

Chapter 1

Bioinspired Nanocomposites for Orthopedic Applications

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1. Introduction

An estimated 1.5 million individuals in the United States suffer a fracture caused by some form of bone disease annually.¹ Most adverse effects of bone diseases relate to fractures. Osteoporosis is a leading underlying cause of bone fractures which affect both males and females at all ages, although to varying degrees. Other bone disorders, such as Paget's disease, osteogenesis imperfecta, rickets, and osteomalacia also have adverse influences on bone structure, strength, and density, and subsequently lead to bone fractures.

Orthopedic prostheses are often required to repair or replace damaged bone tissue due to those diseases, injuries or genetic malformations. In 2001, about 165,000 hip joints and 326,000 knees were replaced in hospitals in the United States according to the National Center for Health Statistics.² Direct care expenditures for fractures such as surgery and therapy cost approximately 18 billion dollars per year in the United States. Indirect costs such as lost productivity for patients may add billions of dollars to this figure.¹ In the coming decades, these costs could increase in double or triple if surgical removal and revisions become necessary after implantation when an orthopedic implant fails under physiological loading conditions. A majority of those patients who receive an orthopedic implant may have to undergo several revision surgeries in their lifetime since the average longevity of current orthopedic implants is only 10 to 15 years.³ Therefore, in order to decrease patient discomfort and costs, designing the next generation of

orthopedic prostheses with improved clinical efficacy and longer effective lifetimes is a principal task of researchers in the biomaterials field.

Over the past 25 years, researchers have been interested in applying composites to satisfy a wide diversity of biomedical demands considering that living tissue are composed of composites with a number of levels of hierarchy. In almost all biological systems a range of properties is required, such as physicochemical properties, mechanical properties, and biological activity, which are all of great importance to the clinical success of biomaterials. The development of bioinspired nanocomposites offers the great promise to improve the efficacy of current orthopedic implants. Specifically, for organic/inorganic biocomposites, it is possible to obtain a wide range of mechanical and biological properties by modifying the type and distribution of inorganic phase in the organic matrix and hence to optimize the performance of the biomedical devices and their interaction with the host tissues. A wide variety of biocomposites have been synthesized and fabricated for various biomedical applications during these years. The general class of organic/inorganic nanocomposites is a fast growing area of research. Significant effort is focused on the ability to obtain control of the nano-scale structures via innovative synthetic approaches. The properties of nano-composite materials depend not only on the properties of their individual components but also on their fabrication techniques which have significant influences on the structure, morphology, distribution of phases and interfacial characteristics of nanocomposites.

For potential applications to be successful, full advantage must be taken of the comprehensive properties of biocomposites and the advanced manufacturing techniques to meet the needs of biomedical applications. This chapter systematically addresses nanocomposites applied to repair or replace damaged bone tissue in a comprehensive manner, and emphasizes on the influence of nanotechnology on fabrication of nanocomposites and their applications in tissue engineering.

This chapter focuses on three main areas. First, it introduces natural bone and widely used synthetic composites in natural bone repair. Second, the requirements of biocomposites in nano-scale structures for

tissue engineering applications are described. The third area concerns manufacturing techniques of various bioinspired nanocomposites, including examples of the design and fabrication of three-dimensional composite scaffolds for tissue engineering applications.

2. Basic Science of Bone

One approach to develop better orthopedic materials is to mimic or closely match the composition, microstructure and properties of natural bone. Bone has a varied arrangement of material structures at different length scales which work in concert to perform diverse mechanical, biological and chemical functions; such as structural support, protection and storage of healing cells, and mineral ion homeostasis.

Scale is very important in describing hierarchical architecture of bone and understanding relationship between structures at various levels of hierarchy. There are 3 levels of structures: (1) the nanostructure (a few nanometers to a few hundred nanometers), such as non-collagenous organic proteins, fibrillar collagen and embedded mineral crystals; (2) the microstructure (from 1 to 500 micrometers), such as lamellae, osteons, and Haversian systems; (3) the macrostructure, such as cancellous and cortical bone. These three levels of oriented structures assemble into the heterogeneous and anisotropic bone, as shown in Fig. 1.

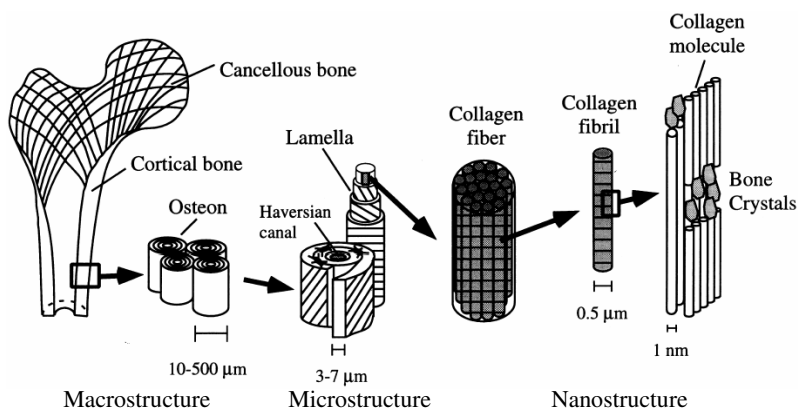


Figure 1. Schematic structure of a human femur. (Adapted and redrawn from⁴).

In this manner, it is important to first understand the nanostructured components of bone.

2.1. Bone Is a Nanostructured Composite

Natural bone is a composite material composed of organic compounds (mainly collagen) reinforced with inorganic compounds (minerals). The most prominent structures seen at nano-scale are the collagen fibers, surrounded and infiltrated by minerals. Bone builds its hierarchical architecture from these nanostructured building blocks. The detailed composition of bone differs depending on species, age, dietary history, health status and anatomical location. In general, however, the inorganic phase accounts for about 70% of the dry weight of bone and the organic matrix makes up the remainder.⁵

2.1.1. Organic Phase: Collagen Nanofibers and Noncollagenous Proteins

Approximately 90% of the organic phase of bone is Type I collagen; the remaining 10% consists of noncollagenous proteins and ground substances.

Type I Collagen found in bone is synthesized by osteoblasts (bone-forming cells) and is secreted as a triple helical procollagen into the extracellular matrix, where collagen molecules are stabilized by cross-linking of reactive aldehydes among the collagen chains. Generally, each of the 12 types of collagen found in body consists of 3 polypeptide chains composed of approximately 1,000 amino acids each. Specifically, Type I collagen (molecular weight 139,000 Daltons) possesses 2 identical $\alpha 1(I)$ chains and 1 unique $\alpha 2$ chain; this configuration produces a fairly rigid linear molecule that is 300 nm long.⁶ The linear molecules (or fibers) of Type I collagen are self-assembled in triple helix bundles having a periodicity of 67 nm, with 40 nm gaps (called hole-zones) between the ends of the molecules and pores between the sides of parallel molecules, as shown in Fig. 2. The collagen fibers provide the framework and architecture of bone while the hydroxyapatite (HA) crystals located in the fibers and between the fibers.

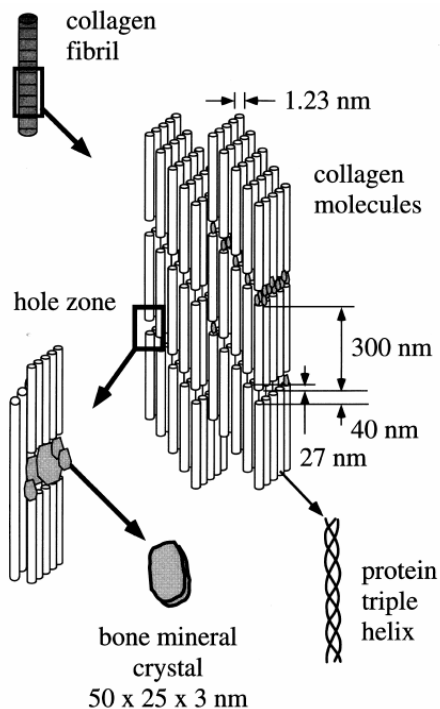


Figure 2. A schematic diagram illustrating the assembly of collagen fibers and bone mineral crystals. (Adapted and redrawn from⁴).

