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Functions and Requirements of Synthetic Scaffolds in Tissue Engineering

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3.1 Introduction

Tissue engineering is an interdisciplinary field that has emerged to address the needs created by a number of interrelated problems including the shortage of donor organs, donor site morbidity, and failure of mechanical devices [1]. These imperfect solutions continue to given great impetus to the relatively new field that applies the principles of engineering and the life sciences toward developing biological substitutes for the restoration, maintenance, or improvement of tissue function [1]. Most tissue engineering techniques utilize a 3D porous scaffold seeded with cells. These scaffolds play a vital role in the development of new tissue.

The goal of this review is to discuss the functions and requirements of scaffolds in tissue engineering as well as their modification. Different types of synthetic scaffolds are discussed in detail along with their methods of fabrication.

3.2 Functions and Requirements of Scaffolds

Scaffolds serve numerous functions critical for the success of tissue regeneration, which include the following [2–4]:

1. Serving as space holders to prevent encroachment of tissues from the immediate vicinity into the affected site
2. Providing a temporary support structure for the tissue that they are intended to replace
3. Creating a substrate for cells to attach, grow, proliferate, migrate, and differentiate on
4. Serving as a delivery vehicle for cells, facilitating their retention and distribution in the region where new tissue growth is desired
5. Providing space for vascularization, neotissue formation, and remodeling to occur
6. Enabling the efficient transport of nutrients, growth factors, and blood vessels and removal of waste material

In order for scaffolds to perform the earlier functions, they need to meet some basic requirements, which necessitate them to [3,5–7]

1. Be biocompatible, that is, not produce an unfavorable physiological response
2. Be biodegradable, that is, get broken down eventually and eliminated from the body via naturally occurring processes
3. Degrade at a rate proportional to the regrowth of new tissue
4. Have mechanical properties that are consistent with the tissue they are replacing
5. Have the desired surface properties to enable cell attachment, growth, proliferation, and differentiation as well as extracellular matrix (ECM) formation

6. Have the optimum architectural properties in terms of pore size, porosity, pore interconnectivity, and permeability and to allow for efficient delivery of nutrients, growth factors, and blood vessels and removal of waste
7. Be easily processed into 3D complex shapes in a well-controlled and reproducible manner

3.2.1 Biocompatibility

Biocompatibility is the primary requirement for any type of scaffold. The scaffold is required to elicit a beneficial response from the cells with which it is seeded and an appropriate immune response from the host tissue on implantation, meaning that the interactions that take place between the scaffold, cells, and host tissue should be favorable without any potential for harm due to induced cytotoxicity, generation of an adverse immune response, or activation of the blood clotting or complement pathways [8]. A number of factors contribute to the kind of tissue response generated by the biomaterial including the shape and size of the implant, its chemical reactivity, the mechanism, rate and by-products of degradation, site of implantation, and the host species [8]. Taking it a step further, one can expand the meaning of the term biocompatibility to include biofunctionality, which indicates the ability of the material to support and promote cell–material interactions according to the local tissue-specific application [9].

3.2.2 Biodegradation

Since synthetic scaffolds serve as temporary structures that are replaced by native tissue subsequently, they need to be gradually removed from the implant site by a process commonly referred to as biodegradation. The terms biodegradable, bioresorbable, bioerodible, and bioabsorbable are often used incorrectly and/or interchangeably in tissue engineering literature [10]. Biodegradable indicates breakdown due to macromolecular degradation caused by biological elements resulting in fragments or other degradation by-products that are not necessarily eliminated from the body; bioresorbable implies complete elimination of foreign material and bulk degradation by-products via resorption within the body, that is, by natural pathways like filtration or metabolization; bioerodible signifies surface degradation, whereas bioabsorbable means dissolution in body fluids without any polymer chain cleavage or decrease in molecular mass [11].

Generally, polymers of the poly(α-hydroxy acid) group undergo bulk degradation. Thus, their molecular weight commences to decrease immediately upon contact with aqueous media, but their mass reduces much more slowly owing to the time required by molecular chains to decrease to a size appropriate for them to freely diffuse out of the polymer matrix. This phenomenon results in an initially delayed but then rapid disintegration of the implant accompanied with a simultaneous increase in the release of acidic degradation by-product. In vivo, not only can this result in inflammatory reactions, but the sudden drop in pH can further compromise the biocompatibility of the implant unless there is sufficient buffering provided by the surrounding body fluids and vasculature [10,12]. Filler materials influence the degradation mechanism by preventing autocatalytic effect of the acidic end groups that occurs as a result of polymer chain hydrolysis [7]. Thus, in order to control acceleration of acidic degradation, researchers in the musculoskeletal tissue engineering arena have incorporated filler materials like tricalcium phosphate (TCP) [13]

or Bioglass® [14] or basic salts [12] into the polymer matrix to produce a composite material with the idea that the resorption products of these additives will buffer the acidic resorption by-products of the original polymer matrix thereby restoring biocompatibility and preventing inflammation [10].

3.2.3 Matching Rates of Degradation of Scaffold and Regrowth of New Tissue

Matching the rate of scaffold degradation with regrowth of new tissue criterion is extremely important but difficult to achieve. It is essential for the scaffold to gradually transfer the function of load bearing and support to the newly growing tissue, especially in musculoskeletal applications where the scaffolds are generally subjected to higher loads compared to other areas. Ideally, the rate of bioresorption or bioerosion or biodegradation of the scaffold should match the rate of regrowth of new tissue at the site of scaffold placement in order to provide an almost seamless transition of load from the disintegrating scaffold to the strengthening, developing tissue without compromising the integrity of the implant. There are drawbacks associated with either a very fast rate of scaffold degradation or a very slow rate, relative to the rate of regrowth of new tissue. If the rate of scaffold disintegration is high, the newly forming tissue will be suddenly exposed to forces greater than what it can tolerate as it will not have had enough time to get conditioned to bear the new forces and can thus be adversely affected. On the other hand, if the rate of scaffold degradation is extremely slow, it can result in stress shielding of the growing tissue, thereby protecting it from the forces that are meant to strengthen it during its development, thereby making it more susceptible to injury later on. Hutmacher [10] has outlined two strategies for selection of the polymeric scaffold material in musculoskeletal tissue engineering applications depending on the time up to which the scaffold needs to assume the role of load bearing. In the case where the scaffold material is required to play the major supporting role till the time the construct is completely remodeled by the host tissue, it needs to be designed to retain its strength till the time the developing tissue can begin to assume its structural role. In the case of bone, the scaffold is required to retain its mechanical properties for at least 6 months, that is, 24 weeks (3 weeks for cell seeding, 3 weeks for the growth of premature tissue in a dynamic environment, 9 weeks for the growth of mature tissue in a bioreactor, and 9 weeks in situ) after which it will gradually start losing its mechanical properties and should be metabolized by the body without a foreign body reaction after 12–18 months. In the second strategy, the scaffold plays the primary role of mechanically supporting cell proliferation and differentiation only till the time that the premature tissue is placed in a bioreactor, after which the function is taken over by the ECM secreted by the cells while the scaffold degrades. Thus, careful selection of various parameters related to a scaffold and its composition, depending on the size of defect and anatomical location, is crucial. These include hydrophobicity, crystallinity, mechanical strength, molecular weight, and kind of breakdown.

The use of composite materials is now on the rise in order to tailor degradation rates and resorption kinetics [10]. Shikinami et al. [15] used a composite of uncalcined and unsintered hydroxyapatite (HA) with poly-L-lactide (PLL) to not only gain better control over resorption but also to enhance mechanical strength. Roether et al. [16] fabricated poly(DL-lactic acid) (PDLLA) foams coated with and impregnated by bioactive glass (BIOGLASS) as scaffolds for bone tissue engineering. The BIOGLASS coating on the pore walls affected the rate and extent of polymer degradation by acting as a protective barrier against hydrolysis [16,17]. The rapid exchange of protons in water for alkali in glass provides a pH buffering effect at the polymer surface, thereby slowing down degradation as a result of small pH

changes during dissolution of BIOGLASS [18]. However, Ang et al. [19] found that when HA was incorporated as a filler in a polycaprolactone (PCL) matrix, the matrices with higher concentrations of HA degraded much faster than those with a lower concentration, although their mechanical properties and bioactivity improved initially. This could be attributed to the random hydrolytic chain cleavages in the amorphous regions of the PCL scaffold or the increase in hydrophilicity imparted by the addition of HA to the PCL scaffolds that were placed in a highly basic medium of 5 M NaOH to actually accelerate the slow degradation rate of PCL [19]. Even in controlled settings, the chemical and mechanical degradation of a polymer can vary significantly between species, individuals, and anatomic locations, thus making it extremely difficult to define an ideal degradation rate [2]. Most design strategies favor extending degradation time over months to minimize the risk of early construct failure rather than the risk of delayed resorption [2].

3.2.4 Mechanical Properties

A scaffold seeded with cells and growth factors is commonly referred to as a construct. The mechanical properties (strength, modulus, toughness, and ductility) of the construct should match those of the host tissue as closely as possible at the time of implantation so that tissue healing is not compromised by mechanical failure of the scaffold before new tissue generation occurs [2,10]. Freeman et al. [20] combined the techniques of polymer fiber braiding and twisting to fabricate a poly(L-lactic acid) (PLLA) braid-twist scaffold for anterior cruciate ligament reconstruction. This addition of fiber twisting to the braided scaffold resulted in significantly better mechanical properties (ultimate tensile strength, ultimate strain greater toe region) over scaffolds that were braided. Webster et al. [21] advocate the use of nanostructured HA as the foundation for bone tissue engineering since in addition to being mechanically robust, the nanophase substrates enhance adhesion and other osteoblast functions as well as provide chemical and structural stability. Horch et al. [22] incorporated functionalized alumoxane nanoparticles into poly(propylene fumarate)/poly(propylene fumarate)-diacrylate (PPF/PPF-DA) to attain a composite with up to a threefold increase in flexural modulus compared to the polymer resin. They attributed the significant improvement in flexural properties to the uniform dispersion of nanoparticles within the polymer as well as greater covalent interactions between the functionalized surface of the filler and polymer chains. Thus, researchers are altering existing methods to optimize the desired properties by modifying some aspect of scaffold fabrication, be it combining techniques or using combinations of polymers or reducing the size of the components.

