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Polymeric Biomaterials for Scaffold-Based Bone Regenerative Engineering

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Abstract

Reconstruction of large bone defects resulting from trauma, neoplasm, or infection is a challenging problem in reconstructive surgery. The need for bone grafting has been increasing steadily partly because of our enhanced capability to salvage limbs after major bone loss. Engineered bone graft substitutes can have advantages such as lack of antigenicity, high availability, and varying properties depending on the applications chosen for use. These favorable attributes have contributed to the rise of scaffold-based polymeric tissue regeneration. Critical components in the scaffold-based polymeric regenerative engineering approach often include (1) the existence of biodegradable polymeric porous structures with properties selected to promote tissue regeneration and while providing appropriate mechanical support during tissue regeneration, (2) cellular populations that can influence and enhance regeneration, and (3) the use of growth and morphogenetic factors which can influence cellular migration, differentiation, and tissue regeneration in vivo. Biodegradable polymers constitute an attractive class of biomaterials for the development of scaffolds due to their flexibility in chemistry and their ability to produce biocompatible degradation products. This paper presents an overview of polymeric scaffold-based bone tissue regeneration and reviews approaches as well as the particular roles of biodegradable polymers currently in use.

Lay Summary

Biomaterials have become an indispensable tool used in biomedical applications ranging from scaffolds for regenerative engineering to controlled drug delivery and immunomodulation. Regenerative engineering is a developing multidisciplinary field of research that employs the principles of advanced materials science, stem cell science, physics, developmental biology, and clinical translation for the regeneration of damaged tissues. In this field, biomaterials can play a major role. Degradable polymeric biomaterials can be excellent components for developing 3D porous structures used as scaffolds for tissue regeneration.

Keywords Biomaterials · Biodegradable polymers · Regenerative engineering · Cell-material interactions

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Introduction

The use of polymeric materials as scaffolds in tissue regeneration is well known [1, 2]. Recent progress in material design and fresh perspectives on material-cell interactions present new opportunities for the development of systems for bone tissue regeneration with diverse properties [1, 3]. Bone tissue regeneration often utilizes polymeric scaffolds, which act as physical support for regenerating tissue [4]. Biological tissues are complex structures with unique cell compositions, chemistry, and mechanical properties [5]. Therefore, it is important that when biomaterials are used in engineering scaffolds, they are optimized to meet the desired requirements for tissue regeneration [6–8]. Various functionalities can be conferred on polymers to tailor their degradation rates, environmental sensitivities, and mechanical properties [9]. With this design

flexibility, different materials with a wide range of properties can be produced to meet the ever-changing needs of regenerative engineering [4, 9–11]. Regeneration of bone tissues with scaffolds which can also be combined with cells and/or suitable biochemical signals has been a successful paradigm [1, 9]. Bone constitutes a widely investigated tissue in regenerative engineering [12]. Several bone graft options such as autografts, allografts as well as a variety of bone graft substitutes are currently available for the surgeons [8, 13]. Autografts and allografts are extensively used, and their biological activity has been found to be due to two primary functions: osteoinduction and osteoconduction [8, 14, 15]. Osteoinduction is driven by the use of graft-derived proteins called bone morphogenetic proteins (BMPs) [14, 16–18]. Among biological bone grafts used, autograft is still considered as the gold standard and has been used in diverse clinical situations such as fracture nonunions and revision of total joint replacements [8, 16]. However, autogenous grafting has several complications such as donor site morbidity and limited availability for harvest [14, 16]. The use of allograft is advantageous in this regard because there is no second procedure needed to remove and transfer a part of the patient's native bone or tissue [16]. Even though allogenic bone grafts have some advantages over autografts, lower osteoinductivity, the risk of disease transmission, and immune rejection raise concerns [14, 16]. These limitations fueled the quest for new alternatives and led to the development of synthetic bone graft substitutes [1]. Bone substitutes, in general, are natural, synthetic, or composite materials used to fill bone defects for the promotion of bone healing [13]. Among these biomaterials, biodegradable polymers constitute an attractive class of synthetic bone graft substitutes. They are often employed for the development of 3D porous scaffold structures [19]. The nature and properties of the 3D scaffolding matrices can be an essential factor for the success of the implant [1]. In bone regenerative engineering, the scaffolds are designed in general to occupy the space and volume of the defect and often provide mechanical integrity [13, 20]. An ideal synthetic biomaterial for orthopedic applications should be biocompatible, have desired mechanical properties, should degrade in a controlled fashion timed to match the rate of bone tissue regeneration, have resorbable degradation products, be osteoconductive, and allow for neovascularization [1, 13, 14, 16, 19, 21]. There has been a continuing effort focused on polymeric material design and the study of material-cell interactions aimed at creating improved polymeric matrices for bone regeneration. Materials interactions can be considered in two ways. "Material dynamics" describes how the material affects the surrounding tissues and "material kinetics" describes how the tissue in the microenvironment affects material properties [5]. Thus, material design has focused on personalizing the synthesis of biodegradable polymers with customized and tailored properties to match specific tissue types and disease states [5, 19]. This approach may ultimately ensure enhanced material performance and positive clinical results.

Concept and Recent Advances in Bone Regenerative Engineering

Scaffold-based bone regenerative engineering ideally utilizes a biomimetic scaffold that provides a structural guide for tissue regeneration [1, 16, 19]. In this approach, biodegradable polymers play a vital role as substrates for tissue development [1, 19, 22]. The main purpose of this approach is to provide a flexible toolbox for bone grafting and regeneration of complex tissues and organs [4, 9]. This toolbox combines the fields of advanced materials science and engineering, stem cell science, physics, developmental biology, and clinical translation [1]. In an idealized scenario, an appropriate scaffold with the right geometry and architecture is ensured by advanced material science and engineering [1]. The scaffold ideally is expected to provide adequate initial mechanical support, structural integrity, and dimensional stability, while modulating the cellular activities by presenting chemical and biochemical cues with precise spatial and temporal control [23]. The use of bioactive molecules to induce tissue regeneration and bone healing can be an important aspect of bone tissue regeneration and can be informed through lessons coming from morphogenesis and developmental biology [1, 8, 16]. Growth is important as regards to tissue induction and complex tissue regeneration [1]. Mechanical simulation can have a positive impact on tissue regeneration and bone healing [19]. Stem cells are now understood to be important in tissue regeneration [24–26]. Currently, stem cells are being investigated for the repair and regeneration of cartilage, bone, ligament, tendon, and muscle tissue due to their potential to replicate and develop into many different cell types [27]. They are also capable of releasing bioactive substances such as growth factors, cytokines, and chemokines for the growth and migration of cells [28]. Clinical and translational efforts in stem cell use present important possibilities for bringing discoveries to the bedside. Polymeric biomaterials continue to have an important role in new and innovative approaches to tissue regeneration as they present possibilities for precise tunability regarding degradability, biocompatibility, and mechanical properties [19].

Bone Tissue—Composition, Structure, and Mechanics

Bone is a highly specialized connective tissue which consists of a structural framework of a mineralized matrix and a heterogeneous cell population [19, 20, 29–31]. It is characterized by its marked rigidity and hardness. The hardness of bone is due to the deposition of complex mineral substances, calcium hydroxyapatite composed of calcium, phosphorus, magnesium, fluoride, and other ions in trace amounts, within the soft

organic matrix of collagen, which is responsible for the toughness and viscoelasticity [13, 20]. Based on the general shape, bone can be categorized into long bones (femur and tibia), short bones (wrist and ankle), and flat bones (skull vault), and irregular bones [32]. There are two types of bone tissue: the cortical bone (compact) and the trabecular bone (cancellous) (Fig. 1). Cortical bone constitutes 80% of the total human skeleton by mass. The compact bone consists of closely packed Haversian systems with a central Haversian canal surrounded by concentric rings (lamellae) of the matrix [34, 35]. It is a very dense material with 5 to 10% porosity. As its name implies, cortical bone forms the cortex, or outer shell, of most bones. Cortical bone is much denser, harder, and stiffer than trabecular bone, and it is important in maintaining the structural and mechanical functions of the skeleton [35]. On the other hand, trabecular bone is commonly found at the end of long bones, which is covered by hard outer cortical bone. Trabecular bone has a loosely organized porous matrix where collagen fibrils form concentric lamellae (see Fig. 2). It is highly porous with 50–90% porosity and plays an important role in maintaining metabolic activity [32, 35]. A periosteum covers the outer surface of bones. Periosteum consists of an inner layer that contains committed osteogenic cells and an outer fibrous layer. It provides vascular and nerve supply to bones and serves as attachment sites for surrounding tendons and muscles. The inner vascular thin layer lining the marrow cavity is called endosteum, which also contains bone precursor cells [35].

Bone is an anisotropic material with mechanical properties that are dependent on the orientation at which the forces are applied [36]. The compressive moduli of cortical bone and trabecular bone are in the range of 17–20 and 0.02–0.9 GPa, respectively. The compressive strengths of cortical bone and trabecular are 100–230 and 2–40 MPa, respectively. The tensile moduli of cortical bone and trabecular bone are in the range of 7–30 and 0.05–0.1 GPa (50–100 MPa), respectively. The tensile strengths of compact bone and trabecular bone are 80–150 and 1–10 MPa, respectively [20, 29, 37, 38].

The cortical and the trabecular bone are similar in composition but differ in the microstructural arrangement. Because of the composite nature of bone, it is characterized just like other composite materials. The mechanical properties of bone will, therefore, depend on the individual components (collagen and hydroxyapatite component). Collagen possesses Young's modulus of 1–2 GPa and an ultimate tensile strength of 50–1000 MPa, compared to the hydroxyapatite component, which has Young's modulus of ~ 130 GPa and an ultimate tensile strength of ~ 100 MPa [39]. The complex nature and arrangement of the collagen and the minerals have hence made the mechanical characterization of bone elusive. Thus, the physical and mechanical properties are not fully understood [31].

Bone Cells

Four major types of bone cells participate in the production, maintenance, and modeling of the bone matrix: osteoblasts, osteocytes, bone lining cells, and osteoclasts (see Fig. 3) [35, 36]. The interaction and communication among these cells are crucial for the maintenance of healthy bone tissue. Osteoblasts, osteocytes, and bone lining cells are derived from mesenchymal stem cells (MSCs) found in the bone marrow or the periosteum, whereas osteoclasts originate from the hematopoietic stem cells in the blood [30].

Osteoblasts are the mature bone-forming cells which are responsible for bone matrix synthesis, deposition, and its subsequent mineralization [40]. As illustrated in Fig. 3, they are cuboidal cells that are clustered in layers on the bone surface. Coordinated efforts of osteoblasts generate the lamellar structure of bone matrix [35]. Throughout the differentiation process, osteoblasts secrete and produce a characteristic extracellular matrix whose constituents are indicators of osteoblastic phenotype [30].

Type I collagen makes up 90% of the bone matrix (osteoid) that undergoes subsequent mineralization to form mineralized tissue [13, 35, 41]. Other noncollagenous proteins include osteopontin, osteonectin, bone sialoprotein, and osteocalcin [13]. Alkaline phosphatase is present in osteoblasts and is involved in the mineralization process [42]. Some of the osteoblasts are then entrapped within the formed matrix and become osteocytes; some remain on the bone surface and become flattened bone lining cells; others undergo apoptosis and disappear [41]. The activity of osteoblasts in bone formation can also be mediated by a number of growth factors: fibroblast growth factor (FGF), transforming growth factor-β (TGF-β), BMPs, insulin-like growth factor (I and II) (IGF), and platelet-derived growth factor (PDGF) [13, 35, 36, 43].

Osteocytes originate from the osteoblasts entrapped within the newly formed bone matrix, which ultimately becomes calcified [35]. Osteocytes are stellate shaped and responsible for the maintenance of mineralized bone via their limited ability to synthesize and resorb the matrix (Fig. 3) [30].

