CHAPTER 14

Biodegradable Injectable Systems for Bone Tissue Engineering

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14.1 Introduction

Natural bone has an intrinsic ability to heal itself, and this regenerative capacity is diminished with age, illness, or injury.[1] The current approaches for the treatment of critical-sized bone defects include the use of natural bone grafts or metallic prosthetic implants. Natural grafts can be categorized based upon their tissue source as autografts, allografts, and xenografts. Autografts currently serve as the gold standard for bone implantation, and are harvested from the patient's own body, which reduces the risk of graft rejection. Although, autografts are widely used in clinical applications, problems such as donor site morbidity, the invasive nature of surgery, long recovery times, and bone graft availability of a desired size and shape have restricted the use of autografts in orthopedic applications.[2,3] In the United States (US), there have been over a million surgical procedures involving large bone defects due to trauma, non-union healing fractures, or resection requiring the use of bone grafts. As the US population ages, there has been an increase in demand for bone grafts, and these surgical procedures have placed a large burden on the healthcare industry

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totaling over 5 billion dollars annually.[4,5] Thus, a clinical need exists for the development of alternative methods to regenerate bone to meet the shortage in bone grafts and address the limitations of the current available treatment choices.

Tissue engineering is a multidisciplinary field, which applies principles from material science, chemical engineering, and biological life sciences to develop alternatives to restore, improve, or maintain diseased or damaged tissue.[6] The foundation of tissue engineering relies on four key elements: cells, scaffolds, signals, and bioreactors.[7,8] In the general scheme for tissue engineering, cells are seeded onto a three-dimensional (3D) scaffold, a tissue is cultivated *in vitro*, proper signals are supplemented to the system, and finally the construct is implanted into the body as a prosthesis.[8] The general scheme for the key elements involved in the tissue engineering paradigm is illustrated in Figure 14.1.

The cells used in tissue engineering applications can be isolated from autologous, allogenic, or xenogenic sources, and may be tissue specific, stem cells, or progenitor cells. The harvested cells are then isolated and expanded *in vitro*. Scaffolds, which mimic the native extracellular matrix and provide a substrate for cell growth, can be composed of either a natural or synthetic material, and fabricated into a fibrous, foam, hydrogel, or capsule architectures. Signals are also introduced to the system for enhanced cell adhesion, proliferation, and differentiation within the construct. Bioreactors are often utilized to mimic the dynamic conditions inside the body, and provide many

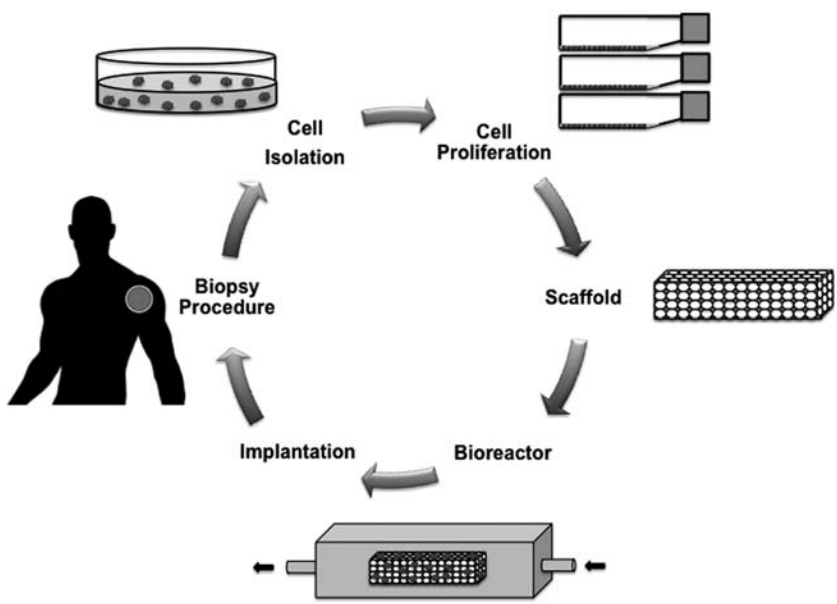

Figure 14.1 A representative schematic describing key elements of the traditional tissue-engineering paradigm.

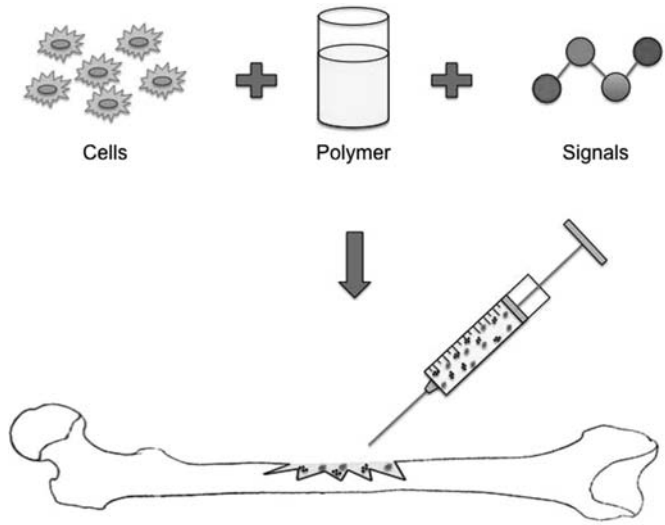

Figure 14.2 Schematic representing the concept behind injectable bone tissue engineering.

benefits such as improved mass transport and the application of mechanical stimuli to the developing tissue.[8,9]

Recently, many tissue engineering designs using injectable, in situ forming systems have been reported for orthopedic applications with many advantages over previous methods. Unlike tissue engineering approaches that utilize pre-fabricated scaffolds, injectable systems have garnered great interest within the field as a unique therapeutic method for difficult to reach areas of the body using minimally invasive procedures, and show the ability to conform to any shape irrespective of the defect geometry. Furthermore, injectable systems can be used as fillers to reinforce the mechanical properties of diseased/ injured bone and as a competent carrier of cells and therapeutic agents such as drugs and growth factors (Figure 14.2).[10–12]

14.2 Rationale and Requirements for Injectable Bone Tissue Engineering

Native bone is a complex tissue to engineer and understanding the structural, physical, chemical, biological, and cellular properties of natural bone is imperative to design a new approach that will fulfill not only the basic principles of tissue engineering, but also address the unique challenges of designing a tissue engineered bone. The following section will highlight the major criteria necessary in the design and development an ideal biodegradable injectable tissue engineered bone substitute.

14.2.1 Injectability and In Situ Cross-linking

The basic requirements for the selection of scaffold material for tissue engineering applications are challenging and multi-faceted. For injectable-based systems, the precursor solution of the polymer should be easily injectable through the required delivery system in order to facilitate the minimally invasive surgical procedure. The polymer viscosity prior to cross-linking plays a critical role in determining the injectability of the system. To ensure the injectability of the system, many in situ cross-linkable polymers are dissolved in biocompatible solvents such as water, *N*-methyl-2-pyrrolidone, and dimethyl sulfoxide. In addition to solvent solubility, the time required for polymer cross-linking should be within an appropriate time window to avoid surgical difficulties and failure of the implant. For example, slower cross-linking times may encourage unwanted migration of the material from the defect area, whereas extremely rapid cross-linking times may hinder the surgical ease of implantation.[13] Many factors such as the intensity of cross-linking stimuli, concentration of the initiating system, type of cross-linker, and material functionality play a major role in the cross-linking time of any injectable system.

