easily eliminated from the site of implantation. The degradation rate of the implanted structure must be synchronized with the reconstruction rate of the damaged tissue in order to have it completely removed once the repairing of the damage is fully done [18]. Degradation profile of various polymers is tabulated in Table 1.

Table 1: Summary of Degradation Span of Different Polymers

Sr. #	Polymers	Degradation time (days)	Reference
1	Collagen	14 – 168	[20]
2	PLGA	Manageable	[21]
3	DL-PLA	360-480	[21]
4	L-PLA	>720	[21]
5	PGA	120-360	[21]
6	PCL	>720	[21]
7	HA	Years (poor degradation)	[20]
8	TCP	56 – 168	[20][12] https://creativecommons.org/licenses/by-nc/4.0/legalcode https://creativecommons.org/licenses/by-nc/4.0/legalcode

1.1.2.2 MECHANICAL STRENGTH:

The scaffold must have mechanical properties to provide necessary strength to the cells seeded onto it against the tensile pressure of the native tissue environment. The provision of biomechanical function of the scaffold should be progressive from minimal level right after implantation to a structure fully integrated with the surrounding host tissue at the complete

restoration of the normal tissue function [18]. Mechanical strength profile of different synthetic polymers is summarized in Table 2.

Table 2: Summary of Mechanical Strength Profile of Different Polymers

Sr. #	Polymers	Tensile strength (MPa)	Modulus (GPa)	Reference
1	PLGA	41.4 - 55.2	1.4 – 2.8	[21][13] http://www.frontiersin.org/AuthorConditions.aspx)
2	DL-PLA	27.6 – 41.4	1.4 – 2.8	[21][13]
3	L-PLA	55.2 – 82.7	2.8 – 4.2	[21][13]
4	PGA	>68.9	>6.9	[21][13]
5	PCL	20.7 – 34.5	0.21 – 0.34	[21][13]
6	PDLLA	-	1.9 – 2.4	[22]
7	PPF	-	2 - 3	[22]

1.1.2.3 MICROSCOPIC AND MACROSCOPIC STRUCTURE:

The microscopic and macroscopic structure of the construct should be reproducible. The seeding site for cell/drug should have a high surface to volume ratio. This will endorse their optimum anchorage, proliferation, migration and differentiation [6, 18, 19].

1.1.2.4 POROSITY:

The porosity of the scaffolds must be high with an appropriate pore size. The porosity comprised of three factors i) the magnitude of the porosity ii) the distribution of the pore size iii) and the interconnectivity of the pores. This is essential for an optimum anchorage and adhesion of the cells seeded onto it. This also aggravates metabolite movement. The ideal porosity for an optimum interaction and integration with the host tissue has been demonstrated as, optimum pore size of 5 μm for neovascularization , 5–15 μm for fibroblast ingrowth, 20 μm for the ingrowth of

hepatocytes, 200–350 μm for osteoconduction, and 20–125 μm for regeneration of adult mammalian skin. Pore interconnectivity is also critical to ensure that all cells are within 200 μm from blood supply in order to provide for mass transfer of oxygen and nutrients [6, 18, 19]. Ideal pore size of scaffolds for different regeneration sites is tabulated in Table 3.

Table 3: Ideal Pore Size for the Different Regeneration Sites

Sr. #	Regeneration Site	Ideal Pore Size
1	Neovascularization	5 μm
2	fibroblast ingrowth	5–15 μm
3	ingrowth of hepatocytes	20 μm
4	Osteoconduction	200–350 μm
5	Adult mammalian skin	20–125 μm

1.1.2.5 LOADING CAPACITY:

In order to have a controlled and uniform drug release pattern over an extended duration the scaffold should have a maximum drug loading capacity. The drug should be uniformly distributed throughout the scaffold and an immediate shattering of it should be obviated [18].

1.1.2.6 STABILITY:

The scaffold must maintain its stability i.e. physical motif, chemical composition, and functional integrity over a durable period [18].

1.1.2.7 BINDING AFINITY:

The binding affinity of the drug with the scaffold should neither be too high to block drug release nor too low to enhance likelihood of dose dumping. It must be adjusted to a modest level [18].

1.1.3 PREPARATION MATERIALS FOR SCAFFOLDS:

The crucial step in tissue engineering is the choice of the right material for preparation of scaffolds with ideal properties in order to produce optimum results [21] . The criteria to choose the material for producing scaffolds is the chemistry of the material, molecular weight, structure and shape of the material, hydrophilicity and hydrophobicity properties, property of lubricity, surface energy, water absorption capacity, mechanism of degradation and erosion. There are three types of materials used for the preparation of scaffolds. Natural polymers, Synthetic polymers and bio ceramics [6]. Each material possess some specific properties required in an ideal scaffold but none offer all the required properties therefore a hybrid of different materials to unite the individual properties can produce the one with optimum properties [23].

1.1.3.1 NATURAL POLYMERS:

Natural polymers are derived from natural resources and therefore have the edge of familiarity with the biological environment [23]. Advantages of natural biomaterials are biocompatibility, biodegradability and a better cellular interaction [23] [6]. Due to these properties the stimulation of immune response to the implanted construct is less likely [23]. Disadvantages of these biomaterials are likelihood of pathogen transmission, lack of mechanical strength and variation in quality from batch to batch [23] [21]. They can be classified into three categories.

i) Proteins

ii) Polysaccharides

iii) Polynucleotides.

a) Proteins include silk, gelatin, collagen, fibrinogen, elastin, keratin, actin and myosin.

b) Polysaccharides include cellulose, chitin, amylose, dextran, glycosaminoglycans.

c) Polynucleotides used as natural polymers to fabricate scaffolds include DNA and RNA [6].

Natural polymers are used for the repairing of nerves , replacement of skin, reengineering of cartilage and bone [21].

1.1.3.2 SYNTHETICS POLYMERS:

Synthetic polymers are developed in order to overcome the limits of natural polymers. They are synthesized to have controlled and desired properties as a substitute to natural polymers [23]. They are FDA approved materials and can tailor desired properties to the implanted construct [21]. These polymers have good porosity, mechanical strength, rather cheap cost and good degradation time, consistent large batch production and long shelf life as an asset to them. They can be classified as follows.

i) Polyethylene Glycol (PEG)

ii) Poly α- hydroxyesters

a) Polyethylene Glycol (PEG) is an FDA approved synthetic biomaterial and has tissue engineering applications. It possesses many useful properties i.e. good biocompatibility, less toxicity, good hydrophilicity, good solubility in organic solvents and property of non-immunogenicity. Besides, modification of its hydroxyl group could further amount to mechanical strength property [23].

b) Poly α- hydroxyesters include poly-lactide (PLA), poly-glycolide (PGA) and copolymers thereof i.e. PLGA and PCL. PGA possesses the quality of high degree crystallization. PCL has good solubility in many organic solvents. PLLA has high mechanical strength. PDLLA endures lower mechanical strength and is amorphous in

structure [23]. PHA is another synthetic polymer and it belongs to microbial polyesters class [6].

Some limits of polymers are the release of acidic degradation products, mechanical stiffness and unavailability of an active chemically reactive site for easy attachment of drugs or cross linkers [21].

1.1.3.3 BIOCERAMIC MATERIALS:

Bioactive ceramic materials are inorganic materials having pivot composition various proportion of Ca^{2+} and PO_4^3 [23]. They include hydroxyapatite (HAP) and TCP, some compositions of phosphate glass & silicate and glass ceramics [6]. These materials are biocompatible in nature, non-immunogenic and able to form chemical bond with the tissue of the host but have the drawback of brittleness and stiffness nature [23]. Some properties of different polymers are tabulated in Table 4.

Table 4: Summary of the Properties of Scaffold Preparation Biomaterials

Sr. #	Polymers	Characteristics
1	Natural Polymers	Poor mechanical strength Poor load bearing capability Good biocompatibility Fast degradation
2	Synthetic polymers	Good mechanical strength Good elasticity Good load bearing ability Good biocompatibility Slow degradation
3	Bioceramics	Poor mechanical strength Brittle in nature Poor elasticity Poor load bearing ability Bioactive Slow degradation (rapid than synthetic polymers)

The composite formulations of these materials in appropriate proportion to incur required properties to scaffolds could overcome the limitations to their ideal proficiency [6] [24]. Some composite scaffolds that have been worked on are summarized in Table 5.