Noncollagenous proteins, for example, growth factors and cytokines (such as insulin-like growth factors and osteogenic proteins), bone inductive proteins (such as osteonectin, osteopontin, and osteocalcin), and extracellular matrix compounds (such as bone sialoprotein, bone proteoglycans, and other phosphoproteins as well as proteolipids) provide minor contributions to the overall weight of bone but have major contributions to its biological functions, such as regulate the size and orientation of the minerals, serve as a reservoir for calcium and phosphate ions, etc. During new bone formation, noncollagenous proteins are synthesized by osteoblasts and mineral ions (such as calcium and phosphate) are deposited into the hole-zones and pores of the collagen matrix to promote HA crystal growth. The ground substance is formed from proteins, polysaccharides and mucopolysaccharides which

acts as a cement, filling the spaces between collagen fibers and HA crystals.

2.1.2. Inorganic Phase: Hydroxyapatite Nanocrystals

The inorganic or mineral component of bone is primarily crystalline hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ or HA. Plate-like HA nanocrystals of bone locate at the discrete spaces (hole zones) within the collagen fibrils, thereby limiting the possible primary growth of the mineral crystals, and forcing the crystals to be discrete and discontinuous. The mineral crystals grow with a specific crystalline orientation, that is, the c axes of the crystals are roughly parallel to the long axes of the collagen fibrils.⁷ The average lengths and widths of the plates are 50 x 25 nm. Crystal thickness is 2-3 nm.⁴

Small amounts of impurities which affect cellular functions may be present in the mineralized HA matrix; for example, magnesium, strontium, sodium, or potassium ions may replace calcium ions, carbonate may replace phosphate groups, whereas chloride and fluoride may replace hydroxyl groups. Because the release of ions from the mineral bone matrix controls cell-mediated functions, the presence of impurities may alter certain physical properties of bone such as solubility and consequently important biological aspects which are critical to normal bone function. For example, magnesium present in the mineralized matrix may enhance cellular activity and promote growth of HA crystals and subsequent new bone formation.¹

In conclusion, bone itself is a nanostructured composite composed of nanometer sized HA well-dispersed in a mostly collagen matrix (Fig. 2). Although the inorganic and organic components of bone have structural and some regulatory functions, the principal regulators of bone metabolism are bone cells which will be discussed in section 2.4.

2.2. Microstructure and Macrostructure of Bone

At the microstructural level, bone consists of two structures: woven and lamellae. Woven bone is immature or a primitive form of bone and is normally found in the metaphyseal region of growing bone as well as in

fracture callus and diseased (such as Pagetic) bone. Woven bone is composed of relatively disoriented coarse collagen fibers and thus has isotropic characteristics. In contrast, lamellae bone is a more mature bone that results from the remodeling of woven or previously existing bone. Bone lamellae with approximate 3-7 μm in thickness is highly organized and contains stress-oriented collagen fibers which lies in parallel in each lamella and results in anisotropic properties with greatest strength parallel to the longitudinal axis of the collagen fibers. These collagen fibers change the orientation from one lamella to another, which is described figuratively as a twisted plywood or helicoidal structure.⁸ Lamellae bone is formed into concentric rings (approximately 4-20 rings) called osteons with a central blood supply called a Haversian system.

At the macrostructure level, bone is distinguished into the cortical (or compact) and cancellous (or spongy) types. In cross-section, the end of a long bone such as the femur has a dense cortical shell with a porous, cancellous interior.⁹ Flat bones such as the calvaria have a sandwich structure: dense cortical layers on the outer surfaces and a thin, reinforcing cancellous structure within. Cancellous bone is characterized by a three-dimensional sponge-like branching lattice structure with 50 to 90% porosity and large pores which are up to several millimeters in diameter. Cancellous bone, primarily found at the epiphyses and metaphyses of both long and cuboidal bones, approximates an isotropic material and mainly receives compression under physiological loading conditions. In contrast, cortical bone is characterized by less than 30% porosity and is composed of small pores up to 1 mm in diameter. Compact bone, primarily found at the diaphysis of long bones such as the femur and the tibia, is highly anisotropic with reinforcing structures along its loading axis. In general, cancellous bone is much more active metabolically, is remodeled more often than cortical bone, and is therefore “younger” on average than cortical bone.

2.3. Mechanical Properties of Bone

Cortical bone is usually more dense and, thus, mechanically stronger than cancellous bone. The relative density and some mechanical

Table 1. Relative density and mechanical properties of healthy human bone. (Adapted and redrawn from¹⁰⁻¹²).

	Cancellous Bone	Cortical Bone (Longitude)	Cortical Bone (Transverse)
Relative Density	0.05-0.7		0.7-1.8
Elongation (%)	5-7		1-3
Elastic Modulus (GPa)	0.1-0.5	17-30	7-13
Ultimate Tensile Strength (MPa)	2-20	130-150	50-60

properties of bone are shown in Table 1. These properties also change with sex, age, dietary history, health status, and anatomical locations. The anisotropic ratio for whole bone is usually between 2.1 and 2.6 in longitudinal and transversal directions.¹⁰ Diseased bone usually has lower density and weaker mechanical properties than respective healthy bone.

2.4. Bone Remodeling and Bone Cells

It is not only the complex architecture of natural bone that makes it difficult to replace, but also its dynamic ability. Bone has the ability regenerate when damaged and also to remodel when the loading conditions change, for example, the mass of bone mineral can be increased with exercise, making bones less likely to fracture.¹³ Therefore, it is important to understand how bone cells coordinate during this bone remodeling process.

Bone as a living organ can change in size, shape, position, and properties by its remodeling process throughout their lifetimes to respond to different kinds of stress produced by physical activity or mechanical loads. Therefore, bone has the capability of self-repairing under excessive mechanical stresses by activating the remodeling process through the formation of a bone-modeling unit (BMU). This process

involves three major types of bone cells: osteoblasts (bone-forming cells), osteocytes (bone-maintaining cells), and osteoclasts (bone-resorbing cells).

Figure 3 depicts how bone cells cooperate in the bone remodeling process.¹⁴ Osteoclasts are activated by growth factors, cytokines, and proteins present in the bone matrix to resorb old bone. Osteoblasts are then activated by growth factors such as insulin-like growth factors I and II secreted by osteoclasts and/or osteocytes to deposit calcium-containing minerals. Osteocytes regulate new bone formation by modulating osteoblast differentiation from non-calcium depositing to calcium depositing cells through secretion of growth factors such as insulin-like growth factor I and the tissue growth factor β .¹⁵

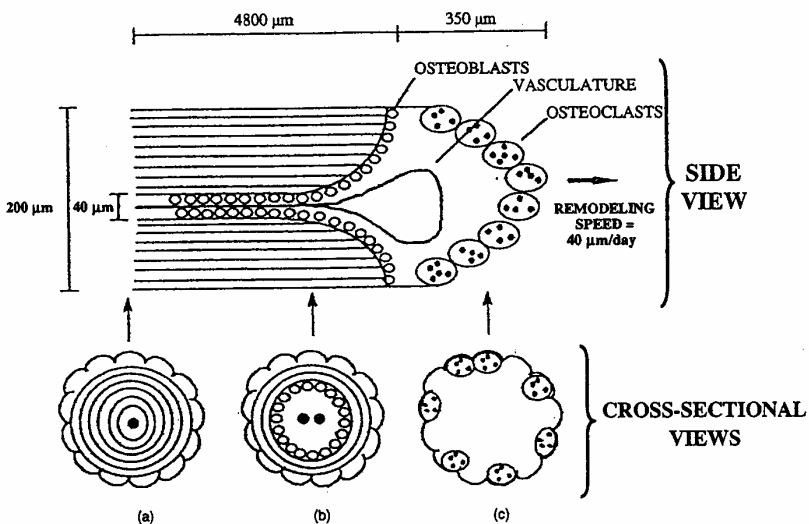


Figure 3. Schematic diagram of coordinated bone cell functions that maintain homeostasis during bone remodeling. (Adapted and redrawn from¹⁴).