Bone lining cells exhibit a thin, flat, and elongated morphology. They are inactive cells on the bone surfaces that are neither being formed nor being resorbed [32, 35]. Though originated from osteoblasts, bone lining cells have less cytoplasm and fewer organelles. The functions of these lining cells are still under investigation [32]. Recent studies have shown that bone lining cells can communicate with the entrapped osteocytes and contribute to the anchorage of hematopoietic stem cells and their subsequent differentiation into osteoclasts. Besides, these lining cells secrete matrix metalloproteinases to remove the thin layer of osteoid covering the mineralized matrix. These actions are essential for attracting osteoclasts to attach to specific bone sites to initiate bone resorption. After

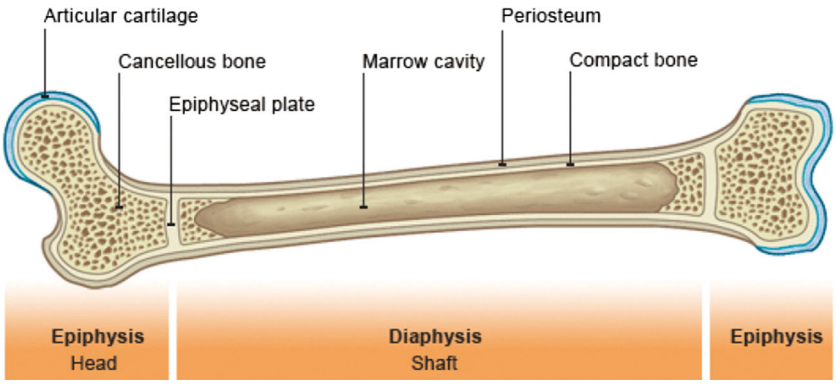


Fig. 1 Diagram showing cortical and trabecular bone [33]

remodeling, a collagen layer is secreted by the bone lining cells to cover the bone surface [32].

Osteoclasts are bone-dissolving cells that carry out the resorption of mineralized tissue [32, 35]. During bone resorption, osteoclasts attach directly to the active sites of the bone surface. They have two distinct plasma membrane regions: a ruffled border where resorption takes place and a sealing zone that forms a connection between the osteoclast and underlying matrix. Also, in the sealing zone, the osteoclasts secrete hydrochloric acid to acidify and dissolve the hydroxyapatite crystals which constitute the mineral portion of the extracellular bone matrix [44]. Following further proteolysis of the bone matrix by the released enzymes and collagenase, the matrix is degraded and dissolved. The matrix degradation products are then removed from the resorption lacuna and transported into the extracellular space through the basolateral membrane of the osteoclasts [30, 44].

The *hierarchical structures* of bone at different length scales are of great importance in maintaining the chemical, mechanical, and biological properties of bone [34]. As summarized in Fig. 4, the structural hierarchy of bone architecture includes five levels according to the length scales: (1) the macrostructures of cortical bone and trabecular bone, (2) the microstructures (10–500 μm) of the osteons and trabeculae, (3) the submicrostructures (1–10 μm) of bone lamella, (4) the nanostructures (from a few hundred nanometers to 1 μm) of collagen fibrils, and (5) the subnanostructures (below a few hundred nanometers) of collagen molecules, bone crystals, and noncollagenous organic proteins. All these five levels of structural components are arranged in a coordinated fashion to make bone material heterogeneous and anisotropic [31, 34, 46].

A good understanding of the bone anatomy, properties, and internal organization will ensure ideal selection of polymeric materials with optimal characteristics for bone tissue regeneration. Natural and synthetic polymers that have been investigated for bone tissue regeneration are discussed in the next section.

Biodegradable Polymers as Biomaterials for Regenerative Engineering

Polymeric materials account for more than half of the biomaterials market [13, 19, 47, 48], and this market will continue to grow due to the high demand of polymeric biomaterials for biomedical applications. In 2016, the biomaterial market was USD 70.90 billion, and it is predicted that the market will hit USD 400 billion by 2020 [49].

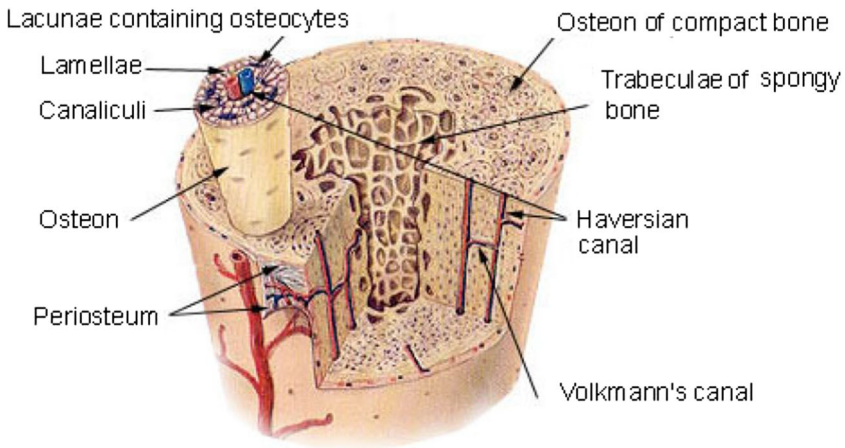


Fig. 2 Bone matrix arranged in the form of concentric rings, lamellae, centered on Haversian canals to form osteons [33]

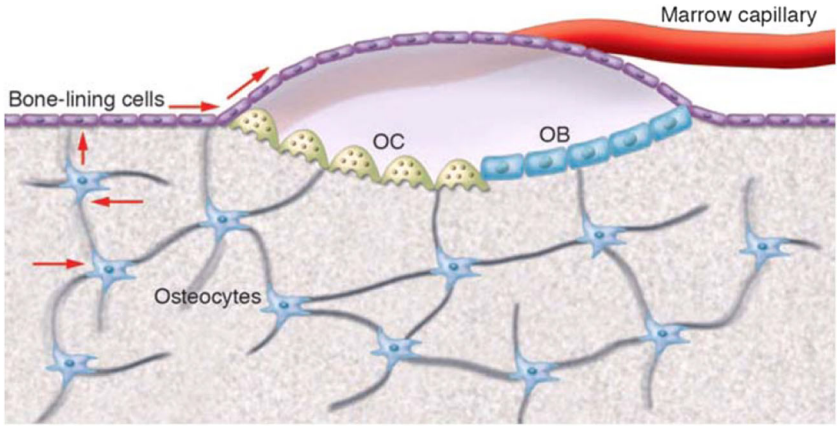


Fig. 3 Bone cell types and organization. Osteoblasts (OB), bone lining cells, and osteoclasts (OC) reside on the bone surface, whereas osteocytes are in the interior of the bone matrix. The gap junctions between all cells might provide a pathway as indicated by the arrows for the signals transduced from osteocytes in the bone matrix to OB and OC on the bone surface [30]. Reproduced with permission from ref. [30]. Copyright 2008 American Society for Clinical Investigation

A wide variety of both natural and synthetic polymers have been investigated for the design and fabrication of scaffolds for bone tissue regeneration [16, 19, 31, 50]. Material selection for this tissue construct has been based on the functional properties such as degradation patterns and mechanical properties [1]. Since biomaterials are intended to interact

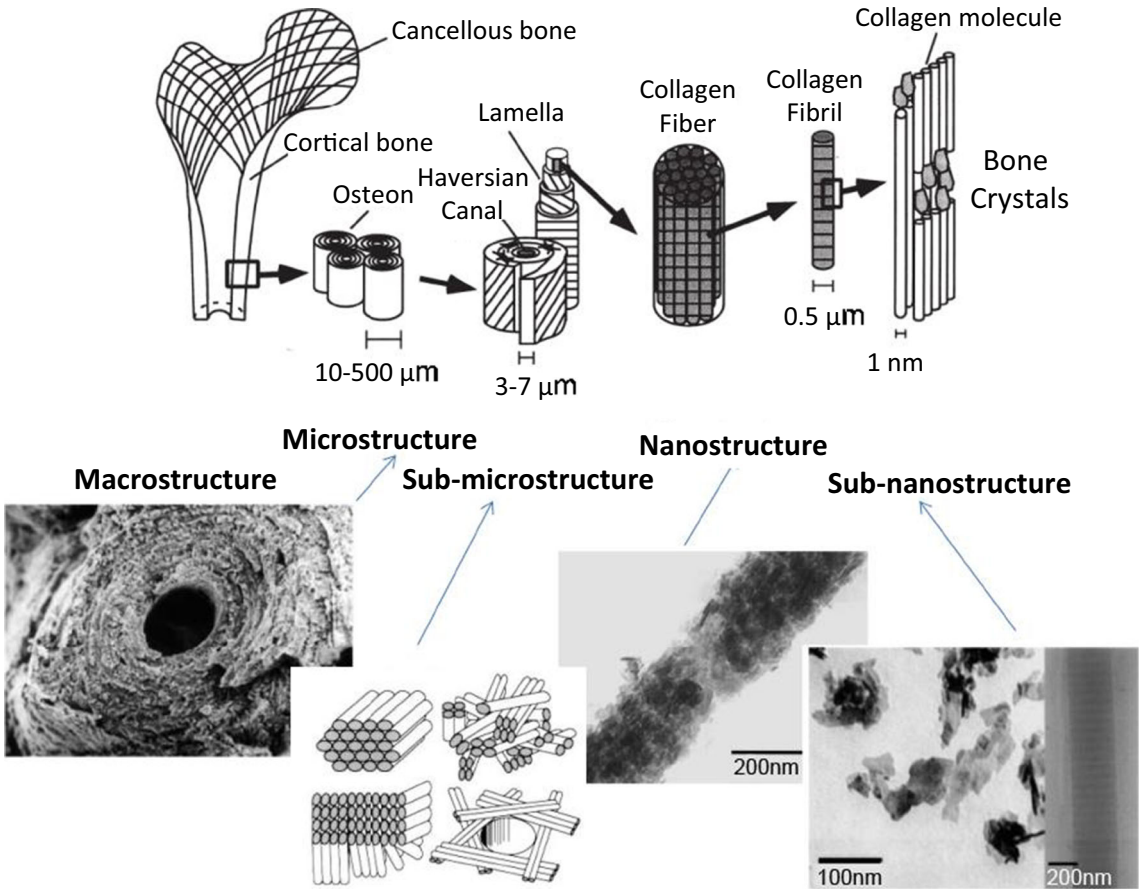


Fig. 4 Hierarchical structure of bone. Fibers, laminae, and pores are present at different size scales resulting in various macro- to subnanostructures. Such material hierarchical arrangement is essential for the mechanical functions of bone [36, 45]. Reproduced with permission from refs. [36, 45], respectively. Copyright 1997 Elsevier and copyright 1998 Annual Reviews

directly with biological systems, the surface chemistry of the selected materials also plays a significant role [51]. Biocompatibility is a vital attribute a material must possess to qualify as a biomaterial which is the feature of being benign with surrounding biological systems and eliciting minimal to mild tissue responses [19]. Factors such as chemical, physical, and biological properties and shape and structure of the implant influence the tissue response to a biomaterial [1, 9, 52]. For biodegradable biomaterials, it is important to demonstrate biocompatibility over time [4]. Ideal biodegradable biomaterials for regenerative engineering should have the following features: (1) Upon implantation, the material should not instigate a sustained inflammatory and toxic response [8, 53]; (2) the degradation rate of the material should allow for the healing or regeneration process to occur [8, 9]; (3) the products of degradation should be nontoxic and easily resorb and be excreted [9, 47]; (4) the material should have appropriate initial mechanical properties, and changes in mechanical strength with degradation should correspond with healing or regeneration process [1, 9, 47]; and (5) the material should be processable for the intended application [1, 14, 16, 19].

Some inherent polymer properties can influence biocompatibility of polymeric materials: these include material chemistry, molecular weight, solubility, shape, and structure of the implant; hydrophilicity/hydrophobicity; lubricity; surface energy; water absorption; degradation rates; and erosion mechanism [14, 19, 31]. No single polymer class possesses ideal properties for all applications. Therefore, a number of polymer-based systems have been designed and studied [2, 9, 19, 47, 54]. Polymeric materials with a wide range of mechanical and degradation properties may be required to mimic the properties of various tissues [1].

Synthetic and natural polymers have been extensively used in tissue regeneration as biodegradable scaffolds [4, 9–11, 39, 52]. Biodegradable scaffolds act as a supportive and temporary template for cell attachment and subsequent tissue development. Biodegradability of the polymer is vital as the cells are allowed time to produce their extracellular matrix and eventually replace the scaffold. The hydrolytic or enzymatic sensitivity of the bonds influences the biodegradation of polymeric materials [55]. Natural polymers were the first to be explored in clinical applications that require degradation of implanted materials [2, 47, 48]. The availability and concentration of enzymes at a given implantation site have massive effects on the rate of in vivo degradation of degradable polymers [17, 56]. Degradation rate can also be influenced via chemical modification of these polymers [47]. Natural polymers have numerous advantages such as bioactivity, biomimetic surfaces that contain particular amino sequences that facilitate cell adhesion and cell differentiation, triggered degradation, and natural remodeling. However, they also have some limitations such as immunogenic response, composition, microbial contamination, weak mechanical strength, and uncontrollable degradation associated with natural polymers [2, 19, 48].

On the other hand, synthetic biomaterials can have defined chemical properties, predictable mechanical properties and degradation rates, and batch-to-batch uniformity [2, 19, 48]. Thus, a high level of control over properties to match specific applications is possible. For medical implantation, synthetic degradable polymers may be considered to be advantageous in regard to natural degradable polymers due to their minimal site-to-site and patient-to-patient variations [10]. For example, the successful application of poly(glycolic acid) as the first synthetic-based degradable suture has spurred the design and development of a variety of biodegradable polymers with a wide range of properties [19].