Table 5: Summary of Some of Existing Composite Scaffolds

Sr. #	Scaffold Composite	Fabricating Technique	Targeted Tissue	Mfg. Year	Reference
1	Alginate-halloysite	Freeze drying	Bone	2015	[25]
2	Silk fibroin-gelatin-nanoHA	Freeze drying	Bone	2015	[26]
3	Nano HA-Chitosan-Alginate	Freeze drying	Bone	2015	[27]
4	Chitosan-TCP	Agglomeration of CS and CS/TCP microspheres	Bone	2015	[28]
5	Chitosan-gelatin-agarose-halloysite	Freeze drying	Vessel	2016	[29]
6	Collagen-HA-chitosan-TCP-ginseng compound K	Freeze drying	Bone	2016	[30]
7	Polyamide 6,6-chitosan	One step co-electrospinning	Bone	2016	[31]
8	PCL-HA	Electrospinning	Neuron	2016	[32]
9	Beta chitin-gelatin-nano HA	Freeze drying	Bone	2016	[33]
10	Chitosan-nanodiopside-nano HA	Freeze drying	Bone	2016	[34]
11	PCL-gelatin	Electrospinning	Blood vessel endothelial layer	2017	[35]
12	Graphene oxide-nanoHA	Self essembly	Bone	2017	[36]
13	Silk fibroin-PGS, Chitosan-PGS	Particulate leaching + Freeze drying	Skin	2017	[37]
14	Chitin-chitosan-nano diopside	Freeze drying	Bone	2017	[38]
15	Collagen-carbon-chitosan-HA	Freeze drying	Bone	2018	[39]
16	Chitosan-nano diopside-gelatin	Freeze drying	Bone	2018	[40]
17	Calcium silicate-silk fibroin-sodium alginate	Freeze drying	Bone	2018	[41]

18	CNC-PLA	In-situ polymerization method	Bone	2019	[42]
19	PLA-PBS-CNF	Electrospinning	Vessels	2019	[43]
20	PVA-TCP	Fused deposition modeling	Bone	2019	[44]
21	Fibrin-PU-MWCNT	Electrospinning	Neuron	2019	[45]
22	Bioactive glass-PLLA-PCL	Polymer coating	Bone	2019	[46]
23	Starch-PCL	Solvent casting salt leaching	Bone	2019	[47]
24	UPCL-PCL-PANI	Electrospinning	Neuron	2019	[48]
25	BC-PCL	3D-bioprinting	Skin	2019	[49]
26	HEC-PVA	Freeze drying	Bone	2019	[50]
27	PCL/DCPD	Electrospinning	Bone	2019	[51]
28	Nano HA-chitosan-CMC	Freeze drying	Bone	2019	[52]

1.1.4 FABRICATION TECHNIQUES FOR SCAFFOLDS:

The preparation of scaffolds can be acquired by several techniques. Each technique has some advantages and some disadvantages. The selection of the technique to be adopted depends upon the type of material chosen as well as the properties desired in the scaffolds. Different conventional and modern techniques for the fabrication of tissue engineering scaffolds are summarized in Figure 9.

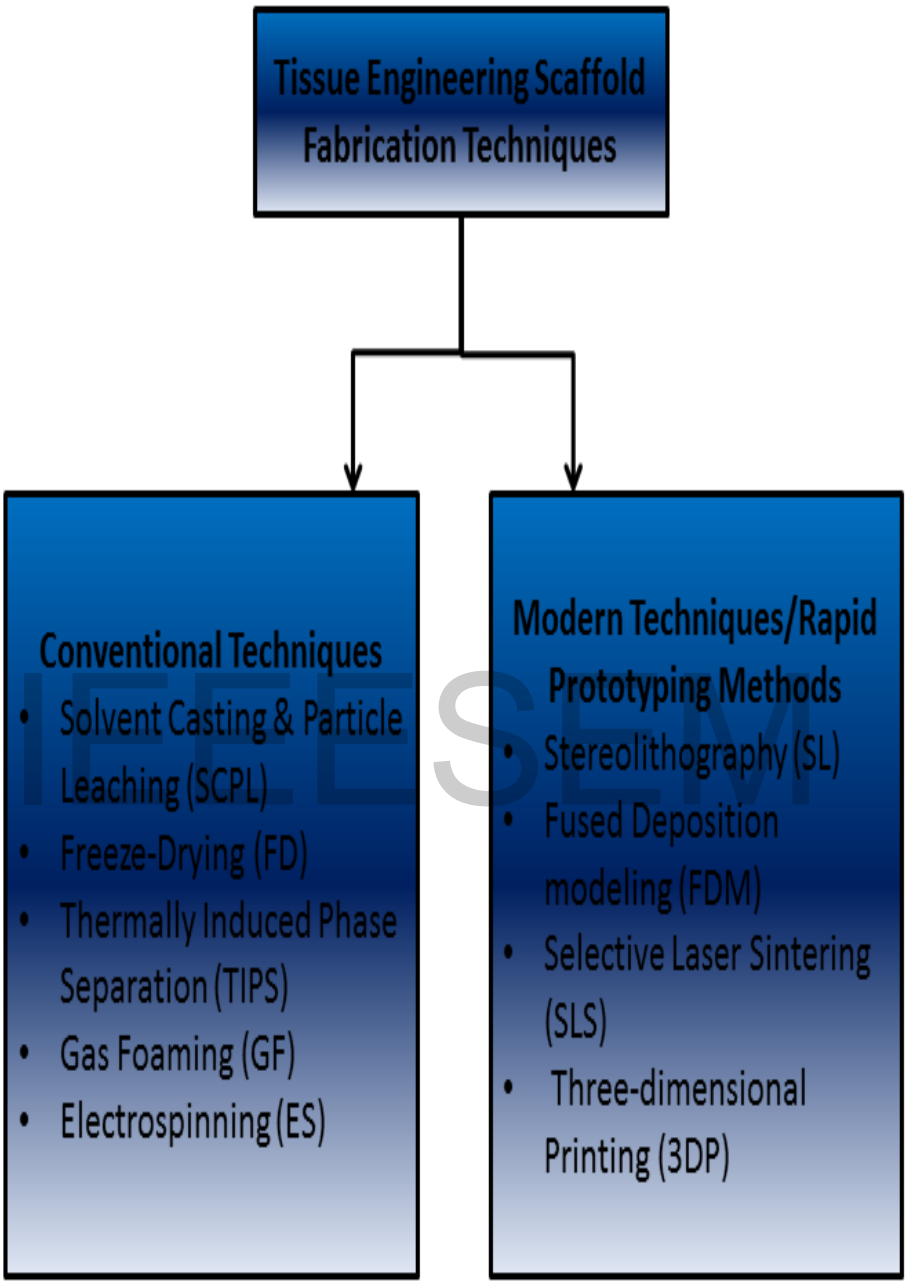

Figure 9: Summary of Tissue Engineering Scaffolds Fabrication Techniques

1.1.4.1 SOLVENT CASTING AND PARTICULATE LEACHING TECHNIQUE*:*

It involves dissolution of the polymer into an organic solvent and then evaporation of the solvent through either casting into a mold or freeze drying. Leaching out of salt particles is conducted through selective dissolution which produces porous substrate. The advantage of this technique is the high porosity i.e. 93% with a 500µm while the disadvantage is its restricted use to produce thin membrane wafers merely. It could be overcome by laminating the membranes of the polymers together to create three dimensional structures of desired credentials [21].

1.1.4.2 SOLVENT-INDUCED PHASE SEPARATION OR THERMALLY INDUCED PHASE SEPARATION TECHNIQUE:

This technique is based on the thermodynamic separation mechanism. It involves the splitting of the solvent solution of the polymer which homogenous yet into two phases i.e. a phase rich in polymer and the other poor in polymer. This is achieved via exposing the solution to another immiscible solvent [24]. Dissolution of the polymer is conducted in a solvent i.e. phenol in molten form, dioxane while keeping the temperature relatively low. Reducing the temperature induces phase separation either liquid-liquid phase separation or solid-liquid separation. In the wake of the removal of the solvent rich phase through sublimation process a porous scaffold is produced [21]. This technique is to be combined with other techniques i.e. salt leaching to obtain the required porosity [53].

1.1.4.3 POLYMER MELT BY FOAMING TECHNIQUE:

Preparation of the scaffold through polymer melt by foaming technique involves exposure of the polymer is made to carbon dioxide gas at a high pressure. The melt of the polymer dissolves CO_2 into it at high temperature and pressure. The process could produce the construct with the morphology of the cells having the size 10µm with homogeneous nucleation or utilize

heterogeneous nucleation to produce cells sized in the range of 30-700 µm. the advantages of the technique is that the resources i.e. CO_2 is readily available and its removal prior to cell seeding is not requisite. Disadvantages of this technique is that it interconnectivity is not achieved to the optimum level which could be overcome by combining it with particulate leaching technique to enhance porosity of the final construct [53].

1.1.4.4 ELECTROSPINNING TECHNIQUE:

This technique uses electricity for the fabrication of nano fibrous scaffolds. The technique is easy and fast to apply but pore distribution is still a challenge to this technique which limits its application.

1.1.4.5 RAPID PROTOTYPING (RP) TECHNIQUE:

is also referred to as solid free form fabrication (SFF) technique. This works on the principle of fabricating a 3D structure adopting a computer aided design (CAD) model. The construct is developed in a manner of layer by layer where its morphological properties, chemical make-up and mechanical strength are controlled with absolute precision [24]. This technique is advantageous in the manner that the porosity as well as the stability of the construct is well controlled while the restriction of the selection of the polymer material limits its usage [53].

1.1.4.6 STEREOLITHOGRAPHIC PRINCIPLE:

Another technique utilizes stereo lithographic principle. It works by triggering a chemical reaction in the polymer material with the help of electromagnetic radiation. The formed layer is lowered and new material is exposed to the light beam and that's how chain reaction is produced leading to the construction of 3D scaffold structures [53].

1.1.4.7 SHAPE DEPOSITION:

The manufacturing method called shape deposition involves the formation of scaffold layers through computer controlled machine. Preformed layers of building block unit material are joined together manually to give rise to 3D structure. This innovative technique yet has rare applications in tissue engineering [53].