14.2.2 Mechanical Properties

Biomaterials implanted inside the body are often subjected to a mechanically dynamic environment, and must be able to sustain and recover from repetitive deformations while allowing material/ tissue integration without irritating surrounding tissues.[14] To complicate matters, the mechanical properties of natural bone vary greatly depending on the location and function of the native bone tissue (Table 14.1). Thus, the mechanical properties of tissue engineered bone constructs should be given special attention in that the given materials are used for multiple purposes such as load bearing and tissue regeneration, and closely match the mechanical properties of natural bone as closely as possible to avoid problems associated with compliance mismatch and load shielding, which may lead to implant failure.[15,16]

In addition to preventing graft failure, many groups have shown that the mechanical properties of scaffolds may also play an important role in the biological outcome of the implanted device.[18,19] Scaffold mechanical properties have been shown to have an influence on the severity of the host inflammatory response, angiogenesis, and wound-healing properties.[20] Injectable materials should withstand and transfer the mechanostimulation needed to induce

Table 14.1 Mechanical properties for the various sections of native bone.[17]

Bone tissue	*Mechanical strength*		*Young's modulus*
	Tensile	*Compressive*	
Cortical bone (longitudinal)	78–151 MPa	131–244 MPa	17–20 GPa
Cortical bone (transverse)	55–66 MPa	106–131 MPa	6–13 GPA
Cancellous bone	5–10 MPa		50–100 MPa

metabolic activities of all types of bone cells including osteoblasts, osteocytes, osteoclasts, and osteoprogenitors. For example, osteocytes under mechanical stimulation can release osteoblastic factors to accelerate bone formation, whereas in the absence of mechanical cues, osteocytes have been shown to undergo apoptosis and recruit osteoclasts, resulting in bone resorption.[21] Furthermore, it has been reported that under fluid flow stimulation, osteoblasts have been shown to reorganize their cytoskeleton, up-regulate transmembrane focal adhesion proteins, and express osteoblast-specific proteins such as osteopontin involved in extracellular matrix adhesion.[17,22,23] Thus, in order to simulate the dynamic mechanical physiological environment, injectable materials should possess similar mechanical properties compared to native bone tissue.

14.2.3 Porosity

The porosity of the scaffold architecture is another important design requirement, which plays a critical role in the outcome of a tissue engineered graft. Cells seeded inside the scaffold rely heavily on the void spaces within the construct for cellular in-growth, vascularization, and the exchange of nutrients and waste products.[24] Thus, the implanted graft should have a highly organized, porous, and interconnected structure. Porosities of more than 90% and pore sizes greater than 300 μm are preferred for cellular penetration and vascularization while maintaining scaffold mechanical integrity.[25–29] Recent research has shown that nanoscale architectures of the scaffold can also influence the cellular behavior of the underlying material.[17] In particular to injectable materials, in situ porous generating techniques have been limited to gas foaming[30] and particulate leaching.[31] The foremost challenge for the success of injectable systems still lies in the creation of injectable scaffolds with an interconnected and homogeneous porous network.

14.2.4 Biodegradation

Tissue engineered devices should be fully resorbed by the body to match the rate of neotissue formation, and cleared from the body through normal physiological functions through a process known as biodegradation. Biodegradation is a process by which polymers are chemically reversed into their precursor monomers, which can be accomplished through dissolution, hydrolysis, and enzymatic activities.[32] Degradation mechanisms for polymers used in tissue engineering are categorized as either bulk degradation or surface erosion. Most polyesters, polyether-esters, and polyester-amides rely on bulk degradation and follow first-order profiles, whereas polyanhydrides and polyorthoesters degrade through surface erosion and yield zero-order profiles.[17]

Injectable polymers used as bone scaffolds are composed of cross-linked networks, which degrade through several mechanisms. Degradation of these networks primarily relies on the nature and location of the degradable groups and cross-linkable moieties. In the case of poly(propylene fumarate) (PPF) and poly(ethylene glycol maleate citrates) (PEGMC), the cross-linkable and

degradable moieties are found alternating along the polymer backbone chain. As the polymer network degrades, it is broken down into multiple kinetic chains and the starting monomers. Lactic acid-based injectable materials are degraded into the original core molecules connected by the degradable units and kinetic chains formed during the free-radical polymerization of the photoreactive groups.[33] Finally, enzymatically cross-linked polymers are degraded through the pendant reactive groups along the proteolytic degradable polymeric backbone.[34]

In addition to chemical structure, the polymer crystallinity, crystal structures, molecular orientation, melting temperature (T_m), glass transition temperature (T_g), cross-linking density, external particulates present in the polymer network, and micro- and nanoscale structure of the scaffold also influence the degradation rate.[35]

14.2.5 Cellular Behavior

Bone is made up of 60% inorganic minerals (calcium phosphate), 30% organic (collagen type I, osteonectin, osteocalcin, osteopontin, proteoglycans, and glycoproteins), and 10% of cellular components (osteoblasts, osteocytes, and osteoclasts). Every component has a critical role for the healthy regeneration and function of bone. For all bone tissue engineering applications, the implanted graft material should be tightly integrated with the surrounding bone tissue to provide a suitable cellular environment for the production extracellular matrix proteins. As mentioned above, materials utilized in tissue engineering applications should mimic the natural extracellular matrix in order to provide mechanical support and regulate cell behavior including cell anchorage, segregation, communication, and differentiation.[36] In general, polymers used in injectable bone tissue engineering should provide suitable functionalities (carboxylic and hydroxyl groups) for facile modification of biomolecules onto the surface and into the bulk of the material to guide cellular behavior.

For example, arginine-glycine-aspartic acid (RGD), a short peptide sequence, has been shown to mediate cell attachment to various extracellular matrix proteins such as fibronectin, vitronectin, bone sialoprotein, and osteopontin.[37] More recently, a collagen-mimetic peptide sequence, glycine-phenlalanine-hydroxyproline-glycine-glutamate-arganine (GFOGER), has been reported to enhance osteoblast functionality and osseointegration *in vivo*.[38] Injectable polymers such as poly(propylene fumarate-co-ethylene glycol), photo-cross-linked poly(ethylene glycol), alginate and poly(*N*-isopropylacrylamideacrylic acid) hydrogels were functionalized with RGD peptide, and demonstrated an improvement in osteoblast attachment and spreading within the materials.[39–42]

Apart from these primary cellular responses, the materials should also contain cellular integrin-binding sites and growth factor-binding sites to promote osteoconductivity, osteoinductivity, and osseointegration. The inorganic component of bone not only promotes osteoblast adhesion and migration/

infiltration (osteoconduction), but also provides strength to the bone tissue. In case of neotissue formation, osteoinduction and angiogenesis are two critical processes of the tissue regeneration. Hence, numerous polylactide- and glycolide-based polymer composites are under active investigation as scaffolding materials for bone tissue engineering.[43–46] Transcriptional growth factors, such as transforming growth factor beta (TGF-b), bone morphogenic proteins (BMPs), platelet derived growth factors (PDGFs), and insulin-like growth factors (IGFs), are reported to have major role on the osteoinductivity of the MSCs.[47–50] Similarly, growth factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) are major cues for neovascularization.[51–53] To introduce these mobile cues to the polymer matrix, various drug delivery systems are constructed in the injectable system. Later sections of this chapter will discuss in detail the existing injectable delivery systems for these key factors.