A number of researchers use natural scaffolds composed of either a biopolymer formed through traditional methods into a scaffold or a decellularized construct [3]. The natural tissue is often processed to overcome immunogenetic responses and to stabilize the ECM components [3]. However, the processing significantly alters the mechanical properties making them less desirable [3]. Researchers are now exploring the use of ECM components as raw materials that can be more controlled through the manufacturing process [23].

Controlling the mechanical properties of the construct over time is extremely challenging. Scaffolds made of metal and ceramics do not degrade and would provide ideal mechanical characteristics in specific circumstances, but in general would compromise tissue repair and function due to stress shielding, possible fracture at the tissue–implant interface, and diminished space for new tissue growth due to the presence of the permanent implant [2]. Generally, polymers lack bioactive function, that is, the ability to produce a strong interfacial bond with the growing tissue, for example, with bone tissue via the

formation of a biologically active apatite layer [7,24]. This compounded with the flexible and weak nature of polymers limits their ability to meet the mechanical demands in surgery and the local environment thereafter, prompting the use of composites that comprise biodegradable polymers and bioactive ceramics [7,24]. Certain ceramic materials such as HA, TCP, and BIOGLASS form strong bonds with bone tissue through cellular activity in the presence of physiological fluids and are hence referred to as *bioactive* [16,25].

Another technique used to improve the load-bearing characteristics of the construct involves use of a bioreactor to mechanically precondition the implant and thus better prepare it to bear the loads it will be subjected to after implantation, in an efficient manner. Tillman et al. [26] used a bioreactor to precondition tissue-engineered blood vessels for use in an arteriovenous application subjected to hemodynamic and mechanical challenges from chronic dialysis access. A bioreactor can be defined as any device that tries to mimic and reproduce physiological conditions in order to maintain and encourage cell culture for tissue growth [27]. Bilodeau et al. [27] have written an excellent review on the different characteristics of bioreactors designed to grow cartilage, bone, ligament, cardiac and vascular tissue, cardiac valve, and liver. They have discussed how mechanical stresses generated within bioreactors influence the quality of the ECM in the case of bone, ligament, and cartilage and how other aspects like cell proliferation and differentiation are influenced more in the case of the other tissues. Androjna et al. [28] investigated the effect of mechanically conditioning small intestine submucosa (SIS) scaffolds with and without tenocytes, in vitro within bioreactors, for enhancing tendon repair. They found the biomechanical properties (e.g., stiffness) of the cell-seeded scaffolds to increase as a result of cell tensioning due to cyclic loading as compared to unseeded scaffolds and no-load or static-load constructs (with or without cells). Reorganization of the matrix may have also contributed to this increased stiffness as the application of mechanical load may have reoriented the collagen architecture along the axis of the applied load [28]. Mahmoudifar et al. [29] seeded chondrocytes on polyglycolic acid (PGA) scaffolds that were cultured in recirculation column bioreactors to produce cartilage constructs. The flow of media through the construct generates shear forces that provide mechanical stimuli to cells thereby improving the quality of cartilage produced [29,30]. Also, hydrostatic pressure produces compressive forces that are beneficial for cartilage formation [30]. Jeong et al. [31] successfully subjected smooth muscle cell-seeded poly(lactide-co-caprolactone) (PLCL, 50:50) scaffolds to pulsatile strain and shear stress in a pulsatile perfusion bioreactor to stimulate vascular smooth muscle tissue development and retainment of their differentiated phenotype. Mechanical signals play a vital role in the engineering of constructs for cardiac tissue as well [32]. Akhyari et al. [33] subjected a cardiac cell-seeded gelatin matrix to a cyclical mechanical stretch regimen that not only improved cell proliferation and distribution but also increased the mechanical strength of the graft by an order of magnitude. Cell and tissue remodeling are important for achieving stable mechanical conditions at the implant site [10]. Thus, it is necessary for the construct to maintain sufficient integrity during the in vitro and/or in vivo growth and remodeling phase [10].

3.2.5 Surface Properties

Most conventional polymers do not adequately meet the surface requirements of scaffolds thereby necessitating the modification of the surface of a biomaterial that already exhibits good bulk properties and biofunctionality [34–36]. Moroni et al. [37] developed a novel system to create scaffolds for cartilage repair with a biphasic polymer network made of poly([ethylene oxide] terephthalate-co-poly[butylene] terephthalate) (PEOT/PBT) to obtain

a shell–core fiber architecture, where the core provided the primary mechanical properties and organization to the scaffold while the shell worked as a coating to enhance the surface properties. The shell polymer contained a higher molecular weight of poly(ethylene glycol) (PEG) segments that were used in the copolymerization as well as a greater weight percentage of the PEOT domains relative to the core [37]. Liu et al. [38] fabricated surface-modified nanofibrous PLLA scaffolds using gelatin spheres as porogen. Gelatin molecules adhered to the scaffold surface during fabrication. This surface modification significantly improved initial osteoblast adhesion and proliferation as well as stimulated greater matrix secretion. Li et al. [39] explored a novel approach for the relatively uniform apatite coating of thick polylactic glycolic acid (PLGA) scaffolds even deep within the interior to enhance its osteoconductivity. They first coated apatite on the surface of paraffin spheres of the required size, which were then molded into a foam. PLGA/pyridine solution was made to penetrate the interspaces among the spheres. Cyclohexane was used to dissolve the spheres, resulting in highly porous PLGA scaffold with controlled pore size and excellent interconnectivity having a uniform apatite coating on the pore surface. Cai et al. [40] modified the surface of PDLLA scaffolds, prepared via thermally induced phase separation (TIPS), with baicalin using a physical entrapment method, in order to increase bone formation potential and biocompatibility that were histologically evaluated using a rabbit radialis defect model in vivo. Baicalin is a flavonoid compound and purified form of a Chinese herbal medicinal plant and possesses antioxidant as well as anti-inflammatory properties.

The local chemical environment controls the interactions between cells and scaffolds that occur at the surface. Generally, all implanted materials get immediately coated with proteins and lipids, which mediate the cellular response to these materials. Finally, it is the interaction between the scaffold surface and the biomolecules that adsorbs on it that dictates the net effect [2]. Koegler et al. [41] patterned the scaffold's surface chemistry and architecture to study cell response as it would facilitate the orderly development of new tissue. They evaluated how rat osteoblasts responded to PLGA scaffolds modified with poly(ethylene oxide) (PEO) and found that higher PEO concentrations decreased adhesion, proliferation, spreading, and migration but enhanced alkaline phosphatase (ALP) activity.

Surface modification plays a very important role in tissue engineering techniques employing thin films or membranes. Tiaw et al. [42] subjected ultrathin PCL films to femtosecond and excimer laser ablation in order to produce drilled-through holes and blind holes, respectively, so as to enhance permeability for applications like epidermal tissue engineering. Laser treatment had made the membrane more hydrophilic, thereby paving the way for further study in the area of membrane tissue engineering. Nakayama et al. [43] studied the effect of micropore density of scaffold films used in cardiovascular tissue engineering applications. They micropatterned four regions of a polyurethane (PU) film with different pore densities and used this to cover a stent that was implanted in arteries in a canine model as an in vivo model of transmural tissue ingrowth. Thrombus formation was maximum in nonporous regions and micropore regions of lowest density. They also found the thickness of the neointimal wall to decrease with a rise in micropore density.

3.2.6 Architectural Properties

The architectural properties of a scaffold mainly dictate the transport that occurs within it, which is primarily a function of diffusion. The transport issues comprise delivery of oxygen and other nutrients, removal of waste, transport of proteins, and cell migration, which in turn are governed by scaffold porosity and permeability [44]. The size, geometry, orientation, interconnectivity, branching, and surface chemistry of pores and channels directly

affect the extent and nature of nutrient diffusion and tissue ingrowth [45,46]. Generally, living tissue is observed in the outer regions of scaffolds, whereas the interior fails to support viable tissue due to lack of adequate diffusion [47]. This may arise due to the fact that as cells within the pores of the scaffold begin to proliferate and secrete ECM, they simultaneously begin to block off the pores, thereby reducing the supply of nutrients to the interior. The formation of this surface layer of tissue with sparse matrix in the interior has been referred to as the *M&M effect*, referring to the popular brand of candy having a hard crust and soft core [44].

3.2.6.1 Pore Size and Shape

A scaffold cannot be completely solid as cells need to grow within it and these cells need to be supplied with nutrients. Thus, the need for a scaffold to have holes or pores or channels seems obvious but not so obvious is what their shape and dimensions should be. The pore size should at least be a few times the size of the cells that will be seeded on it to provide enough space for the entry and exit of nutrients and waste, respectively. Also, blood vessels and growth factors may need to enter the construct as well. There is no common pore or channel size range that is suitable for all types of tissue growth as cells making up different tissues have different dimensions. Sosnowski et al. [48] prepared PLL/PLGA scaffolds from microparticles with a bimodal pore size distribution. Macropores in the 50–400 μm range promoted osteoblast growth and proliferation within the scaffold, whereas micropores in the range of 2 nm–5 μm in the scaffold walls allowed for diffusion of nutrients and metabolites as well as products of polyester hydrolysis. Draghi et al. [49] used three different porogens (gelatin microspheres, paraffin microspheres, and salt crystals) to fabricate scaffolds from commonly used biodegradable materials via the solvent casting/porogen leaching technique to see which allowed maximum control over scaffold morphology. Although all the porogens contributed to producing highly porous scaffolds, microsphere leaching produced well-defined spherical pores that resulted in better mechanical properties and lesser flow resistance.

Researchers have fabricated scaffolds with different pore sizes or even a range of pore sizes within the same scaffold to see their effect on cell growth and to mimic certain types of tissues. Oh et al. [50] fabricated cylindrical PCL scaffolds with gradually increasing pore sizes along the longitudinal axis using a novel centrifugation method to evaluate the effect of pore size on cell–scaffold interaction. The pore sizes within the scaffold gradually increased from 88 to 405 μm and the porosity from 80% to 94% due to the gradual increment of centrifugal force along the cylindrical axis. Chondrocytes, osteoblasts, and fibroblasts were evaluated for their interaction in vitro with this PCL scaffold and in vivo using calvarial defects in a rabbit model. The scaffold section having pore sizes in the 380–405 μm range showed better chondrocyte and osteoblast growth, while the 186–200 μm range was better suited for fibroblast growth. Moreover, the scaffold section with a 290–310 μm range pore size seemed to be best suited for new bone formation. This shows the existence of pore ranges that are ideal for the growth of some cell types and that this range can change while the cells differentiate to form tissue.