2.4.1. Osteoblasts

Osteoblasts are located on the periosteal and endosteal surfaces of bone with an average diameter of 10 to 50 μm and contribute to new bone synthesis. Fig. 4 schematically describes the time course of osteoblast

proliferation and differentiation on a newly implanted biomaterial. After initial adhesion to the surface of an implant, osteoblasts actively proliferate and express genes for Type I collagen, vitronectin, and fibronectin. At the end of proliferation, the extracellular matrix development and maturation begin and osteoblasts start to differentiate from non-calcium to calcium depositing cells. Alkaline phosphatase activity and mRNA expression for proteins (such as osteopontin, and collagenase) are increased tenfold. As the mineralization process begins and mineral nodules form, osteoblasts synthesize and deposit bone sialoprotein, osteocalcin (a calcium-binding protein), and other matrix proteins. Osteocalcin interacts with HA and is thought to mediate coupling to bone resorption by osteoclasts and bone formation by osteoblasts and/or osteocytes.

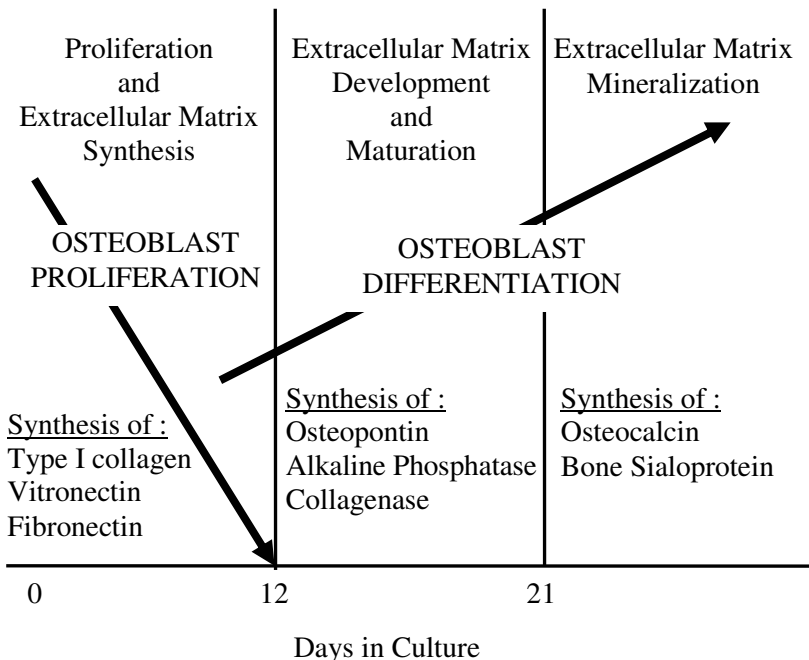


Figure 4. Time course of osteoblast functions on a newly implanted biomaterial. (Adapted and redrawn from¹⁶).

2.4.2. Osteocytes

Osteocytes are mature osteoblasts embedded in mineralized bone matrix and also contribute to new bone synthesis but to a lesser extent than osteoblasts. The principal difference between osteocytes and osteoblasts is their relative location in bone. Osteocytes are arranged concentrically around the central lumen of an osteon and in between lamellae (Fig. 1). Osteocytes possess extensive long branches with which they establish contacts and communications with adjacent osteocytes through small channels called canaliculi. Due to their three-dimensional distribution and interconnecting structure, osteocytes are believed to be sensitive to physiological stress and strain signals in bone tissue and help to mediate or balance (i) osteoblastic activity to deposit new bone and (ii) osteoclastic activity to dissolve old bone.

2.4.3. Osteoclasts

Osteoclasts are derived from pluripotent cells of bone marrow and lie in the regions of bone resorption in pits called Howship's lacunae. Osteoclasts, primarily responsible for bone resorption, are distinguished by their large size which is up to 100 μm in diameter and their multiple nuclei which could be up to 100 per cell. When osteoclasts sweep across disrupted bone surfaces to dissolve bone, they first form ruffled cell membrane edges to increase their total surface area of attachment onto the resorptive surfaces. Then, osteoclasts produce tartrate-resistant acid phosphatase (also known as TRAP) which results in the release of hydrogen ions through the carbonic anhydrase system and subsequently decreases the pH of the local environment. The lowered pH increases the solubility of HA crystals and the organic component of bone matrix are removed lastly by acidic proteolytic digestion.

Importantly, the extent of bone remodeling that occurs at an implant surface will determine the fate of the prosthetic device. For example, loosening and failure of the implant may result from either: (1) little or no remodeling in the bone surrounding an implant, which may lead to malnourished juxtaposed bone, or (2) too much remodeling in the bone surrounding an implant, which may lead to excessive bone resorption, or osteolysis.

3. Problems of Current Bone Substitutes

Traditionally, autografts, allografts, xenografts and metal implants have been used to repair fractures and other bone defects. However, these substitutes are far from ideal as each has its own specific problems and limitations.¹⁷

3.1. Autografts

Autograft is the tissue removed from one portion of the skeleton and transferred to another location in the same individual. It is commonly taken in the form of cancellous bone from the patient's iliac crest, but compact bone can be used as well.¹⁸ Historically, autografts have been the gold standard of bone replacement for many years because they provide osteogenic cells as well as essential osteoinductive factors needed for bone healing and regeneration. However, autografts are always associated with donor shortage and donor site morbidity, which severely limit its applications. The number of patients requiring a transplant far exceeds the available supply of donor tissue.¹⁹ New technology is needed to reduce this deficit.

3.2. Allografts and Xenografts

Allograft is the tissue transplanted between genetically non-identical members of the same species while a xenograft is the tissue transplanted between members of different species. Clearly, allografts and xenografts have the risk of disease transmission and immune response.^{20,21}

3.3. Metal and Metal Alloys

Due to the above stated issues with autografts, allografts, and xenografts, synthetic materials such as metals have been the material of choice for numerous orthopedic applications for a long time. However, metal and metal alloys can not perform as well as healthy bone and can not remodel or self-repair with time because they do not exhibit the physiological, dynamic and mechanical characteristics of true bone.

Table 2 highlights some physical and mechanical properties of metals which are currently used for orthopedic implants. Obviously, metals have much higher density and mechanical properties than true bone previously listed in Table 1.

Table 2. Selected physical and mechanical properties of metal alloys. (Adapted and redrawn from²²).

	Stainless Steel (316L Annealed)	CoCrMo(F75 Cast)	Ti6Al4V
Density (g/cm ³)	8	8.3	4.42
Elastic Modulus (GPa)	193	220	100
Yield Strength (MPa)	172	450	795
Ultimate Tensile Strength (MPa)	485	655	860
Elongation (%)	40	8	10

Mismatches in the mechanical properties of metallic implants and physiological bone result in “stress shielding” problems.²³ That is, the implanted material shields the healing bone from mechanical loading, resulting in necrosis of the surrounding bone and subsequent implant loosening. This condition creates clinical complications and necessitates additional surgery to remove implants and necrotic bone tissue. In addition to the “stress shielding” problems, insufficient osseointegration or lack of strongly bonded bone to the material surface may also lead to either loosening of implants or ingrowth of fibrous tissue. Both outcomes may consequently lead to clinical failure and further revision surgery.

All these clinical problems that are major obstacles to overcome emphasize a critical need for novel synthetic bone substitutes with similar structure, properties, and functions as physiological bone.

4. Bone Tissue Engineering: Promises and Challenges

Bone tissue engineering, which typically involves the assembly of bone structures by combining bone cells and scaffolds, offers a

promising opportunity for bone regeneration in a natural way. Tissue engineering is a new evolving discipline that has been described as: “the application of principles of engineering and life sciences towards a fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain, or improve tissue function”.²⁴ In tissue engineering, tissue substitutes are constructed in the laboratory by combining living cells with artificial components such as biomaterials which are subsequently introduced into a patient to create, repair or replace natural tissues and/or organs. Fig. 5 shows the bone tissue engineering concept using a hypothetical example of a femur.²⁵ Ideal scaffolds should be biodegradable and are designed as a temporary 3D mirror matrix, onto which cells grow and regenerate the needed tissues. The scaffolds will resorb after fulfilling the template functions and thus nothing foreign will be left in these patients.