14.2.6 Biocompatibility

Injectable systems must be compatible with cells, tissues, and bodily fluids in order to function properly and avoid future complications. Any leachable compounds and degradation products should not hinder the process of tissue formation.[54] Since injectable materials are often used to deliver cells and sensitive compounds, the cross-linking process after injection should occur under physiologically accepted conditions. While designing any injectable scaffolds, careful consideration towards the toxicity, carcinogenicity, and chronic inflammatory response induced by the material, cross-linking system, solvents, and the degradation products should be given special attention.

14.3 Network Formation

Numerous injectable biomaterials have been recently reported in the scientific community as a non-invasive or minimally invasive technique for the regeneration and healing of defective tissues. To induce in situ cross-linkability in the polymeric system, monomers liable to mild cross-linking strategies should be incorporated within the polymer chains. In general, these systems consist of injectable and monodisperse polymeric chains with sites for three-dimensional network expansion under specific stimuli. The ability of biomaterials to behave as suitable tissue-specific injectable materials depends entirely upon the underlying cross-linking mechanism of solidification.[24] Biomaterial scientists are actively exploring cross-linking mechanisms based upon chemical cross-linking and physical gelation strategies. Chemical cross-linking is a network formation method achieved by covalently bonding two or more monomers. On the other hand, physical gelation can be achieved when two or more polymeric chains come to assemble due to the physical interaction of the chains such as ionic, hydrophobicity, or self-assembly in response to the surrounding environmental stimuli such as pH, temperature, and polymer precipitation.

Chemically cross-linked networks provide higher cross-linking densities to the polymer network, are more favorable for the sustained release of therapeutics, and allow for the fabrication of scaffolds with enhanced mechanical properties. However, the toxicity of the chemical cross-linking agents used may adversely affect cell behavior and the incorporated bioactive molecules. On the other hand, physical gelation of the network may avoid the use of cross-linking agents, but shows a limited performance in their physical properties. In the next sections, we will discuss the mechanisms involved in the solidification of injectable materials.

14.3.1 Free Radical Polymerization (FRP)

FRP consists of two components: (1) a radical-generating initiator system and (2) radical-liable monomers or oligomers. The most common radical-generating initiator systems used in biomaterials are high-energy gamma rays, ultraviolet light sensitive photo initiators,[55] and redox initiators.[56] Due to their ability to cross-link under physiological conditions, the latter two have received increased attention in the field of biomaterials. However, all these initiating systems rely on the same principle in that the generation of free radicals to initiate the cross-linking process is required. Once the free radicals are generated, the monomers or oligomers containing radical-liable moieties (usually vinyl and thiol groups) will go under further propagation steps. During propagation, these radical initiators homolytically cleave the radical-liable moieties to induce the cross-linking propagation. Finally, two radicals in the propagating polymer chains bond covalently to terminate the cross-linking process (Figure 14.3).

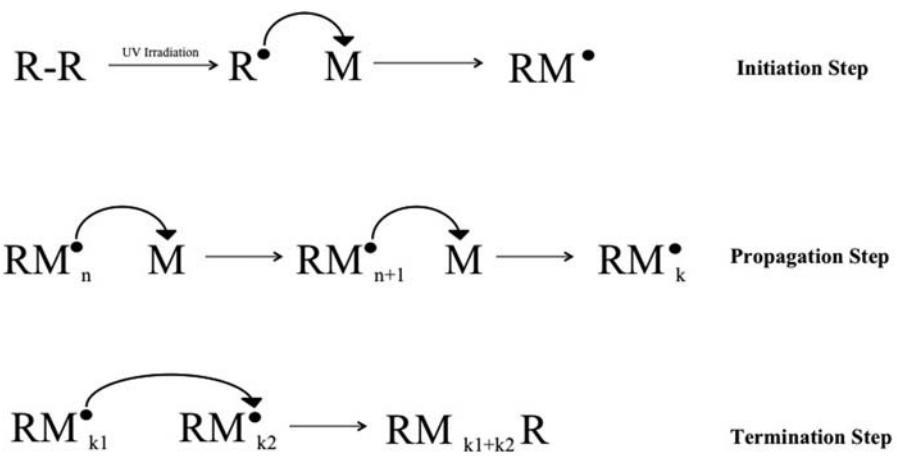

Figure 14.3 Representative schematic depicting the steps involved in free radical polymerization network formations.

Thus, the concentration of initiator, radical-liable moieties, and nature of solvent system all have a collective influence over the rate, stability, and kinetics of network formation. These parameters, in turn, reflect on the overall performance of the material in terms of cross-linking density, mechanical properties, and degradation profiles. Distinct differences in the application of systems cross-linked through photo-initiated and redox-initiated mechanisms have been reported in various literatures.[57–59] For example, redox-initiated systems are more favored in areas of limited light penetration and where homogeneous cross-linking of the network is preferred, whereas, photo-initiated systems are more favored where temporal and spatial control is required to develop complex structures such as patterned surfaces.

14.3.2 Chemical Cross-linking Systems (CCS)

CCS can be introduced into injectable materials by separately modifying soluble polymer chains with a pair of molecules that have specific affinity to each other. When these modified polymeric chains are injected simultaneously to the cross-linking site, they undergo rapid cross-linking to give rise to a covalent cross-linked network. The most commonly used pairs of molecules with specific affinities towards each other are *N*-hydroxysuccinimide (-NHS) to amine ($-NH_2$),[60] 1,4-addition of a doubly stabilized carbon nucleophile to an α,β-unsaturated carbonyl compound (Michael-type addition reaction),[61] and alkyne to azide (click chemistry).[62] In these types of reactions, the rate, stability, and kinetics of network formation are solely dependent upon the strength of the affinity of one molecule to its counterpart.

Another class of injectable materials using CCS is enzymatically cross-linked polymers. These polymers contain pendant phenol groups that undergo self-cross-linking in the presence of hydrogen peroxide (H_2O_2) and horseradish peroxidase (HRP). Phenol groups can be introduced to polymer chains such as chitosan,[63] gelatin,[64] hyluronic acid,[65] and dextran[66] by reacting their pendent carboxylic groups or amine groups with tyramine or 3,4-hydroxyphenylpropionic acid (HPA). This cross-linking strategy is inspired by the fact that treatment of proteins with peroxidase and H_2O_2 causes oxidation of phenol groups of tyrosine residues, resulting in cross-linking of protein molecules to form dityrosine and tertyrosine.[34] It has been reported that the cross-linking rate of the system is dependent on the concentration of HRP, H_2O_2, and phenol functionality. Gel formation is decreased with increasing HRP concentrations and decreasing H_2O_2 concentrations. An excess amount of H_2O_2 can oxidize HRP that results in deactivation of the cross-linking ability.[34] Besides HRP, another typical example of CCS is transglutaminase (TG) mediated glutaminamide-functionalized poly(ethylene glycol) (PEG) and poly(lysine-co-phenylalanine), which utilize calcium ions as cofactors.[67]