Woodfield et al. [51] investigated the ability of anisotropic pore architectures to control the zonal organization of chondrocytes and ECM components in scaffolds made of poly(ethylene glycol)-terephthalate-poly(butylene terephthalate) (PEGT/PBT). They used a 3D fiber deposition technique to produce scaffolds with either uniformly spaced pores (fiber spacing of 1 mm and pore size of 680 μm diameter) or pore size gradients (fiber spacing of 0.5–2 mm and pore size range of 200–1650 μm diameter), but having a similar overall

porosity of about 80%. They found the gradient to promote anisotropic cell distribution similar to that found in the upper, middle, and lower zones of immature bovine articular cartilage, irrespective of whether the method of cell seeding was static or dynamic. Additionally, they discovered a direct correlation between the zonal porosity and both DNA and glycosaminoglycan (GAG) content. Also, Harley et al. [52] produced cylindrical scaffolds with a radially aligned pore structure having a smaller mean pore size and lesser porosity toward the outside. Increasing the spinning time and/or velocity caused the formation of a large inner diameter hollow tube and a gradient of porosity along the radius due to increased sedimentation. Thus, an important underlying trend is the need for scaffolds to have an appropriate porosity.

3.2.6.2 Porosity

Porosity is the amount of void space within the scaffold structure. Several studies have reiterated the need for scaffolds to possess high porosity and high surface-area-to-mass ratio for promoting uniform cell delivery and tissue ingrowth [53,54] as well as to have an open pore network for optimal diffusion of nutrients and waste [55]. Another study indicated that a scaffold should ideally possess a porosity of 90% to allow for adequate diffusion during tissue culture and to provide adequate area for cell–polymer interactions [56]. However, Goldstein et al. [57] have suggested that polylactic–polyglycolic acid (PLG) scaffolds be prepared with a porosity not exceeding 80% for implantation into orthopedic defects as it would otherwise compromise the scaffold integrity. Thus, in case of polymeric scaffolds, there may be a conflict between optimizing porosity and maximizing mechanical properties. Moreover, Agrawal et al. [58] found that lower initial porosity and permeability result in a faster rate of degradation for PLG scaffolds and lower mechanical properties during the initial weeks. Wu et al. [59] investigated the effects of porosity (80%–95%) and pore size (50–450 μm) on the degradation of 85/15 PLGA scaffolds, performed in phosphate-buffered saline (PBS) at 37°C up to 26 weeks. Scaffolds possessing a higher porosity or smaller pore size degraded more slowly than those with a lower porosity or larger pore size as the latter had thicker pore walls and smaller surface areas that prevented the diffusion of acidic degradation products resulting in greater acid-catalyzed hydrolysis.

Thus, in view of these contradictory factors, there is a need to optimize scaffolds for bone regeneration based on their specific mechanical requirements balanced with their desired useful life and diffusion characteristics. This could be achieved by optimizing porosity with respect to nutrient availability and using it with biomaterials that can provide adequate mechanical properties. Lin et al. [60] developed a general design optimization strategy for 3D internal scaffold architecture to have the required mechanical properties and porosity simultaneously, using the homogenization-based topology optimization algorithm for bone tissue engineering. Howk et al. [61] showed that it was possible to increase the porosity and strength of a bone tissue engineering scaffold through simple iterations in architectural design using computer-aided design (CAD) software and finite element analysis. The goal of their optimization was to maintain the strength of a design constant while increasing its porosity. Xie et al. [62] selected mechanoactive scaffolds that respond to applied compression stress without undergoing permanent deformation for engineering functional articular cartilage from a biomechanical point of view and then determined the best porosity. They used PLCL sponges (pore size, 300–500 μm; porosity, 71%–86%) as mechanoactive scaffolds and determined that the lower their porosity, the nearer their mechanical properties came to those of native cartilage. Hence, the scaffold with a porosity of 71% was found to be the best suited for cartilage regeneration. Moroni et al. [63]

varied pores in size and shape by altering fiber diameter, spacing, as well as orientation and layer thickness using the 3D fiber deposition method in order to study their influence on dynamic mechanical properties. They observed a reduction in elastic properties like dynamic stiffness and equilibrium modulus as well as an increase in viscous parameters like damping factor and creep unrecovered strain as porosity increased.

3.2.6.3 Pore Interconnectivity

It is not sufficient for a scaffold to be porous but the pores in the scaffold need to be interconnected for efficient delivery of nutrients to the interior and removal of waste to the exterior of the scaffold. Pore interconnectivity also has implications as far as the transport of proteins, cell migration, and tissue ingrowth are concerned.

Griffon et al. [64] found chondrocyte proliferation and metabolic activity to improve with increasing interconnected pore size of chitosan sponges. Lee et al. [65] produced poly(propylene fumarate) (PPF) scaffolds with controlled pore architecture to study the effects of pore size and interconnectivity on bone growth. They fabricated scaffolds with three pore sizes (300, 600, and 900 μm) and randomly closed 0%, 10%, 20%, or 30% of the pores. Porosity and permeability decreased as the number of closed pores increased, especially when the pore size was 300 μm, as a result of low porosity and pore occlusion. Suh et al. [66] compared the proliferation of chondrocytes on equally porous (95%) PLG scaffolds prepared by the solvent casting and particulate leaching (SCPL) technique using two different porogens: salt and gelatin. The scaffolds produced using gelatin exhibited better cell attachment and proliferation, and this was attributed to better pore interconnectivity at the same porosity. Hou et al. [67] suggested that the extraction of salt particles in a salt leaching process implied that the resulting pores were interconnected. However, the complete removal of the salt does not necessarily ensure a permeable structure as there might be dead-end spaces with only a single opening thereby not permitting end-to-end interconnectivity of the whole structure [44].

Traditional scaffold manufacturing techniques have been modified to increase pore interconnectivity. Murphy et al. [68] imparted improved pore interconnectivity to PLGA scaffolds by partially fusing the salt before creating the polymer matrix via either the solvent casting/salt leaching process or the gas foaming/salt leaching process. Gross et al. [69] made spheroid salt particles in a flame and sintered them to produce an interconnected salt template, which was filled with a carbonated fluorapatite powder and polylactic polymer to form a composite scaffold. A larger pore size was possible with the use of large spherical salt particles, and this technique could be used to successfully produce scaffolds with good interconnectivity and graded pore sizes. Hou et al. [70] fabricated highly porous (93%–98%) and interconnected scaffolds by freeze-drying polymer solutions in the presence of a leachable template followed by leaching of the template itself. Sugar or salt particles were fused to form the well-connected template, the interstices of which were filled with a polymer solution in solvent, followed by freeze-drying of the solvent and subsequent leaching of the template. This resulted in relatively large interconnected pores based on the template and smaller pores resulting from the freeze-drying process.

Darling et al. [71] and Wang et al. [72] used micro computed tomography (microCT) to quantify pore interconnectivity within their PCL scaffolds for bone tissue engineering that were manufactured by a type of solid free-form fabrication (SFF) technique called precision extrusion deposition (PED). They achieved pore interconnectivity greater than 98% in their scaffolds. Moore et al. [73] also used microCT followed by a custom algorithm to nondestructively quantify pore interconnectivity. The program calculated accessible

porosities over a range of minimum connection sizes. The accessible porosity varied with connection size as a function of porogen content. However, microCT is still not widely available and researchers have improvised, like Li et al. [74] who appreciated the difficulty in obtaining 3D information about pore interconnectivity through 2D images and devised a rather simple unique experiment to verify the same. They soaked porous HA in black pigment dispersion and centrifuged it. After removing the pigments, they sectioned, dried, and pictured the sample and found black-colored pores to be accessible either directly or via adjacent pores.

3.2.6.4 Permeability

Permeability is a measure of the ease with which a fluid can flow through a structure. Generally, an increase in porosity leads to an increase in permeability, but for this to happen, the pores need to be highly interconnected [44]. One of the authors (Agrawal) has previously shown that scaffolds can possess different permeabilities while maintaining similar porosity [58,75]. Thus, permeability should be treated as an independent scaffold design parameter. A high permeability can produce superior diffusion within the scaffold, which would facilitate the inflow of nutrients and the disposal of degradation products and metabolic waste [44]. Permeability is also affected by fluid–material interactions and thus influences the viscoelastic response of a scaffold. This, in turn, affects the fluid pumping movement of the scaffold that is important while designing scaffolds for articular cartilage repair, where mechanotransduction and cell apoptosis may be affected by hydrostatic pressure and flow-induced shear [76].

Scaffold porosity and permeability are clearly related to the physical and mechanical properties possessed by the scaffold. For example, better mechanical properties may be obtained for a scaffold if it is made more solid and less porous. Less obvious is the fact that porosity and permeability can also have a significant impact on the chemical behavior of the scaffold, especially its degradation characteristics [44]. For example, as stated earlier, it has been shown that low-porosity and low-permeability PLG scaffolds degrade faster [58,77]. Also, such scaffolds exhibit a lower decrease in their mass, molecular weight, and mechanical properties under dynamic fluid flow conditions compared to static conditions [58]. This phenomenon has been attributed to the inhibition of autocatalytic degradation due to better diffusion or forced fluid flow.

Li et al. [78] proposed using the permeability/porosity ratio to describe the accessibility of inner voids in macroporous scaffolds as they found porosity and pore size to be inadequate descriptors. The ratio given earlier is an indicator of the percolative efficiency per unit porous volume of a scaffold, where permeability can be termed as the conductance normalized by sample size and fluid viscosity. Good pore interconnectivity could lead to a positive correlation between porosity and permeability. Permeability could represent a combination of five important scaffold parameters: porosity, pore size and distribution, interconnectivity, fenestration size and distribution, and pore orientation.