1.1.4.8 THREE DIMENSIONAL FIBER DEPOSITION TECHNIQUE:

Another method called 3D fiber deposition technique includes conversion of a polymer melt into porous scaffolds fibers through an extruder which is temperature-controlled. The technique is computer controlled. It uses the polymers which are able to melt and hence slowly biodegradable or non-biodegradable, therefore, poor biodegradability is a disadvantage of this technique [53].

1.1.4.9 NON-FUSED LIQUID DEPOSITION MODELING TECHNIQUE:

This technique offers the resolve for the requisite of meltable polymers in the previous technique. It is called non-fused liquid deposition modeling which involves the use of polymers in slurry, solution, paste or dispersion form and formation of scaffolds is conducted through specialized procedures i.e. multiphase jet solidification [53].

1.1.4.10 SELECTIVE LASER SINTERING TECHNIQUE:

Selective laser sintering technique uses laser beam to produce powdered layers. The principle of this technique is to enhance the temperature of the material above the glass transition temperature but below its melting point. It leads to fusion of material and hence layers are produced with the help of a roller. Essential requirements for this technique are the availability of the material with required melting and welding properties [53].

1.1.4.11 THREE DIMENSIONAL PRINTING TECHNIQUE:

A latest innovative technique called 3D printing involves formation of scaffolds layers through bonding of adhesion. The primary layer of powder is poured, onto which solution of binder is spread through ink-jet print. It gives rise to a 2D structure onto which fresh layer of powder material is deposited to bring about 3D structures. It could produce a network channel with a pore diameter 500µm. The availability of the powder material with required particle size to produce good flow properties is limited therefore, the material needs several pretreatments to be used in this technique [53].

1.1.4.12 COMPUTER AIDED TISSUE ENGINEERING TECHNIQUE:

is an advance technique. It works by designing and manufacturing of the 3D tissue scaffold structure through computer-aiding that carries out the principles of solid free form fabrication technique as well as bio blueprint modeling. Since this technique provides more detailed spectacle of various requirements both mechanical and geometrical during different stages of the tissue engineering it is advantageous in the manner that the construct scaffolds created through technique are rather closer to the natural environment [53].

1.1.5 APPLICATIONS OF SCAFFOLDS:

In terms of applications, the scaffolds are divided into two major types; 1) Cell delivery scaffolds that seed cells onto them which lead to new tissue generation. 2) Drug delivery scaffolds which load drugs onto them and lead to its sustained release over an extended period. As cell delivery applications scaffold have found applications in many fields including bone regeneration, development of cartilage, formation of cardiac valves, tendon and ligament repairing, reconstruction of periodontal, vascular implants, nerve tissues regeneration, skin repair and

fixing nasal and auricular malformations [18][11]. As drug delivery scaffolds they are being used in cardiovascular disorders, diabetes, wound healings, tumor treatment, and osteochondrogenesis and in inflammatory diseases i.e. joint pain. It involves a controlled drug delivery for an extended duration. This also incorporates delivery of the genetic material i.e. DNA into the site of action. The delivery of antibiotics through scaffolds prevents the spread of infection after surgery and in chronic diseases [18]. In the future the concept of scaffolds is supposed to introduce innovative applications in developing the understanding of various mechanisms and hence a better treatment to i.e. cancer spread cardiovascular and mental diseases, apoptosis, aging, human development, and many other degenerative conditions[18][11]. Scaffolds could offer all these unique advances in tissue engineering only when they have all ideal characteristics in them which are not very easy. Some of the commercially available scaffolds and their applications are summarized in Table 6.

Table 6: List of Some Commercially Available Scaffolds and Their Uses [54]

Sr. #	Trade Name of the Scaffold	Applications
1	Amvisc, Amvisc plus	Surgical aid in ophthalmic procedures i.e. • Cataract extraction • Intraocular lens implantation • Surgery of glaucoma filtering and corneal transplantation
2	Acufex	Orthopedic surgical instruments, flexible suture material
3	Alzamer	Used in drug delivery and ocular processes
4	Bioanchor	Biodegradable sutures
5	Biobrane & Alloderm	Skin substitutes
6	Biofix	Devices for fixing of bone internally
7	Cameo	Targeted drug delivery of micro hydrophobic drugs
8	Capronor	Contraceptive implant
9	Cytoplast resorb	Membrane for regeneration of tissue
10	Dacron	Used Vascular grafts and in arm fractures
11	Degrapol	In-vivo treatment of biological tissue lesions
12	Dermabond	An FDA approved tissue adhesive
13	Dexon	FDA approved biodegradable sutures
14	Gliadel	Wafer implant used in brain tumor, hodgkin's disease,

		multiple myeloma and non-hodgkin's lymphoma
15	Hyaff	Wound dressing
16	Lupron depot	Carrier for drug delivery
17	Maxon	Suture material
18	Monacryl	Suture material
19	Ossigel	Graft for synthetic bone
20	Polynova	Orthopedic uses
21	Resomer	Bioresorbable Implant
22	Synbiosys	Pain reliever
23	Synvisc, orthovisc	Drug delivery vehicle
24	Transcyte	Bioengineered skin equivalents

1.1.6 CHALLENGES TO IDEAL EFFICIENCY OF SCAFFOLDS:

There are many challenges that limit the ideal efficiency and hence the potential applications of tissue engineering construct.

1.1.6.1 INADEQUATE NETWORK CHANNEL:

Inadequate network channel to support cell and nutrient transport essential to bring about cell growth [55].

1.1.6.2 INADEQUATE MECHANICAL STRENGTH:

Inadequate mechanical strength poses another challenge to the proficiency of the scaffold. The mechanical strength of the regenerating construct is not enough to stand biological tensile force in vivo. Hence the load bearing functions of the scaffolds are compromised.

1.1.6.3 UNATTAINED BIODEGRADATION:

Unattained biodegradation of the implant leads to the dire need of its removal through surgery.

1.1.6.4 LACK OF BIOCOMPATIBILITY:

The toxic degradation products i.e. lack of biocompatibility can provoke immune or inflammatory reaction in vivo [56].

None of the preparatory material meets all the challenges hence composite formulation offers the solution to the problem [56].

1.1.7 RECENT ADVANCES IN THE DOMAIN OF TISSUE ENGINEERING SCAFFOLDS:

The recent work done (2015-2020) in the domain of tissue engineering scaffolds and their limitations are tabulated in Table 7-10.

Table 7: Summary of Some of the Work Done in 2015-2017

Sr. #	Scaffolds	Properties/Innovations	Limitations and Future Directions	Reference
1	Clay nanotube-biopolymer composite scaffolds	• Chitosan-agarose-gelatin scaffolds with halloysite nanotube doping (3% and 6%) were fabricated by freeze drying method • Halloysite nanotubes doping added mechanical strength to the constructs and improved their swelling behavior • Cell culture was performed with A549, Hep3B, HepG2, PC3 and HCT116 cells • In-vitro cell culture study illustrated cellular attachment and growth • In-vivo study in rats indicated 6 hours degradation period with a little inflammatory response but not tissue rejection to the implanted construct	• Optimization of the formulation is needed • Extension of the study to clinical trials is yet required to implement its applications to tissue engineering	[29]
2	Surface modified cellulose tissue engineering scaffolds	• Surface of the bacterial cellulose was modified to cationic by GTMAC and anionic by oxidation • Cell culture was performed with MG63 cells • Cationic cellulose scaffold illustrated a 70%	• In-vivo study is yet to be conducted to optimize the results • The existing architecture is 2D which could be applied to produce 3D structure by utilization of advanced fabrication techniques i.e. 3D	[57]

		enhancement in cellular attachment as compared to unmodified cellulose scaffold • While anionic cellulose indicated a decreased cellular adhesion as compared to unmodified cellulose scaffold • Surface modification didn't show any degradation to mechanical strength of the substrate	printing	
3	Chitosan-PVA-Methylcellulose tissue engineering scaffolds	• Methylcellulose (MC) was incorporated at different concentration (25%,50%,75%) to Chitosan-PVA blend and porous scaffolds were produced out of this hybrid by freeze drying method • The formulation with enhanced concentration of MC illustrated optimum porosity, mechanical strength and swelling behavior • The substrate illustrated good cellular viability and bactericidal activity against gram positive (E.coli) and gram negative bacteria (S.aureus)	• Further extension of the study to clinical trials will help extend its practical applications to tissue engineering	[58]
4	PEDOT:PSS bone tissue engineering scaffolds	• Porous and conductive scaffolds of PEDOT:PSS were fabricated by freeze drying method • The constructs showed well interconnected network structure • Cell culture was performed with MC3T3-E1 cells and cell differentiation into mature cells was observed	• In-vivo study is crucial to extend its practical applications	[11]
5	3D- Nano fiber assembled tissue engineering	• Three dimensional nano fiber assembled scaffold of	• In-vivo study is crucial to extend its practical	[59]

	scaffolds	Gelatin-PLA was prepared by joining electrospinning as well as lyophilization technique • The prepared construct turned out to be super-elastic and super-absorbent • Cell culture was performed with L-929 cells which demonstrated cell adhesion, proliferation and infiltration	applications	
6	Alginate-lignin hybrid tissue engineering scaffolds	• Alginate-lignin aerogels are produced as tissue engineering scaffolds with the gelation induced by CO_2 • Macroporosity with well interconnected network was achieved • Constructs exhibited low stiffness and good wet ability • Cell culture study with L929 cells illustrated these aerogels substrates to be non-cytotoxic and possessing good cell adhesion property	• In-vivo study is required to optimize the results of these aerogels to advance their practical applications as tissue engineering scaffolds	[60]
7	Open cellular PLLA tissue engineering scaffolds	• PLLA scaffolds were prepared by combining pressure induced flow and CO_2 foaming technique • The resultant substrate had porous and open cellular architecture with good compressive strength • The cell culture study with MEFs cells illustrated good cellular attachment, proliferation and a supportive nutrient movement	• In-vivo evaluation of these constructs have the potential to advance their application in tissue engineering	[61]
8	Vascularizable tissue engineering scaffolds	• PVA integrated porous scaffold was fabricated • The developed construct was perfused in a	• In-vivo evaluation of these constructs have the potential to advance their application in tissue engineering	[62]