14.3.3 Thermally Induced Gelation Systems (TGS)

TGS are widely famous for their use in polymers, which have a unique ability to undergo sol-to-gel and gel-to-sol phase transitions as a function of temperature. These polymers are amphiphilic in nature (composed of both hydrophilic and hydrophobic segments), and display a sol-to-gel transition, which is attributed from the balance between intermolecular forces and hydrophobic section aggregation. The molecular weight of these segments is solely responsible for the sol-gel performance of these copolymers. For example, copolymers of methoxy poly(ethylene glycol) (MPEG)-poly(ε-caprolactone) (PCL) shows both gel-to-sol and sol-to-gel transition at elevated temperatures. Copolymers composed of MPEG ($M_n = 2000$) are in a gel state at 10 °C, and transition from gel to sol with increasing temperature.[68] When the MPEG segment molecular weight is reduced ($M_n = 750$), the material is liquid at room temperature, and transitions from sol to gel with increasing temperatures. In the case of copolymers with MPEG ($M_n = 750$), the sol-to-gel phase transition temperature decreased substantially with increasing molecular weight of PCL.[69] Various other PEG-based biodegradable copolymers have been reported to exhibit thermoresponsive properties. Linear or star-shaped copolymers such as poly(ethylene glycol-L-lactic acid) and poly(ethylene glycol-DL-lactic acid-co-glycolic acid)[70–72] have shown a sol-to-gel transition as the temperature is decreased. A more recently reported copolymer MPEG-b-(PCL-ran-PLLA) has been shown to display a liquid state at room temperature, and transitions to a gel state precisely at body temperature (37 °C), which is a major advantage for use in injectable therapies.[73]

Another class of TGS polymers is *N*-isopropylacrylamide-based copolymers that display a sol-to-gel transition as the temperature is elevated above their lower critical solution temperature (LCST). The drastic difference in solubility above the LCST is due to the entanglement and the gradual collapse of polymeric chain. Various copolymers have been reported to induce biodegradation to this polymeric system. The factors determining the gelation process include polymer concentration, molecular weight, and chemical structure of the copolymer. Other typical examples of thermosensitive polymers are poly(ethylene oxide) and poly(propylene oxide) copolymers (poloxamers or pluronics),[74] cellulose derivatives,[75] chitosan,[76] and gelatin[77] have also been widely explored as thermoresponsive injectable materials in tissue engineering and drug delivery.

14.3.4 Self-assembly Systems (SAS)

Most of the SAS have been reported in amphiphilic polymers, which show the ability to self-assemble due to the desolvation, collapse, and intermolecular association of the hydrophobic portions of monomers.[78] In the case of charged (anionic, cationic, or zwitterionic) amphiphiles, further stability and structural specificity can be designed using intermolecular polar interactions, such as electrostatic and hydrogen bonding.[79] Various biologically inspired materials

such as peptide- and protein-based systems have been reported with distinct amphiphilic properties for use in tissue regeneration and growth factor delivery. For example a self-assembling peptide-based hydrogel has been reported with the property of low viscosity at certain shear stress demonstrating the ability to be injectable. Following injection, the hydrogel recovers to its gel form to solidify in the defect cavity.[80] Others have demonstrated the three-dimensional encapsulation of biologically active molecules such as bone morphogenetic protein-2 (BMP-2)[81] and fibroblast growth factor (bFGF)[82] by mixing the suspension of these molecules in aqueous solution of amphiphilic peptide that undergo self-assembly to form a mechanically stable hydrogel.

Water-insoluble biodegradable polymers have also been injected in solutions with water-miscible, physiologically compatible solvents to show self-assembly via phase segregation. Following injection, the solvent diffuses into the tissue space and water diffuses to the polymer matrix. This results in the precipitation of water-insoluble polymer into a matrix at the injection site. The solvent systems that have been reported based on this approach are propylene glycol, acetone, 2-pyrrolidone, tetrahydrofuran, *N*-methyl-2-pyrrolidone, and dimethyl sulfoxide.[83] The rate of precipitation of the polymer depends upon many factors such as the concentration of the polymer in solvent, the molecular weight of the polymer, the solvent used, and the addition of a surfactant.[84]

14.3.5 Ion-mediated Gelation Systems (IGS)

IGS rely on the ability of di- or trivalent cations to form ionic interchain bridges between the polymeric chains. Alginate is the most wildly used polymer that has ability to cross-link via calcium or zinc cations. Structurally, alginate is a linear polysaccharide composed of homopolymeric blocks of 1,4-linked b-D-mannuronic (M) acid and a-L-guluronic (G) acid residues in various proportions and sequential arrangements.[85] These di- or trivalent cations have been reported to cross-link through different sites of the alginate chain.[86] However, calcium ions are more selective in the cross-linking ability through the polyguluronic acid block (GG) in a planar geometry. On the other hand, zinc cations are reported to be less selective for the cross-linking sites resulting in more extensive cross-linked alginate hydrogels.[87] The rate and kinetics of cross-linking are highly influenced by the concentration of multivalent cations and G-block segment sequences. However, higher concentrations of the alginate polymer chains also lead to a decreased cross-linking rate.[88]

Table 14.2 provides a summary.

14.4 Injectable Ceramics

Natural bone is made of 60% of inorganic calcium phosphate minerals.[15,25] To this end, many researchers have developed synthetic bone substitutes based upon ceramics to better mimic the natural composition of bone. Ceramics have been widely used for orthopedic and dental applications, and have been used

Table 14.2 Biodegradable injectable system properties.

Network Formation	*Material*	*Application*	*Reference*
Ceramic setting	Calcium phosphate	Bone substitute	89–92
Free radical polymerization	Alginate	Cell encapsulation	93–95
	Chitosan	Ligament tissue	96–98
	Hyaluronic acid	Cartilage tissue	99–101
	Poly(ethylene glycol) based	Bone tissue	16,102,103
	Poly(L-lactide) based	Bone substitute	104–106
	Poly(vinyl alcohol)	Wound tissue	107,108
	Poly(propylene fumarates)	Bone tissue	30,32,41,54, 56,109–113
	Poly(alkylene maleate citrates)	Cell delivery Drug delivery Bone substitute	114,115
Chemical cross-linked systems	Chitosan	Cartilage tissue	63
	Dextran	Tissue engineering Protein delivery	66
	Gelatin	Cell delivery	64
	Hyaluronic acid	Protein delivery	65
	Poly(ethylene glycol) based	Bone tissue	52,61,116
Thermally induced gelation systems	Chitosan	Cardiac tissue Neural tissue Bone tissue	76,117–119
	Poloxamers or Pluronics	Lung tissue Bone tissue	74,120,121
	MPEG-PCL		68,69
	MPEG-b-(PCL-ran-PLLA)		73
	PEG-co-poly(a-hydroxy acid)	Drug delivery Protein delivery Cell delivery	70–72
Self-assembly systems	RGD-based fibers	Bone tissue	122
	Poly(DL-lactide-co-caprolactone)		83
	Peptide-based hydrogel	Bone tissue	123,124
Ion-mediated gelation systems	Alginate	Bone tissue	40,125–127

dating as far back as 1892 in the development of plaster of Paris ($CaSO_4$). The currently available bone cements, which are both injectable and biodegradable, can be organized into three categories: calcium phosphate cements (CPCs), bioglass, and bioactive glass cements.[128,129]

CPCs are widely used as bone substitutes and for augmentation in orthopedic applications due to their close resemblance to the mineral component of natural bone.[92] CPCs are a powder phase of calcium and/or phosphate salts,

which set at body temperature when mixed in an aqueous phase. CPCs such as apatites including hydroxyapatite (HA), carbonated apatite (CA), and calcium-deficient hydroxyapatite (CDHA) can be further categorized as apatite or brushite cements depending on their rate of resorption.[128,130] Apatite cements have higher mechanical strength but slower degradation rate than the brushite-based cements.[128] In order to use CPCs in bone tissue engineering applications, it is imperative to incorporate macroporosity in the material to allow for tissue and new blood vessel formation. Recently, many groups have used mannitol,[131] sodium bicarbonate,[132] and albumin[133] as porogens to incorporate interconnected macropores in CPCs.