Wang et al. [79] wanted to optimize scaffold morphology for connective tissue engineering to overcome the problem of disproportionately high tissue formation at surfaces of scaffolds grown in bioreactors relative to their interior. Thus, they determined geometric parameters for PEGT/PBT scaffolds using scanning electron microscopy (SEM), microCT, and flow permeability measurements and then seeded fibroblasts on these scaffolds under dynamic flow conditions for 2 weeks. Only scaffolds with an intermediate pore interconnectivity supported homogeneous tissue formation throughout the scaffold with complete filling of all pores. Hollister et al. [80] used an integrated image-based design along with

SFF to create scaffolds with the desired elasticity and permeability from a variety of biomaterials including degradable polymer, titanium, and ceramics to fit any craniofacial defect. The scaffolds supported significant bone growth in minipig mandibles for a range of pore sizes from 300 to 1200 μm. Huang et al. [81] used scaffolds made of chitosan and PLGA with longitudinally oriented channels running through them to serve as guides for nerve generation. They found chitosan to be a better scaffold for nerve guidance compared to PLGA owing to its high permeability and characteristic porous structure.

In addition to traditionally used direct permeation experiments as conducted by Spain et al. [82] and Li et al. [78], researchers have begun to use magnetic resonance imaging (MRI) and microCT for measuring permeability as well. Neves et al. [83] used MRI to determine construct permeability to a low-molecular-weight magnetic resonance (MR) contrast agent and correlate the findings with measurements of cell growth and energetics. They used perfusion bioreactors to seed mature sheep meniscal fibrochondrocytes on polyethylene terephthalate (PET) fabric to produce bioartificial meniscal cartilage constructs. Knackstedt et al. [84] used microCT with a resolution of 16.8 μm to measure a number of structural characteristics like pore-volume-to-surface-area ratio, pore size distribution, permeability, tortuosity, diffusivity, and elastic modulus of coral bone graft samples.

3.2.7 Scaffold Fabrication

The successful generation of completely functional tissues should be addressed not only at the microscale to expose the cells to an environment conducive to their optimal functioning but also at the macroscale for the tissue to possess suitable mechanical properties, facilitate nutrient transport, and promote coordination of multicellular processes [85].

3.2.7.1 Solvent Casting and Particulate Leaching

One of the most commonly used scaffold fabrication techniques is solvent casting followed by particulate leaching, wherein the pore size of the resulting scaffold is controlled by the size of the porogen, and porosity is controlled by the porogen/polymer ratio. This method involves mixing a water-soluble porogen in a polymer solution followed by casting the mixture into a mold of the desired shape. The solvent is removed by evaporation or lyophilization and the porogen is leached out by immersion in deionized water. Organic solvents may be used with non-water-soluble porogens including certain nanofillers [86]. Widmer et al. [87] used solvent casting followed by extrusion, in order to form a tubular construct, and then leached the salt to generate PLGA and polylactic acid (PLA) scaffolds with a pore size of 5–30 μm and porosity in the 60%–90% range. Although salt is the most commonly used porogen, sugar as well as gelatin [38,66] and paraffin spheres [88] are also used and these are sometimes modified to enhance scaffold functionality [39]. In case paraffin spheres are used as the porogen, the solvent used is organic (like hexane) [86,88] and not water. This method is the most widely used owing to its simplicity. However, natural porogen dispersion allows little control over the internal scaffold architecture and pore interconnectivity. Also, the thickness of the scaffold that can be fabricated by this method is hindered by difficulty removing the porogen from deep within the scaffold interior [89]. This has led to the modification of the SCPL technique to produce a greater pore interconnectivity in some cases [68,75,88,90,91] and to new techniques like rapid prototyping (RP), also known as SFF, in others [46,92–94]. Agrawal et al. [75] modified the technique by vibrating the mold while dissolving the salt, thereby preventing the particles from settling due to gravity, thereby enhancing permeability of the scaffold by creating better pore interconnectivity and more even distribution of pores.

3.2.7.2 Gas Foaming

The gas foaming technique can be used to fabricate highly porous scaffolds in the absence of organic solvents. Carbon dioxide (CO_2) generally acts as the *porogen* in this method in its normal gaseous [86,95,96] or subcritical [97,98] or supercritical form [99–101]. Solid polymeric disks when exposed to high-pressure CO_2 at room temperature get saturated with the gas. The solubility of the gas in the polymer is rapidly decreased by reducing the pressure to atmospheric levels, creating a thermodynamic instability for the dissolved CO_2 resulting in the nucleation and growth of gas bubbles in the interior of the polymer matrix. Mooney et al. [95] created PLGA scaffolds with a pore size of about 100 μm and porosity up to 93% using this method. However, this method resulted in a relatively nonporous skin layer due to rapid diffusion of the dissolved CO_2 from the surface and closed pore structure with limited pore interconnectivity. These drawbacks were improved upon by combining the above process with particulate leaching [96,102–104]. Harris et al. [96] compression molded PLGA and salt particles and then subjected them to gas foaming so that the salt particles subsequently leached out leaving behind a macroporous foam with good interconnectivity. Kim et al. [105] found that PLGA/HA scaffolds fabricated by gas foaming and particulate leaching enhanced bone regeneration compared to scaffolds fabricated by SCPL.

3.2.7.3 Emulsion Freeze-Drying

The emulsion freeze-drying process involves creation of an emulsion by homogenization of a polymer solution and water mixture that is rapidly cooled to lock in the liquid-state structure [89]. The solvent and water are then removed by freeze-drying [89]. The disadvantage of this technique is that it yields scaffolds with a closed pore structure [106]. Whang et al. [90] investigated the effect of median pore size and protein loading on protein release kinetics from emulsion freeze-dried PLGA scaffolds. The profiles indicated an initial burst followed by a slower sustained release. The scaffold tortuosity and partition coefficient for protein adsorption significantly reduced protein diffusivity. The activity of the released protein was demonstrated by the successful delivery of recombinant human bone morphogenetic protein-2 (rhBMP-2) from the scaffold to an ectopic site in a rat [107]. Moshfeghian et al. [108] evaluated the formation of chitosan/PLGA scaffold using controlled-rate freezing and lyophilization. The microarchitecture of the scaffold was significantly influenced by the solvent and freezing temperature. Controlling the concentration of chitosan yielded scaffolds with a porosity exceeding 90%.

3.2.7.4 Cryogelation

Cryogelation is a technique that utilizes moderate freezing and thawing steps to produce scaffolds that exhibit tissue-like elasticity and large interconnected pores and can withstand elongation and torsion deformations [109,110]. This process involves exposing soluble polymeric gel precursors (monomers, initiator, polymers) to moderate freezing that expels the nonfrozen components as ice crystals form [109,110]. Cross-linking polymerization occurs in the nonfrozen channels around the ice crystals resulting in a scaffold with interconnected macropores after the ice melts [110]. In this technique, ice crystals serve as porogen substrates [109]. Hwang et al. [111] noted that increasing the rate of nucleation of ice crystals compared to the rate of gelation resulted in more homogenous, interconnected macropores. Reichelt et al. [112] produced cryogels from frozen methacrylate, acrylate,

and PEG solutions that were exposed to electron beam irradiation before being cooled and rinsed. The group altered the characteristics of the cryogels by varying the molar mass of the PEG molecules [112].

3.2.7.5 Thermally Induced Phase Separation

TIPS involves dissolution of the polymer in a solvent at a high temperature followed by a liquid–liquid or solid–liquid phase separation induced by lowering the solution temperature [86,89]. Subsequent use of sublimation causes removal of the solidified solvent-rich phase, resulting in a porous scaffold with good mechanical properties [89]. TIPS has been used to fabricate scaffolds covering a wide range of polymers and composites: from the regular PLLA, PDLLA, PDLLGA [113], and PLGA [114] to the more sophisticated poly(ester urethane) urea/collagen [115], amorphous calcium phosphate/PLLA [116], and PDLLA/BIOGLASS [117], to name a few. Rowland et al. [118] fabricated a PLGA/PU composite scaffold using TIPS showing how this process can bring together two very different polymers, whose morphology can be manipulated by controlling the phase separation behavior of the initial homogeneous polymer solution. Helen et al. [119] found composite PDLLA/BIOGLASS foams prepared by TIPS to provide a suitable microenvironment for the culture and proliferation of bovine annulus fibrosus (BAF) cells as well as the production of sulfated glycosaminoglycans (sGAGs), collagen type I and collagen type II, providing preliminary evidence of their suitability for the treatment of intervertebral disks with damaged annulus fibrosus regions. Gong et al. [120] used TIPS to produce PLLA scaffolds, which were filled with chondrocytes entrapped in agar hydrogel, thereby resulting in an implant with suitable mechanical properties and macroscopic shape while possessing an interior that is analogous to native ECM. Mo et al. [121] used TIPS to produce a porous PCL solution coating on the outside of a PLGA fiber braided tube to produce a PCL/PLGA composite tubular scaffold for small-diameter blood vessel tissue engineering. The porous PCL coating was used with the intention of providing a surface suitable for cell attachment, proliferation, and tissue regeneration. Cao et al. [122] compared the in vitro and in vivo degradation properties of PLGA scaffolds produced by TIPS and SCPL. TIPS produced far less changes in dimension, mass, internal architecture, and mechanical properties compared to SCPL over a 6-week period. Morphometric comparison indicated slightly better tissue ingrowth accompanied with a greater loss of scaffold structure in SCPL scaffolds. Chun et al. [123] fabricated PLGA scaffolds using TIPS for the controlled delivery of plasmid DNA over a period of 21 days. The various parameters in TIPS fabrication directly affecting pore structure and pore interconnectivity, such as polymer concentration, solvent/nonsolvent ratio, quenching methods, as well as annealing time, were also examined to determine their effects on the sustained release of plasmid DNA. Shao et al. [124] assessed the effect of temperature on the nanomechanics and morphology of scaffolds formed using TIPS with PLLA. They found that lower temperatures produced nanofibrous scaffolds with alternating distribution of higher and lower adhesion forces on the surface and demonstrated increased plasticity and viscoelastic properties [124].

3.2.7.6 Gravity and Microsphere Sintering

Qiu et al. [125] sintered HA-coated hollow ceramic microspheres that were developed in rotating-wall vessels, in order to create microcarriers for 3D bone tissue formation. Borden et al. [126] randomly packed PLGA microspheres to form a gel microsphere matrix, which had a high Young's modulus but a pore system less optimal for bone growth, and a sintered

microsphere matrix, which had mechanical properties in the midrange of cancellous bone accompanied with a well-connected pore system. The sintered microsphere matrices were created by thermally fusing the PLGA microspheres into a 3D array without any HA. They went on to study the osteoconductivity and degradation profile of these scaffolds by evaluating how osteoblasts and fibroblasts interacted with these scaffolds and performing degradation studies [127,128]. The group went on to evaluate the matrices' efficacy by using it in a 15 mm ulnar defect in rabbits and found that it supported significant formation of bone at the implant–bone interface [129]. Jiang et al. [130] fabricated composite chitosan/PLGA scaffolds by sintering and found osteoblast-like cells to proliferate better on these as compared to PLGA scaffolds. The presence of chitosan on the microsphere surfaces upregulated gene expression of ALP, osteopontin, and bone sialoprotein as well as increased ALP activity. Kofron et al. [131] developed tubular PLGA/HA sintered microsphere matrices using solvent evaporation for bone regeneration. The tubular composites were made to more closely mimic the bone marrow cavity and were found to have mechanical properties similar to cylindrical composites of the same dimensions.