		bioreactor and it produced an endothelium which was hierarchically branched		
9	Chitosan-chitin nanocrystal tissue engineering scaffolds	• Chitosan-chitin scaffolds were prepared by freeze drying technique • The resultant constructs exhibited better compressive strength and porosity with well interconnectivity • Cell culture with MC3T3-E1 cells illustrated good cell attachment and proliferation while the constructs showed low cell toxicity and good biocompatibility	• In-vivo evaluation of these constructs have the potential to advance their application in tissue engineering	[25]
10	Bacterial cellulose-alginate composite tissue engineering scaffolds	• Novel bacterial cellulose-alginate (N-BCA) composite scaffolds were fabricated by lyophilization followed by surface modification with ca^{2+} and then a second lyophilization • The resultant scaffolds exhibited 6.5times better swelling behavior as compared to that of the bacterial cellulose scaffolds • The substrates illustrated improved porosity, no cellular toxicity and an enhanced cellular attachment and proliferation with L929 cell culture study	• In-vivo study is needed to extend its practical applications	[63]

Table 8: Summary of Some of the Work Done in 2018

Sr. #	Scaffolds	Properties/Innovations	Limitations and Future Directions	Reference
1	Esophageal Tissue	• Electrospun poly-amide-6	• Endothelial cells of human	[64]

	Engineering Scaffolds	scaffold was prepared to imitate esophageal ECM • Mechanical strength of the construct turned out to be greater than native ECM • In-vitro assessment of Edipose mesenchymal stromal cells as well as bone marrow mesenchymal stromal cells of human exhibited biocompatibility with the substrate	Umbilical vein resulted in inadequate cell growth • In-vivo assessment needs to be performed	
2	Vasculature tissue engineering scaffolds	• Collagen scaffold was generated by dellularization of tendon and cross-linking with bovine elastin for tissue engineering of the vessels • The construct with 15% elastin concentration exhibited optimum mechanical strength • Cell culture study with human smooth muscle cells and endothelial cells showed more than 80% viability	• In-vivo analysis is requisite to be performed for evaluating lasting functionality of these constructs	[65]
3	Nerve Tissue Engineering Scaffolds	• PCL/gelatin scaffold with coating of platelet-rich plasma were fabricated by electrospinning technique • Cell culture was performed with SADS cells of human scalp adipose tissue after being treated with insulin, indomethacin and isobutylmethylxanthine • The result indicated the expression and differentiation of early neuronal markers i.e. NEUN and mature neuronal markers i.e., MAP2, TAU while mature as astrocyte marker i.e. GFAP was not exhibited	• In-vivo assessment of the results of these template architectures is needed to advance its applications in nerve tissue engineering	[66]

4	Skin Tissue Engineering Scaffolds	• Different types of skin tissue engineering scaffolds were fabricated using collagen type I, composite of collagen type I and PCL, composite of collagen type I and PLLA, hybrid of PCL and PLLA, hybrid of collagen type I , PCL and PLLA by freeze drying technique • Cell culture study was performed with AT-MSCs. • Collagen type I scaffold exhibited best cellular anchorage and tissue development • PCL-PLLA scaffold showed optimum mechanical strength • PCL-PLLA-Collagen type I hybrid scaffold expressed best porosity and well interconnected network structure	• Optimization of the formulation and in-vivo analysis is needed to further in this horizon	[67]
5	Bone Tissue Engineering Scaffolds	• Porous composite scaffolds were prepared with 45S5 bioactive glass and cross linked with collagen utilizing foam replica technique • Collagen layer was a few micrometers that added a 5-fold increment to compressive strength of the construct • Macroporous substrate exhibited positive cellular viability, adherence and proliferation with MG63 cell culture	• In-vivo study of the functionality of the construct is required to rationalize its applications	[68]
6	Neural Tissue Engineering Scaffolds	• Neural tissue engineering scaffolds were fabricated with PCL , Polypyrrole and chitosan using electrospinning technique	• In-vivo study has not been performed which is needed to extend the practical applications of this substrate for neural tissue engineering	[69]

		• In-vitro cell culture study was performed with PC12 cells. • PCL-chitosan-polypyrrole composite scaffold illustrated enhanced hydrophillicity, good cellular adherence and advanced proliferation		
7	Bone and Neural Tissue Engineering Scaffolds	• Scaffold for bone and neural tissue engineering were fabricated by thermally induced phase separation technique using a various proportioned compositions of a new synthetic polymer PPF-co-PLGA and a binary solvent dioxane:water • The formulation with 8-10% polymer and 80/20 - 82/18% solvent concentration showed optimum results • In-vitro study was performed with MC3T3-E1 and PC12 cells for bone and neural cell culture respectively • Cell toxicity study illustrated good cell adhesion and proliferation and prove these construct promising for bone and neural tissue engineering	• In-vivo study of these templates is needed for further advancement in their practical applications	[70]
8	Cartilage Tissue Engineering Scaffolds	• Cartilage tissue engineering scaffolds were fabricated through freeze drying technique using hyaluronic acid, graphene oxide and tannic acid while iron chloride as cross linker • The prepared scaffolds illustrated improved mechanical strength and porosity while biological	• Biological study of these templates is needed to excel in their practical applications	[71]

		study was not performed		
9	Heart valves Tissue Engineering Scaffolds	• Nano fibrous scaffolds were prepared using silk fibroin and LDI-PEUU by electrospinning technique • Cytotoxicity study was conducted on these novel composite scaffolds with human umbilical vein endothelial cells • The formulation with the ratio SF/LDI-PEUU 40:60 illustrated optimum results • Cell culture test showed good viability and cell proliferation	• In-vivo study has not been performed which is needed to extend the practical applications of this substrate for heart valve tissue engineering	[72]
10	Soft and Hard Tissue Engineering Scaffolds	• Scaffolds using collagen with quaternary ammonium organosilane (QOS) as cross linker at various concentration (0.1-10)% were fabricated electrospinning method • Cell culture was performed with hFOb and hDF cells • The formulation with 0.1% cross linker concentration illustrated enhanced cell proliferation • The QOS cross linking also incorporated anti-infective properties	• In-vivo study of these template architectures is needed to extend their practical applications	[73]

Table 9: Summary of Some of the Work Done in 2019

Sr. #	Scaffolds	Properties/Innovations	Limitations and Future Direction	Reference
1	Corneal tissue engineering scaffolds	• Some corneal scaffolds providing native corneal environment include poly(L-lactic acid), PLGA, poly(L-lactide-co-DL-lactide) • Boston Kpro type I is the most commonly used keratoprostheses • Complications in this	• Post-operative complications are a limit to the approach which are needed to be overcome in order to make further advancement in this horizon	[74]

		horizon are the rejection, infection and recurrence of the original injury • Post-operative management is essential for the success of host-implant junction suturing		
2	Periodontal tissue reengineering scaffolds	• The properties of different natural, synthetic materials, bioceramics as well as various composites thereof are expanded and their applications in periodontal tissue engineering are summarized • 3D bioprinting technique to fabricate periodontal tissue engineering scaffolds is reviewed along with the briefing over multiphasic scaffolds and concept of compartmentalization in the scaffolds to control spatiotemporal events and to avoid the ankylosis of the tooth root	• Inadequately studied biological, mechanical and degradation properties • Inadequate reporting of clinical applications • Non-validated clinical trials • Testing performed on only small animals • Composite scaffolds with combination of different fabricating techniques along with new insertion methods are needed to be developed to overcome limitations in this regard, avoid postsurgical morbidities and for producing more promising results	[75]
3	Bladder tissue reengineering scaffolds	• An overview of recent progress in the field of bladder tissue engineering is given along with brief over clinical trials results • Decellularized scaffolds for bladder tissue reengineering are small intestine submucosa (SIS) , Bladder acellular matrix graft (BAMG) • Naturally derived material scaffolds include collagen-gel scaffolds, Silk-fibrion scaffolds • Synthetic material scaffolds include PEG hydrogel scaffolds	• Inadequate innervation and vascularization • Inadequate control of design-of-materials before implantation • Bladder tissue-engineering scaffolds with controlled topography and angiogenesis factors are yet required to be produced in future	[76]