Bioglass is a member of the family of bioactive glass composed of silica (SiO_2), sodium oxide (Na_2O), calcium phosphate (CaP), and phosphoric anhydride (P_2O_5). Bioglass-like CPCs have excellent biocompatibility and have shown the ability to grow apatite layers on the surface resulting in better bone-implant integration. In addition, bioglass also promotes osteoblast attachment, proliferation, differentiation, enzymatic activity, and angiogenesis. Studies have shown that the degradation products of 45S5® Bioglass up-regulates the gene expression that controls osteogenesis and production of growth factors.[134] The degradation rates of bioglass are tailored to meet specific bone tissue engineering applications by changing the composition and processing environment. However, the mechanical properties of bioglass are plagued by low fracture toughness and poor mechanical strength, which makes it unsuitable for use in load-bearing applications.[129,130] 45S5® Bioglass is clinically available as Perioglas™ for treating periodontal disease, and Novabone™ as a filler for treating bone defects.[25] Bioglass ceramics such as apatite/wollastonite (A/W) glass exhibit better mechanical properties than the parent glass and sintered crystalline ceramics.[25,129] A/W glass have higher mechanical strength, excellent biocompatibility, and are used as fillers for bone defects caused by iliac grafts and as artificial vertebrae. A/W glass ceramics also show better bioactivity than sintered hydroxyapatite (HA).[135,136] Table 14.3 is a summary.

14.5 Injectable Cell Vehicles

The incorporation of cells into tissue engineered scaffolds has been based upon the use of two different approaches: (1) surface seeding of cells on prefabricated scaffolds and (2) encapsulation of cells in a 3D scaffold. While the use of surface seeding onto prefabricated scaffolds allows for the design of a precisely controlled porous network using different scaffold fabrication methods such as particulate leaching, gas foaming, and thermally phase-induced separation, scaffolds fabricated through this route cannot be implanted in a minimally invasive manner. On the other hand, the encapsulation of relevant cells into a 3D matrix is an inherently mild process, and can be used for injectable applications where the cells are mixed with the liquid cross-linkable solution and administered in a minimally invasive manner to the desired site *in vivo*. Since the liquid will diffuse and conform to the shape of the defect, the adhesion of scaffold to the tissue is improved when compared to prefabricated scaffolding

Table 14.3 Cement-based injectable system mechanical properties and applications.

Cement material	*Compressive strength*	*Application*	*Reference*
Hydroxyapatite	>400 MPa	Bone filler and prostheses coating materials	25,137
Biosorb (β-TCP)	15–150 MPa	Bone filler	130
Calcibon®	4–7 MPa	Bone substitute material	130
ChronOS Inject	7.5 MPa	Bone remodeling and cyst treatment	130
Bone Source™	26 MPa	Craniotomy cuts and cranial defects	128,138
Norian® SRS	50 MPa	Bone fractures	128,139,140
Cementek	20 MPa	Bone substitute material	130
45S5®Bioglass	~500 MPa	Bone filler, Middle ear prostheses, Periodontal disease	25,141
A/W glass ceramic	1080 MPa	Artificial vertebrae, Bone fillers, Intervertebral discs	136,137

approaches.[142,143] Polymeric networks that have the ability to uptake large quantities of water and demonstrate elastic properties are termed hydrogels, and have been used extensively in tissue engineering applications due to their close resemblance to native tissues. Hydrogels can be broadly classified based upon their nature of origin such as natural hydrogels and synthetic hydrogels. The following section summarizes the ongoing research on cell encapsulation for bone tissue engineering using injectable and biodegradable hydrogels.

14.5.1 Naturally Derived Hydrogels

Chitosan, alginate, and hyaluronic acid are all polysaccharide-based hydrogels similar to native ECM. Chitosan is a natural biopolymer with a striking resemblance to mammalian glycosaminoglycans. Alginate is non-mammalian polysaccharide that can be cross-linked under mild conditions with low toxicity. Alginate beads have been used to encapsulate MC3T-E1 osteoblasts, which were then mixed with calcium phosphate cement and chitosan-calcium phosphate cements. The alginate beads were found to improve cell viability significantly by protecting the cells from the cement hardening reaction.[144,145] Alginate gels encapsulated with murine embryonic stem cells (mESCs) were cultured in a rotary cell culture microgravity bioreactor. The resultant 3D mineralized constructs were found to have attributes of osteogenic lineage as well as the mechanical strength and mineralized Ca/P deposition.[146] Calcium alginate core of mineralized alginate/chitosan capsules were used to encapsulate human osteoprogenitor cells (STRO-1^+) and at the end of 7 days of *in vitro* subculture indicated the maintenance of osteoblastic phenotype. New bone formation with type I collagen matrix was seen when these polysaccharide capsules encapsulated with human bone marrow cells and rhBMP-2 were

implanted in nude mice.[147] Thermoresponsive hydroxybutyl chitosan (HBC) was evaluated as an injectable therapeutic treatment of degenerated intervertebral discs (IVD). HBC gels were encapsulated with human mesenchymal stem cells (hMSCs), human annulus fibrosus cells (hAFC) and human nucleus pulposus cells (hNPs) and provided a suitable environment for the survival of the disk cells and hMSCs in a metabolically active and proliferative state.[148] Recent strategies involving hyaluronic acid for cell encapsulation for bone tissue engineering applications have involved modifying hyaluronic acid with methacrylates and thiols. *In vivo* studies using acrylated hyaluronic acid injected into rat calvarial (skull) defects showed that human mesenchymal stem cells (hMSCs) in the presence of bone morphogenetic factor-2 (BMP-2) demonstrated the ability to differentiate into specific cells such as endothelial and osteoblast cells.[145,149]

14.5.2 Synthetic-based Hydrogels

Poly(ethylene glycol) (PEG)-based hydrogels have been extensively studied as cell encapsulating networks for bone tissue engineering applications. Although PEG based hydrogels are highly hydrophilic, and have shown to resist protein adsorption and cell adhesion, studies have shown that osteoblasts and chondrocytes can survive in such hydrophilic conditions without any added biological cues.[143,150,151] Poly(ethylene glycol diacrylate) (PEGDA) was used to encapsulate hMSCs, and facilitate differentiation into osteoblasts in the presence of osteogenic differentiation media consisting of ascorbic acid, dexamethasone, and β-glycerophosphate.[152–154] PEG-based hydrogels were modified by using RGD peptide sequence to improve osteoblast attachment, proliferation, and differentiation.[155] When RGD-modified PEGDA hydrogels were encapsulated with bone marrow stromal stem cells (bMSCs), the osteogenic activity of the cells improved and peaked at an optimum peptide concentration.[156] The addition of phosphate-containing molecule methacrylate phosphate (EGMP) improved hMSCs adhesion by promoting the mineralization within the hydrogel.[157]

The degradation profile of PEG hydrogels can be controlled by the addition of degradable linkages such as poly(α-hydroxy esters) or peptides that can be cleaved enzymatically.[149,150] Osteoblasts encapsulated in hydrogels through the copolymerization of poly(lactic acid)-b-poly(lactic acid) with PEGDA showed elevated ECM production in terms of osteopontin, type I collagen, and calcium phosphatase deposition.[149,151,158]

A new class of synthetic injectable and biodegradable hydrogels using fumaric acid has been developed, which includes poly(propylene fumarate) (PPF), poly(propylene fumarate-co-ethylene glycol) (Poly(PF-co-EG)), and oligo(poly(ethylene glycol) fumarate) (OPF).[159–164] Poly(alkyl fumarates) can be easily cross-linked with itself or in the presence of a cross-linking agent in situ to form a degradable polymeric network. The *in vitro* osteogenic differentiation of MSCs encapsulated in OPF gels (PEGDA mol. wt. 3 K and 10 K) in the presence of dexamethasone showed increased calcium content and

Table 14.4 Biodegradable injectable cell delivery vehicles for bone tissue engineering.