3.2.7.7 Rapid Prototyping/Solid Free-Form Fabrication

RP or SFF techniques involve building 3D objects using layered manufacturing methods and offer several advantages over the traditional porogen leaching method, mainly independent control over the micro- and macroscale features enabling fabrication of complex structures customizable to the shape of the defect or injury [85,132]. Yang et al. [45] have reviewed the advantages and limitations of various RP techniques. Leong et al. [133] have tabulated the pros and cons of the conventional methods and discussed the capabilities and limitations of the important RP techniques. The process, in general, comprises the design of a scaffold model using CAD software, which is then expressed as a series of cross sections [134]. Corresponding to each cross section, the RP machine lays down a layer of material starting from the bottom and moving up a layer at a time to create the scaffold. Each new layer adheres to the one below it, thereby providing integrity to the finished product. Agrawal et al. [5] and Yang et al. [135] have provided comprehensive reviews weighing the pros and cons of traditional scaffold materials and fabrication methods. The different types of techniques encompassed by SFF include fused deposition modeling (FDM), PED, selective laser sintering (SLS), stereolithography (STL), multiphoton polymerization (MPP)/two-photon polymerization (2PP), and 3D printing (3DP) [46,136].

FDM [6,94,137–139] utilizes a moving nozzle that extrudes a polymeric fiber in the horizontal plane, and once a layer is completed, the plane is lowered and the procedure is repeated. PED is very similar to FDM, except that scaffold material in the form of granules or pellets is directly extruded and deposited in the form of fibers without the need of having to change these into precursor filaments as is the case with FDM [72].

Pressure-assisted microsyringe (PAM) [85] is like FDM but requires no heat and has greater resolution but cannot incorporate micropores using particulate leaching owing to the syringe dimensions. This method involves the deposition of polymer solution in solvent through a syringe fitted with a 10–20 μm glass capillary needle. The solvent acts as the binding agent, and the size of the polymer stream deposited can be altered by varying the syringe pressure, solution viscosity, tip diameter of the syringe, as well as speed of the motor [140].

SLS [141–144] involves building objects by sintering powder on a powder bed using a beam of infrared laser. The laser beam interacts with the powder to increase the local temperature to the glass transition temperature of the powder, causing the particles to fuse to each other as well as the layer underneath [45]. Laser power and scanning speed

affect sintering significantly [145]. Also, control over the finished product can be achieved by varying the laser processing parameters as these, in turn, control the degree of particle fusion and porosity [145].

STL [146–148] uses an ultraviolet (UV) laser beam to selectively polymerize a liquid photocurable monomer, a layer at a time [134]. The CAD data guide the UV beam onto the liquid surface, which is then lowered to enable the liquid photopolymer to cover the surface. Arcaute et al. [148] encapsulated human dermal fibroblasts in bioactive PEG hydrogels that were photo-cross-linked using STL.

MPP/2PP [136,149] uses tightly focused femtosecond-pulse-induced photomodification reaction in a confined volume to form micro- or nanostructures directly from a CAD model. A spatial resolution down to 100 nm is possible for polymeric structures [136].

Three-dimensional printing involves ink-jet printing of a binder onto a ceramic [150,151], polymer [134,152,153], or composite [154,155] powder surface, one layer at a time. The movement of the jet head, which dispenses the binder, is controlled by the CAD cross-sectional data. Adjacent powder particles join as the binder dissolves [134]. Indirect 3DP is sometimes used in order to overcome few of the pitfalls of 3DP. Lee et al. [156] used indirect 3DP, where the molds were printed first and the final material was cast into the mold cavity, in order to overcome some of the limitations of 3DP. These include higher pore sizes, due to the need to increase the thickness of each incremental layer to the porogen size range that can eventually compromise layer-to-layer connectivity resulting in lamination defects [156]. Also, shape complexity when powder material requires an organic solvent as the liquid binder, custom machines, proprietary control software, and extensive operator expertise make 3DP a helpful but sometimes difficult technique to employ [156].

Some researchers have combined two or more manufacturing techniques in order to optimize their scaffold designs. Taboas et al. [46] coupled SFF with conventional sponge scaffold fabrication techniques (phase separation, emulsion–solvent diffusion, and porogen leaching) to develop methods for casting scaffolds possessing designed and controlled locally as well as globally porous internal architectures. Dellinger et al. [157] used an SFF technique based on the robotic deposition of colloidal pastes to produce HA scaffolds of different architectures with porosities spanning multiple length scales. Macropores (100–600 μm) were obtained by spacing the HA rods appropriately, whereas micropores (<30 μm) were produced by including polymer microsphere porogens in the HA paste and controlling the sintering of scaffolds. Moroni et al. [37] combined 3D fiber deposition and phase separation to create a shell–core fiber architecture by viscous encapsulation resulting in scaffolds with a biphasic polymer network.

Inspired by developmental biology, Varghese et al. [158] have combined RP procedures with cell encapsulation to print viable free-form structures using customized ink-jet printers, with the hope that this method might provide the required signals, rules, and framework for hierarchic self-assembly. They *printed* bovine aortic endothelial cells in culture media (which they termed *bioink*) onto an alginate-coated frame that they used as a scaffold, to generate a 50 mm long tube with an outer diameter of 4 mm. Smith et al. [159] have coextruded cells suspended in polymers using a direct-write 3D bioassembly tool to create viable, patterned tissue engineering constructs. Mironov et al. [160,161] have introduced the futuristic concept of organ printing, which is the computer-aided, jet-based, 3D engineering of living human organs, to overcome the obstacles of generating vascularized organs. They propose using a cell printer capable of printing single cells, cell aggregates, and gels on *printing paper* comprising sequentially arranged layers of a thermoreversible gel. Sun et al. [162] have given a broad overview of computer-aided tissue scaffold design, including biomimetic modeling as well as 3D cell and organ printing.

The main advantage of RP techniques is their ability to finely control the microstructure and macrostructure of scaffolds and thus produce complex topographies from a computer model; their main drawbacks are the low resolutions achievable by the current systems and the types of polymeric materials that can be used [45]. Sachlos et al. [134] have not only discussed the conventional scaffold fabrication techniques and their drawbacks but have also described various SFF techniques and how they can overcome current scaffold design limitations. Tsang et al. [85] have discussed the various fabrication techniques by dividing them based on their mode of assembly, that is, fabrication with heat, binders, light, and molding, whereas Hutmacher et al. [163] have described SFF techniques by dividing them based on their processing technology. Yeong et al. [164] have well articulated the various RP techniques and their emerging subbranches as well as compared these methods and tabulated their strengths and weaknesses.

3.2.7.8 Hydrogels

Quite often, acellular scaffolds are designed to provide the required mechanical properties, but they end up being difficult to populate with cells uniformly, while constructs that are able to successfully achieve uniformly high cell distribution owing to their high porosity end up being mechanically weak [85]. Thus, researchers are increasingly trying to combine structural stability with high cell density while maintaining an in vivo–like environment to achieve the best of both worlds by fabricating hydrogels, which are cross-linked networks of hydrophilic polymers that are capable of absorbing large amounts of fluid. Hydrogels can be degradable or nondegradable, and their water content influences the viability of encapsulated cells and, thus, the rate of tissue development [165]. They are increasing in popularity due to their high water content and mechanical properties that are similar to soft tissues like cartilage [166]. Solid scaffolds provide a substrate for cells to adhere to where as liquid and gel scaffolds function to physically entrap cells [167]. Hydrogels can be formed in situ within a defect site and cells can be encapsulated during the hydrogel formation process. Their mechanical properties can be controlled by altering the comonomer composition, changing the cross-linking density, and modifying the polymerization conditions (reaction time, temperature, and amount and type of solvent) [168].

Photopolymerization is a commonly used technique for making hydrogels. Visible or UV light can react with certain light-sensitive compounds called photoinitiators to form cross-linked hydrogels in vitro, in vivo, or in situ. Thus, photopolymerization offers several advantages over traditional polymerization methods, namely, spatial as well as temporal control over polymerization, curing rates from less than a second to a few minutes at room or physiological temperatures, minimal heat production, as well as the ability to form complex shapes that adhere and conform to the defect site [166]. Although biological systems put constraints on the use of photopolymerization in vivo, owing to the limits of acceptable temperatures, pH, and toxicity of most monomers and organic solvents, these can generally be overcome by the implementation of mild polymerization conditions (low light intensity and organic solvent levels, short irradiation time, and physiological temperature) [166].

Although it may seem like hydrogel-based scaffold systems are at a disadvantage as far as mechanical properties of the skeletal system are concerned, they do provide an environment for accelerated tissue formation that in turn provides the desired mechanical stability [167]. Ferruti et al. [169] found amphoteric poly(amidoamine) (PAA)-based hydrogels containing carboxyl and amino groups in their repeating units to have a good potential as scaffolds, based on their cytocompatibility with fibroblasts as well as

noncytotoxic degradation products, but their mechanical properties needed improvement. They further modified the PAA hydrogels by introducing side guanidine groups to improve cell adhesion and proliferation and found the mechanical properties to improve when a second PAA carrying primary amino groups was used as a cross-linking agent [170]. Hydrogels are quite often modified with cell adhesion peptides to enhance cell attachment and spreading [171–173]. Sannino et al. [174] combined the photo-cross-linking reaction with a foaming process in order to induce an interconnected porosity within PEG-based hydrogels that had been modified with peptide sequences for enhancing cell adhesion.