Currently, the scientific challenges of bone tissue engineering are: (i) developing suitable 3D scaffolds that act as templates for cell adhesion, growth and proliferation in favored 3D orientations and (ii) understanding cell functions on these scaffolds.²⁶ The scaffolds provide the necessary support for the cells to proliferate and differentiate, and their architectures define the ultimate shapes of new bones. Over the past decade, one of the main goals of bone tissue engineering has been to develop biodegradable materials as bone substitutes for filling large bone defects. In addition, such scaffolds must allow for proper diffusion of oxygen and nutrients to cells embedded into the scaffold as well as proper diffusion of waste from the cells. The final goal is to return full biological and mechanical functionality to a damaged bone tissue.

Scaffolds, as essential components for tissue engineering strategies, must have a series of suitable properties for bone regeneration purposes. Successful design of scaffolds involves comprehensive consideration of macro and micro-structural properties of the scaffolds and their interactions with natural tissue at nano-scale range. Such properties affect not only cell survival, proliferation, signaling, growth, and differentiation but also their gene expression and the preservation of their phenotype, which eventually determines clinical healing success or failure.

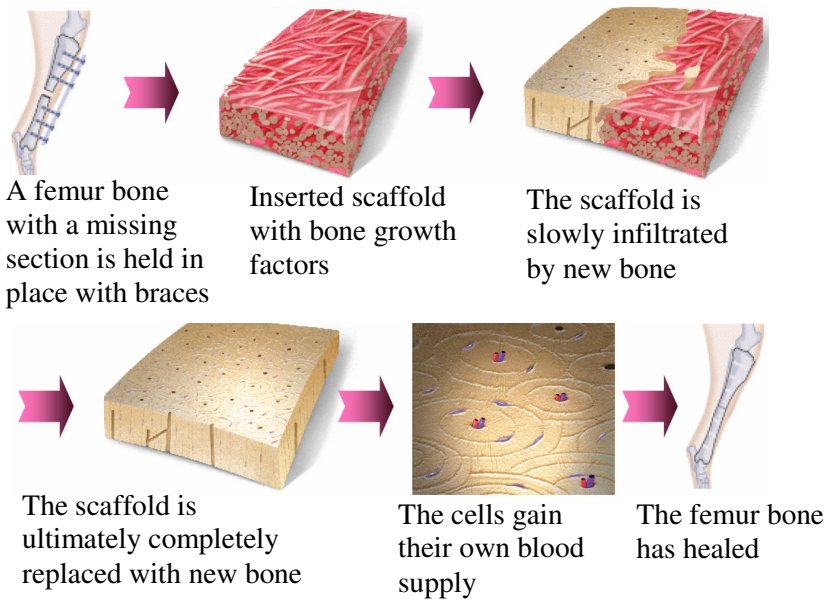


Figure 5. Schematic diagram of bone tissue engineering concept. (Adapted and redrawn from²⁵).

4.1. Essential Requirements for Bone Scaffolds

When developing a scaffold for use in orthopedics, the properties highlighted in the following sections are critical considerations.

4.1.1. Biocompatibility

The scaffolds should be biocompatible to the cells and be well integrated into the host tissue without eliciting an immune response, cytotoxicity, or formation of scar tissue.¹⁸ Factors that determine biocompatibility can be affected by scaffold or polymer synthesis and fabrication techniques. For example, residual chemicals involved in polymer processes (such as organic solvents, initiators, stabilizers, cross-linking agents, or unreacted monomers) may leach out of the scaffold once implanted. Therefore, not only the intact biomaterial, but also any leachable components and degradation products, must be biocompatible. Specifically, the release of

acidic by-products from some scaffold materials, cause tissue necrosis or inflammation due to a quick drop in local pH.²⁷

4.1.2. Biodegradability

The scaffolds should be biodegradable and bioresorbable with a controllable degradation and resorption rate to match cell/tissue growth *in vitro* and *in vivo*. The degradation rate of the scaffolds and the rate of new tissue formation must be coupled appropriately to each other in such a way that by the time the injury site is totally regenerated, the scaffold is totally degraded. The degradation rate of a scaffold can be altered by many factors such as its structure and molecular weight of the component materials. The scaffold structures (such as surface-to-volume ratio, porosity, pore size and shape) play a role in degradation kinetics, as does scaffold geometry. *In vivo*, the choice of implantation site, the amount of mechanical loading, and the rate of metabolism of degradation products also influence the degradation time of the scaffolds.

4.1.3. Mechanical Properties

The scaffolds should have adequate mechanical properties to match the intended site of implantation. *In vitro*, the scaffolds should have sufficient mechanical strength to withstand hydrostatic pressures and to maintain spacing required for cell in-growth and matrix production.²⁸ *In vivo*, because bone is always under physiological stresses (such as compression, tension, torsion, and bending), the mechanical properties of the implanted scaffolds should closely match those of living bone so that an early healing of the injured site can be made possible.

4.1.4. Surface Properties

The scaffolds should have appropriate surfaces to favor cell attachment, proliferation and differentiation. Surface properties, both chemical and topographical, can control and affect bioactivity and osteoconductivity. Chemical properties are related to the ability of proteins to initially adsorb and subsequently for cells to adhere to the material surface. Topographical properties are of particular interest when

osteoconductivity is concerned. Osteoconduction is the process by which osteogenic cells migrate to the surface of the scaffold through a fibrin clot, which is established right after the material implantation. This migration of osteogenic cells through the clot will cause retraction of the temporary fibrin matrix. Hence, it is of the utmost importance that the fibrin matrix is well secured to the scaffolds, otherwise, when osteogenic cells start to migrate, the fibrin will detach from the scaffolds due to wound contraction. As opposed to a smooth surface it has been previously shown that a more “rough” surface will be able to imprison the fibrin matrix and hence facilitate the migration of osteogenic cells to the scaffold surface.^{29,30}

4.1.5. Osteoinductivity

Osteoinduction is the process by which mesenchymal stem and pluripotent osteoprogenitor cells are recruited to a bone healing site. It is the hope that they are then stimulated to the osteogenic differentiation pathway. However, when the portion of bone that requires regeneration is large, natural osteoinduction combined with a biodegradable scaffold may be not enough. Therefore, the scaffold itself should be osteoinductive to promote bone formation. Recombinant human bone morphogenetic proteins (rhBMPs), such as rhBMP-2 and rhBMP-7, were found osteoinductive and capable of inducing new bone formation. Recent researches demonstrated that combining rhBMPs with the scaffolds could significantly increase osteoinductivity of the scaffolds and hence promote new bone growth.³¹

4.1.6. Interconnected Three-Dimensional Structures

The scaffolds should be three-dimensional and highly porous with appropriate scaled interconnected pores to favor vascularization, tissue integration, and flow transport of nutrients and metabolic waste. Pore size is a very important property because the scaffolds with large void volume and large surface-area-to-volume ratio maximize space to help cells, tissues, and blood vessels penetrate. To attain a high surface area per unit volume, however, smaller pores are preferable as long as the pore size is greater than the diameter of osteoblasts (typically 10 μm). If

the pores employed are too small, pore occlusion by the cells may happen. This will prevent cellular penetration and neovascularization of the inner areas of the scaffold. It is reported that interconnected larger pores facilitate diffusion and cell migration within the scaffolds, improving nutrient supply and waste removal, and thus increasing the viability of cells at the center of the scaffolds.²⁷ Currently, researchers are still searching for optimal pore size and shape for various tissue engineering applications. It is also crucial to control the suitable porosity of scaffolds by adjusting available fabrication techniques to match the porosity of true bone. Importantly, the porosity, pore structures, and pore size affect the mechanical and biological properties of scaffolds.

4.1.7. Feasible Fabrication Techniques and Sterilizability

The scaffolds should be fabricated reproducibly on a large scale from versatile processing techniques for a variety of shapes and sizes to match bone defects in the patients. As with all implanted materials, the scaffolds must be easily sterilizable to prevent infection. The method of sterilization, however, must not interfere with bioactivity of biomaterials or alter their chemical composition, which could influence their biocompatibility or degradation properties.

Bearing these requirements in mind, several popular materials for bone tissue engineering applications will be further discussed in the next section.