Material	*Cell*	*Reference*
Alginate beads	MC3T-E1 osteoblasts	144,145
Alginate gels	Murine embryonic stem cells	146
Alginate/chitosan capsules	Human osteoprogenitor cells (STRO-1$^+$)	147
	C2C12 myoblasts	147
	Bone marrow stromal cells	147
	Adipocytes	157
EGMP-containing PEGDA	hMSCs	157
Hydroxylbutyl chitosan gels	hMSCs	148
	hNPCs	148
	hAFCs	148
Hyaluronic acid gels	hMSCs	145,149
OPF/PEGDA	Rat MSCs	164
OPF	Rat bMSCs	165
PEGDA	hMSCs	152
PEGDA modified with RGD	Osteoblasts	155
PEGDA modified with poly(α-hydroxy ester)	Osteoblasts	149,151, 158
PPF	MSCs	166,167
PhosPEG	MSCs	168–170

osteopontin production with increased swelling behavior.[62,63] Rat bMSCs encapsulated in RGD-modified OPF gels supported osteogenic differentiation in the absence of any supplements (dexamethasone and β-glycerol phosphate).[165] PPF has also been investigated as a cell carrier in bone regeneration applications. Initial cell viability was increased when MSCs were encapsulated in gelatin microcapsules before adding them to the cross-linking PPF.[166,167]

Phosphoester hydrogels are photopolymerizable phosphate-containing PEG hydrogels (PhosPEG) that were designed to undergo degradation via hydrolysis at the phosphoester linkage. PhosPEG hydrogels were photo-cross-linked from the macromer precursor of poly(ethylene glycol)-di-[ethylphosphatidyl (ethylene glycol) methacrylate]. The presence of alkaline phosphatase (ALP) enhanced degradation by cleaving the phosphoester groups in the PhosPEG network, demonstrating enzymatic degradation. The by-products of the enzymatic degradation react with the calcium ion in the media promoting autocalcification and promoted osteogenic differentiation of encapsulated MSCs.[149,168–170] The incorporation of hydroxyapatite (HA) in thermosensitive poly(isopropylacrylamide-co-acrylic acid) (p(NiPAAm) with rabbit MSCs and bone morphogenic factor (BMP-2) showed increased osteogenic differentiation and extracellular matrix production.[171] Table 14.4 is a summary.

14.6 Injectable Drug Delivery Systems

Many of the previous injectable systems for bone tissue engineering have mainly relied on the material chemistry and physical properties to control

cellular adhesion, proliferation, and differentiation.[172–174] However, bone regeneration is a complex process governed by an intricate interplay between various growth factors and cytokines to guide the healing process.[175] As this complex cascade of biological events for bone regeneration is further understood, new therapeutics and therapeutic release strategies are continually emerging towards the development of an ideal injectable system. Preferably, the administration of bioactive molecules or drugs from the delivery system should be precisely controlled to provide the appropriate dose over the therapeutic time frame to match the dynamic physiological needs of the regenerating tissue. Due to the hydrolytically unstable ester,[176,177] ether-ester,[178,179] anhydride,[180] or amide functional groups,[175,181] biodegradable polymers have been extensively researched in the area of controlled delivery of bioactive molecules and drugs.[182] Detailed reviews highlighting the concepts behind drug delivery for bone tissue engineering are available,[183–185] and the following section will briefly discuss the localized delivery of antibiotics and growth factors from injectable delivery systems.

14.6.1 Antibiotic Delivery

Infections associated with implanted devices are a significant challenge in the field of orthopedic tissue engineering.[186] Osteomyelitis is a deep bone infection caused by staphylococci and it often leads to bone loss and the spread of bacterial infection to the surrounding tissues. Treatment of this type of infection has proven difficult due to the short half-life of antibiotics, inadequate blood circulation to the infected area, and the systemic toxicity of the antibiotic, which limits the use of high systemic dosages.[187] Since the overall success of implanted materials is largely dependent on the prevention of bacterial in-growth to the defect site, many groups have focused on the localized delivery of antibiotics through injectable cement and polymer delivery systems. Early strategies to treat osteomyelitis have been researched since the mid-1990s, and relied on local antibiotic treatment through the release of antibiotics from non-biodegradable poly(methyl-methacrylate) (PMMA) cement carriers.[188] However, a major disadvantage to this approach is the non-biodegradable nature of the drug delivery vehicle, which requires a second surgery to remove the PMMA beads.[189] Recent efforts to treat orthopedic infections have now moved to the use of injectable biodegradable cements and polymer systems.

Many of the injectable cement-based systems used in the treatment of orthopedic infections, such as calcium phosphate cements (CPCs),[190] β-dicalcium silicate (β-Ca_2SiO_4),[191] and hydroxyapatite cements (HACs),[192] have relied on the local delivery of antibiotics such as gentamicin or cephalexin monohydrate to increase the antibacterial activity against *E. coli* and *S. aureus* strains *in vitro*. A study led by Joosten *et al.*[192] evaluated the effects of gentamicin release from a HAC both *in vitro* and *in vivo*. Bone infections were induced into the right tibia of 'New Zealand' rabbits, and treated with HAC-loaded gentamicin. The *in vivo* results confirmed that no histopathological evidence of infection was found for

the HAC/gentamicin-treated animals, whereas different stages of chronic osteomyelitis were found in all control groups.

Although antibiotics have been incorporated into many commercially available types of cement, the high curing temperatures required and poor release kinetics of the antibiotic have driven researchers to develop other delivery vehicles.[185] Peng *et al.*[193] have recently developed a novel thermosensitive implant composed of poly(ethylene glycol) monomethyl ether (mPEG) and poly(lactide-co-glycolic acid) (PLGA) copolymer (mPEG-PLGA) drug delivery system. The thermosensitive behavior of this system allows for the efficient loading of the drug or bioactive molecule without the use of harsh conditions, which can cause denaturing, aggregation, and undesirable chemical reactions. Similar to the control teicoplanin-loaded PMMA bone cements, the mPEG-PLGA hydrogel containing teicoplanin was effective in treating osteomyelitis in rabbits *in vivo*. Other strategies to treat osteomyelitis have been evaluated using poly(sebacic-co-ricinoleic-ester-anhydride) containing gentamicin, which increases in viscosity and becomes a semisolid gel when exposed to an aqueous environment.[187,194] Published studies have indicated a positive effect on established osteomyelitis in a rat model; however, these studies did not show complete eradication of the infection.[194]