Several groups are working on making hydrogels with synthetic copolymers [175] or a combination of natural and synthetic polymers [176,177]. Martens et al. [175] photoencapsulated chondrocytes in a PEG–polyvinyl alcohol (PVA) copolymer network and found DNA, GAG, and total collagen content to increase with culture time, resulting in homogeneously distributed neocartilageneous tissue at the end of 6 weeks. Hiemstra et al. [178] prepared PEG–PLA hydrogels to draw upon the excellent antifouling properties and renal clearance below 30 kDa of PEG and biodegradability of PLA, as well as the biocompatibility of both, in order to engineer cartilage. Cascone et al. [179] prepared blends of nonbiodegradable PVA with different biological macromolecules like hyaluronic acid, dextran, and gelatin to improve the biocompatibility of PLA and thereby produce bioartificial hydrogels as potential tissue engineering scaffolds. A unique property about hydrogels that is being increasingly exploited by tissue engineers is the ability to make them bioresponsive and, thus, *intelligent biomaterials* [180]. Wang et al. [180] synthesized a phosphoester-PEG (PhosPEG) hydrogel encapsulating marrow-derived mesenchymal stem cells (MSCs) for engineering bone. The rate of hydrolytic degradation of these phosphor-containing hydrogels increased in the presence of ALP, a bone-derived enzyme. The presence of phosphorus also increased mineralization and PhosPEG was also found to increase gene expression of bone-specific markers.

Rapid prototyping techniques have also begun to be used for fabricating hydrogels. Landers et al. [181] used 3D plotting, which is 3D dispensing in a liquid medium, to fabricate thermoreversible hydrogel scaffolds with a specific external shape and well-defined internal pore structure. They were also able to surface coat the scaffold to facilitate cell adhesion and growth. Dhariwala et al. [182] entrapped Chinese hamster ovary cells in a PEO hydrogel scaffold formed using STL. The cytotoxic effect of the initiator was minimized by using 50 μL of the initiator per milliliter of medium, and the exposure time to the UV laser for the in vitro cytotoxicity experiments was longer than what would generally be used, giving the authors confidence in the low cytotoxic effect of the initiator. However, the elastic modulus was found to be comparable to values of soft tissue like breast tissue and not cartilage. Arcaute et al. [148] also used STL to fabricate PEG hydrogels encapsulating human dermal fibroblasts, with at least 87% found to be viable up to 24 h after fabrication.

Hoffman [183] has given an excellent overview of the important physiochemical parameters and properties of hydrogels relevant to their use as matrices in tissue engineering applications along with discussing their pros and cons. Drury et al. [184] have given a comprehensive review of hydrogels used as tissue engineering scaffolds. They have discussed the main synthetic and natural polymers used for making hydrogel scaffolds along with scaffold design variables and have identified three categories of scaffold applications: space filling agents, bioactive molecule delivery, and cell/tissue delivery. Brandl et al. [185] have described a rational approach for designing hydrogels for tissue engineering applications with an emphasis on physical properties and outlined their impact on cell function and tissue morphogenesis.

3.2.7.9 Electrospinning

Another scaffold fabrication technique receiving increasing importance is that of electrospinning of nano- and microfibers for the production of scaffolds due to their resemblance scalewise to native ECM [186,187]. In this process, nanometer- or micrometer-scale diameter polymer fibers are produced using electrical forces [187,188]. When an applied electric field of high voltage creates large enough forces at the surface of a polymer solution to overcome the surface tension, an electrically charged jet is ejected that solidifies into an electrically charged fiber [188]. Branching and drawing of the fiber occur between the ejection point and a collecting unit some distance away. The fibers collect on a unit of various shapes in a nonwoven mat. Controlling the extrusion rate, solution concentration, applied voltage, collecting unit, distance to the collector, and environmental conditions affects the fiber diameter, mechanical properties, and morphology [187]. While aliphatic polyesters are commonly used for this method of fabrication, recent advances have resulted in increased use of natural polymers such as elastin and collagen as well as polysaccharides [189–191]. Aliphatic polyesters have been used with a variety of cell types for diverse applications including vasculature, bone, neural, and tendon/ligament tissue engineering [192], for example, PLLA for neural stem cell adhesion and differentiation; [193] PLGA for viability, growth, and differentiation of human MSCs [194]; and PCL for human dermal fibroblast adhesion [195] and contractile cardiac myocyte adhesion [196] as well as bone formation from MSCs [197]. Li et al. [186] studied the interaction of fibroblasts and bone marrow–derived MSCs on an electrospun 500–800 nm diameter PLG nanofibrous structure. Since pores in the structure were formed by randomly oriented fibers lying loosely upon one another, the cells while migrating through the pores could possibly push aside the surrounding unresisting, but mechanically strong, fibers thereby causing the pore to expand [186]. The authors hypothesized that this type of dynamic scaffold architecture allowed cells the freedom to adjust the pore diameter according to their liking and also let them pass through relatively small pores but cautioned that their theory needed further investigation. Li et al. [198] also evaluated electrospun 700 nm diameter PCL nanofibrous scaffolds for their ability to retain the functionality of chondrocytes and proposed their use as suitable scaffolds for cartilage tissue engineering. Yoshimoto et al. [199] too successfully cultured rat MSCs on electrospun 400 nm (±200 nm) diameter PCL scaffolds to show their potential as suitable scaffolds for bone tissue engineering. However, they found the fibers to have varying diameters along their lengths and irregular surfaces. Chen et al. [200] were able to achieve nanofibers up to a diameter of 117 nm but found them to contain beads, which in turn adversely affected cell adhesion and growth kinetics prompting them to conclude that the uniformity and diameter of the fibers played a crucial role in modulating cell attachment and proliferation. In spite of these minor drawbacks, nanofibers hold a great promise as potential scaffolds owing to their high porosity and high surface-area-to-volume ratio, which are favorable parameters for cell attachment, growth, and proliferation in addition to possessing favorable mechanical properties [186].

Deng et al. [201] investigated the morphology and biocompatibility of PLA–HA hybrid nanofibrous scaffolds prepared via electrospinning. They found the surface of the fibers to be coarse due to the formation of a new COO- surface bond and saw improved MG-63 cell attachment and proliferation compared to pure PLA scaffolds. Meng et al. [202] found mouse embryonic fibroblasts (NIH 3T3) cells to adhere and grow more effectively on electrospun poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV)/collagen composite nanofibrous scaffolds relative to PHBV nanofibrous scaffolds. Li et al. [203] investigated the cytocompatibility of a coelectrospun PLGA, gelatin, and alpha-elastin composite

scaffold (PGE) as a potential material for engineering of soft tissues like the heart, lung, and blood vessels. Pan et al. [204] created a highly porous electrospun dextran/PLGA scaffold by physically blending the two polymers and characterized it for different cellular responses using dermal fibroblasts from the point of view of using this composite in enhancing the healing of chronic or trauma wounds. Townsend-Nicholson et al. [205] employed a coaxial needle arrangement wherein a concentrated living cell suspension flowed through the inner needle and a medical-grade, highly viscous poly(dimethylsiloxane) (PDMS) with low electrical conductivity flowed through the outer needle, to form cell-containing composite microthreads. This bionanofabrication process did not seem to affect cell viability postelectrospinning, demonstrating the feasibility of using coaxial electrospinning to fabricate active biological scaffolds.

Natural polymers, most notably collagen and elastin, are increasingly electrospun due to the biomimetic properties offered. One of the challenges of electrospinning natural polymers involves the solvent used in the solution. A volatile solvent is necessary for the fibers to form and collect properly, yet the natural polymers have been reported to denature in many of the volatile solvents traditionally used in electrospinning [206]. There are also complications associated with the long-range electrostatic interactions and counterions present in certain biopolymer solutions [23]. To overcome these complications, researchers have altered the solution components as well as the electrospinning environment [23].

Researchers have studied different aspects of the electrospinning process to see how varying certain parameters associated with fiber production affect their properties. Thomas et al. [207] found that differences in collector rotation speeds affected tensile strength and modulus of aligned nanofibrous PCL meshes for bone scaffolds, due to increased fiber alignment and packing as well as decrease in interfiber pore size at higher uptake rates. Similarly, Li et al. [208] found that nanofiber organization was greatly influenced by the speed of the rotating target: the greater the speed of rotation, the better the fiber alignment, which in turn had a profound effect on mechanical properties. Pham et al. [209] utilized a multilayering technique to construct a PCL scaffold comprising alternate layers of electrospun micro- and nanofibers to combine their advantages in a single structure. Microfibers offer the advantage of providing a greater pore size that facilitates cellular penetration and diffusion of nutrients within the structure, while nanofibers provide a larger surface area for attachment and cell spreading [209]. Li et al. [210] characterized the physical and biological properties of six commonly used poly(alpha-hydroxy esters). Moroni et al. [211] studied the effect of different fiber diameters and their surface nanotopology on cell seeding, adhesion, and proliferation. They found smooth fibers with a diameter of 10 μm to support optimal cell seeding and adhesion within the range analyzed, while nanoporous surfaces were found to significantly enhance cell proliferation and spreading. Vaz et al. [212] used sequential multilayering electrospinning (ME) to produce a bilayered tubular scaffold comprising an outer stiff and aligned fibrous PLA layer and an inner pliable and randomly oriented fibrous PCL layer for engineering a blood vessel.

Researchers have also attempted to incorporate nanoparticles within the fibers or use nanocomposites to enhance the abilities of electrospun scaffolds. Wutticharoenmongkol et al. [213] fabricated novel electrospun scaffolds for bone tissue engineering using a PCL solution containing nanoparticles of calcium carbonate or HA, and these were successfully evaluated in vitro for attachment, proliferation, and ALP activity using human osteoblasts. Lee et al. [214] combined a nanocomposite technique along with electrospinning to produce a scaffold with two pore sizes: nanosized pores for transport of nutrients and waste and microsized pores for cell infiltration and blood vessel invasion. This was achieved by incorporating nanosized montmorillonite platelets into PLLA solution that

was subsequently electrospun and subjected to cold compression molding followed by salt leaching/gas foaming to get the microsized pores. Thomas et al. [215] created a nanocomposite scaffold by electrostatic cospinning of nanofibrous PCL and nanohydroxyapatite (nanoHA) to better mimic the features of natural ECM.