Based on the mode of degradation, degradable polymeric biomaterials can be broadly classified into two categories: (1) hydrolytically degradable polymers and (2) enzymatically degradable polymer [57]. Hydrolytically degradable polymers possess hydrolytically labile chemical bonds that can undergo hydrolysis without secondary influence [58]. These bonds include esters, ortho esters, anhydrides, urethanes, urea, carbonate, polyphosphazene, etc. These linkages can be very prone to hydrolysis without the aid of enzymes [2, 19, 47, 48], whereas enzymatically degradable polymers have bonds that while technically hydrolytically active require catalysis to degrade meaningfully under physiological conditions. Polymers with amide or ether linkages fall under this category and have much lower hydrolytic degradation rates than the hydrolytically degradable ones [58].

Synthetic Polymeric Biomaterials

Most synthetic polymeric biomaterials degrade hydrolytically because of the presence hydrolytically labile chemical bonds in their backbone except the ones with amide and ether bonds [59, 60]. Synthetic polymers for biomedical applications are produced via two general routes: step-growth (condensation) polymerization and chain-growth (addition) polymerization [2, 48]. The step-growth technique involves condensation of two difunctional or multifunctional monomers to form linear high molecular weight polymers or network polymers (in the case of multifunctional monomers) [61]. The molecular weight builds up slowly [62]. Synthetic polymers such as polyesters, poly(ortho esters), polyamides, polyanhydrides, and polyurethanes are produced using step-growth condensation polymerization [63]. Ring-opening polymerization is a form of chain-growth polymerization that has been extensively investigated for the development of hydrolytically sensitive polymers such as polyesters and polyphosphazenes [37, 63–66]. Many studies have demonstrated the feasibility of employing polymer cross-linking in the development of cross-linked synthetic degradable polymers and hydrogels

for biomedical applications. Furthermore, there is an increasing interest in the microbial biosynthesis of biodegradable polymers. This technique is used in producing polyhydroxyalkanoates via bacterial fermentation of sugar or lipids [64, 67–70]. Some of the clinically relevant synthetic polymers will be discussed in the following sections.

Poly(α-esters)

Poly(α-esters) are an important subgroup of synthetic biodegradable polymers, and they are characterized by their ability to be hydrolyzed through ester linkage in their backbone [71]. Poly(α-esters) are thermoplastic polymers that include polylactide, polyglycolide, and their copolymers, polydioxanone, polycaprolactone, and poly(trimethylene carbonate). Due to the reversibility of the esterification process used in making polyesters, they are regarded to be degradable [71]. However, polyesters with long aliphatic chains between ester linkages may not be suited for tissue regeneration purposes. In contrast, the degradation time of the polyesters with reasonably short aliphatic chains between ester linkages matches the time frame for most biomedical applications [2]. For example, -CH_2- groups affect hydrophilicity of polymers, which hinder degradation in water. The cyclic compound in aromatic ester also makes polymers hard to degrade due to the hydrophobicity of the phenyl group [50]. The chemistry and the synthetic flexibility of polyesters make them an outstanding class of polymers [50, 65, 70]. Ring-opening and condensation polymerization are commonly used routes in making poly(α-esters), in which a variety of monomers are utilized [50, 65, 70]. Some poly(α-esters) can also be developed using microbial biosynthesis [50, 65, 70]. Poly(α-hydroxyl acid) such as poly(glycolic acid) and stereoisomeric forms of poly(lactic acid) are the most extensively investigated polymers in the class of poly(α-esters). In the 1960s, glycolides blazed the trail by becoming the first synthetic polymeric biomaterial to be used in the development of medical suture. After that, various aliphatic polyesters have been developed as biodegradable biomaterials and are being used extensively as medical implants because of their excellent biocompatibility and controllable degradation pattern [2, 19, 48, 50, 71].

Polycondensation of difunctional monomers is also used to synthesize polyesters. This technique is employed through the self-condensation of hydroxyl acids, diacids with diols, acid anhydrides with diols, diacid chloride with diols, or by the ester interchanges (transesterification) reaction of diesters and diol [72]. The low molecular weight associated with polycondensation route has limited its use for biomedical applications. However, polycondensation of cyclic monomer yields relatively higher molecular weight materials as compared to polycondensation of traditional monomer [71–73].

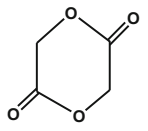
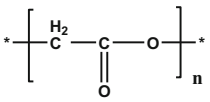
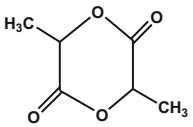
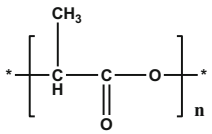
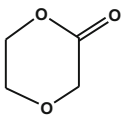
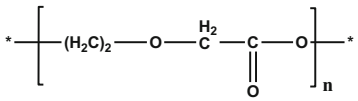
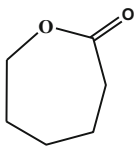
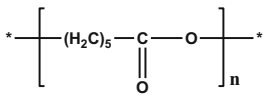
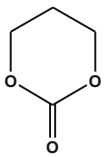
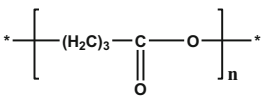
High molecular weight polyesters can be attained using ring-opening polymerization of cyclic lactones. This method has evolved into an efficient one-pot polymerization route for making high molecular weight homo and co polyesters [72]. Ring-opening polymerization (ROP) is more beneficial and commercially viable than polycondensation because its synthetic route proceeds under milder reaction conditions and shorter reaction time without producing any by-products [74]. ROP is another form of chain-growth polymerization in which the reactive center lies on the terminal end group of a polymer chain, and more cyclic monomers can be added by ring opening and addition of the broken bond [74, 75]. These cyclic monomers are usually difunctional when they open up the ring which has been under strain. The addition of a small amount of a nucleophilic reagent (Lewis base) as an initiator can facilitate the opening process. This is termed anionic ring-opening polymerization (AROP). Thus, polycaprolactone is produced using AROP. The use of a small amount of an electrophilic reagent (Lewis acid) as an initiator is also feasible. In this case, it is called cationic ring-opening polymerization (CROP). CROP is much harder to occur with cyclic monomers with small ring strain than large ring size. Small rings with greater ring strain like 4-, 6-, and 7-membered rings of cyclic esters polymerize readily through CROP [50, 71, 75, 76]. The structure of the different types of cyclic lactones and their corresponding homopolymers are represented in Table 1. Lactide, glycolide, and caprolactone are among the most investigated cyclic monomers used for aliphatic polyester synthesis utilized in tissue regeneration applications [2, 48].

The mechanism of the hydrolytic erosion of biodegradable polymers can be broadly classified into bulk and surface erosion [2, 10, 48]. Poly(α-ester) is an example of a bulk eroding polymer [77]. In bulk erosion, the polymer undergoes degradation with significant decrease in molecular weight and corresponding material properties (such as mechanical properties) as a function of degradation time [2, 48, 77]. Numerous investigations have been carried out on homopolymers and copolymers of poly(α-esters) to study their potentials as biomaterials for biomedical applications. The synthesis, functional properties, and biomedical applications of some of these polymers will be reviewed in the following section.

Polyglycolide

Polyglycolide (PGA) is credited as being among the first biodegradable synthetic polymers used in biomedical applications [78, 79]. It is a semicrystalline polymer with a crystallinity of about 44–55%. It possesses a high tensile modulus and hardly dissolves in an organic solvent. Polyglycolide has a glass transition in the range of 35–40 °C and a melting point that sits above 200 °C. PGA has an orthorhombic unit cell with dimensions of $a = 5.22\ \text{Å}$, $b = 6.19\ \text{Å}$, and $c(\text{fiber axis}) = 7.02\ \text{Å}$. The high melting point of PGA is attributed to the stabilized

Table 1 Cyclic lactones and their corresponding homopolymer [10]

Cyclic Lactone	Linear Homopolymer
 Clycolide	 Poly(glycolide)
 Lactide	 Poly(lactide)
 Dioxanone	 Poly(dioxanone)
 Caprolactone	 Poly(caprolactone)
 Trimethyl carbonate	 Poly(trimethyl carbonate)

crystal lattice that results from the tight molecular packing and proximity of the ester groups [80]. PGA has specific gravities of 1.707 for a perfect crystal and 1.05 for an amorphous material [55, 81]. For a 100% crystallized PGA, a previous study reported that PGA has the enthalpy of fusion of 12 kJ/mol (45.7 cal/g) [82].

The biomedical PGA is produced via ring-opening polymerization of cyclic dimers of glycolic acid [55]. Ring-opening polymerization utilizes numerous catalysts, and this includes organometallic compounds and Lewis acids. For biomedical applications, stannous chloride dihydrate or trialkyl aluminum is preferred. The use of stannous chloride dihydrate (in the presence of alcohol) renders it as a cationic melt polymerization, whereas the use of trialkyl aluminum will ensure nucleophilic attack of a carbanion on one of the glycolide carbonyls. Lauryl alcohol is added to control the molecular weight during polymerization [83].

PGA was used to make the first resorbable sutures called DEXON which is a fiber made from the melting spinning of PGA chips [84]. This process involves heating PGA to a temperature above its glass temperature and stretching it to several hundred percentages of its original length. Heating must be carefully done and placed under control to ensure minimal or no shrinkage and excellent dimensional stability. Surgical sutures produced with PGA usually lose their mechanical strength typically over a period of 2 to 4 weeks after implantation [79].

For scaffold-based tissue regeneration, nonwoven PGA fabrics have been extensively explored as biomaterials due to its excellent biocompatibility, degradability, initial mechanical properties, and cell viability on the matrices [14]. Extrusion, injection molding, compression molding, particulate leaching, and solvent casting are some of the processing techniques used in making PGA parts for biomedical applications [9]. Polyglycolide owes its excellent mechanical properties to its high crystallinity. The modulus of a self-reinforced PGA form is superior to other clinically relevant biodegradable polymers [18]. Its modulus is approximately 12.5 GPa [10].

Polyglycolide undergoes bulk erosion through random cleavage of its ester linkages of the backbone [2, 48, 85]. Under physiological conditions, PGA is broken down to glycolic acids which can enter the tricarboxylic acid cycle and be removed from the body as water and carbon dioxide [2, 9, 47, 48, 86], and some of the glycolic acids are also excreted through urine [86]. Enzymes with esterase can also cause PGA to degrade which is attributable to why in vivo degradation of PGA is found to be faster than degradation in vitro [87]. The high sensitivity to hydrolysis and low solubility of PGA can limit its applications [86]. Therefore, copolymers of PGA with more hydrophobic polymers (such as PLA) have been investigated to address inherent disadvantages. This has paved the way for additional applications in tissue regeneration [65].

Polylactide

Due to the chiral nature of lactic acid, polylactic exists in four morphologically distinct polymers that stem from two stereoisomeric forms of lactic acid: L-lactide and D-lactide. Semicrystalline polymers are obtained from the polymerization of L-lactide and D-lactide. Amorphous polymers, however, are obtained from the racemic (D, L)-lactide and mesolactide. Poly(L-lactide) (PLLA) and poly(D-lactide) PDLA are stereo-regular polymers; D, L-PLA is a racemic polymer derived from a mixture of D- and L-lactic acid; and meso-PLA can be obtained from D, L-lactic acid [70, 76].

PLLA is a crystalline polymer with a crystallinity of about 37%. Its crystallinity has some practical implication on its uses [88]. The molecular weight of PLLA and the processing method have a considerable effect on its crystallinity [71]. In fact, the higher the molecular weight, the lower the crystallinity [89]. It is thought to be much easier for polymer molecules to rearrange and realign in an orderly fashion at the lower molecular weight PLLA. This systematic arrangement leads to higher crystallinity [89]. Processing conditions such as fast and slow cooling also have an impact on the crystallinity of PLLA [90]. The quicker the cooling (i.e., quenching), the lower the crystallinity. Slow cooling provides sufficient time for the crystals to grow. PLLA has a glass transition temperature of 60–65 °C and a melting temperature of 175 °C [91]. PLLA degrades slower than PGA because it is more hydrophobic. The hydrophobicity is attributed to the presence of an extra methyl group in lactic acid [9]. PLLA dissolves more readily in organic solvent than PGA. Due to its crystallinity, PLLA has a high modulus (about 4.8 GPa) and good tensile strength (65 MPa), and hence, it is preferred for load-bearing applications that demand high mechanical strength and toughness [92]. In orthopedic and dental applications, PLLA polymers have been utilized as fixation device like screws, pins, washer darts, and arrows in reconstructive surgeries including those of the mandibular joint; facelifts; thoracic, hand, leg, finger, and toe fractures; ligament reconstruction procedures; soft and hard tissue fixations; alignment of osteochondral and bone fragments; meniscus repair; and hyaline cartilage fixation [93]. As a result of its hydrophobicity and slow degradation, it takes 2 and 5.6 years for complete in vivo resorption of high molecular weight PLLA [48, 94]. The crystallinity of the polymer and the porosity of its matrix influence the degradation rate. In as much as the strength of the polymer diminishes after 6 months under hydrolysis, the mass remains intact for an extended period of time [2, 10, 11, 19, 39, 50, 76, 77]. On that account, the development of copolymers with glycolide or DL-lactide was carried out with the aim of customizing the properties for enhanced performance and clinical results.