14.6.2 Growth Factor Delivery for Osteogenesis

Growth factors are signaling polypeptides that bind to specific receptors of the target cell and are crucial in controlling important cellular functions.[175] After a bone fracture, the locally produced cytokines and growth factors direct the migration, adhesion, proliferation, and differentiation of osteoprogenitor cells into specific lineages as well as extracellular matrix production at the defect site.[195,196] Unfortunately, growth factors are plagued with a relatively short biological half-life, and a major challenge has been to design a delivery system which can administer a prolonged sustained release to maintain the activity of the growth factors.[183] Many osteoinductive growth factors have been identified such as fibroblast growth factors (FGFs),[197] insulin-like growth factors (IGFs),[198] epidermal growth factors (EGFs),[199] and platelet-derived growth factors (PDGFs).[200] However, a majority of the growth factors utilized in injectable systems for orthopedic applications have been primarily been devoted towards the development of new bone formation through the use of the transforming growth factor beta superfamily.[175,201–203]

A number of delivery systems have been developed for the controlled release of bone morphogenic protein-2 (BMP-2). For example, calcium phosphate cement-based materials have shown the ability to deliver recombinant human bone morphogenic protein-2 (rhBMP-2) to increase alkaline phosphatase (ALP) activity in MC3T3-E1 cells *in vitro*,[119] and enhance bone formation both ectopically[204] and in an ulna osteotomy model.[91] In addition to cement-based systems, polymeric materials have also been heavily researched as potential delivery vehicles of BMP-2. Saito *et al.* have developed a temperature sensitive poly(D,L-lactic acid-polyethylene glycol) (PLA-PEG) block copolymer as an

injectable delivery system for rhBMP-2, and was able to form new bone on the surface of murine femur 3 weeks after injection.[205] Hosseinkhani *et al*. have reported on the controlled release of BMP-2 using a novel injectable peptide amphiphile (PA) system, which has the ability to form a three-dimensional nanofibrous scaffold by mixing the PA aqueous solution with the BMP-2 suspension.[206] This system was able to induce significant increase in homogenous ectopic bone formation subcutaneously in the back of rats when compared to BMP-2 injection alone.

Delivery methods based on microparticle and nanoparticle designs have gained increased attention in the delivery of growth factors to induce osteogenesis with smaller amounts of BMP-2 and with improved release over more sustained times. Magnetic liposomes,[207] collagen minipellets,[208] cationic nanoparticles,[209] and poly-ε-caprolactone microparticles[210] have all been reported as vehicles with a more uniform release of growth factors to increase osteogenesis. Research groups have also investigated the controlled release of an osteogenic peptide, TP508, loaded poly(D,L-lactic-co-glycolic acid) (PLGA) microparticles added to a mixture of poly(propylene fumarate) (PPF). The PLGA TP508 loaded microparticles showed release of the osteogenic factor for up to 28 days, and also serve as a sacrificial porogen in the PPF matrix once degraded.[211] Radiographic, microtomograph, and histological results all confirmed that the PLGA/PPF system was shown to enhance the bone consolidation process in a rabbit model of distraction osteogenesis when compared to TP508 saline solutions and dextran only.[212] Table 14.5 shows selective experimental results of orthopedic therapeutic carrier systems.

Table 14.5 Selective experimental results of orthopedic therapeutic carrier systems.

Carrier material	*Therapeutic agent*	*Matrix type*	*Cell/animal model*	*Reference*
β-Dicalcium silicate	Gentamicin	Cement	L929 cells	191
Calcium alginate	hIGF-1	Hydrogel	Goat (meniscus)	213
Calcium phosphate	Bisphosphonate	Cement	Rat (femur)	214
	bFGF	Cement		215
	BMP-2	Cement		215
	Cephalexin	Cement	S. aureus	190
	Gentamicin	Cement	S. aureus	189,192
	rhBMP-2	Cement	Primate (fibular)	216
	rhBMP-2	Cement	Primate (vertabrate)	217
	rhBMP-2	Cement	Rabbit (radius)	218
	rhBMP-2	Cement	Rabbit (subcutaneous)	204
	rhBMP-2	Cement	Rabbit (ulnar)	91
	Salmon-calcitonin	Cement	Rat (abdomen)	219
	TGF-beta 1	Cement		215,220

Table 14.5 (*Continued*)

Carrier material	*Therapeutic agent*	*Matrix type*	*Cell/animal model*	*Reference*
Cationic nanoparticles	OP-1	Nanoparticle	Rat (intramuscular)	209
Chitosan/alginate	BMP-2	Hydrogel	Mouse (subcutaneous)	221
Chitosan/inorganic phosphates	BMP-2	Composite	Rat (calvarial)	222
Cholesterol-bearing pullulan	W9-peptide	Nanogel	Mouse (subcutaneous)	223
E-matrix	rhBMP-2	Scaffold	Rat (spinal)	224
Elastin-like polypeptides	Vancomycin	Hydrogel		186
Hydroxyapatite	Gentamicin	Cement	Rabbit (tibia)	192
Magnetic liposome	rhBMP-2	Nanoparticle	Rat (femur)	207
mPEG-PLGA	Teicoplanin	Nanoparticle	Rabbit (femur)	193
N-isopropylacrylamide		Hydrogel	Rat (intramuscular)	225
Nanobone putty	hBMP-2	Putty	Mouse (intramuscular)	226
Nanohydroxyapatite	Amoxicillin	Microsphere	MG63 cells	227
Oligo(poly(ethylene glycol) fumarate)	TGF-beta 1	Hydrogel	Mesenchymal stem cells	56
Peptide amphiphile	BMP-2	Scaffold	Rat (subcutaneous)	81
PLA-PEG	rhBMP-2	Hydrogel		205
PLGA	Dexamethasone	Nanoparticle	Rat (cranial)	228
	rhGDF-5	Composite		229
	Vancomycin	Microparticle		230
PLGA/CaP	TGF-beta 1	Microsphere	Rat (skull)	231
PLGA/hydroxyapatite	Alendronate	Microsphere	hFOB	232
PLGA-mPEG	Teicoplanin	Hydrogel	Rabbit	193
Poly(NiPAAm-co-AAc)/HA	BMP-2	Composite	MSC	233
Poly(propylene fumarate)/calcium phosphate	Ginsenoside Rg1	Cement	HUVEC	234
Poly(sebacic-co-ricinoleic-ester-anhydride)	Gentamicin	Hydrogel	Rat (tibia)	187,194
Polyurethane	PDGF	Scaffold	MC3T3-E1 Cells	235
	Tobramycin	Scaffold	S. aureus	236
Starch-poly-ε-caprolactone	BMP-2	Microparticle	C2C12 Cells	210
Tricalcium phosphate	Platelet-rich plasma	Composite	Goat (tibia)	89
Tricalcium phosphate/alginate	IGF-1	Composite	MG-63 and Saos-2	237
Tricalcium phosphate/collagen	rh-PDGF	Microparticle	Rat (tibia)	238

14.7 Citric Acid-based Systems

In 2004, Yang *et al*. synthesized the first citric acid-based biomaterial through a convenient polycondensation reaction between citric acid and 1,8-octanediol to create poly(octamethylene citrate) (POC).[239] The resulting biodegradable, soft, and elastic material was shown to cover a wide range of mechanical properties, degradation profiles, and surface energies, which are all important in controlling the biological response to an implanted material. The excellent biocompatibility, hemocompatible nature, and tunable mechanical properties of POC drove Yang *et al*. to utilize the material primarily for small diameter vascular grafts[240] and medical device coatings.[241] Qiu *et al*. later proposed to combine POC and HA to create a composite (POC-HA) that would have the desired characteristics of a bioceramic suitable for orthopedic tissue engineering.[242] Bone screws fabricated from POC-HA composites displayed improved processability, mechanical properties, and degradation kinetics over previous biodegradable composites. However, the previous design required harsh processing conditions (>120 °C) for polymer network formation rendering them unable to be used in injectable strategies.