4.2. The Choices of Materials for Bone Scaffolds

The selection of the most appropriate material to produce a scaffold to be used in bone tissue engineering applications is a very important step towards the construction of a successful tissue-engineered product. As mentioned, the properties of constituent materials will determine, to a great extent, the properties of the scaffolds. So far, a wide variety of natural and synthetic biomaterials, such as polymers, ceramics, and a combination of them, have been studied for bone tissue engineering applications. Table 3 highlights some physical and mechanical properties of materials of particular interest for bone repair.

Table 3. Material properties. (Redrawn from³²⁻³⁵.)

Materials	Density (g/cm ³)	Elastic Modulus (GPa)	Ultimate Strength (MPa)
Polymers			
Polyethylene (PE)	0.91-0.96	0.88-1	30-35 (Tensile)
Poly(methyl methacrylate) (PMMA)	1.15-1.2	2.1-3.4	22-48 (Tensile) 64-103 (Compressive)
Tyrosine-derived Polycarbonate	1.2	1.2-1.5	51-67 (Tensile)
Ceramics			
Alumina	3.8-4.0	365-380	6-55 (Tensile) 1000-2700 (Compressive)
Zirconia	5.7-5.95	190-210	>300 (Tensile) 1500-2000 (Compressive)
HA	3.15-3.22	40-117	8-50 (Tensile) 100-294 (Compressive)
Composites			
Epoxy/carbon fiber	1.55-1.63	46-215	579-1240 (Tensile)
Polypropylene fumarate/ Tricalcium phosphate (PPF/TCP)	N/A	0.4-1.2	16.7-17.9 (Compressive)
Bioglass [®]	2.2-3.7	35	42-84 (Tensile)

4.2.1. Biodegradable Polymers

Bioresorbable natural and synthetic polymers have attracted increasing attention for their use as scaffold materials in the last ten years.³⁶ Many

practical advantages arise because these polymers such as PLGA (poly-lactide-co-glycolide) allow for precise control of chemical composition (e.g., the lactide/glycolide ratio in the PLGA copolymers), crystallinity, molecular weight, molecular weight distribution, as well as microstructure and macrostructure (including porosity).³⁷⁻³⁹ This allows adequate control of scaffold properties (such as degradation rate and mechanical strength), thus creating optimal conditions for cell survival, proliferation, and subsequent tissue formation. The degradation products of these polymers can be removed by natural metabolic pathways.

The most commonly used synthetic polymers are biodegradable aliphatic polyesters. Poly(glycolic acid) (PGA, also called as polyglycolide), poly(lactic acid) (PLA, also called as polylactide), and their copolymers poly(lactic-co-glycolic acid) (PLGA, also called as poly-lactide-co-glycolide), as a family of aliphatic polyesters, are some of the most popular scaffold polymers.⁴⁰⁻⁴²

PLGA was originally developed for use in resorbable surgical sutures and biodegradable drug delivery systems. These polymers (PLA, PGA, and PLGAs) are approved by the U.S. Food and Drug Administration (FDA) for certain human clinical applications. The first commercial suture, Dexon[®] (composed of poly-lactide-co-glycolide), was available in 1970 and the first FDA-cleared drug product was the Lupron Depot drug-delivery system (TAP Pharmaceutical Products Inc.; Lake Forest, IL) which was a controlled release device for the treatment of advanced prostate cancer that used biodegradable microspheres of 75/25 lactide/glycolide to administer leuprolide acetate over periods of time up to 4 months (replacing daily injections). Since then there has been intensive development of medical devices composed of PGA, PLA, and their copolymers.⁴³ The use of biodegradable polymers in orthopedic devices for fixation of fractures of long bones was first clinically implemented in Finland in 1984.^{44,45} Since the 1990s, the applications of PLA, PGA, and PLGAs in tissue engineering have been investigated extensively.⁴⁶

DL-Lactides and glycolides are polymerized via a cationic ring-opening reaction in the presence of stannous octoate as a catalyst to form a random copolymer called poly(DL-lactide-co-glycolide) or PLGA. A representative polymerization reaction is shown in Fig. 6. PLGA

gradually degrades into the endogenous natural metabolites lactic acid and glycolic acid by non-enzymatic hydrolysis of ester bonds in its backbone.^{47,48} The polymers that undergo hydrolytic cleavage tend to have more predictable degradation rates *in vivo* than polymers whose degradation is mediated predominantly by enzymes because the levels of enzymatic activity may vary widely not only among different patients but also among different tissue sites in the same patient. But, the availability of water is virtually constant in all soft/hard tissues and varies little from patient to patient. The degradation products of PGA, PLA and PLGA are nontoxic, natural metabolites, and are eventually eliminated from the body in the form of carbon dioxide and water.

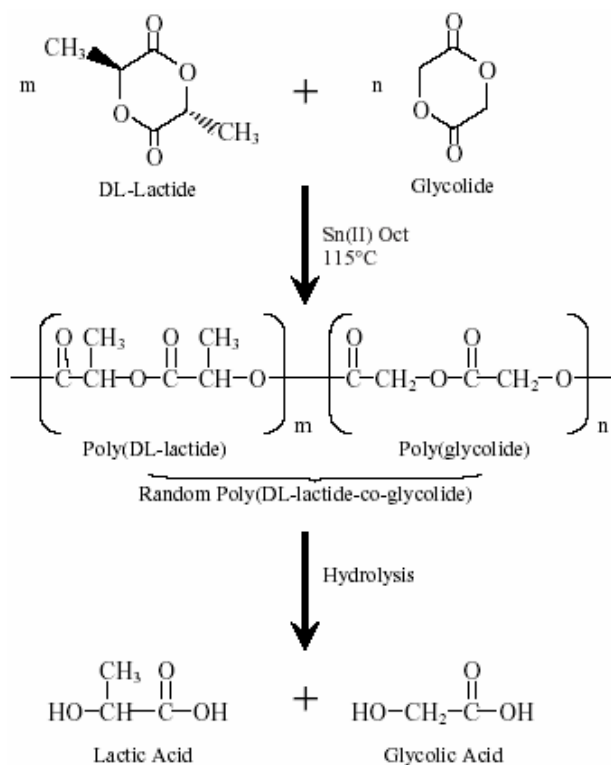


Figure 6. Synthesis of poly(DL-lactide-co-glycolide) (PLGA) and decomposition into respective acids by hydrolysis.

The PLGA degradation process has been divided into four steps that begins at the outer perimeter of the device and moves gradually into the interior, followed by catastrophic disintegration.⁴⁹ In step 1, water diffuses into the polymer and hydrolytic random chain scission of ester bonds start. In step 2, differentiation between the surface and interior begins, with a drastic decrease in molecular weight in the inner part of the matrix, where the acidic environment accelerates the degradation. In step 3, low-molecular-weight oligomers begin to diffuse through the thinning outer layer, and when the molecular weight of these oligomers is low enough to allow the solubilization in the medium, weight loss begins. In the final step 4, a polymer shell remains after the oligomers have solubilized and slow degradation of the shell takes place. Degradation of PLGA demonstrated random scission mode under normal conditions (i.e. in water or phosphate buffer medium of pH 7.4 at 37 °C), while unzipping mode (chain-end scission) under harsh conditions (such as high acidity, high temperature, or high energy radiation).⁵⁰ Clearly, this complex degradation process indicates the difficulties in controlling the release rate.

The degradation rate of these polymers, such as PGA, PLA, and PLGA, even can be tailored to satisfy the requirements from several weeks to several years by altering the ratio of polylactic to polyglycolic acid, molecular weight, molecular weight distribution, crystallinity, hydrophilicity, pH of the surrounding fluids, as well as specimen size, geometry, porosity, surface properties and sterilization methods.⁵¹ The degradation rate becomes slower as the molecular weight becomes higher. The lower the crystallinity, the higher is the chance of penetration of water molecules to initiate hydrolysis of the chains. Gamma irradiation used for sterilization at doses of 2-3 MRad can result in significant backbone degradation since aliphatic polymers are sensitive to radiation damage. These materials are usually sterilized by exposure to ethylene oxide. Unfortunately, the use of ethylene oxide gas represents a serious safety hazard as well as potentially leaving residual traces in the polymeric devices. They must be degassed for extended periods of time.