For poly(DL-lactide) (PDLLA), the random arrangement of the L- and D-lactide units results in an amorphous morphology. It has a glass transition of approximately 60 °C [2, 92]. PDLLA shows relatively inferior strength (1.9 GPa) as compared to the PLLA due to its amorphous nature. Under hydrolysis, the strength of PDLLA is gone within 1–2 months and a significant loss of mass is exhibited within 12–16 months [95]. Being an amorphous polymer with a faster rate of degradation compared to PLLA, it is an ideal material for drug delivery applications where it is essential to have a homogeneous dispersion of active species within the polymer [96]. It can also be used to develop low strength scaffolding material for tissue regeneration [11].

Polylactides hydrolyze through bulk erosion mechanism by a nonspecific cleavage of ester linkages along the backbone [77]. The degradation results in lactic acid, which is nontoxic and a by-product of human metabolism. There is usually a decrease in pH and autocatalytic acceleration of the degradation rate due to the higher number of carboxylic end groups [97]. The lactic acid is eventually broken down into water and carbon dioxide through an acid cycle. Degradation preferentially takes place in the amorphous domain first before the crystalline region [95].

In spite of its excellent mechanical strength, processability, biocompatibility, good degradation rates, and nontoxic degradation products, PLA-based materials suffer from the lack of ideal surface chemistry that could aid cell adhesion and proliferation [2, 14, 48, 77]. Several solutions have been devised which include the use of plasma treatment, protein adsorption, and surface modification with bioactive moieties to surmount this issue and to ensure enhanced osteoconductivity for bone tissue regeneration [93].

Poly(lactide-co-glycolide)

Poly(lactide-co-glycolide) (PLGA) is one of the most widely investigated copolymers of polyesters [79]. PLGA with a wide range of properties has been developed and employed in many biomedical applications including tissue regeneration [54]. Physicomechanical properties of the PLGA copolymer vary

greatly with the ratio of glycolic acid to lactic acid [54, 75]. There is no linear correlation between the composition of the individual components (ratio of glycolic acid to lactic acid) and the overall physicomechanical properties of PLGA copolymer [79]. Copolymerization disrupts the crystallization of the polymer components, leading to a decrease in the degree of crystallinity of the overall copolymer [7, 86, 89]. The change in morphology is accompanied by an increase in the amorphous domain thereby causing a faster hydration and hydrolysis. Thus, PLGA copolymer tends to have more rapid degradation than either PGA or PLA [98, 99]. Different ratios of PLGA are in the market and are investigated for a wide variety of biomedical applications. PLGA undergoes bulk erosion through the hydrolysis of the ester bonds. Several parameters such as the LA/GA ratio, molecular weight, and the shape and structure of the matrix can determine the degradation rate of PLGA. The popularity of PLGA is ascribed in part to the FDA approval in human use, its excellent processibility which makes it easy for its fabrications into many different shapes, controllable degradation rate, and their success in previous biomedical applications [100].

Many different studies have also shown that PLGA possesses excellent cell adhesion and proliferation making it one of the most ideal and suitable biomaterials for scaffold-based tissue regeneration [65, 101]. However, acidic accumulation of lactic and glycolic acids can cause inflammatory responses in the region of the implant [102]. These acidic degradation products from bulk erosion of PLGA copolymer often sometimes cause unexpected structure failure and foreign body reactions [22, 102]. Thus, the development of polymeric biomaterials with neutral degradation products and bioactivity is attractive for bone tissue regeneration. Studies have shown that the incorporation of other polymers (polyphosphazene) or substance (alkaline salts) can alleviate the issue of acid accumulation upon degradation [22, 103].

Polycaprolactone

Polycaprolactone (PCL) is an aliphatic, semicrystalline polyester that has widespread use in biomedical applications [2, 21]. It has attracted enormous attention due to its commercial value as it is made by the ring-opening polymerization of an inexpensive monomer (ε-caprolactone) [59, 72, 104]. Its high processibility, excellent solubility in a wide range of organic solvents, and ability to form a miscible blend with other biologically relevant polymers made it stand out among other polymers. Its melting and glass transition temperatures are 55–60 and −60 °C, respectively. When exposed to physiological conditions (such as in the human body), the polymer degrades hydrolytically due to the presence of hydrolytically active ester linkage; however, the rate at which it degrades is prolonged (2–3 years). Gradual degradation, high drug permeability, and nontoxicity have made PCL a good candidate for engineering long-term drug/vaccine delivery system. This polymer was used to make Capronor®, a long-term contraceptive, that has been designed for a long-term zero-order release of levonorgestrel [11]. PCL possesses a low tensile strength (approximately 23 MPa) and an extremely high elongation at breakage (< 700%) [50]. PCL has been under numerous investigations for use as scaffolds for tissue regeneration due to its biocompatibility [105]. Chiari et al. [106] studied the use of PCL-hyaluronic-based composite matrix as a potential substitute for the meniscus. An investigation is ongoing for the design of PCL-calcium phosphate composite as appropriate scaffolds for bone tissue regeneration. In a recent study, Jang et al. [107] demonstrated the feasibility of using MSC-laden polycaprolactone/collagen scaffold for bone tissue regeneration. The scaffold was designed using PCL micro-/nanofibers, collagen, and cell-laden alginate struts. A cell-free scaffold was also designed with the compositions and used as a control. Results revealed that the cell-laden PCL-based scaffold provided more rapid and broader osteogenesis than the control scaffold [105].

Due to the low degradation rate of PCL, investigators have explored the design of PCL-based copolymers with better properties. Co-polymers of ε-caprolactone with DL-lactide have resulted in a system with fast degradation rate [105]. Also, fibers obtained from the copolymerization of ε-caprolactone with glycolide possess less stiffness compared to those made of polyglycolide which is known commercially as monofilament (MONACRYL®) [10, 105].

Polyhydroxyalkanoate (Bacteria Polyesters)

Polyhydroxyalkanoates (PHA) are linear polyesters produced by fermentation of sugar or lipids with the aid of bacteria. Materials with a wide range of properties have been created within this family using more than 150 different monomeric units [108]. These properties can vary from being thermoplastic to being elastomeric and with melting points ranging from 40 to 180 °C. Poly-3-hydroxybutyrate (PHB) is the most common and widely used of the PHAs (Fig. 5) produced using Ralstonia eutrophus (*Cupriavidus necator*) [109], Methylobacteria rhodesianum [69], or *Bacillus megaterium* [69]. PHB is a semicrystalline isotactic polymer that degrades via surface erosion mechanism. Degradation of this polymer occurs through the cleavage of the ester linkage, and the melting point is between 160 and 180 °C [110]. PHB can also be synthesized using several chemical synthetic routes. Previous studies have demonstrated the feasibility of using the ring-opening polymerization of optically active b-butyrolactone to produce an identical PHB to the bacterial one. Brittleness and rigidity of PHB can sometimes be a drawback in its use [111, 112]. Therefore, some studies have focused on the improvement of PHB properties by incorporating a co-monomer like 3-hydroxyvalerate [108, 109]. Poly(3-hydroxybutyrate-

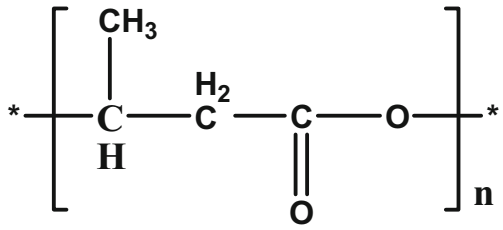


Fig. 5 Chemical structure of poly(3-hydroxybutyrate) (PHB) [10]

co-3-hydroxyvalerate) (PHBHV) is a semicrystalline polymer just like the PHB. However, the melting point is much lower than PHB, and the glass transition temperature is in the range of − 5 to 20 °C. These improved properties would perhaps widen its application scope. PHB and its copolymer PHBHV can easily be dissolved using a wide range of solvents. They have excellent processability and can be processed into different geometries such as films, sheets, spheres, and fibers. Piezoelectricity is another remarkable property of the PHBHV copolymer that makes it a promising biomaterial for orthopedic applications since electrical stimulation can aid in bone tissue regeneration. Wang et al. [113] showed that the use of PHB-based scaffolds for human bone marrow stromal cells was possible. In the study, terpolyester of the composition poly(3-hydroxybutyrate-3-hydroxyvalerate-3-hydroxyhexanoate) (P(HB-HV-HHx)) was compared to PLA and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (P(HB-HHx)) for their function in differentiating the human bone marrow stromal cells into nerve cells. Results showed that there were better cell adhesion, proliferation, and differentiation for the terpolyester than the other two. In a recent study, Zheo et al. [114] designed drug delivery composite scaffolds for bone tissue regeneration using a 3D bioplotter. The compositions of the well-defined porous scaffold were P(HB-HHx) and mesoporous bioactive glass. Results showed enhanced bioactivity and osteogenic properties, including fast apatite-forming ability, and promotion of human bone marrow-derived MSC adhesion, proliferation, alkaline phosphatase (ALP) activity, and bone-related gene expression. There was also an indication of the ability of the composite to stabilize the pH of the environment with increasing PHBHHx ratio. This is unusual features among polyesters that have the lingering issue of acidic degradation products [115].

The degradation products of PHB include D-(−)-3-hydroxy-butyric acid which is typically found in low quantity in the blood (concentrations between 0.3 and 1.3 mM) [115]. Owing to its high crystallinity, PHB degrades at a slow rate as compared to synthetic polyesters. In contrast, PHBHV copolymer degrades faster because of being less crystalline; however, the amount of HV in the copolymer has no direct relationship with degradation rate [69]. This polymer loses its mass during degradation via zero-order release kinetics. Since surface erosion proceeded through zero-order release kinetics and coupled with the hydrophobicity of the polymer, PHB undergoes surface erosion. This makes it a possibly important candidate for drug delivery use. Currently, there are ongoing investigations to increase the degradation rate of these polymers by blending them with more hydrophilic polymers or other low molecular weight additives to improve water penetration and facilitate degradation [64].

Poly(anhydrides-co-imide)

Polyanhydrides are surface-eroding polymers made by either melt condensation or solution polymerization [116]. High molecular weight polymers can be obtained using melt condensation of dicarboxylic acid monomers with excess acetic anhydride and a catalyst to facilitate the reaction. Other techniques that can be used to make anhydrides are as follows: ROP of anhydrides, interfacial condensation, dehydrochlorination of diacids and diacid chlorides, or by the reaction of diacyl chlorides with coupling agents such as phosgene or diphosgene [96, 117]. It is prone to hydrolysis due to the presence of highly sensitive aliphatic anhydride bonds on the polymer backbone. Its inherent surface erosion is attributed to the presence of a hydrolytically active backbone and the hydrophobicity of the polymer that prevents the permeation of water to the matrix [118]. As surface-eroding polymers, they undergo a linear mass loss during erosion. For this reason, polyanhydrides are considered as ideal candidates for drug delivery applications. The reduced mechanical properties have limited polyanhydrides and are not suitable for load-bearing applications, such as for bone tissue regeneration. Poly[1,6-bis(carboxyphenoxy) hexane] has Young's modulus of 1.3 MPa, and this is much lower than the modulus of human cancellous bone [119]. The deficiency and limitation in the strength of polyanhydrides led to the development of poly(anhydride-co-imides) whose mechanical properties were significantly enhanced as a result of the presence of the imide segments in the backbone. The poly(anhydride-co-imides) were found to degrade sequentially by the cleavage of the anhydride bonds first, followed by the hydrolysis of the imide bonds [120, 121]. Laurencin et al. [122, 123] evaluated the mechanical performance and biocompatibility of a wide range of poly(anhydride-co-imides), such as poly[pyromellitylimidoalanine-co-1,6-bis(p-carboxyphenoxy) hexane] (PMAala:CPH) (Fig. 6) as scaffolds for bone tissue regeneration. A rat tibial model was utilized to investigate the osteocompatibility of the polymers. While the untreated defects healed in 12 days, it was observed that the one treated with poly(anhydride-co-imides) exhibited endosteal growth as early as day 3 and formation of cortical bone around the implanted matrices by day 30. The outcome suggested good osteocompatibility of the matrices as compared to the untreated controls [122, 123]. Based on the results of the mechanical examination studies, polymers made with succinic acid trimellitylimidoglycine and trimellitylimidoalanine

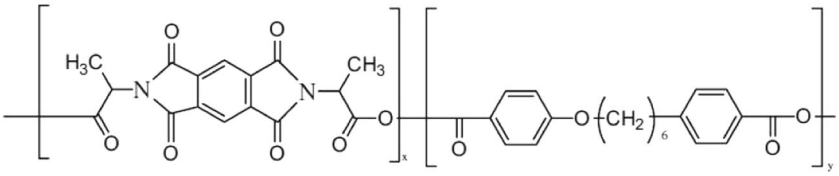


Fig. 6 Diagram showing the poly[pyromellitylimidoalanine-co-1,6-bis(p-carboxyphenoxy) hexane] structure [10]

possess compressive strength on the order of 50–60 MPa. These results once again indicated that poly(anhydride-co-imides) could be ideal materials for bone tissue regeneration [119].