		• Composite scaffolds include Collagen with PLGA and collagen with PGA for bladder tissue engineering • Successful scaffolds for bladder tissue engineering reported are PGA: PDLLG for dogs and Cell seeded collagen-PGA for human		
4	Intestinal tissue reengineering scaffolds	• Gut-on-a-chip device leads to micro level understanding of the cellular responses, drug/nutrient transport and gene/protein engineering • Some decellularized intestinal scaffolds are: decellularized porcine intestinal mucosa, decellularized human small intestine, decellularized porcine ileum • Natural polymer intestinal scaffolds are: collagen-chitosan composite, chitosan hydrogel, silk fibroin • Some synthetic material intestinal scaffolds are: PGA and PLLA composite, PCL, PGS and PLLA composite	• Incomplete understanding of the complex anatomy and function of the intestine • Utilization of the devices i.e. endoscopic, laparoscopic for minimal invasive delivery is needed • Patient-specific therapy needs to be developed	[77]
5	Cardiac muscle reengineering scaffolds	• Control of the diameter as well as morphology of the fiber to enhance its natural ECM imitation. • Integration with nobel metals e.g. Gold, Platinum and carbon nanoparticles for modifying mechanical properties and conductivity. • Impregnation of scaffolds with electronic mesh	• Further improvement of yet inadequate mimicking of natural cardiac ECM, control and monitoring of the in-vivo functionality of the construct • Complete control of the functionality of the tissue engineering scaffold by the interspersed algorithm integrating set of parameters i.e. pH, temperature,	[78]

		network having elecrodes for non-invasive online control and monitoring of tissue engineering	pressure etc and acting based on the data collected out of it, with no requisite for human external intervention	
6	Pancreatic muscle reengineering scaffolds	• Practical demonstration of the requirement of ECM for the survival of pancreatic beta cells • Coating of the natural polymer microcapsules by Poly-L-ornithine (PLO) to reduce immune reaction and improve mechanical strength • Several synthetic polymers polydimethylsiloxane (PDMS), Poly (D,L-lactide-co-E-caprolactone) (PDLLCL), Polytetrafluoroethylene (PTFE) studied and found applications in pancreatic tissue engineering. • Encapsulation of decellularized ECM as tissue engineering engrafts into the hydrogels for immunosuppression were studied and found fruitful	• Further improvement of yet Inadequate understanding of the characteristics of the ECM of pancreas and function of its molecules	[79]
7	Tendon reengineering scaffolds	• An overview and crucial discussion on existing biomaterials and tissue-implant based techniques for tendon tissue engineering has been provided • Electrospun devices and imprinting technology have potential for future applications in this field • Many hydrogel scaffolds i.e. collagen hydrogel, alginate hydrogel, fibrin hydrogel, chitosan-collagen hydrogel along	• Inadequate clinical applications • Inadequate understanding and hence imitation of the natural ECM and the cellular events behind the growth of tendon • The implantable device with non-toxic, non-immunogenic and adequate mechanical strength is needed to be developed	[80]

		with alignment with PLLA etc are there for wound healing or other applications but none has been reported for tendon repair • Some currently available products have potential for tendon tissue engineering i.e. Collagen Matrix Inc, Avitene, Bicol which are all collagen sponges. • Decellularized allografts and xenografts are promising approaches for tendon tissue engineering		
8	Ligament reengineering scaffolds	• Biological scaffolds, biodegradable and tissue engineered non biodegradabe scaffolds are used for ligament tissue engineering • Poor Mechanical strength, unpredictable degradation rate, storage time, sterility, biocompatibility, inadequate understanding of the phenomenon "lag" are yet limitations in this area	• Inadequate understanding and hence control over predictability of wound healing, degradation rate, storage time, sterility, biocompatibility, lag phenomenon which needed to be overcome to further in this domain	[81]
9	Epidermal tissue reengineering scaffolds	• Bio artificial skin is the very first tissue engineering product. • Scaffolds incorporating dermal components i.e. fibroblasts produce full-thickness skin equivalents • Scaffolds incorporating live epidermis produce split-thickness skin which is used in chronic wound closures and reconstructive surgeries (excessive burns) • Some products i.e. fibrin glue, hyaluronic acid, gelatin and a composite of	• Bio artificial skin is very costly that causes limit to its abundant application • Research needs to expand on incorporating other components into it i.e. melanocytes, immune cells etc. and use of artificial skin to release insulin • Disparity in the permeability of cultured skin and in-vivo epidermis is needed to be overcome	[82]

		collagen/HA and gelatin scaffolds are there for epidermal tissue engineering • Skin-on-chip microdevices could be used for cell response measurement i.e. contact dermatitis sensitization measurement		
10	Optic nerve tissue reengineering scaffolds	• Remedy of optic nerve injury through scaffolds includes the self-assembling synthetic biomaterials that form porous scaffolds. It provides optimal microenvironment for retinal exon reconstruction • An example includes a composite scaffold of polycaprolactone and poly-gamma-benzyl-L-glutamate fabricated through three dimensional electrospinning techniques along with combined cultured RGC progenitors.	• Future directive in this domain is incorporation of cessation of degeneration process into the scaffold along with its regenerative function	[83]
11	Bone Tissue Engineering Scaffolds	• Graphene oxide and amine modified graphene oxide incorporated chitosan-gelatin scaffolds with glutaraldehyde as cross linking agent were fabricated through freeze drying technique for bone tissue engineering • Cell toxicity study was conducted with MG63 cells • These modified scaffolds illustrated improved physicochemical characterization as well as better cellular	• In-vivo analysis is needed to further the advancement of its practical implementation in tissue engineering	[84]

		viability		

Table 10: Summary of Some of the Work Done in 2020

Sr. #	Scaffolds	Properties/Innovations	Limitations and Future Direction	Reference
1	Bone Tissue Engineering Scaffolds	• Chitosan + 45S5 bioactive glass composite scaffolds were fabricated through one pot electrophoretic deposition method • Testing indicated non-cytotoxic and antibacterial activity which proved them promising for BTE	• In-vitro testing is needed to optimize the product and then in-vivo testing and clinical trials are yet needed to use this product commercially	[85]
2	Bone Tissue Engineering Scaffolds	• Hydroxyapatite + bioglass scaffolds for BTE were fabricated • Antimicrobial and non-immunogenic properties of this scaffolds were its crucial properties	• Clinical trials are yet needed to be performed to substantiate the findings and be used commercially	[86]
3	Bone Tissue Engineering Scaffolds	• PLA + Akermanite (Ak) scaffolds were fabricated for BTE • The mechanical strength of these scaffolds i.e. 45±3MPa turned out to be nearly equivalent to the strength of human bone cortical	• Optimized proportion of the two polymers in the composite scaffolds has been identified i.e 20% Ak which is to be further strengthened by clinical trials of the optimized product	[87]
4	Bone Tissue Engineering Scaffolds	• PCL+ PVA + gelatin + HA scaffolds were fabricated for BTE • Mechanical strength and cellular mineralization was significantly improved	• Clinical trials of the product are not yet performed	[88]
5	Bone Tissue Engineering Scaffolds	• PLA and SBA-15 ceramic composite scaffolds were fabricated for BTE • These scaffolds indicated non-cytotoxicity in in-vitro testing	• Product optimization is needed to be further strengthened by clinical trials	[89]
6	Bone Tissue Engineering Scaffolds	• PLLA + CHWs were fabricated for BTE • At 0-20% CHWs, the	• At 40% CHWs increase, the scaffolds showed decreased compressive strength and affinity for cells but an	[90]

		scaffolds showed improved compressive strength, hydrophilicity and cellular attachment, growth and differentiation	increased anti-inflammatory property • Product optimization along with clinical trials are needed	
7	Bone Tissue Engineering Scaffolds	• CLN–nHA/CS–G composite scaffolds were fabricated for BTE • The scaffolds showed improved pore size , swelling ability and biocompatibility	• Degradation rate and mechanical strength of the scaffolds were not optimum • Optimization of the product coupled with clinical trials is needed	[91]
8	Bone Tissue Engineering Scaffolds	• ACGO composite scaffolds were fabricated through freeze drying technique • The scaffolds showed improved mechanical strength, shape retention capability, porosity as well as biocompatible properties	• Degradation rate of the scaffolds didn't turn out optimum • Optimization of the product yet needed • Clinical trials of the optimized product are needed before making it available for use commercially	[92]
9	Bone Tissue Engineering Scaffolds	• PCL + HAp incorporated with SiO_2 scaffolds were fabricated for BTE. • The optimized product with 10% by weight SiO_2 and PCL:HAp in 75:15 showed good pore size, mechanical strength, cellular proliferation and non-cytotoxic properties	• In-vivo optimization and Clinical trials are needed to be performed to strengthen the results obtained for in-vitro studies	[93]
10	Bone Tissue Engineering Scaffolds	• PLLA + gMgOs and PLLA + gHNTs were fabricated for BTE • The former scaffold showed improved cellular adhesion, proliferation and differentiation while later improved mechanical strength	• Optimization of the product is needed uniting, mechanical strength and cellular adhesion, proliferation and differentiation together in one product. • Clinical trials of the optimized product are needed before making it available commercially	[94]

2 CONCLUSION:

Tissue engineering claims the promise to resume quality life that becomes compromised due to improperly or dysfunctional functional tissue/organs. Provision of a 3D artificial substrate that mimics host's native biological environment for cells growth and their differentiation into tissues/organs replaces the need of external organ transplantation. Down the ages many fabrication materials and techniques have been introduced to produce a tissue engineering construct with all ideal properties but yet there are many challenges that are limiting its practical applications. These challenges are met by combining the ideal properties of different individual biomaterials and fabricating techniques into composite formulations which manifest all required traits. Over last five years many formulations have been claimed for the engineering of various tissue organs i.e. bone, cardiovascular, bladder, skeletal, neurons etc. However, in-vivo studies in this domain have not yet seen many advances. Because of this, tissue engineering scaffolds have yet very few practical applications. In order to widen the sphere of practical applications of tissue engineering scaffolds it is now required to extend their in-vivo studies and clinical trials.