Polymer crystallinity is a measure of the alignment of polymeric chains along each other. The presence of bulky side groups, branches and freely mobile atoms (like oxygen in the backbone bonds) adversely

influences the alignment of neighboring chains and thus crystallinity. Because lactic acid has a chiral center, PLA can exist in four stereoisomeric forms, poly(L-lactic acid), poly(D-lactic acid), meso-poly(DL-lactic acid), and the racemic mixture of poly(L-lactic acid) and poly(D-lactic acid). Stereoregular poly(L-lactic acid) is semicrystalline, while the racemic poly(DL-lactic acid) is amorphous.

In the same conditions, hydrophilic PGA degrades faster in aqueous solutions or *in vivo* than the hydrophobic PLA because the adsorption of water molecules is higher into the chain of the former polymer, although the ester bonds in them have about the same chemical reactivity towards water. The extra methyl group in the PLA repeating unit (compared with PGA) makes it more hydrophobic, reduces the molecular affinity to water, and thus leading to a slower hydrolysis rate. Therefore, it seems that the higher the glycolic acid content, the faster the degradation rate. However, the lifetime of PLGA becomes shortest at PLA/PGA ratio of 50/50, because the more crystalline domains of PGA form as the amount of glycolic acid in the copolymer increases.⁵² In the crystalline state, the polymer chains are densely packed and organized to resist the penetration of water. Consequently, backbone hydrolysis tends to only occur at the surface of the crystalline regions, which takes a much longer time than hydrolysis in amorphous polymer or in the amorphous regions of a semicrystalline polymer.

The mechanical properties of biodegradable polymers depend on its chemical structure, crystallinity, molecular weight, or molecular orientation. Table 4 highlights the mechanical properties of selected biodegradable polymers.^{9,12,53,54}

Clearly, degradation leads to a loss of mechanical properties and an increase in crystallinity as a result of content loss. PGA loses mechanical integrity between two and four weeks while PLA takes many months or even years to lose mechanical integrity *in vitro* or *in vivo*.^{55,56} The amorphous regions of semicrystalline polymers are subjected to degradation earlier than the crystalline regions, leading to an increase in crystallinity. Heterogeneity index (HI, M_w/M_n), an indicator of molecular weight distribution, increases upon PLGA degradation, indicating a faster decrease in M_n (number average molecular weight) in comparison to a decrease in M_w (weight average molecular weight).

Table 4. Mechanical properties of selected biodegradable polymers.

Polymers	Elastic Modulus (GPa)	Tensile Strength (MPa)	Ultimate Elongation (%)
PGA(polyglycolide)	>6.9	>68.9	15-20
PLLA(semicrystalline)	2.4-4.2	55.2-82.7	5-10
PDLLA(amorphous)	1.4-2.8	27.6-41.4	3-10
PLGA	1.4-2.08	41.4-55.2	3-10
PCL(poly(ϵ -caprolactone))	0.21-0.34	20.7-34.5	300-500

There are other aliphatic polyesters, such as poly(ϵ -caprolactone) (PCL), which is also used in bone tissue engineering applications.⁵⁷ PCL degrades at a significantly slower rate than PLA, PGA, and PLGA⁵⁸⁻⁶⁰. A slow degradation rate makes PCL less attractive for general tissue engineering applications, but more attractive for long-term implants and controlled drug release applications. PCL-based copolymers have recently been synthesized to improve degradation properties.⁶¹ Poly(propylene fumarate) (PPF) is also an important synthetic biodegradable polymer and can degrade through hydrolysis of the ester bonds similar to glycolide and lactide polymers.⁶² The mechanical properties of PPF can vary greatly according to the synthesis method and the cross-linking agents used.⁶³

Naturally derived polymers, such as collagen, have also been used for scaffold fabrication.⁶⁴⁻⁶⁶ Collagen is a fibrous protein and a major natural extracellular matrix component. On one hand, collagen (as the most popular natural polymer for tissue regeneration by far) has very attractive biological properties (such as biocompatibility) desirable for bone tissue engineering applications; on the other hand, there are concerns over collagen because of poor handling and poor mechanical properties to support bone loading requirements. Denatured collagen (gelatin) has also been processed into porous materials for bone tissue repair.⁶⁷⁻⁶⁹ To increase the strength of these natural materials, they are often combined with ceramics.⁷⁰

4.2.2. Bioactive Ceramics

The main advantage of using ceramics lies in their high cytocompatibility with bone cells. For bone tissue engineering, alumina, zirconia, titania, and calcium phosphates (such as calcium tetrachlorophosphate ($\text{Ca}_4\text{P}_2\text{O}_9$), tricalcium phosphate (TCP, $\text{Ca}_3(\text{PO}_4)_2$), hydroxyapatite (HA, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) and its derivatives, as well as their combinations) are the most common types of bioceramics that have been used to fabricate scaffolds for bone tissue regeneration.^{71,72} These ceramics are widely considered to be osteoconductive because their surface properties support osteoblast adhesion, growth, and differentiation and are also reported to be osteoinductive as a result of their capacity to bind and concentrate bone morphogenetic proteins (BMPs) *in vivo*.⁷³ Moreover, selected ceramics, such as HA and TCP, due to their chemical and structural similarity to the mineral phase of native bone, can react with physiological fluids and form tenacious bonds to hard and soft tissues through cellular activity, thus classifying them as “bioactive”.^{74,75} In addition, the dissolution rate of HA depends on its crystallinity, and therefore, it can be controlled to be compatible with the rate of new bone growth. The dissolution of HA crystals has been observed to be much slower than amorphous HA.⁷⁶

Due to their excellent bioactivity, these ceramics are often used as coating materials to modify surfaces of traditional metal implants for the purpose of improved bonding to juxtaposed bone (i.e. osseointegration). Various techniques, such as sputtering, electron beam deposition, and plasma spraying, have been used to deposit calcium phosphate and HA on metal substrates. So far, plasma spraying has been most widely used and commercialized because of its simplicity and cost effectiveness. Regardless of the coating technique, the amorphous HA coating generally has a high dissolution rate in aqueous solution than crystalline HA coating. Although the biocompatibility of metal implants can be improved using bioactive ceramic coating, the intrinsic problems of metallic implants (such as much higher stiffness than bone) still can not be solved by these coating methods.

The clinical applications of these bioactive ceramics in large bone defects repair have been limited because of their intrinsic brittleness,

difficulty in deforming and shaping, and poor mechanical properties preventing them sustain the mechanical loading needed for bone remodeling.

4.2.3. Ceramic/Polymer Biocomposites

The design of ceramic/polymer composites offers an exceptional approach to combine the advantages of bioactive ceramics and biodegradable polymers to optimize physical, mechanical, and biological properties of scaffolds for bone regeneration. In the past few years, the development of ceramic/polymer composites as scaffold materials for bone tissue engineering has attracted more and more attention.^{38,77,78}

First, in ceramic/polymer composites, osteoblast functions can be enhanced from better cell seeding and growth environments due to improved osteoconductivity properties provided by the bioactive ceramic phase.⁷⁹⁻⁸³ For example, Ma et al. prepared highly porous PLA/HA composite scaffolds with a thermal-induced phase separation technique and demonstrated that osteoblast survival percentages and proliferation rates in the PLA/HA scaffolds were higher than in the pure PLA scaffolds.⁸³

Second, ceramic particles (such as Bioglass[®], HA and TCP) used as inclusions in biodegradable polyesters can provide a pH buffering effect at the polymer surface and tailor the desired degradation and resorption kinetics of the polymer matrix; thus, preventing acceleration of polymer degradation, avoiding the formation of an unfavorable environment for the cells, and reducing side-effects (such as inflammation) from acidic degradation by-products.³⁸

Third, the stiffer particulate ceramic phase in polymer composites is important for improving scaffold mechanical properties.⁸⁴⁻⁸⁷ The addition of biodegradable polymers such as PLA, PGA, and PLGA to calcium phosphate ceramics would allow for better manipulation and control over both the macro- and microstructure in shaping composites to fit bone defects. Furthermore, biodegradable polymers can be used as binders for HA or TCP to reduce the brittleness of the ceramics.^{88,89} For example, Thomson et al. demonstrated that the compressive yield strength increased from 0.95 ± 0.11 MPa for PLGA foams to 2.82 ± 0.63 MPa for

foams with PLGA/HA fiber weight ratios of 7/6.⁷⁸ Moreover, Marra et al. reported that the Young's modulus increased from 2.5 ± 0.7 MPa to 12.5 ± 3.2 MPa when 10 wt. % HA was incorporated into a PCL/PLGA blend with a weight ratio of 10/90.⁸⁰ Wei et al. have also demonstrated that the compressive modulus of HA/PLA scaffolds increased with HA content.¹⁷ Specifically, the modulus increased from 4.3 MPa for the plain PLA scaffolds to 8.3 MPa when the weight ratio of HA to PLA was 50/50.¹⁷

These ceramic/polymer biocomposites demonstrated many promising advantages over traditional single phase materials for orthopedic applications; however, they are still not ideal because they can not mimic many aspects of natural bone, especially nano-scale features of bone.