Electrospun scaffolds have also found applications in the cardiovascular and skeletal muscle tissue engineering area. Van Lieshout et al. [216] compared an electrospun valvular scaffold and a knitted valvular scaffold, both made from PCL, for their suitability in engineering of the aortic valve and concluded that the ideal scaffold would need to have the strength of the knitted structure combined with the cell-filtering ability of the spun structure. Zong et al. [217] examined the growth of cardiomyocytes on electrospun nanostructured PLGA membranes with different compositions to assess cell attachment, structure, and function on these potential heart tissue constructs. Riboldi et al. [218] evaluated the suitability of a commercially available electrospun degradable block polyesterurethane called DegraPol® as a scaffold for skeletal muscle tissue engineering by characterizing their morphological, degradative, and mechanical properties. Electrospinning has found a particular use in tissue-engineered vascular grafts (TEVGs) due to the ability to tailor the graft by adjusting the electrospinning parameters and the ease of producing tubular constructs [219]. TEVG has been electrospun using a wide range of both synthetic and natural polymers in an effort to withstand the mechanical constraints of a vascular graft while promoting cell organization [219].

Electrospun scaffolds have also begun to be used for releasing drugs, growth factors, and DNA. Kim et al. [220] have successfully demonstrated the incorporation and sustained release of a hydrophilic antibiotic from electrospun PLGA-based nanofibrous scaffolds without the loss of structure and drug bioactivity. Luu et al. [221] have successfully demonstrated the incorporation and controlled release of plasmid DNA from an electrospun synthetic polymer/DNA composite demonstrating its potential use for therapeutic gene delivery. The synthetic polymer comprised PLGA random copolymer and PLA–PEG block copolymer.

Other publications have discussed electrospun scaffolds in detail. For example, Nair et al. [222] have reviewed recent advances in the development of synthetic biodegradable nanofibrous scaffolds fabricated via electrospinning. Boudriot et al. [223] have reviewed the spinning parameters relevant for making scaffolds as well as discussed scaffolds composed of nanofibers. Teo et al. [224] have discussed the ECM and how electrospinning techniques combined with surface modification and cross-linking of nanofibers can help one tailor the scaffold to meet the requirements of the tissue they wish to regenerate. Electrospinning is not the only process by which nanofibrous scaffolds can be produced. Smith et al. [225] have discussed how self-assembly, electrospinning, and phase separation can produce nanofibrous scaffolds spanning the entire range of sizes of ECM collagen.

3.3 Modification of Scaffolds

Scaffolds can also be modified to deliver biomolecules like proteins and growth factors as well as drugs [226,227]. Growth factors are polypeptides that either stimulate or inhibit cellular activities like proliferation, differentiation, migration, adhesion, and gene expression [228]. Growth factors can be incorporated directly into the scaffold during or after fabrication. Sheridan et al. [103] and Farokhi et al. [229] incorporated vascular endothelial

growth factor (VEGF) into PLG scaffolds during the fabrication process and released it in a controlled manner. The released VEGF was found to retain over 90% of its bioactivity. Hu et al. [230] incorporated the osteoinductive growth factor bone morphogenetic protein (BMP) into composite scaffolds made of hydroxyapatite/collagen (HAC) and PLA, which were implanted in diaphyseal defects of dogs. Histological studies revealed that BMP not only promoted osteogenesis but also caused an accelerated degradation of the scaffold material. Williams et al. [142] seeded PCL scaffolds fabricated via SLS with BMP-7-transduced fibroblasts and implanted these constructs subcutaneously in mice to evaluate the biological properties. Histological evaluation and microCT analysis confirmed the generation of bone. Grondahl et al. [231] modified PHBV with acrylic acid by graft copolymerization. PHBV is used in bone tissue engineering owing to its biocompatibility, favorable degradation characteristics, suitable mechanical properties, and support of osteoblast attachment. Acrylic acid was used to induce surface hydrophilicity, to eventually improve HA growth, and increase cell compatibility. Moreover, the carboxylic acid groups that were introduced on the PHBV surface by acrylic acid were linked to glucosamine, which is a model biomolecule, to show the ability of the material to be modified for tissue engineering applications. Similarly, Ma et al. [232] introduced a stable collagen layer on the PLLA scaffold surface, via grafting of poly(methacrylic acid) (PMAA), in order to incorporate basic fibroblast growth factor (bFGF) to improve biocompatibility, enhance cell growth, and more closely mimic the natural ECM. Ennet et al. [104] incorporated VEGF either directly into PLGA scaffolds or preencapsulated in PLGA microspheres that were used to fabricate the scaffolds using gas foaming. The preencapsulation led to VEGF being embedded more deeply within the scaffold, thereby resulting in a delayed release. In vivo, the released VEGF significantly enhanced local angiogenesis with negligible amounts being released in the systemic circulation. Park et al. [233] coencapsulated bovine chondrocytes and gelatin microparticles loaded with transforming growth factor-β1 (TGF-β1) in a novel injectable hydrogel composite oligo(poly(ethylene glycol) fumarate) (OPF) for growing cartilage.

Protein and drug delivery using scaffolds for the purpose of enhancing cellular activity and treating local acute inflammation, respectively, are also being pursued. Lenza et al. [234] developed bioactive scaffolds that would allow the incorporation and delivery of proteins at controlled rates for the promotion of cell function and growth of soft tissue. Yoon et al. [102] fabricated dexamethasone-containing porous PLGA scaffolds by a gas foaming/salt leaching method to create a biodegradable stent for reducing intimal hyperplasia in restenosis. Dexamethasone, which is a steroidal anti-inflammatory drug, was slowly released from the PLGA scaffold in a controlled manner for over a month without showing an initial burst release and was successful in drastically suppressing the proliferation of lymphocytes and smooth muscle cells in vitro.

A severe drawback to direct protein delivery is rapid degradation in vivo and limited stability even if encapsulated in a polymeric delivery vehicle [235]. A promising solution is the use of localized gene therapy to promote the creation of the required growth factor at the specific site of interest [235]. Thus, scaffolds can also be used as vehicles for gene delivery to promote localized transgene expression for inducing formation of functional tissue [236]. Jang et al. [236] employed substrate-mediated delivery that involves immobilization of DNA complexes on the polymer surface for eventual delivery to cells growing on the polymer. They studied the immobilization of polyethylenimine (PEI)/DNA complex and eventual cellular transfection on PLG scaffolds. With this technique, they were able to uniformly distribute the DNA throughout the scaffold, thereby transfecting more than 60% of the cells using low quantities of DNA at the surface.

3.4 Types of Scaffold Materials

Scaffold materials are either fabricated from synthetic polymers or derived from naturally occurring ones. Synthetic polymers have the advantage of possessing highly controllable properties of strength, rate of degradation, and microstructure as well as batch-to-batch consistency [89,237]. Naturally derived materials possess the potential advantage of biological recognition that might enhance cell attachment and function [89]. However, they have certain disadvantages as well, which include limited control of mechanical properties, biodegradability and batch-to-batch consistency, limited availability contributing to their being expensive, possible exhibition of immunogenecity, and possession of pathogenic impurities [89]. Velema et al. [8] have written an excellent review discussing three important types from the two main classes of naturally derived polymers that are used for fabricating scaffolds. These are polysaccharides (alginate, chitosan, and hyaluronan) and fibrous proteins (collagen, silk fibroin, and elastin). We will only be discussing synthetic polymers here. Nair et al. [238] have very elegantly discussed the primary synthetic and natural polymers used for tissue engineering and drug delivery, while Gunatillake et al. [237] have detailed the major classes of synthetic biodegradable polymers in tissue engineering and discussed them with regard to synthesis, properties, as well as their biocompatibility and biodegradability. Holland et al. [239] have discussed the different synthetic degradable polymers used for drug delivery in bone tissue engineering applications, while Agrawal et al. [5] have discussed the major biodegradable synthetic polymers for musculoskeletal tissue engineering applications and their methods of fabrication. Chen et al. [240] have provided an extensive review of elastomeric biomaterials for tissue engineering with copious references as well.

3.4.1 Polyesters

The most commonly used synthetic polymers are the aliphatic (α-hydroxy) polyesters [240]. These include PGA, PLA, and their copolymer PLGA, which have been widely used in a number of clinical applications, mainly as resorbable sutures as well as plates and fixtures for fracture fixation devices [237,238]. PGA is highly crystalline with a melting point exceeding 200°C and a glass transition temperature (T_g) around 35°C–40°C [237,238]. Owing to its high crystallinity, it possesses high tensile strength and modulus as well as low solubility in most organic solvents. PLA is generally used in the form of PLLA and PDLLA to fabricate scaffolds. PLLA is semicrystalline with the degree of crystallinity depending on the molecular weight and processing parameters. It too possesses high tensile strength and modulus and has a melting point of 170°C and a T_g of 60°C–65°C. PDLLA, on the other hand, is amorphous with a T_g of 55°C–60°C. The chemical structures of PLA and PGA are similar except that PLA has a pendant methyl group making it more hydrophobic, and thereby more resistant to hydrolytic attack, than PGA. This produces differences in the degradation kinetics of the two and thus the degradation rate of their copolymer (PLGA) is controlled by the ratio in which these two are present [5]. PLA, PGA, and PLGA undergo bulk degradation by random hydrolysis of their ester linkages, whereby material is lost from the entire polymer volume simultaneously due to penetration of water into the bulk of the scaffold. PLA degrades to form lactic acid, which is normally present in the body, and enters the tricarboxylic acid (TCA) cycle to be excreted as water and carbon dioxide. PGA is broken down by hydrolysis and nonspecific esterases and carboxypeptidases. The glycolic acid monomer is either excreted via urine or enters the TCA cycle [5].

Some of the disadvantages of the aliphatic polyesters include inferior mechanical properties, release of acidic degradation products, and limited processability [237].

PCL is a semicrystalline polyester with a T_m of 60°C and a T_g of −60°C. PCL degrades at a much slower rate compared to the other aliphatic polyesters and has hence been widely used in the area of long-term controlled drug delivery. It is flexible, easy to process, and structurally and thermally stable while simultaneously being less sensitive to environmental changes [94,240]. It possesses an exceptional ability to form blends with a variety of polymers thereby making it a widely used material for scaffold fabrication, especially pertaining to cartilage and bone tissue engineering applications [6]. PCL degrades hydrolytically via both bulk and surface erosion with 5-hydroxyhexanoic acids (caproic acids) being the degradation product [19].