3 FUTURE DIRECTIONS:

The field of tissue engineering has overcome the conventional limitations incurred to it over the ages i.e. inadequate mechanical strength to bear cellular process pressure, inadequate interconnected porous web to facilitate cell adhesion and proliferation, lack of biocompatibility and non-synchronized biodegradation of the implant. Many formulations recently developed claim to render a solution to these challenges; however, the domain yet finds very limited practical applications. This is because a well-collaborated effort in this domain is needed. The formulations that claim to overcome conventional limitations are now required to be tested in-

vivo and undergone clinical trials in order to utilize their benefit practically and restore quality life. There is yet further need to experiment hybridization of various techniques and biomaterials to unite their exclusive valuable properties into one ideal product. This needs to be then furthered with in-vivo testing and clinical trials to bring their use in practical domain.

ABBREVIATIONS:

PBS – Polybutylene Succinate

CNF - Cellulose Nano Fibrils

PLA - Polylactic Acid

PVA – Polyvinyl Alcohol

TCP - Tricalcium Phosphate

CNC - Cellulose Nano Crystals

MWCNT - Multiwalled Carbon Nanotube

PU - Polyurethane

UPCL - Urethane Polycaprolacone

PANI - Polyaniline

BC - Bacterial Cellulose

HEC - Hydroxylethyl Cellulose

DCPD - Dicalcium Phosphate Dehydrate

PGS - Poly Glycerol Sebacate

GO – Graphene Oxide

AUTHOR CONTRIBUTIONS:

The manuscript was written through contributions of all authors. All authors have given their approval to the final version of the manuscript.

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REFERENCES:

[1] L. E. Dickinson and S. Gerecht, "Engineered biopolymeric scaffolds for chronic wound healing," *Frontiers in Physiology,* vol. 7, p. 341, 2016.
[2] S. Salminger, A. Roche, A. Sturma, J. Mayer, and O. Aszmann, "Hand transplantation versus hand prosthetics: pros and cons," *Current surgery reports,* vol. 4, p. 8, 2016.
[3] N. J. Collins, R. H. Bridson, G. A. Leeke, and L. M. Grover, "Particle seeding enhances interconnectivity in polymeric scaffolds foamed using supercritical CO2," *Acta Biomaterialia,* vol. 6, pp. 1055-1060, 2010.
[4] T. M. Snyder, K. K. Khush, H. A. Valantine, and S. R. Quake, "Universal noninvasive detection of solid organ transplant rejection," *Proceedings of the National Academy of Sciences,* vol. 108, pp. 6229-6234, 2011.
[5] Y. Liu, D. Cai, J. Yang, Y. Wang, X. Zhang, and S. Yin, "In vitro hemocompatibility evaluation of poly (4-hydroxybutyrate) scaffold," *International journal of clinical and experimental medicine,* vol. 7, p. 1233, 2014.
[6] D. Brahatheeswaran, Y. Yasuhiko, M. Toru, and K. D. Sakthi, "Polymeric scaffolds in tissue engineering application: a review," *International Journal of Polymer Science,* vol. 2011, pp. 1-19, 2011.
[7] A. Venezuela, "Porous Titanium Scaffolds Produced by Powder Metallurgy for Biomedical Applications," *Materials Research,* vol. 11, pp. 1-00, 2008.
[8] P. X. Ma, "Scaffolds for tissue fabrication," *Materials today,* vol. 7, pp. 30-40, 2004.
[9] S. M. Willerth and S. E. Sakiyama-Elbert, "Combining stem cells and biomaterial scaffolds for constructing tissues and cell delivery," *StemJournal,* pp. 1-25, 2008.
[10] S. Udhayakumar, K. G. Shankar, S. Sowndarya, S. Venkatesh, C. Muralidharan, and C. Rose, "l-Arginine intercedes bio-crosslinking of a collagen–chitosan 3D-hybrid scaffold for tissue engineering and regeneration: in silico, in vitro, and in vivo studies," *RSC Advances,* vol. 7, pp. 25070-25088, 2017.
[11] A. G. Guex, J. L. Puetzer, A. Armgarth, E. Littmann, E. Stavrinidou, E. P. Giannelis*, et al.*, "Highly porous scaffolds of PEDOT: PSS for bone tissue engineering," *Acta biomaterialia,* vol. 62, pp. 91-101, 2017.
[12] D. Mondal, S. J. Dixon, K. Mequanint, and A. S. Rizkalla, "Mechanically-competent and cytocompatible polycaprolactone-borophosphosilicate hybrid biomaterials," *Journal of the mechanical behavior of biomedical materials,* vol. 75, pp. 180-189, 2017.
[13] T. Momose, H. Miyaji, A. Kato, K. Ogawa, T. Yoshida, E. Nishida*, et al.*, "Collagen hydrogel scaffold and fibroblast growth factor-2 accelerate periodontal healing of class ii furcation defects in dog," *The open dentistry journal,* vol. 10, p. 347, 2016.
[14] J. S. Castro, L. V. Tapia, R. A. Silveyra, C. A. Martinez, and P. A. Deymier, "Negative impact of paclitaxel crystallization on hydrogels and novel approaches for anticancer drug delivery systems," in *Current Cancer Treatment-Novel Beyond Conventional Approaches*, ed: IntechOpen, 2011.
[15] J. D. Patient, H. Hajiali, K. Harris, B. Abrahamsson, C. Tannergren, L. J. White*, et al.*, "Nanofibrous scaffolds support a 3D in vitro permeability model of the human intestinal epithelium," *Frontiers in pharmacology,* vol. 10, p. 456, 2019.

[16] K. Wei and I.-S. Kim, "Fabrication of nanofibrous scaffolds by electrospinning," *Advances in Nanofibers,* vol. 1, p. 103, 2013.
[17] W. Wang, Y. Liu, C. Yang, X. Qi, S. Li, C. Liu*, et al.*, "Mesoporous bioactive glass combined with graphene oxide scaffolds for bone repair," *International journal of biological sciences,* vol. 15, p. 2156, 2019.
[18] T. Garg, O. Singh, S. Arora, and R. Murthy, "Scaffold: a novel carrier for cell and drug delivery," *Critical Reviews™ in Therapeutic Drug Carrier Systems,* vol. 29, 2012.
[19] B. Dhandayuthapani, Y. Yoshida, T. Maekawa, and D. S. Kumar, "Polymeric scaffolds in tissue engineering application: a review," *International journal of polymer science,* vol. 2011, 2011.
[20] K. Leong, s. C. Chua, N. Sudarmadji, and W. Yeong, "Engineering functionally graded tissue engineering scaffolds," *Journal of the mechanical behavior of biomedical materials,* vol. 1, pp. 140-152, 2008.
[21] S. Yang, K.-F. Leong, Z. Du, and C.-K. Chua, "The design of scaffolds for use in tissue engineering. Part I. Traditional factors," *Tissue engineering,* vol. 7, pp. 679-689, 2001.
[22] M. Okamoto and B. John, "Synthetic biopolymer nanocomposites for tissue engineering scaffolds," *Progress in Polymer Science,* vol. 38, pp. 1487-1503, 2013.
[23] B. Chang, N. Ahuja, C. Ma, and X. Liu, "Injectable scaffolds: Preparation and application in dental and craniofacial regeneration," *Materials Science and Engineering: R: Reports,* vol. 111, pp. 1-26, 2017.
[24] M.-W. Sa and J. Y. Kim, "Design of multi-scaffold fabrication system for various 3D scaffolds," *Journal of Mechanical Science and Technology,* vol. 27, pp. 2961-2966, 2013.
[25] M. Liu, H. Zheng, J. Chen, S. Li, J. Huang, and C. Zhou, "Chitosan-chitin nanocrystal composite scaffolds for tissue engineering," *Carbohydrate polymers,* vol. 152, pp. 832-840, 2016.
[26] A. Teimouri, M. Azadi, R. Emadi, J. Lari, and A. N. Chermahini, "Preparation, characterization, degradation and biocompatibility of different silk fibroin based composite scaffolds prepared by freeze-drying method for tissue engineering application," *Polymer degradation and stability,* vol. 121, pp. 18-29, 2015.
[27] H.-L. Kim, G.-Y. Jung, J.-H. Yoon, J.-S. Han, Y.-J. Park, D.-G. Kim*, et al.*, "Preparation and characterization of nano-sized hydroxyapatite/alginate/chitosan composite scaffolds for bone tissue engineering," *Materials Science and Engineering: C,* vol. 54, pp. 20-25, 2015.
[28] M. Kucharska, K. Walenko, M. Lewandowska-Szumieł, T. Brynk, J. Jaroszewicz, and T. Ciach, "Chitosan and composite microsphere-based scaffold for bone tissue engineering: evaluation of tricalcium phosphate content influence on physical and biological properties," *Journal of Materials Science: Materials in Medicine,* vol. 26, p. 143, 2015.
[29] E. A. Naumenko, I. D. Guryanov, R. Yendluri, Y. M. Lvov, and R. F. Fakhrullin, "Clay nanotube–biopolymer composite scaffolds for tissue engineering," *Nanoscale,* vol. 8, pp. 7257-7271, 2016.
[30] T. Muthukumar, A. Aravinthan, J. Sharmila, N. S. Kim, and J.-H. Kim, "Collagen/chitosan porous bone tissue engineering composite scaffold incorporated with Ginseng compound K," *Carbohydrate polymers,* vol. 152, pp. 566-574, 2016.
[31] B. K. Shrestha, H. M. Mousa, A. P. Tiwari, S. W. Ko, C. H. Park, and C. S. Kim, "Development of polyamide-6, 6/chitosan electrospun hybrid nanofibrous scaffolds for tissue engineering application," *Carbohydrate polymers,* vol. 148, pp. 107-114, 2016.
[32] E. Entekhabi, M. H. Nazarpak, F. Moztarzadeh, and A. Sadeghi, "Design and manufacture of neural tissue engineering scaffolds using hyaluronic acid and polycaprolactone nanofibers with controlled porosity," *Materials Science and Engineering: C,* vol. 69, pp. 380-387, 2016.
[33] A. Teimouri and M. Azadi, "β-Chitin/gelatin/nanohydroxyapatite composite scaffold prepared through freeze-drying method for tissue engineering applications," *Polymer Bulletin,* vol. 73, pp. 3513-3529, 2016.