5. Nanocomposites: Next-Generation Materials in Orthopedics

5.1. Rationale and Evidence

As mentioned, natural bone is a nanostructured composite composed of a polymer matrix (mainly collagen) reinforced with nanometer-sized ceramic particles (mainly carbonated HA). Recent researches in bone regeneration suggested that better osteoconductivity would be achieved if synthetic materials were fabricated to resemble bone in terms of its nano-scale features.^{90,91} For example, Du et al. synthesized nano-HA/collagen composites with a porous microstructure similar to bone and these materials promoted the deposition of a new bone matrix. Furthermore, they showed that osteoblasts within this biologically-inspired composite eventually acquired a three-dimensional polygonal shape that integrated with juxtaposed bone fragments.^{90,91}

Moreover, nano-sized HA in bone has other special properties due to its small size and huge specific surface area. Specifically, Webster et al. reported significant increase in initial protein adsorption and subsequent osteoblast adhesion on the nano-sized ceramic materials compared to respective traditional micron-sized ceramic materials (such as HA, titania, and alumina).⁹²⁻⁹⁴ Scaffold materials with surface properties

similar to physiological bone (characterized by surface grain sizes in the nanometer regime) would aid in the formation of new bone at the tissue/biomaterial interface.⁹⁵ Therefore, it is clear that one approach for the design of next generation scaffold materials should incorporate nano-dimensional structures in an effort to mimic natural bone.

Orthopedic implants with surface properties that promote cell and tissue interactions that lead to implant osseointegration are needed. Surface properties such as area, charge and topography depend on the grain size of a material; in this respect, nanophase materials, which, by their very nature, possess higher surface area with increased portions of surface defects such as edge-corner sites and grain boundaries, have an advantage that currently remains largely unexplored for biomedical applications. Surface roughness determined by grain size, crystallinity, and microporosity influence interactions (such as adsorption and/or configuration or bioactivity) of select proteins and subsequent cell adhesion.^{92,96,97} Liu et al reported that nanophase titania/PLGA composites which had closest surface roughness to natural bone demonstrated greatest osteoblast adhesion and subsequent calcium-containing mineral deposition.⁹⁸ Wei et al. demonstrated greater initial protein adsorption important for osteoblast adhesion on nano-HA/PLA porous scaffolds than on respective micro-HA/PLA scaffolds.¹⁷

Few studies have addressed the mechanisms of enhanced osteoblast activity on nanophase materials. One set of *in vitro* studies pinpoints grain size in the nanometer regime as the major parameter for enhancing ceramic cytocompatibility. For example, compared to respective conventional, larger grain size, ceramic formulations, enhanced adhesion of osteoblasts and decreased adhesion of fibroblasts (cells that contribute to fibrous encapsulation and callus formation events that may lead to implant loosening and failure) have been observed on nanophase alumina, titania, and HA.⁹² In fact, decreasing alumina grain size from 167 to 24 nm increased osteoblast adhesion 51% and at the same time decreased fibroblast adhesion 235% after 4 hours.⁹³

Investigations of the underlying mechanisms revealed that the concentration, conformation, and bioactivity of vitronectin (a protein contained in serum that is known to mediate osteoblast adhesion) was responsible for the select, enhanced adhesion of osteoblasts (a crucial

prerequisite for subsequent, anchorage-dependent-cell functions) on these novel nanophase ceramic formulations. Vitronectin is a linear protein 15 nm in length and preferentially adsorbed to the small pores present in nanophase ceramics, such as 0.98 nm pores for nanophase titania compacts. For example, adsorption of vitronectin was 10% greater on nanophase compared to conventional alumina.⁹⁹ Furthermore, protein conformation plays a critical role in mediating subsequent cell interactions. Increased unfolding of vitronectin adsorbed on nanophase ceramics compared to conventional ceramics was observed.⁹⁹ Vitronectin unfolding promoted the availability of specific cell-adhesive epitopes (such as the RGD sequence) for subsequent enhanced osteoblast adhesion; evidence supporting this claim was provided by competitive inhibition studies.⁹⁹

Importantly, nanophase biocomposites may be synthesized to possess hardness, bending, compressive and tensile strengths that are significantly different than conventional materials but more similar to those of physiological bone. Indeed, greater mechanical properties have been reported for biocomposites with a reduction in ceramic grain size to nanometer range. McManus et al. reported that the bending moduli of composites of PLA with 40 and 50 wt. % nanophase (< 100 nm) alumina, titania and HA were significantly greater than respective composite formulations with conventional coarser grained ceramics. Specifically, compared to a bending modulus of 60 ± 3 MPa for plain PLA and 870 ± 30 MPa for conventional titania/PLA composites with the weight ratio of 50/50, the bending modulus of nanophase titania/PLA composites with the weight ratio of 50/50 was 1960 ± 250 MPa, which were on the same order of magnitude of healthy trabecular bone.²³

Mechanical deformation theory indicates that high-volume fraction of interfacial regions compared to bulk materials leads to increased deformation by grain-boundary sliding and short-range diffusion-healing events as grain size is reduced, and thus increased ductility in nanocrystalline ceramics. Compared to conventional ceramics, nanophase ceramics possess increased surface roughness resulting from both decreased grain size and decreased diameter of surface pores. Moreover, nanophase ceramics possess enhanced surface wettability due

to greater surface roughness and greater numbers of grain boundaries on their surfaces.

Nanostructured biocomposites provide alternatives not yet fully explored for orthopedic applications. They may be fabricated to possess similar microarchitecture as that of healthy, physiological bone. Their improved mechanical properties and biocompatibility promise improved orthopedic efficacy in the future.

5.2. Fabrication Techniques of Biocomposite Scaffolds

The commonly accepted concept defines nanomaterials as those materials with basic structural units in the range of 1-100 nm (nanostructured). Nanomaterials exhibit enhanced magnetic, catalytic, optical, electrical, and mechanical properties when compared to conventional formulations of the same material.¹⁰⁰⁻¹⁰² However, to date, relative few advantages of nanocomposites have been taken for orthopedic applications due to the limitation of available fabrication techniques. Therefore, it is important to review the promise of fabrication techniques used for making nanostructured bone substitutes or scaffolds.

Biocomposites can be fabricated with different technologies. The selection of the most appropriate manufacturing technology is also influenced by the relatively low volumes of the production and relatively low dominance of the manufacturing cost over the overall cost of the device. In the body, tissues are organized into three-dimensional structures as functional organs and organ systems. To engineer functional tissues and organs successfully, the scaffolds have to be designed to facilitate cell distribution and guide tissue regeneration in three dimensions.

5.2.1. Solvent-Casting/Particulate-Leaching

Solvent-casting/particulate-leaching (SC/PL) technique has been widely used to fabricate 3D porous polymer scaffolds for tissue engineering applications. Salt (Sodium Chloride) is the most commonly used particulate (also called porogen) because it is easily available and very easy to handle. Briefly, this technique involves producing a suspension

of polymer composites in a solvent. Salt particles are ground and sieved into small particles and those of the desired size (most researchers used 100-200 μm range particles) are transferred into a mold. A polymer suspension is then cast into the salt-filled mold. The solvent is then removed by evaporation in air and/or in vacuum. After the evaporation of the solvent, the salt crystals are leached away by immersion in water to form a porous structure, as schematically shown in Fig. 7. In this technique, the pore size can be controlled by the size of the porogen particles and the porosity can be controlled by the salt/polymer composite ratio.