Cross-linked Polyanhydrides

An alternative approach has been employed to improve and maximize the mechanical competence of polyanhydrides by adding acrylic functionality to the monomeric units to form injectable and photocross-linkable polyanhydrides [37]. In the case of irregularly shaped bone defects, the irregular shapes can be filled up using injectable anhydrides due to the shear-thinning and viscoelastic properties of the polymer. The polymer becomes less viscous under shear and retains its rigidity when the shearing is withdrawn. The product generated from the degradation of the polymers is benign and made up of the corresponding diacid molecules and water-soluble linear methacrylic acid molecules [124]. There have been investigations on the development of cross-linkable matrices with appropriate thickness for orthopedic applications [2, 58]. A variety of initiator-accelerator systems and energy sources have been employed in this regard; 1.0 wt% camphorquinone (CQ) and 1.0 wt% ethyl-4-N, N-dimethyl aminobenzoate (EDMAB) with 150 mW/cm^2 of blue light were proved to be the most effective composition for the photopolymerization of these polymers. Figure 7 shows the structure of the polymers poly(sebacic acid) (PSA) and poly(1-3-bis(p-carboxyphenoxy)propane) (PCPP) and poly(1-6-bis(p-carboxy phenoxy)hexane) (PCPH). Similar to the conventional polyanhydrides, the type and nature of the monomeric units influence the mechanical strength and degradation rates of the cross-linked polyanhydrides. Langer et al. demonstrated that this class of polymers possesses compressive strength within the lower range of cancellous bone (30–40 MPa) [125].

Poly(propylene fumarate)

Co-polyester poly(propylene) fumarate (PPF) is another cross-linkable, biodegradable, high strength polymeric biomaterials designed for orthopedic applications. A number of techniques used for the synthesis of PPF have been reported [126, 127]. The most commonly used technique is the two-step reaction (Fig. 8) of diethyl fumarate and propylene glycol through a bis(hydroxypropyl) fumarate diester as intermediate [127, 128].

PPF is known to undergo bulk erosion through the hydrolysis of the ester bonds, and several factors (such as molecular weight, types of curing agent, and cross-link density) can influence degradation time. The molecular weight is dependent on the reaction and temperature. PPF degrade into benign products called fumaric acid, a component of the Krebs cycle and 2-propanediol, a common diluent of drug formulations. PPF is associated with a rather low molecular weight, whereas the presence of unsaturated carbon-carbon bonds in fumaric acid units allows for cross-linking of the polymer into a covalent polymer network. This cross-linking improves the material properties (such as mechanical strength) [127, 129]. Several investigations have been carried out on the development of a mechanically competent and biodegradable PPF using cross-linking or designing PPF-based composites that have ceramic materials as second phase (filler) [129]. Although PPF can undergo self-cross-linking, a wide range of cross-linking agents have been explored with PPF to form cross-linked [130], degradable polymer networks. For instance, cross-linked networks of PPF with N-vinyl pyrrolidinone [131], poly(ethylene glycol)-dimethacrylate [132], PPF-diacrylate [133], and diethyl fumarate [134] have been developed. The cross-linked matrices exhibited a compressive strength in the order of 1–12 MPa which is dependent on the composition and conditions of polymerization [135]. The combination with appropriate initiator results in an injectable PPF-based polymer solution, which can set in situ and be molded into the desired shape. Temenoff and Mikos [129] demonstrated that PPF composites with ceramics, such as tricalcium phosphate or calcium sulfate, were mechanically (2–30 MPa) suitable for orthopedic applications. PPF's injectability, biocompatibility, and biodegradability have made them promising candidates for bone tissue engineering applications.

Polyphosphazene

Polyphosphazene polymers are among the few inorganic-organic hybrid polymers that have been thoroughly investigated as potential biomaterials for bone tissue regeneration [136]. They are polymers with a unique inorganic backbone of alternating phosphorus and nitrogen atoms with two organic units attached to the phosphorus groups. The character of the inorganic backbone and the structure of the organic side determine the properties of these polymers. The general structure of polyphosphazene is shown in Fig. 9 where R can be organic or organometallic side groups. Polyphosphazene polymers

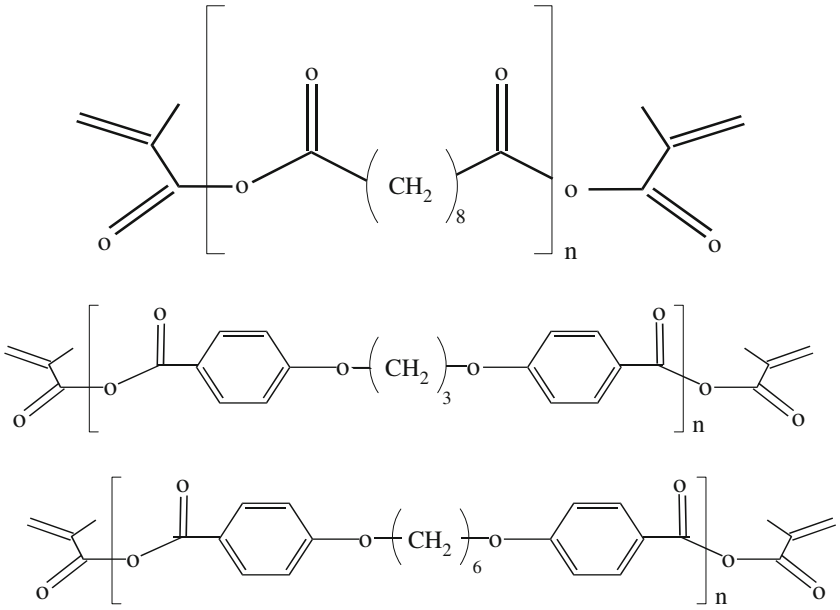


Fig. 7 Diagram depicting the structure of photocross-linked polymers: (a) poly(sebacic acid) (PSA), (b) poly(1-3-bis(p-carboxyphenoxy)propane) (PCPP), and (c) poly(1-6-bis(p-carboxy phenxoy)hexane) (PCPH) [10]

have been in existence since the mid-1960s [66], but it was more recently that biodegradable polyphosphazenes were developed [66]. A few specialized polyphosphazenes, particularly those with amino acid ester, peptide, glucosyl, glyceryl, glycolate, lactate, and imidazole, side groups have been developed extensively as matrices for bone regeneration [22, 23]. These side groups have been found to sensitize the polymer backbone to hydrolysis. The reactivity of phosphorous-chlorine bonds has made way for the synthesis of a wide variety of polyphosphazene polymers so far [136].

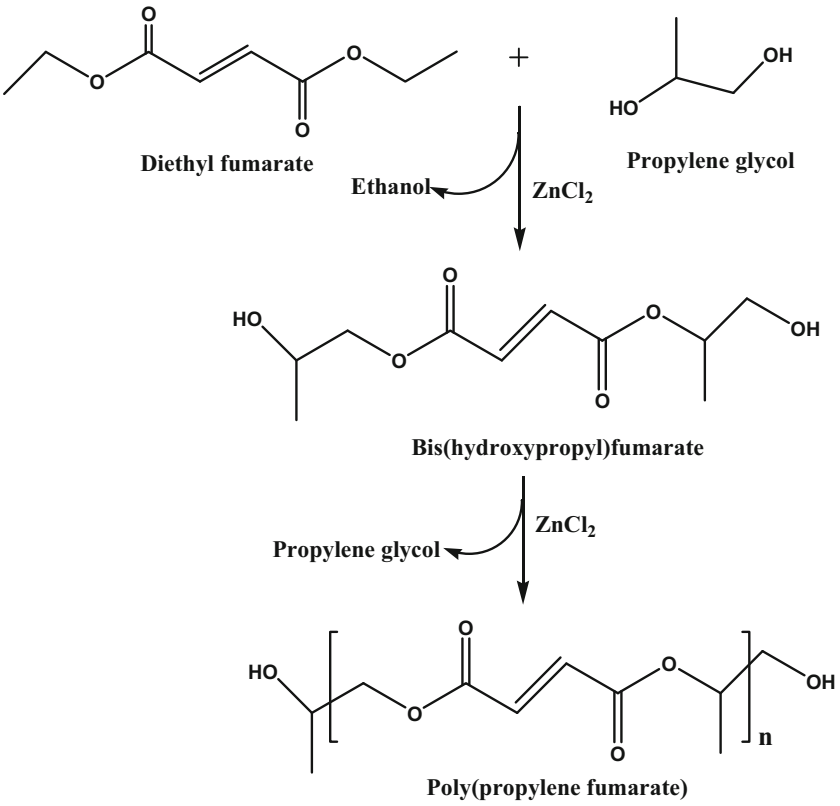


Fig. 8 Polymerization of poly(propylene fumarate) from diethyl fumarate and propylene glycol by the two-step procedure [127]

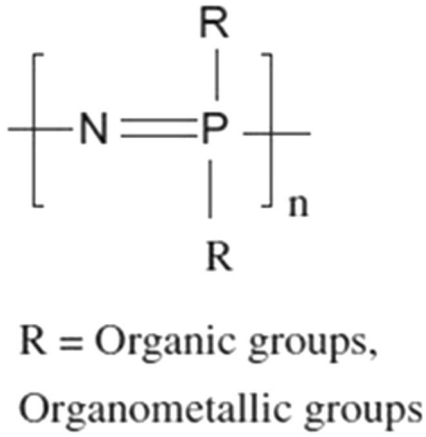


Fig. 9 General structure of polyphosphazene [66]

The most widely used route to poly(organo) phosphazenes is via a two-step reaction process starting from the commercially available cyclic trimer hexachlorocyclotriphosphazene (HCCTP) [136]. As shown in Fig. 10, the first step involves the synthesis of linear poly(dichloro)phosphazene (PDCP) which can be achieved via the controlled thermal ring-opening polymerization of cyclic trimer at 250 °C under vacuum [66, 136]. The ring-opening polymerization can be carried out at a lower temperature (200 °C) with the aid of a Lewis acid catalyst, most commonly anhydrous $AlCl_3$. Studies published recently showed that PDCP could be produced by solution state method that makes use of trichlorobenzene as a solvent, at 214 °C, with $CaSO_4{\cdot}2H_2O$ as a promoter and HSO_3 (NH_2) as a catalyst [22]. The second step involves the macromolecular nucleophilic substitution of the chlorine units with any of the wide range of organic side groups. Sequential substitutions are usually employed when dealing with different side groups. The bulkier ones are substituted first followed by the less bulky ones just to avoid steric hindrance [137].

An alternative method explored for the synthesis of polyphosphazenes with defined and well-controlled structure is the living cationic polymerization process phosphoranimines at ambient temperature. The reaction mechanism involves catalyzed condensation of monomer trichloro(trimethylsilyl) phosphoranimine, $(CH3)_3Si–N=PCl_3$ with loss of $(CH_3)_3SiCl$. This reaction can take place at room temperature, and there is usually a good handle on the molecular weight, molecular weight distributions, and chain lengths. The living cationic polymerization has provided a springboard for the development of phosphazene-based block copolymers with PLA and PGA [66].

Biodegradable polyphosphazene polymers are regarded as unique and interesting biomaterials for bone tissue regeneration due to their exceptional design flexibility. Incorporation of different side groups can adjust and modulate the degradation rates, hydrophobicity/hydrophilicity, and mechanical properties of the polymers [11]. For example, amino acid ester side groups will induce hydrolysis within the polymer backbone, while the presence of hydrophobic phenylphenoxy side groups will shed water thereby retarding hydrolysis.