[34] A. Teimouri and M. Azadi, "Preparation and characterization of novel chitosan/nanodiopside/nanohydroxyapatite composite scaffolds for tissue engineering applications," *International Journal of Polymeric Materials and Polymeric Biomaterials,* vol. 65, pp. 917-927, 2016.

[35] Y.-C. Jiang, L. Jiang, A. Huang, X.-F. Wang, Q. Li, and L.-S. Turng, "Electrospun polycaprolactone/gelatin composites with enhanced cell–matrix interactions as blood vessel endothelial layer scaffolds," *Materials Science and Engineering: C,* vol. 71, pp. 901-908, 2017.

[36] W. Nie, C. Peng, X. Zhou, L. Chen, W. Wang, Y. Zhang*, et al.*, "Three-dimensional porous scaffold by self-assembly of reduced graphene oxide and nano-hydroxyapatite composites for bone tissue engineering," *Carbon,* vol. 116, pp. 325-337, 2017.

[37] X. Zhang, C. Jia, X. Qiao, T. Liu, and K. Sun, "Silk fibroin microfibers and chitosan modified poly (glycerol sebacate) composite scaffolds for skin tissue engineering," *Polymer Testing,* vol. 62, pp. 88-95, 2017.

[38] A. Moatary, A. Teimouri, M. Bagherzadeh, A. N. Chermahini, and R. Razavizadeh, "Design and fabrication of novel chitin hydrogel/chitosan/nano diopside composite scaffolds for tissue engineering," *Ceramics International,* vol. 43, pp. 1657-1668, 2017.

[39] S. Türk, I. Altınsoy, G. Ç. Efe, M. Ipek, M. Özacar, and C. Bindal, "3D porous collagen/functionalized multiwalled carbon nanotube/chitosan/hydroxyapatite composite scaffolds for bone tissue engineering," *Materials Science and Engineering: C,* vol. 92, pp. 757-768, 2018.

[40] A. Teimouri, S. Roohafza, M. Azadi, and A. N. Chermahini, "Fabrication and characterization of chitosan/gelatin/nanodiopside composite scaffolds for tissue engineering application," *Polymer Bulletin,* vol. 75, pp. 1487-1504, 2018.

[41] A. Zheng, L. Cao, Y. Liu, J. Wu, D. Zeng, L. Hu*, et al.*, "Biocompatible silk/calcium silicate/sodium alginate composite scaffolds for bone tissue engineering," *Carbohydrate polymers,* vol. 199, pp. 244-255, 2018.

[42] W. Luo, L. Cheng, C. Yuan, Z. Wu, G. Yuan, M. Hou*, et al.*, "Preparation, characterization and evaluation of cellulose nanocrystal/poly (lactic acid) in situ nanocomposite scaffolds for tissue engineering," *International journal of biological macromolecules,* vol. 134, pp. 469-479, 2019.

[43] T. Abudula, U. Saeed, A. Memic, K. Gauthaman, M. A. Hussain, and H. Al-Turaif, "Electrospun cellulose Nano fibril reinforced PLA/PBS composite scaffold for vascular tissue engineering," *Journal of Polymer Research,* vol. 26, p. 110, 2019.

[44] G. Chen, N. Chen, and Q. Wang, "Fabrication and properties of poly (vinyl alcohol)/β-tricalcium phosphate composite scaffolds via fused deposition modeling for bone tissue engineering," *Composites Science and Technology,* vol. 172, pp. 17-28, 2019.

[45] E. Hasanzadeh, S. Ebrahimi-Barough, E. Mirzaei, M. Azami, S. M. Tavangar, N. Mahmoodi*, et al.*, "Preparation of fibrin gel scaffolds containing MWCNT/PU nanofibers for neural tissue engineering," *Journal of Biomedical Materials Research Part A,* vol. 107, pp. 802-814, 2019.

[46] M. J. Woźniak, A. Chlanda, P. Oberbek, M. Heljak, K. Czarnecka, M. Janeta*, et al.*, "Binary bioactive glass composite scaffolds for bone tissue engineering—Structure and mechanical properties in micro and nano scale. A preliminary study," *Micron,* vol. 119, pp. 64-71, 2019.

[47] B. Akbari and J. Nourmohammadi, "Nanoclay Reinforced Starch-Polycaprolactone Scaffolds for Bone Tissue Engineering," *Journal of Tissues and Materials,* vol. 2, pp. 55-63, 2019.

[48] M. H. Nazarpak, E. Entekhabi, F. Najafi, M. Rahmani, and M. Solati Hashjin, "Synthesis and characterization of conductive neural tissue engineering scaffolds based on urethane-polycaprolactone," *International Journal of Polymeric Materials and Polymeric Biomaterials,* vol. 68, pp. 827-835, 2019.

[49] E. Altun, N. Ekren, S. E. Kuruca, and O. Gunduz, "Cell studies on Electrohydrodynamic (EHD)-3D-bioprinted Bacterial Cellulose\Polycaprolactone scaffolds for tissue engineering," *Materials Letters,* vol. 234, pp. 163-167, 2019.

[50] F. H. Zulkifli, F. S. J. Hussain, W. Harun, and M. M. Yusoff, "Highly porous of hydroxyethyl cellulose biocomposite scaffolds for tissue engineering," *International journal of biological macromolecules,* vol. 122, pp. 562-571, 2019.

[51] M. A. Taghavi, S. M. Rabiee, M. Jahanshahi, and F. Nasiri, "Electrospun Poly-ε-Caprolactone (PCL)/Dicalcium Phosphate Dihydrate (DCPD) Composite Scaffold for Tissue Engineering Application," *Molecular biotechnology,* vol. 61, pp. 345-354, 2019.

[52] M. H. Ibrahim, J. Ady, D. Hikmawati, and M. Yasin, "Synthesis and Characterization of Nano-Hidroxyapatite/Chitosan/Carboxymethyl Cellulose Composite Scaffold," *Journal of International Dental & Medical Research,* vol. 12, 2019.

[53] T. Weigel, G. Schinkel, and A. Lendlein, "Design and preparation of polymeric scaffolds for tissue engineering," *Expert Review of Medical Devices,* vol. 3, pp. 835-851, 2006.

[54] G. Caetano, R. Violante, A. B. Sant, A. B. Murashima, M. Domingos, A. Gibson*, et al.*, "Cellularized versus decellularized scaffolds for bone regeneration," *Materials Letters,* vol. 182, pp. 318-322, 2016.

[55] H. Bramfeld, G. Sabra, V. Centis, and P. Vermette, "Scaffold vascularization: a challenge for three-dimensional tissue engineering," *Current medicinal chemistry,* vol. 17, pp. 3944-3967, 2010.

[56] R. Nigam and B. Mahanta, "An overview of various biomimetic scaffolds: Challenges and applications in tissue engineering," *Journal of Tissue Science & Engineering,* vol. 5, p. 1, 2014.

[57] J. C. Courtenay, M. A. Johns, F. Galembeck, C. Deneke, E. M. Lanzoni, C. A. Costa*, et al.*, "Surface modified cellulose scaffolds for tissue engineering," *Cellulose,* vol. 24, pp. 253-267, 2017.

[58] K. Kanimozhi, S. K. Basha, and V. S. Kumari, "Processing and characterization of chitosan/PVA and methylcellulose porous scaffolds for tissue engineering," *Materials Science and Engineering: C,* vol. 61, pp. 484-491, 2016.

[59] W. Chen, J. Ma, L. Zhu, Y. Morsi, S. S. Al-Deyab, and X. Mo, "Superelastic, superabsorbent and 3D nanofiber-assembled scaffold for tissue engineering," *Colloids and Surfaces B: Biointerfaces,* vol. 142, pp. 165-172, 2016.

[60] S. Quraishi, M. Martins, A. A. Barros, P. Gurikov, S. Raman, I. Smirnova*, et al.*, "Novel non-cytotoxic alginate–lignin hybrid aerogels as scaffolds for tissue engineering," *The Journal of Supercritical Fluids,* vol. 105, pp. 1-8, 2015.

[61] T. Kuang, F. Chen, L. Chang, Y. Zhao, D. Fu, X. Gong*, et al.*, "Facile preparation of open-cellular porous poly (l-lactic acid) scaffold by supercritical carbon dioxide foaming for potential tissue engineering applications," *Chemical Engineering Journal,* vol. 307, pp. 1017-1025, 2017.