3.4.2 Polyfumarates

PPF is the most widely studied in the category of copolyesters based on fumaric acid. It holds promise as a bone-tissue engineering material [4,241] for filling skeletal defects as it has mechanical properties similar to that of trabecular bone and possesses the ability to cure in situ thereby providing skeletal defects of any shape or size to be filled with minimal intervention [242]. PPF-based polymers are available as injectable systems that employ chemical cross-linking thereby facilitating the treatment of deep crevices in bone and defects of nonuniform shapes by being cross-linked in situ [243]. PPF possesses unsaturated sites in its backbone that are used in cross-linking reactions resulting in complex structures [237]. Since achieving PPF of high molecular weight is difficult owing to side reactions due to the presence of the backbone double bond, ceramics such as TCP, calcium carbonate, or calcium sulfate are incorporated to improve mechanical properties [237,243]. β-TCP not only increased mechanical strength but also acted as a buffer by minimizing pH change during degradation [243]. Peter et al. [244] concluded that injectable PPF/β-TCP pastes could be prepared with handling characteristics suitable for clinical orthopedic applications and found the mechanical properties of the cured composites to be suitable for trabecular bone replacement. They also investigated the in vivo degradation and biocompatibility of PPF/β-TCP composites and found them to elicit a mild initial inflammatory response followed by thin fibrous encapsulation [245] and also found the composite to be osteoconductive in vitro [246]. PPF/β-TCP has also been cross-linked with other polymers, like poly(ethylene glycol) dimethacrylate (PEGDMA) [247], while some PPF composites have been reinforced with nanoparticles [22] in order to enhance their mechanical properties for orthopedic tissue engineering applications. PPF composites have also been successfully employed as carriers of microspheres carrying model drugs [248] or microparticles encapsulating osteoblasts for bone tissue engineering applications [249]. The bioactivity of PPF was found to be augmented in vivo by the incorporation of nanoHA [250], while PPF coated with recombinant human transforming growth factor beta 1 (rhTGF-β1) was found to adequately induce bone formation in the cranium of rabbits [251]. Some novel fumarates, like poly(ε-caprolactone fumarate) [252], poly(ethylene glycol fumarate), and their copolymer, are also under investigation for diverse tissue engineering applications [253]. PPF undergoes bulk degradation by hydrolysis to produce fumaric acid, which is a naturally occurring substance in the TCA cycle (Krebs cycle), and propylene glycol [237].

3.4.3 Polyanhydrides

Polyanhydrides are surface-eroding polymers with low hydrolytic stability making them ideal candidates for drug delivery applications [254]. They possess a highly hydrophobic

backbone that prevents water from penetrating into the scaffold interior and a hydrolytically sensitive anhydride bond that confines the degradation to the surface, resulting in linear mass loss kinetics and zero-order drug release kinetics when used as drug delivery systems [238,255]. Their rapid degradation contributes to their poor mechanical properties, prompting the incorporation of imide segments to create scaffolds from poly(anhydride-co-imide) [256], especially for orthopedic applications [257]. Ibim et al. [258] studied the biocompatibility and osteocompatibility of poly(anhydride-co-imide) and found them to produce endosteal and cortical bone growth and a local tissue response similar to PLGA. They advocated the use of these polymers in weight-bearing orthopedic applications. Burkoth et al. [259] used porogen leaching to create polyanhydride constructs that could be eventually filled with osteoblasts photoencapsulated in a hydrogel to potentially create a synthetic allograft for engineering bone. The degradation rate of polyanhydrides, which degrade by hydrolysis of the anhydride linkage, can be altered by making simple changes to the polymer backbone structure via a judicious choice of the diacid monomer [237]. Combining different amounts of these monomers could produce polymers with custom-designed degradation properties [243].

3.4.4 Poly(Ortho Esters)

Poly(ortho esters) (POEs) undergo surface erosion and the rate of degradation can be controlled by using diols having varying degrees of chain flexibility or with the incorporation of acidic and basic excipients [238]. Andriano et al. [260] found POE to possess better control over polymer mass loss with new tissue formation as well as better structural integrity relative to 50:50 PLGA. Also, bone mineral density in POE scaffolds was found to be 25% higher than PLGA scaffolds, although the amount of bone formed was inconsequential. Ng et al. [261] hypothesized that the appropriate choice of diols and their ratios could result in the formation of POEs that were viscous fluids in the 37°C–45°C range that converted to nondeformable highly viscous materials at or below 37°C. This could be of tremendous use in cases where slightly warmed materials could be injected into the desired site of injury or drug release, where they would eventually solidify at body temperature into, possibly, drug-releasing scaffolds. Incorporation of proteins or antigens could be achieved by simple mixing with the gently warmed polymer without the need of solvents or water. Kellomaki et al. [262] found the rate of hydrolysis of two POEs, as measured by the strength loss of the polymers, to be too rapid for load-bearing orthopedic applications. Ng et al. [261] used POEs containing varying amounts of glycolic acid dimer segments in the polymer backbone to accurately control the erosion rate that proceeds by zero-order kinetics. This polymer, when placed in an aqueous environment, would hydrolyze to produce glycolic acid that would catalyze hydrolysis of the ortho ester linkages of the polymer backbone. Thus, by varying the amount of acid segment in the polymer backbone, one could finely tune the rate of degradation from a few days to several months [238].

3.4.5 Poly(Amino Acids) or Polycarbonates

Synthetic poly(amino acids) are very similar to naturally occurring proteins but possess low degradation rates, unfavorable mechanical properties, and immunogenecity [263]. Thus, amino acids have been used as monomeric building blocks in polymers lacking the conventional backbone structure present in peptides to overcome these drawbacks [263]. Tyrosine-derived polycarbonates are the most extensively studied from this group and possess a T_g in the range of 52°C–93°C and a decomposition temperature

exceeding 290°C [263]. The backbone carbonate bond is hydrolyzed faster than the pendant chain ester bond, except under very acidic conditions (pH ≤ 3) when the rates get interchanged due to acid-catalyzed hydrolysis of the ester bond [264]. Final degradation products of polycarbonates in vitro are desaminotyrosyl-tyrosine and alcohol, while in vivo, one can expect the former to enzymatically degrade into desaminotyrosine and L-tyrosine [263]. From a degradation-biocompatibility perspective, the tyrosine-derived polycarbonates were found to be similar to PLA when studied in a canine bone chamber model [265] and showed good potential as orthopedic implant materials [266]. To decrease the hydrolytic stability of polycarbonates, the carbonyl oxygen was replaced by an imino group resulting in the production of polyiminocarbonates that had hydrolytically degradable fibers that retained the strength of polycarbonates [267]. Polycarbonates having an ethyl ester pendant group have shown to be quite osteoconductive, with good bone apposition and possessing adequate mechanical properties for load-bearing bone fixations [237]. Meechaisue et al. [268] used electrospinning to produce a mat of poly(DTE carbonate) fibers as tissue scaffolding material that supported the adhesion and propagation of three different cell lines.

3.4.6 Polyphosphazenes

Polyphosphazenes have an inorganic backbone of alternating phosphorus and nitrogen atoms, which can be rendered hydrolytically unstable by substituting with appropriate organic side groups on the phosphorus atoms. Their good biocompatibility, synthetic flexibility, hydrolytic instability, nontoxic degradation products, ease of fabrication, and matrix permeability make them highly adaptable for tissue engineering [238], drug delivery [269], and gene delivery [270] applications. The pentavalency of phosphorus in polyphosphazenes provides active sites for attachment of drug molecules. The degradation products of these polymers are phosphates, ammonia, and the corresponding side groups, all of which are neutral and nontoxic [238]. Laurencin et al. [271] modulated cell growth and polymer degradation by varying the nature of the hydrolytically unstable side chains of the polyphosphazenes. They found the polymer to support osteoblast attachment and proliferation showing potential for skeletal tissue regeneration [272]. Ambrosio et al. [273] and Krogman et al. [274] designed blends of polyphosphazenes with PLGA to decrease the acidity of the degradation products of PLGA via the neutralizing effect produced by the degradation products of the polyphosphazene. Polyphosphazene nonwoven nanofiber meshes created by electrospinning were found to promote adhesion and proliferation of osteoblast-like cells [275]. Greish et al. [276] formed composites of HA and polyphosphazenes at physiologic temperatures via a dissolution–precipitation process that resulted in a mildly alkaline environment suitable for deprotonation of the acidic polyphosphazene and formation of calcium cross-links. Carampin et al. [277] used electrospinning to generate flat or tubular matrices of polyphosphazene comprising ultrathin fibers to mimic blood vessels. Neuromicrovascular endothelial cells formed a monolayer on the whole surface after 16 days of incubation, thereby demonstrating the feasibility of the polymer to form human tissues like vessels and cardiac valves.

3.4.7 Composites

As discussed earlier, scaffolds made of composites allow the tailoring of mechanical properties and resorption rates according to the specific needs of the implant site, as well as enhance bioactivity [7,16,24]. The use of composites is mostly in the arena of

musculoskeletal tissue engineering, mainly bone, as that is where tailoring of mechanical properties is most crucial. The most commonly used composite combinations comprise HA, TCP, or BIOGLASS particles or fibers used as fillers or coatings or both in PLA, PGA, or other resorbable polymers [16,24,278]. Zhang et al. [279] incorporated HA into PLLA and PLGA to fabricate scaffolds for bone tissue engineering that had better osteoconductive properties as well as superior buffering capability and improved mechanical properties. Marra et al. [280] used blends of PLGA, PCL, and HA, while Roether et al. [16] fabricated PDLLA foams coated with and impregnated by BIOGLASS as scaffolds for bone tissue engineering. Taboas et al. [46] created biphasic scaffolds with mechanically interdigitated PLA and sintered HA regions having 600 and 500 μm wide global pores, respectively.

3.5 Conclusions

Scaffolds form one of the most important components of a tissue engineering construct. Their functions, requirements, methods of fabrication, modifications, and commonly used synthetic scaffold materials have been discussed with the intention of impressing upon the reader the amount of research that is being done in order to create the ideal scaffold. One must appreciate that the requirements of different tissues in the body are unique, and although most scaffold materials satisfy these requirements to varying degrees, there are some materials or combinations of materials that are better suited for specific applications. It would be extremely helpful, but difficult, to compare the best biomaterial candidates for different tissue applications by considering a common set of parameters and evaluation procedure so as to determine which one works the best. With newly emerging scaffold fabrication techniques like electrospinning as well as the continuous modification of existing methods, like cell and organ printing, along with the emergence of composite and hybrid materials as well as the benefits of adding nanoparticles/nanocomposites, the quest for finding the best scaffold seems to be within reach in the not too distant future.

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