In terms of potential biomedical applications, poly(amino acid ester) phosphazenes have recorded the most success among other biodegradable polyphosphazene investigated. Due to the hydrolytically active nature of amino acid ester, all amino acid ester-substituted polyphosphazenes are degradable with the rate of degradation solely dependent on the type of amino acid esters used. Among the amino acid esters investigated, glycine ethyl ester and glycylglycine ethyl ester-substituted polyphosphazenes showed the fastest degradation [138, 139]. The co-substitution of both amino acid ester groups such as glycine ethyl esters and hydrophobic groups into the polyphosphazene backbone provides a platform for tuning degradation pattern to match a specific time frame [66].

Unlike the polyester family, amino acid ethyl ester-substituted polyphosphazenes undergo hydrolysis generating nontoxic and buffering degradation products composed mainly of phosphates, ammonia, and corresponding side groups. Laurencin and coworkers recently exploited the use of this unique property of polyphosphazenes to fabricate self-neutralizing blend systems by combining polyphosphazenes with poly(lactide-co-glycolide) [139, 140]. A recent study demonstrated that using peptide as side groups as in the case of glycylglycine ethyl ester-substituted polyphosphazenes resulted in polyphosphazene-PLAGA blends with high miscibility. The high miscibility in the blend was attributed to the increased number of hydrogen bonding sites in glycylglycine ethyl ester than in glycine ethyl ester and other forms of amino acid esters [139, 141, 142].

Another unusual characteristic of polyphosphazenes is that the blend systems exhibited a unique erosion profile quite distinct from any biodegradable systems currently available [143]. The systems have an erosion mechanism by which the degradation process changes the polymer from a coherent film to an assemblage of microspheres with interconnected porous structures [143]. Ongoing work has centered on optimizing this unique inherent pore-forming property of polyphosphazene-PLAGA blends for enhanced cell infiltration, tissue ingrowth, and nutrient transport [143]. The in situ pore-forming structures may preclude the need to fabricate porous 3D structures which are known in many cases to have poor initial mechanical properties that are not appropriate for bone tissue regeneration. Laurencin et al. [144, 145] carried out extensive investigations on the in vitro and in vivo biocompatibility of biodegradable polyphosphazene. Using a rat subcutaneous model, most of the amino acid and peptide ester polyphosphazene elicited minimal to mild tissue inflammatory responses. Figures 11 and 12 illustrate the minimal inflammatory response and fibrous capsule formation of polyphosphazene-PLAGA blends as compared to PLAGA [139]. Many of the amino acid and peptide ester

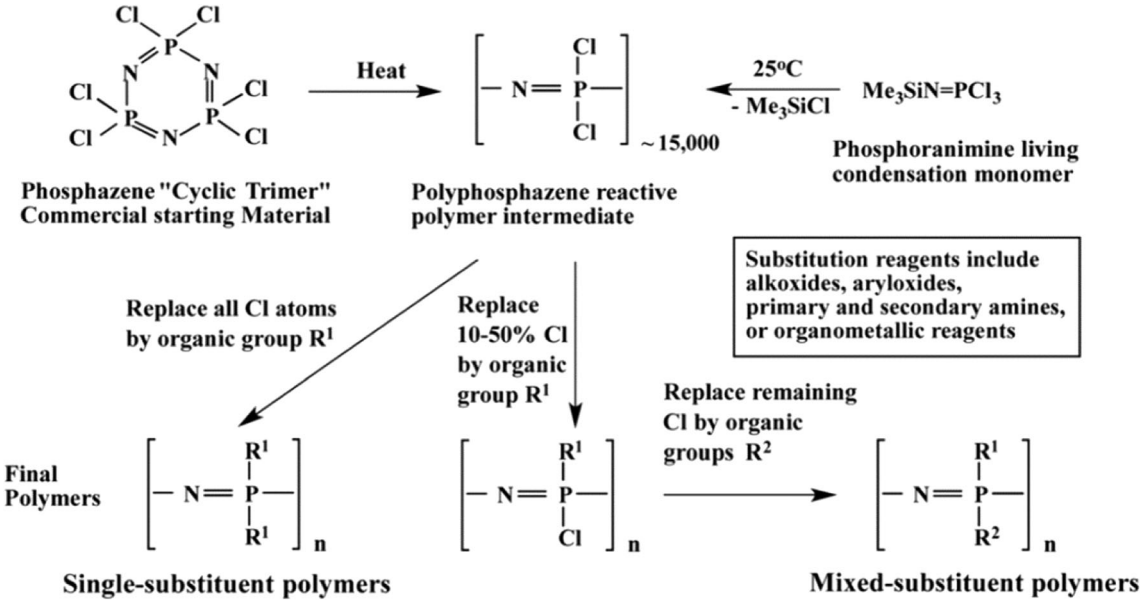


Fig. 10 Synthetic mechanism of polyphosphazene showing the single substituent and mixed substituent polymers [66]

polyphosphazenes and their blends with polyesters have shown excellent osteocompatibility and have been investigated as matrices for bone tissue engineering [22]. Due to the inherent backbone flexibility, the development of polyphosphazenes with high Tg and excellent mechanical properties is challenging. However, previous studies demonstrated the feasibility of overcoming this challenge by carefully co-substituting polyphosphazenes with suitable side groups. Laurencin and coworkers showed that the mechanical properties of the biodegradable polyphosphazene could be improved by substituting in bulkier side groups such as phenylphenoxy into the backbone [23, 139, 142, 146]. We are currently working on the development of mechanically competent biodegradable polyphosphazene for load-bearing applications [147].

Natural Polymers

Biodegradable polymers from natural origins, such as polysaccharides (e.g., cellulose, chitin, and glycosaminoglycan) and proteins (e.g., collagen, silk, fibrinogen, and elastin), are

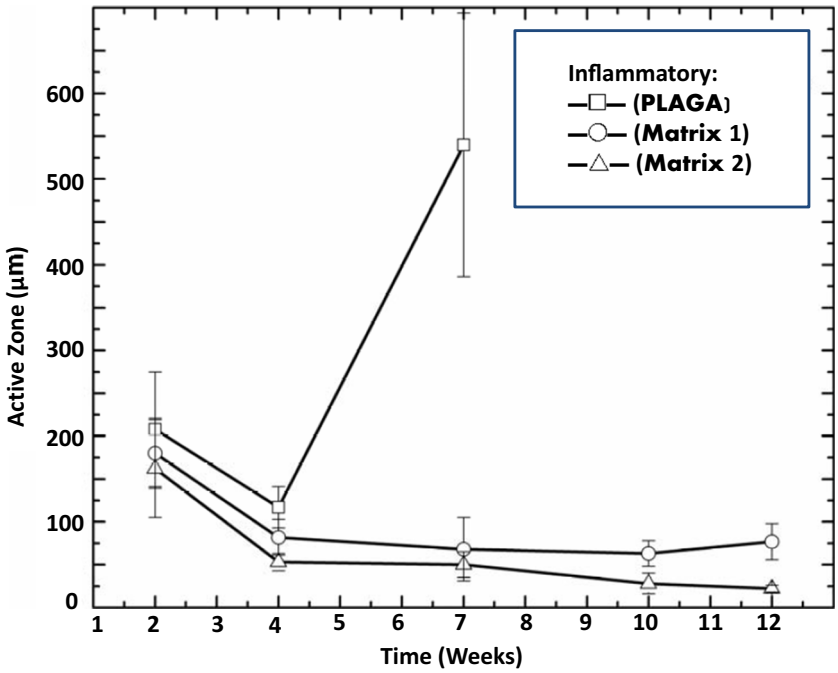


Fig. 11 Time-dependent thickness change of the tissue response for PLAGA and polyphosphazene-PLAGA blends during 12 weeks of implantation. The inflammatory responses for the blends were minimal [139]. Reproduced with permission from ref. [139]. Copyright 2010 Elsevier

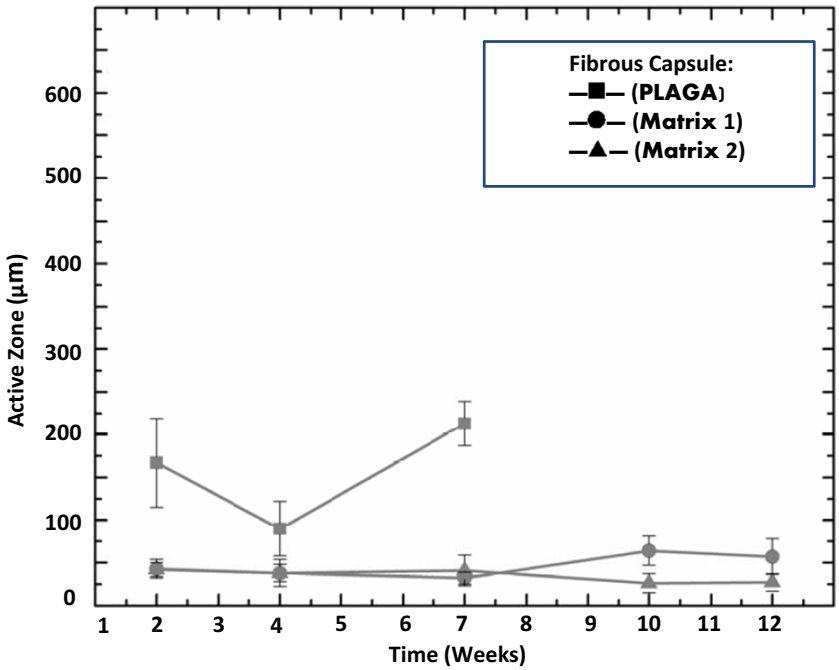


Fig. 12 Time-dependent thickness change of the fibrous capsules for PLAGA and polyphosphazene-PLAGA blends. The thicknesses of the fibrous capsules for the blends were minimal [139]. Reproduced with permission from ref. [139]. Copyright 2010 Elsevier

very similar to the extracellular matrix in terms of chemical composition and biological activity. Due to these similarities, they intend not to cause any toxicity and immunological response when used as implants [12]. The following sections discuss some natural polymers that have been exploited in tissue regeneration.

Proteins and Poly(amino acids)

Proteins are large biomolecules composed of long chain(s) of amino acid units [148]. Protein-based biomaterials are preferred for sutures, hemostatic agents, scaffolds for tissue engineering, and drug delivery vehicles because as a major component of native tissues, they have cell-supportive properties. Besides, proteins are renowned for their naturally controlled degradation [149].

The human body has the inherent ability to synthesize a wide range of proteins, and the intermediates of these protein syntheses pass through four major stages to become functional proteins [148]. The first step proceeds through the formation of a primary structure that entails the linear and sequential connecting of amino acids by peptide bonds. The amino acids constituted in the primary structure then partake in hydrogen bonding to form the secondary structure of protein. The linear primary structure tends to align in the most stable forms—a α-helices or β-pleated sheets. These secondary structures then join together to create 3D tertiary structures which in turn interact with other protein chains to create the more refined 3D quaternary structure of a multiunit protein [150].

Collagen

Collagen is regarded as the most abundant protein in mammals as a result of being the main component of musculoskeletal tissues [2, 14, 16, 34, 48, 151]. Collagen is a rod-shaped polymeric structure with a length of 300 nm and a molecular weight of 300,000. So far, 28 types of collagen have been identified and described with the most common being types I–IV. Type I constitutes over 90% of the collagen in the human body and is the most extensively investigated protein [32, 34]. Type I collagen consists of three polypeptide subunits with similar amino compositions and sequence; 1050 amino acids are contained in each polypeptide with approximately 33% glycine, 25% proline, and 25% hydroxyproline with a relative abundance of lysine [16, 25, 32, 34]. Free amino acid situated in the body is used to biologically synthesize subunit chains of collagen which undergo transcription, translation, and posttranslation modification processes in appropriate cells such as fibroblasts and osteoblasts [25, 32, 34]. The repeating triplets of (glycine-X-Y)n (where X and Y are often proline and hydroxyproline) make up the primary structure of the proteins. The helical structure and the predictable mechanical strength of collagen are attributed to the repeating sequence [152]. The flexibility of the collagen is due to the glycine content, and collagen flexibility increases with increasing content of glycine. The α-chain of collagen is formed by ten of these polypeptide chains which arrange to create a right-handed helical secondary structure. The arrangement of three secondary structures leads to the formation of a left-handed, triple helical tertiary collagen structure. There is a collection of

glycine molecules (the smallest repeating units) toward the inner part of the triple helix [153]. The procollagen molecules are released into the extracellular space to form higher order structures from their immediate self-assembly. These structures can sometimes undergo further modification via cross-linking. The fibrils formed are aligned and oriented differently in different types of tissues to give them the appropriate mechanical strength [32, 34].