[62] A. Tocchio, M. Tamplenizza, F. Martello, I. Gerges, E. Rossi, S. Argentiere*, et al.*, "Versatile fabrication of vascularizable scaffolds for large tissue engineering in bioreactor," *Biomaterials,* vol. 45, pp. 124-131, 2015.

[63] S. Kirdponpattara, A. Khamkeaw, N. Sanchavanakit, P. Pavasant, and M. Phisalaphong, "Structural modification and characterization of bacterial cellulose–alginate composite scaffolds for tissue engineering," *Carbohydrate polymers,* vol. 132, pp. 146-155, 2015.

[64] M. Zhuravleva, Z. Gilazieva, T. E. Grigoriev, A. D. Shepelev, T. Kh. Tenchurin, R. Kamyshinsky*, et al.*, "In vitro assessment of electrospun polyamide-6 scaffolds for esophageal tissue engineering," *Journal of Biomedical Materials Research Part B: Applied Biomaterials,* vol. 107, pp. 253-268, 2019.

[65] S. Ghazanfari, K. A. Alberti, Q. Xu, and A. Khademhosseini, "Evaluation of an elastic decellularized tendon-derived scaffold for the vascular tissue engineering application," *Journal of Biomedical Materials Research Part A,* vol. 107, pp. 1225-1234, 2019.

[66] M. Fesharaki, S. Razavi, L. Ghasemi-Mobarakeh, M. Behjati, R. Yarahmadian, M. Kazemi*, et al.*, "Differentiation of human scalp adipose-derived mesenchymal stem cells into mature neural cells on electrospun nanofibrous scaffolds for nerve tissue engineering applications," *Cell Journal (Yakhteh),* vol. 20, p. 168, 2018.

[67] A. Rahmani Del Bakhshayesh, E. Mostafavi, E. Alizadeh, N. Asadi, A. Akbarzadeh, and S. Davaran, "Fabrication of three-dimensional scaffolds based on nano-biomimetic collagen hybrid constructs for skin tissue engineering," *ACS Omega,* vol. 3, pp. 8605-8611, 2018.

[68] J. Hum and A. Boccaccini, "Collagen as coating material for 45S5 bioactive glass-based scaffolds for bone tissue engineering," *International journal of molecular sciences,* vol. 19, p. 1807, 2018.

[69] A. Sadeghi, F. Moztarzadeh, and J. A. Mohandesi, "Investigating the effect of chitosan on hydrophilicity and bioactivity of conductive electrospun composite scaffold for neural tissue engineering," *International journal of biological macromolecules,* vol. 121, pp. 625-632, 2019.

[70] W. Wu, X. Liu, Z. Zhou, A. L. Miller, and L. Lu, "Three-dimensional porous poly (propylene fumarate)-co-poly (lactic-co-glycolic acid) scaffolds for tissue engineering," *Journal of Biomedical Materials Research Part A,* vol. 106, pp. 2507-2517, 2018.

[71] M. Hunger, P. Domalik-Pyzik, and J. Chłopek, "Sodium hyaluronate/graphene oxide hydrogels for cartilage tissue engineering," *Engineering of Biomaterials,* vol. 21, 2018.

[72] J. Du, T. Zhu, H. Yu, J. Zhu, C. Sun, J. Wang*, et al.*, "Potential applications of three-dimensional structure of silk fibroin/poly (ester-urethane) urea nanofibrous scaffold in heart valve tissue engineering," *Applied Surface Science,* vol. 447, pp. 269-278, 2018.

[73] C. Dhand, Y. Balakrishnan, S. T. Ong, N. Dwivedi, J. R. Venugopal, S. Harini*, et al.*, "Antimicrobial quaternary ammonium organosilane cross-linked nanofibrous collagen scaffolds for tissue engineering," *International journal of nanomedicine,* vol. 13, p. 4473, 2018.

[74] S. Yousaf, S. H. Keshel, G. A. Farzi, M. Momeni-Moghadam, E. D. Ahmadi, I. O. Asencio*, et al.*, "Scaffolds for corneal tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 649-672.

[75] F. Asa'ad, A. Monje, and L. Larsson, "Scaffolds for periodontal tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume One*, ed: Elsevier, 2019, pp. 479-504.

[76] N. Kumar, S. Saxena, V. Kumar, S. Shrivastava, A. K. Gangwar, S. K. Maiti*, et al.*, "Scaffolds for bladder tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 493-548.

[77] N. S. Kanetkar and A. K. Ekenseair, "Scaffolds for intestinal tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 593-632.

[78] R. Feiner, A. Shapira, and T. Dvir, "Scaffolds for tissue engineering of functional cardiac muscle," in *Handbook of Tissue Engineering Scaffolds: Volume One*, ed: Elsevier, 2019, pp. 685-703.

[79] C. Gazia, M. Gaffley, A. Asthana, D. Chaimov, and G. Orlando, "Scaffolds for pancreatic tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 765-786.

[80] H. Capella-Monsonís, S. Guillaumin, S. Ribeiro, S. Korntner, Y. Bayon, and D. I. Zeugolis, "Scaffolds for tendon tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume One*, ed: Elsevier, 2019, pp. 259-298.

[81] A. Oluwadamilola, S. Yousaf, M. Zare, M. Mozafari, M. Youseffi, P. Twigg*, et al.*, "Scaffolds for ligament tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume One*, ed: Elsevier, 2019, pp. 299-327.

[82] S. Kumar, H. J. Kang, and F. Berthiaume, "Scaffolds for epidermal tissue engineering," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 173-191.
[83] J. D. Oliver and K. S. Vyas, "Scaffolds for tissue engineering in optic nerve regeneration," in *Handbook of Tissue Engineering Scaffolds: Volume Two*, ed: Elsevier, 2019, pp. 711-720.
[84] A. Olad and H. B. K. Hagh, "Graphene oxide and amin-modified graphene oxide incorporated chitosan-gelatin scaffolds as promising materials for tissue engineering," *Composites Part B: Engineering,* vol. 162, pp. 692-702, 2019.
[85] L. Bonetti, L. Altomare, N. Bono, E. Panno, C. E. Campiglio, L. Draghi*, et al.*, "Electrophoretic processing of chitosan based composite scaffolds with Nb-doped bioactive glass for bone tissue regeneration," *Journal of Materials Science: Materials in Medicine,* vol. 31, pp. 1-12, 2020.
[86] M. Rizwan, K. Genasan, M. R. Murali, H. R. B. Raghavendran, R. Alias, Y. Y. Cheok*, et al.*, "In vitro evaluation of novel low-pressure spark plasma sintered HA–BG composite scaffolds for bone tissue engineering," *RSC Advances,* vol. 10, pp. 23813-23828, 2020.
[87] M. Arastouei, M. Khodaei, S. M. Atyabi, and M. J. Nodoushan, "Poly lactic acid-akermanite composite scaffolds prepared by fused filament fabrication for bone tissue engineering," *Journal of Materials Research and Technology,* vol. 9, pp. 14540-14548, 2020.
[88] H. Kim, H. Hwangbo, Y. Koo, and G. Kim, "Fabrication of Mechanically Reinforced Gelatin/Hydroxyapatite Bio-Composite Scaffolds by Core/Shell Nozzle Printing for Bone Tissue Engineering," *International journal of molecular sciences,* vol. 21, p. 3401, 2020.
[89] O. A. Chanes-Cuevas, U. Arellano-Sánchez, C. A. Álvarez-Gayosso, F. Suaste-Olmos, E. Villarreal-Ramírez, O. Álvarez-Fregoso*, et al.*, "Synthesis of PLA/SBA-15 Composite Scaffolds for Bone Tissue Engineering," *Materials Research,* vol. 23, 2020.
[90] K. Liu, L. Zhu, S. Tang, W. Wen, L. Lu, M. Liu*, et al.*, "Fabrication and evaluation of a chitin whisker/poly (L-lactide) composite scaffold by the direct trisolvent-ink writing method for bone tissue engineering," *Nanoscale,* vol. 12, pp. 18225-18239, 2020.
[91] A. Sadeghinia, S. Soltani, M. Aghazadeh, J. Khalilifard, and S. Davaran, "Design and fabrication of clinoptilolite–nanohydroxyapatite/chitosan–gelatin composite scaffold and evaluation of its effects on bone tissue engineering," *Journal of Biomedical Materials Research Part A,* vol. 108, pp. 221-233, 2020.
[92] P. Sivashankari and M. Prabaharan, "Three-dimensional porous scaffolds based on agarose/chitosan/graphene oxide composite for tissue engineering," *International journal of biological macromolecules,* vol. 146, pp. 222-231, 2020.
[93] N. A. Pattanashetti, T. Viana, N. Alves, G. R. Mitchell, and M. Y. Kariduraganavar, "Development of novel 3D scaffolds using BioExtruder by varying the content of hydroxyapatite and silica in PCL matrix for bone tissue engineering," *Journal of Polymer Research,* vol. 27, pp. 1-13, 2020.
[94] K. Liu, W. Li, S. Chen, W. Wen, L. Lu, M. Liu*, et al.*, "The design, fabrication and evaluation of 3D printed gHNTs/gMgO whiskers/PLLA composite scaffold with honeycomb microstructure for bone tissue engineering," *Composites Part B: Engineering,* vol. 192, p. 108001, 2020.