To overcome this limitation, our lab has recently developed a new family of in situ cross-linkable citric acid-based polymers, which can be cross-linked through free radical polymerization methods to avoid the use of harsh processing conditions required by the previous design. In this system, citric acid, maleic anhydride or maleic acid, and 1,8-octanediol were reacted together in a convenient polycondensation reaction to produce a biodegradable elastomer, poly(alkylene maleate citrates) (PAMC), which could be cross-linked using UV irradiation or redox systems to form a cross-linked network.[243] Maleic anhydride[244] and maleic acid[245] were both used to introduce a vinyl moiety in order to allow for network formation under mild conditions. Unlike the previous citric acid-based designs, this additional cross-linking method allowed for the preservation of valuable citric acid carboxylic acid and hydroxyl chemistries, which could be later used to conjugate bioactive molecules into the bulk material to control cell behavior.[246] To ensure that cells and sensitive drugs/factors could be incorporated and delivered to the injury site, poly(ethylene glycol) and acrylic acid were introduced into the system to create poly(ethylene glycol) maleate citrate (PEGMC), which allowed for water solubility and faster network formation kinetics.[247] The encapsulation of NIH 3T3 fibroblasts and human dermal fibroblasts showed the cytocompatibility of PEGMC and the controlled drug release using bovine serum albumin demonstrated PEGMC potential as a suitable cell and drug delivery vehicle.

To widen the application of PEGMC, our lab set out to develop an injectable, porous, and strong citric acid based-composite, which could be used as a delivery vehicle for cells and drugs in bone tissue engineering applications. PEGMC was combined with various wt.-% of HA to create PEGMC/HA composites.

The rationale behind this biomaterial design are:

(1) citric acid was chosen as a multi-functional monomer, which could participate in pre-polymer formation using a convenient polycondensation reaction while preserving valuable pendant functionalities
(2) to create a completely water-soluble material, which was injectable and provided a suitable environment for the delivery of sensitive cells/molecules, PEG was chosen a di-functional diol
(3) maleic anhydride introduced a vinyl moiety into the polymer backbone, which allowed for network formation using free radical polymerization to avoid the harsh processing conditions of previous designs
(4) to improve the osteointegration capacity and mechanical properties, HA was incorporated as a composite blend
(5) the pendant carboxylic acid chemistries can react with bicarbonates to induce gas foaming and create an injectable porous material.

This new generation of biodegradable citric acid-based elastomer composite offers many advantages over other injectable cell and drug delivery systems in that the valuable pendant chemistries are preserved during network formation, mechanical strength and osteoconductivity are improved using HA, mild conditions are utilized for network formation to enable the delivery of sensitive cells/biomolecules, and a porous construct can be created after delivery using minimally invasive procedures.

The degradation profiles for PEGMC/HA networks showed increasing mass loss with lower concentration of HA. Mechanical compressive tests showed that the PEGMC/HA networks were elastic and achieved complete recovery without any permanent deformation for hydrated and non-hydrated conditions. Human fetal osteoblast (hFOB 1.19) encapsulated in PEGMC/HA hydrogel composites showed that the cells were viable and functional at the end of 21 days of subculture (Figure 14.4A). ECM production was measured for alkaline phosphatase and calcium content, and both were shown to increase after 3 weeks of culture. SEM/EDX analysis of the constructs showed that the PEGMC/HA films were covered with small cauliflower shaped structures after 7 days of incubation in simulated body fluid (Figure 14.4B).[248,249] The presence of pendant groups in the PEGMC polymer allows for easy modification through the bioconjugation of biological molecules such as type I bovine collagen, and resulted in enhanced cellular attachment and proliferation at the end of day 7 of subculture. Unlike many injectable systems, PEGMC/HA composites could also be fabricated into highly porous architectures from gas foaming techniques in situ (Figure 14.4C). Thus, unlike previous injectable materials, PEGMC/HA composites show great potential as an injectable, porous, and strong cell/drug delivery system for orthopedic applications.

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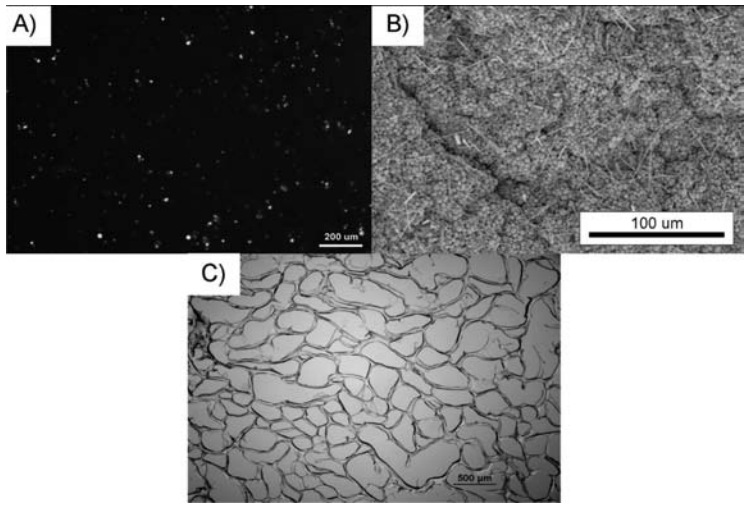

Figure 14.4 (A) Live/dead stain of hFOB 1.19 osteoblasts encapsulated in PEGMC/HA hydrogel after 7 days. (B) Mineralization in SBF for PEGMC/HA composite with 40 wt.% HA at 7 days. (C) 10 µm section of PEGMC scaffold showing the porosity created from a gas foaming technique.

14.8 Future Directions

The use of injectable systems in orthopedic tissue engineering is still in its infancy, and continued advances in biomaterial development and design are required to realize the goal of applying injectable strategies to bone regeneration. Although recent success has been demonstrated in delivering cell and therapeutic agents using injectable-based designs, more studies using synthetic polymer composites to improve the construct mechanical properties while maintaining proper degradation kinetics to match the stringent requirements for bone tissue engineering will be the focus of future studies. In addition to mechanical compliance, research focused on the use of sacrificial porogens to deliver drugs and introduce porosity to promote cell infiltration and the establishment of a vascular network will continue to dominate the future investigations. Thus, as new materials are continually introduced to the field, the growth of knowledge in designing constructs with improved mechanical properties, porosities, and angiogenesis will bring the field closer, developing clinically relevant orthopedic tissues using biodegradable injectable systems.

14.9 Conclusions

The context of this chapter aims to discuss the most recent advances in the use of injectable biodegradable materials for bone tissue engineering. The current clinical need, design criteria, and material property requirements were illustrated followed by an overview of the latest material, cellular, and drug

delivery technologies through the use of injectable systems. Finally, the major roadblocks pertaining to the field and the future perspectives to address the current challenges were described. The ability to design injectable systems shows huge potential for the regeneration of damaged orthopedic tissues through minimally invasive procedures. While the initial studies are encouraging and many injectable materials have shown great promise, the regeneration of mechanically compliant and porous constructs with a vascular supply remains a challenge. The precisely controlled and cooperative interaction between the scaffold material, architecture, therapeutics, and cells is imperative to fully regenerate biologically functional engineered bone. The continued advancement in material chemistry and a greater understanding of cell–matrix interactions, metabolic transport, and the cellular events involved in the body's natural healing response will be significant steps in the translation of tissue engineering research into clinical reality.

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