The degradation of collagen within the body is triggered off by the presence of enzymes, such as collagenases and metalloproteinases, to yield the corresponding amino acids. Collagen has been extensively studied for use in the biomedical field due to its enzymatic degradability and unique physicochemical, mechanical, and biological properties. Collagen can be dissolved using acidic aqueous solutions and can be processed into different forms such as sheets, tubes, sponges, foams, nanofibrous matrices, powders, fleeces, injectable viscous solutions, and dispersions. According to previous studies, enzymatic pretreatment or cross-linking (using various cross-linking agents) tools are employed for fine-tuning or customizing degradation rates [154, 155].

Collagen constitutes the main component of the extracellular matrix and serves as a natural substrate for cell attachment, proliferation, and differentiation, and as such, there is reemergence of interest in the areas of tissue regeneration. The high reactivity of collagen renders it cross-linkable by a variety of cross-linking agents such as difunctional or multifunctional aldehydes, carbodiimides, hexamethylene-diisocyanate, polyepoxy compounds, and succinimidyl ester polyethylene glycol. This cross-linking can be achieved using thermal or high-energy irradiation, as well as by chemical modification, such as succinylated collagen to form collagen gels for use as carriers for drug delivery and as scaffolds for tissue regeneration. Geiger et al. [155] demonstrated the feasibility of using cross-linked absorbable collagen sponges as protein carrier vehicles. In the study, bioactive proteins, such as recombinant human bone morphogenic protein-2 (rhBMP-2), were incorporated into the collagen matrices. As a result of favorable interactions of the collagen matrix with the protein, there was a sustained release of the protein which promoted bone healing [155]. This combination product has been approved by the US FDA to be used in conjunction with a titanium interbody spine fusion cage for anterior lumbar spinal fusion (InFUSE® Bone graft/LT-CAGEs Lumbar Tapered Fusion Device) and is approved in Europe (InductOs®) for the treatment of acute tibia fractures in adults. Absorbable collagen sponges have been extensively investigated for use as scaffolding material for accelerated tissue regeneration due to their excellent biocompatibility, biodegradability, and porosity. A collagen-based composite of fibrillar collagen, hydroxyapatite, and tricalcium phosphate (Collagraft®) is FDA approved for use as a biodegradable synthetic bone graft substitute [156]. Several forms of collagen (including the work of Jang et al. described in the PCL section) have been explored as scaffolds for tissue regeneration.

Bovine or porcine skin or quine Achilles tendons are the primary sources of collagen being used in biomedical applications [157, 158]. The drawbacks of collagen-based biomaterials for its medical use are their mild immunogenicity caused by the composition of the terminal region and the antigenic sites in the central helix [58, 159]. The species from which collagen has been isolated, processing techniques, and the site of implantation result in dissimilar and varying immune response [159, 160]. Due to the nonuniform compositions of collagen, concerns such as pure collagen tends to be expensive, their physicochemical and degradation properties tend to vary, and high risk of infection are prevalent [58, 157, 160].

Polysaccharides

Polysaccharides are carbohydrates (macromolecules) which are composed of sugar molecules (monosaccharides) joined together by the glycosidic linkages. Polysaccharides have seen a massive demand for use as biomaterials due to their unique biological functions ranging from cell signaling to immune recognition. The feasibility of modifying polysaccharides through a synthetic means or synthesizing oligosaccharide moieties to customize biodegradability, coupled with their unique biological functions and excellent processability made them promising natural biomaterials. Polysaccharides can be of human origin or animal origin depending on the source.

Hyaluronic Acid

Meyer and Palmer were the first to isolate hyaluronic acid (HA) in 1934 from the vitreous humor of the eye [161]. There has been an emerging interest in the biomedical field since its discovery. HA belongs to the family of glycosaminoglycans which are linear polysaccharides consisting of alternating units of N-acetyl-D-glucosamine and glucuronic acid and widely distributed throughout connective, epithelial, and neural tissues of vertebrates. HA can be regarded as the largest member of the glycosaminoglycan family with high molecular weight up to several millions [162]. No covalent bonds exist between HA and proteins, and this is uncommon among other members of the glycosaminoglycan family present in the human body, such as chondroitin sulfate, dermatan sulfate, keratin sulfate, and heparin sulfate. HA exhibits unique viscoelastic characteristics when in a viscous form. Dissolution in water usually gives a highly viscous solution that demonstrates viscoelastic behavior and can form 3D structures with strong intramolecular hydrogen bonds. It has been reported that the viscoelastic properties of some tissues like synovial fluid and vitreous humor are as a result of having a high concentration of HA in them [162]. Moreover, a variety of

tissues including articular cartilage, the nucleus pulposus, skin, the cervix, and the glycocalyx of endothelial cells employ HA as a supportive template. Figure 13 shows the structure of HA. Studies have shown that 50% of the HA content in the body is found in the skin and the polymer has a high-life that varies from a few minutes to weeks depending upon the type of tissue it is associated with [10, 60].

Studies have also shown that within the cells, hyaluronan synthase-1 (Has-1, Has-2, and Has-3) aid and direct the synthesis of HA on the cytosal surface of the plasma membrane [163]. HA synthesis during embryogenesis is mainly carried out by Has-2; however, the specific roles performed by Has1 and Has3 are not yet identified [164]. HA are traditionally isolated from rooster combs and bovine vitreous humor. However, HA synthesis using bioprocess and bacterial fermentation is now growing in popularity. Free radicals, such as nitric oxide and MMPs present in the extracellular matrix in the body, aid in the degradation of HA in the body. Endocytosis occurs after degradation. Also, Lysosomal enzymes catalyze the digestion of HA to form mono and disaccharides, which can be further converted into ammonia, carbon dioxide, and water via the Krebs cycle [165].

Earlier studies reported that HA acts as a passive structural component of connective tissues; nevertheless, later studies confirmed it to have active involvement in many biological processes such as modulating cell migration and differentiation during embryogenesis, regulating extracellular matrix organization and metabolism, as well as playing essential roles in metastasis, wound healing, and inflammation [166]. HA possesses other unique properties including its ability to promote angiogenesis, to modulate wound site inflammation by acting as a free radical scavenger, and to be recognized by receptors on a variety of cells associated with tissue repair. HA can be cross-linked using a variety of physical and chemical methods due to its high functionality and charge density [167]. Esterified derivatives like ethyl/benzyl esters (HYAFF®) and cross-linked hyaluronic acid gels are examples of modified HA. These chemical modifications significantly reduce degradation rate. The degradation of (HYAFF®) is hydrolytic via the cleavage of ester bonds without any enzymes. The degradation time varies from 1–2 weeks

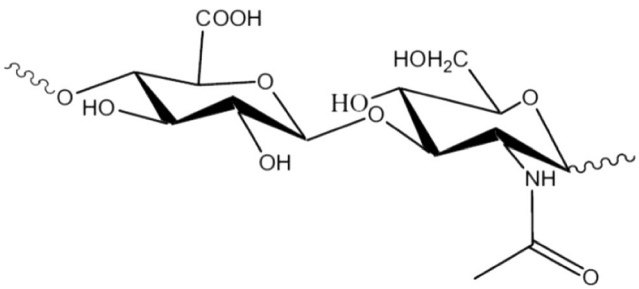


Fig. 13 Chemical structure of hyaluronic acid [10]

to 2–3 months depending on the degree of esterification. The polymeric products of the de-esterification are hydrated and readily soluble with strike resemblance of native HA [168, 169]. The promotion of mesenchymal and epithelial cell migration and differentiation is also carried by HA which leads to collagen deposition. Moreover, HA also supports angiogenesis. These important tissue repair functions coupled with its immunoneutrality makes HA a suitable biomaterial for tissue regeneration applications. Its viscoelastic behavior and aqueous solubility provide HA the required design flexibility for fabrication into different types of porous and 3D structures for these applications. Thus, a viscous formulation of HA containing fibroblast growth factor (OSSIGEL®) is being used as a synthetic bone graft to accelerate bone fracture healing. Similarly, HYAFF® 11 is also being used as a carrier vehicle for a variety of growth factors and morphogens as well as bone marrow stromal cells. Hunt et al. [170] conducted a comparison of HYAFF® 11 with absorbable collagen sponge as a carrier vehicle for osteoinductive protein, rhBMP-2. In the study, HYAFF® 11 carriers showed better healing response than collagen.

Cell-Polymeric Material Interaction

Scaffolds for tissue regeneration are mainly made up of biodegradable polymers which could be synthetic or natural [10, 31, 52]. Biodegradable scaffolds constitute a vital part of tissue regeneration that acts as a temporary template for tissue development [31]. The interaction of cells, mainly stem cells with polymers and the way cells respond when in intimate contact with the material, can define the outcome of the regeneration of tissues [2]. The selection of proper polymers for tissue regeneration should be based on the understanding of the effect of the polymers on cell viability, adhesion, growth, and proliferation [1, 9, 19]. Also, it is essential to have an understanding of the effect of the microenvironment of the tissues on the polymers themselves. Taking into consideration, the material dynamics (effect of polymers on the cells) and material kinetics (effect of the tissue microenvironment on the polymer properties) can provide a great avenue to customizing polymer properties for enhanced material performance and positive clinical results [5]. The basic understanding of cell-material interactions opens new doors for the development of new classes of polymeric biomaterials that may have favorable interactions with cells for enhanced bone tissue regeneration [5].

Protein Adsorption to Polymers

Upon implantation into the body or placing in solution, a polymer is immediately covered with proteins. This enclosure of the polymer happens in a short time interval. The

composition of the protein layer determines the type of subsequent interaction that will take place between the cells and the materials. It was demonstrated in vitro that polymers adsorb immense quantity of proteins [171]. Previous studies showed that the extent of protein adsorption hugely depends on the hydrophobicity/hydrophilicity of the proteins as well as the polymer surface [172–176]. In one study, Absolom et al. [172] used four plasma proteins (fibrinogen, immunoglobulin G (IgG), human serum albumin, and bovine serum albumin) and four different types of small particles as substrates (siliconized glass, Teflon, polyvinyl chloride, and Nylon-6, 6) and showed that maximum adsorption occurred for the most hydrophobic protein surface (fibrinogen), and the least adsorption happened for the most hydrophilic protein surface (bovine serum albumin). Polymer geometry (flat sheet or particle) has no significant effect on the pattern of protein adsorption. It was also observed that low surface tension (hydrophobic) was associated with the highest protein adsorption and the higher surface tension solids related to lowest protein adsorption [177, 178]. In in vivo studies that entail the IP implantation methods, the foreign body response (FBR) is influenced by the adsorption of fibrinogen. The adsorption of fibrinogen causes the denaturation of the proteins which stems from its interaction with the substrate material. Denaturation is vital and has an impactful effect on the subsequent blood surface interaction. Also, the conformational changes induced by the material on the adsorbed proteins might lead to the exposure of cryptic cell adhesion motifs [7, 179, 180]. The denatured proteins may be taken as foreign bodies and can cause an immune response with the infiltration of phagocytic cells such as macrophages and granulocytes [177]. Several overlapping phases are caused by FBR which entail nonspecific protein adsorption and inflammatory cell recruitment—which is mostly neutrophils and macrophages, foreign body giant cells (FBGCs) from macrophage fusion, fibroblast, and endothelial cells. FBGCs' formation on the polymer surface and eventual encapsulation of the implant by a fibrous capsule is the outcome of FBR [178, 181, 182].

There is a modification of interfacial properties of the substrate by the physical adsorption of protein molecules onto a polymer. This modification leads to the various extent of subsequent cell adhesion in which it increases or decreases. For instance, fibrinogen and immunoglobulin G when adsorbed onto a variety of polymer surfaces increase platelet adhesion, whereas a decrease in platelet adhesion is observed with albumin [172]. Proteins adsorbed from local environments tend to mediate interactions between the cells and polymer surfaces [6, 8, 180]. Due to the complexity of these interactions of proteins with the material, polymer surfaces are often pretreated with purified protein solutions. This can represent an ideal biomimetic condition with a stable layer of surface-bound proteins [7, 180].

Effect of Polymer Chemistry on Cell Behavior

As aforementioned, the surface characteristics of a material have a tremendous impact on the behavior and functions of cells. Folkman and Moscona [183] carried out a study where it was demonstrated that the nature of polymer surface plays a significant role in the cell function. In that study, surfaces coated with conventional tissue culture polystyrene (TCP) with various dilutions of polyhydroxyethylmethacrylate (pHEMA) were seeded with cells. The amount of pHEMA added to the surface was directly proportional to the cell spreading as indicated by the average cell height on the surface. A direct correlation holds between the degrees of spreading and average height with cell growth, suggesting that cell proliferation was controlled by cell shape which is defined by the adhesiveness of the surface (Fig. 14). These experiments where two polymers (tissue culture polystyrene (TCPS) and pHEMA) were used to design serials of surfaces with different adhesivity showed that cell function is highly dependent on the nature of the polymer surface. A similar study was carried out by Tamada and Ikada where the relationship between chemical or physical characteristics of the polymer and function (adhesion, growth, and collagen synthesis) of the attached cells was investigated [184]. In the study, fetal fibroblasts from rats were seeded on surfaces made of 13 different polymers. A wide range of surface energies associated with the polymer surfaces were observed, as determined by static water contact angles, from very hydrophilic to very hydrophobic. Results showed that cell adhesion and growth were poor on PVA and cellulose, whereas they were moderate on other surfaces. The cell doubling time was 24 h and slightly slower on

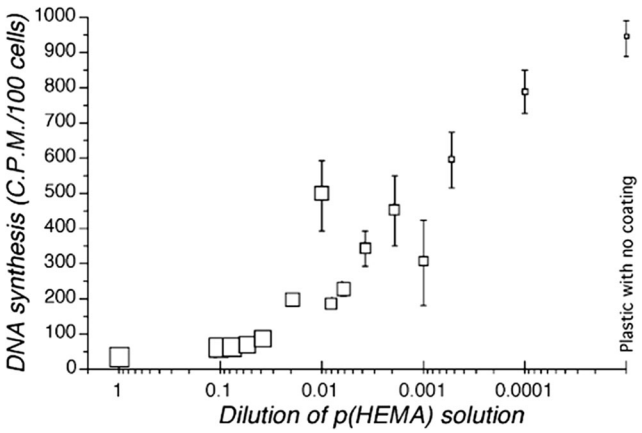


Fig. 14 Effects of polymer surface properties on cell function. Surfaces of the cell culture were made with vapor deposition of pHEMA onto TCPs. Proliferation was determined using the extent of uptake of [^{3}H] thymidine. The relative cell height is represented by the size of the symbol. Cells with small heights indicate significant spreading which correspond to small symbols. Cells having large heights indicate infinitesimal spreading which correspond to large symbols [183]. Reproduced with permission from ref. [183]. Copyright 1978 Springer Nature

very hydrophobic surfaces (e.g., PTFE, PT). Collagen synthesis was positively proportional to the hydrophobicity of the surface.

Surface Modification

Polymers' surface can be modified to give better and desired surface properties for enhanced cell attachment and growth [6]. A perfect example is TCPS (for tissue culture) substrate which is obtained from the modification of polystyrene (PS) by glow discharge or exposure to chemicals like sulfuric acid [185–187]. Exposing the PS surface to sulfuric acid increases the number of charged groups at the surface, which consequently enhances the attachment and growth of several types of cells. This modification process has been used for many other polymers [6, 7, 177, 179, 187–190].

Wettability of the surface can be changed by adding chemical groups, which often have effects on cell adhesion [6, 191]. Alternatively, the surface of whole proteins such as collagen can be immobilized on the surface of the substrate to mimic the ECM environment partially [192]. Collagen and other ECM-based hydrogels have been produced by incorporating proteins during their synthesis or by forming a polymer blend with the proteins and polymerized material such as pHEMA, in the right solvent [193]. Also, smaller bioactive functional groups (such as oligopeptide, saccharides, or glycolipids) have been utilized to modify surfaces [194]. This will circumvent the complexity associated with the compositions of ECM molecules and leads to the production of surfaces that can be characterized easily.

Analysis of active fragments of ECM molecules reveals certain short amino acid sequences which seem to bind to receptors on cell surfaces and mediate cell adhesion. For instance, fibronectin's cell-binding domain contains the tripeptide RGD [195]. Thus, it is well known that RGD sequence is important for cell adhesion and is present in many ECM proteins (e.g., fibronectin, collagen, vitronectin, thrombospondin, tenascin, laminin, and entactin). Furthermore, other sequences such as YIGSR and IKVAV can induce cell adhesion and differentiation [188, 189].

The importance of RGD in cell adhesion to ECM has motivated many investigators to explore the incorporation of this sequence to synthetic polymer substrates. Cell adhesion to nonadhesive or weakly adhesive surfaces can be induced by incorporating a cell-binding peptide to a polymer. Optimization of cell spreading and focal contact formation are practicable by the addition of peptide. ECM molecules are recognized by cell adhesion receptors. Thus, polymer surfaces can be made to be cell selective by using an appropriate cell-binding sequence. This is usually determined by the type of peptide utilized [189, 196]. Furthermore, the chemistry of the functional groups also plays a vital role in cell adhesion. Functionalization or modification of scaffolds with alcohol (R–OH), carboxylic acids (R–COOH), or primary amine (R-NH_2) residues can maximize cell adhesion [197].

Physical cues such as topography, porosity, and matrix stiffness have important effects on the cell-material interactions (Fig. 15). Chemical cues such as the use of chemical functional groups on the material surface can determine the behavior of cells. Furthermore, biological cues such as bioactive small molecules and growth factors can lead to enhanced cellular responses. Incorporation of these cues is of great importance in creating synthetic biomaterials customized to provide optimal performance and positive clinical results [3].

Electrically Charged Polymers

Electroconductive polymers have also been used as substrates for cell growth and tissue formation [198, 199]. Electrical and electrochemical cues can be used to control the orientation and frequency of cell division [200]. Cells experience a specific inducement when exposed to electric fields and potentials. A variety of different cell types (corneal, epidermal, and epithelial cells) can move and migrate directionally, regulate phenotypes of vascular endothelial cells, and regenerate nerve fibers under guidance of the applied electric field. Electric and electromechanical clues are among the most relevant clues in determining tissue functionality in tissues such as muscle, bones, etc. Electroactive materials, especially the piezoelectric ones, have great prospects for novel tissue engineering strategies. Indeed, different parts of the body experience piezoelectricity including bones, tendon, ligament cartilage, skin, dentin, collagen, deoxyribonucleic acids (DNA), and cell membranes [201–203]. Utilization of piezoelectric polymers for tissue engineering applications can be mostly dedicated to bone, neural, and muscle regeneration. PVDF and its copolymers are most investigated piezoelectric polymers for tissue regeneration due to their high piezoelectric response. PVDF serves as a gold standard for proving the concept of mechano-electrical transduction for tissue regeneration, and different sample geometries (such as films, fibers, porous membranes, and 3D porous scaffolds) have been used for different applications in tissue regeneration. Thus, Damaraju et al. [204] fabricated PVDF fibers for bone regeneration and showed that hMSCs attached to the PVDF fibers and presented after differentiation a larger ALP activity and early mineralization when compared with the control. Similar results were obtained when PLLA fibers were seeded with hMSCs and used to study their biocompatibility and suitability for bone differentiation. The applied mechanical stimulus can be vibration, compression, or stretching of the piezoelectric scaffold. PVDF films have been used to investigate the response of bone cells to mechanical stimulation by converse piezoelectric effects. The dynamic mechanical conditions used to achieve the stimulation were alternating sinusoidal current (AC) of 5 V at 1 and 3 Hz for 15 min at each frequency. Formation of new bone in vivo and

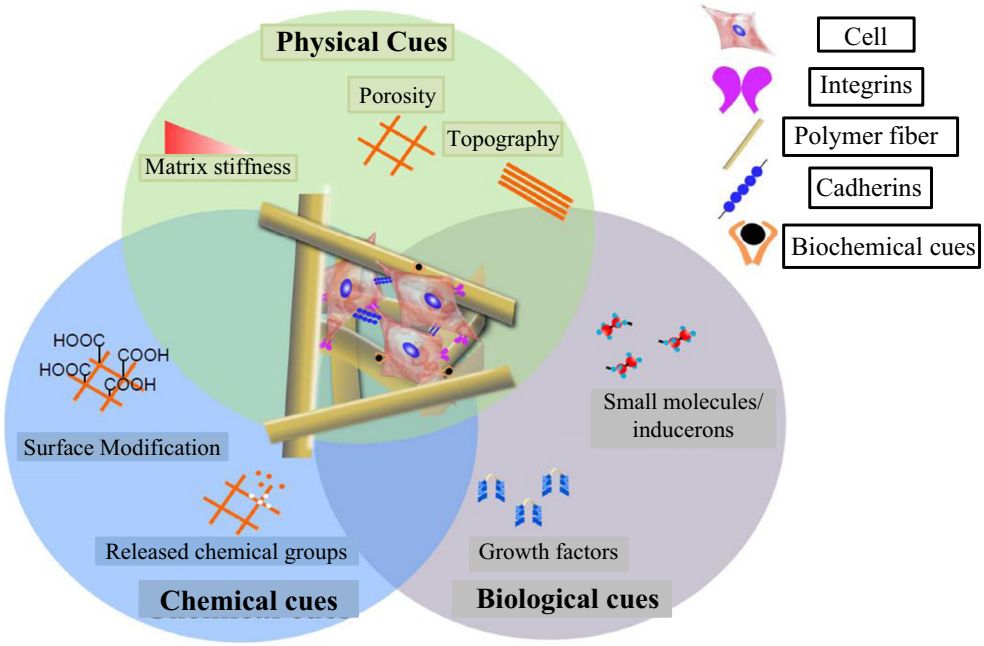


Fig. 15 Design parameters that ensure optimal and favorable cell-material interactions [3]. Reproduced with permission from ref. [3]. Copyright 2016 Springer Nature

the increase in the metabolic activity and gene expression of osteoblasts in culture were observed when the bone was mechanically stimulated [205]. The influence of the same piezoelectric substrate, PVDF film, on the bone response cultivated under static and dynamic conditions was also investigated [206]. When exposed to PVDF films, a different response was exhibited by the MC3T3-E1 osteoblast cell culture [206]. The results showed that the surface charge under mechanical stimulation improves the osteoblast growth [206]. Thus, appropriate electrical stimuli for the growth and proliferation of electrically responsive tissue can be achieved with electroactive polymeric materials, for tissues showing piezoelectric response, such as bone [206, 207]. Osteogenic differentiation of human adipose stem cells was also enhanced by the same dynamic culture, reaffirming that dynamic mechanical stimulus in combination with appropriate osteogenic differentiation media can provide a great avenue to mimic native conditions obtainable in vivo [208]. Furthermore, actuator in the in vivo assays for orthopedic applications has been using a piezoelectric material (PDVF). After 1 month of implantation, it was demonstrated that the converse piezoelectric effect could be used to stimulate the bone growth at the bone-implant interface [209].

Influence of Surface Morphology on Cell Behavior

The behavior and function of cells are highly dependent on the microscale texture of an implanted material [210]. Thus, the optimization of implant surface in a way that could enhance osteogenic differentiation of mesenchymal stem cells is of paramount importance [189, 210]. The behavior of cells on rough surfaces (with edges and grooves) is different from the behavior on smooth surfaces. In most cases, there are orientation and migration of cells along fibers or ridges in the surface. This phenomenon is termed contact guidance from previous studies on neuronal cell cultures [211]. Fibroblasts orient on grooved surfaces [212], with the depth and pitch of grooves playing a significant role in the degree of cell orientation. For an identical surface, there are different degrees of contact guidance for different cells. In other words, cells cultured on identical surface might possess the same contact guidance. Materials with grooves ranging in pitch from 150 to 1000 nm were used to study the adhesion and initial migration of an osteoblast cell line. Optimal cell function was observed on grooves of intermediate spacing [213]. Neves et al. [214] investigated the effect of surface roughness of PCL on the osteogenic differentiation of human bone marrow MSCs. In the study, they prepared PCL material with roughness gradients of average roughness (Ra) varying from the submicron to the micrometer range (0.5–4.7 μm) and mean distance between peaks (RS_m) gradually varying from ~214 to 33 μm. The degree of cytoskeleton spreading, ALP expression, collagen type 1, and mineralization were analyzed. Specific PCL roughness accelerated the osteogenic commitment of human bone marrow MSCs and strongly accentuated the osteogenic differentiation of these cells, as compared to TCPs. These findings suggest that with the adjustment of the surface roughness, it is practicable to optimize substrate surface for improved regenerative performance.

Conclusion

Polymeric scaffold-based bone regenerative engineering is an important and increasingly innovative approach that has the potential to address clinical challenges of bone tissue loss or failure. The success of this approach hugely depends on the currently available biodegradable polymeric biomaterials. Most of the commercially available biodegradable materials are based on natural polymers such as collagen and synthetic polymers such as poly(α-esters). Advances in materials science and engineering are ensuring the development of a wide variety of novel polymeric biomaterials to broaden the scope of this regenerative approach. Understanding of cell-material interactions and the use of technologies that may further personalize design or modify existing materials will ensure improved material performance and positive clinical results in the future.

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