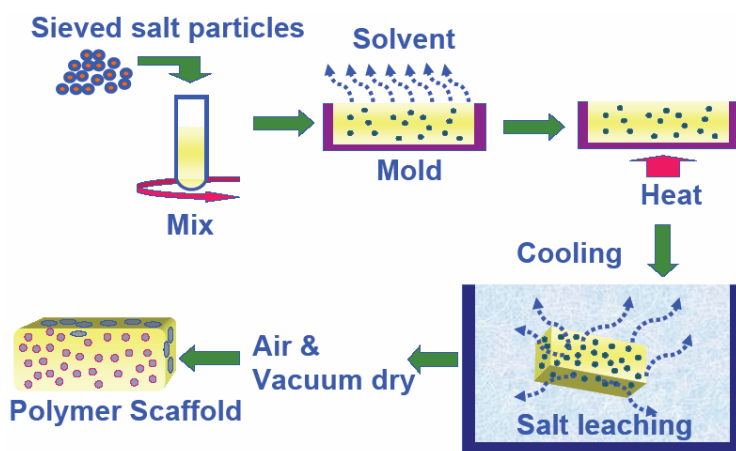


Figure 7. The schematic diagram of solvent-casting particulate-leaching techniques.

Other porogens, such as gelatin, were also studied to replace salt particles. Suh et al. compared the biocompatibility of the two PLGA scaffolds made from either salt porogen (salt scaffold) or gelatin particles (gelatin scaffold) by this technique. Their results demonstrated that the gelatin scaffold showed better attachment of chondrocytes (from knee cartilage) and smooth muscle cells (from bladder) at the initial stage, and both cell types showed much better proliferation of cells during 3 months. Suh et al. believed that the better performance of a gelatin scaffold also contributed to the better connection of pores at the same porosity.¹⁰³

Using waxy hydrocarbons as porogens was also reported in the fabrication of PLA and PLGA scaffolds with up to 87% porosity and pores over 100 μm in diameter.¹⁰⁴ After mixing the waxy hydrocarbon and polymer (dissolved in chloroform) into a paste, the composite was cast into a desired mold. The mold was then immersed in a hydrocarbon solvent (pentane or hexane) to remove the wax without dissolving the PLA or PLGA. The remaining foam was vacuum dried for several days to extract any solvents. Thick samples (up to 2.5 cm) with interconnected pores can be created using this technique. This method also offers the possibility of adding a second phase to the paste to create a composite which could increase the strength or electrical conductivity of the final scaffolds.

Solvent-casting/particulate-leaching technique is easy to carry out in the laboratory and flexible to be combined with other fabrication techniques due to its simplicity and low cost. For example, Wu et al reported a method which combined modified compression molding and conventional particulate-leaching to fabricate complexly shaped 3D porous scaffolds.¹⁰⁵ Briefly, a polymer-particulate mixture was first prepared by the conventional solvent casting method and then compressively molded in a specially designed flexible-rigid combined mold which facilitates shaping and mold release during the fabrication process. The molding was carried out at a moderate temperature, above the glass transition temperature and below the flow temperature of these amorphous polymers. A porous scaffold was then obtained after particulate leaching. Highly interconnected and uniformly distributed pores both in the bulk and on the external surface of the PLA and PLGA auricle-shaped scaffolds were observed, and the porosity could exceed 90%.¹⁰⁵

However, solvent-casting/particulate-leaching technique has four main disadvantages. First, this technique usually involves organic solvents which are not favorable for tissue engineering applications due to potential harmful influences on cells and tissues. Organic solvents also in many cases preclude the possibility of adding pharmacological agents to the scaffold during the fabrication. Secondly, certain critical variables such as pore shape and inter-pore openings are still not well controlled in

this technique. Third, when applying this technique to polymer/ceramic composite scaffolds, the polymer-organic solvent solutions may coat the bioactive ceramic surfaces, hinder their exposure to the scaffold surface, and decrease their direct contact with osteogenic cells which are crucial for osseointegration. The last but not the least, if nanophase ceramic particles were used to make nanocomposite scaffolds in this technique, nanoparticles may interfere the porogen leaching process, which will result in residual porogen particles in the final tissue engineering products, and thus, having adverse effects on their biocompatibility.

5.2.2. Gas-Foaming/Particulate-Leaching

Due to the problems associated with the solvent-casting particulate-leaching technique, gas-foaming/particulate-leaching (GF/PL) was proposed by Mooney et al. without the use of organic solvent, as shown in Fig. 8.¹⁰⁶ Disks comprised of polymer (e.g., PLGA) and salt (NaCl) particles were compression molded at room temperature and subsequently allowed to saturate with high pressure carbon dioxide (CO₂) gas (800 psi). The solubility of the gas in the polymer was then decreased rapidly by bringing the CO₂ pressure back to atmospheric level, which created a thermodynamic instability leading to the nucleation and growth of gas pores in the polymer particles, resulting in the expansion of the polymer particles. The polymer particles fused to form a continuous matrix with entrapped salt particles. The NaCl particles subsequently were leached away in water to yield macropores within the polymer matrix. The overall porosity and level of pore connectivity were regulated by the ratio of polymer/salt particles and the size of salt particles. Both the compressive modulus (289 ± 25 kPa versus 159 ± 130 kPa) and the tensile modulus (1100 ± 236 kPa versus 334 ± 52 kPa) of the scaffolds formed with this approach were significantly greater than those formed with a standard solvent casting/particulate leaching process.

Gas-foaming/particulate-leaching technique could be directly applied to fabricate nanophase ceramic/polymer composite scaffolds without major modifications. For example, Kim et al fabricated nano-HA/PLGA composite scaffolds for bone tissue engineering using this technique.¹⁰⁷

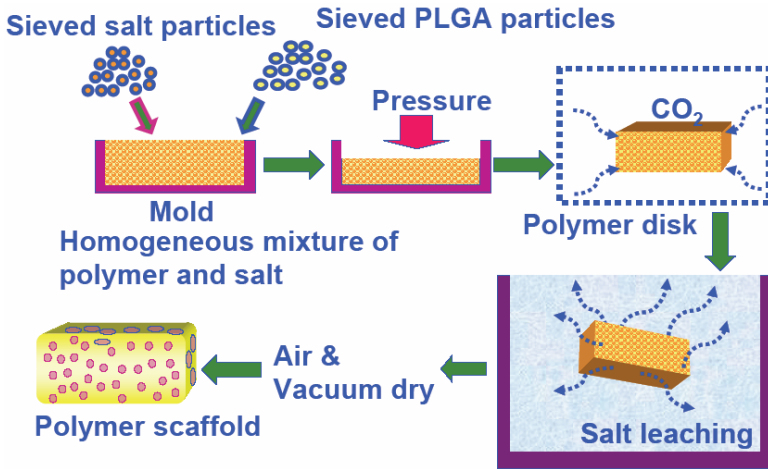


Figure 8. The schematic diagram of the gas-foaming/particulate-leaching technique.

HA particles with approximately 100 nm in size rather than micro-sized particles, were used to fabricate the composite scaffolds to increase the HA exposure to the scaffold surface without increasing the amount of HA. The GF/PL method exposed HA nanoparticles at the scaffold surface significantly more than the conventional SC/PL method does. The GF/PL scaffolds showed interconnected porous structures without a skin layer and exhibited superior enhanced mechanical properties to those of scaffolds fabricated by the SC/PL method. Both types of scaffolds were seeded with rat calvarial osteoblasts and cultured *in vitro* or were subcutaneously implanted into athymic mice for eight weeks. The GF/PL scaffolds exhibited significantly higher cell growth, alkaline phosphatase activity, and mineralization compared to the SC/PL scaffolds *in vitro*. Histological analyses and calcium content quantification of the regenerated tissues five and eight weeks after implantation showed that bone formation was more extensive on the GF/PL scaffolds than on the SC/PL scaffolds. Compared to the SC/PL scaffolds, the enhanced bone formation on the GF/PL scaffolds may have resulted from the higher exposure of HA nanoparticles at the scaffold surface, which allowed for direct contact with the transplanted cells and stimulated the cell proliferation and osteogenic differentiation.

However, the both SC/PL and GF/PL techniques have limited range of pore sizes (i.e., 100-500 μm) in the composite scaffolds, as shown in Fig. 9.¹⁰⁷

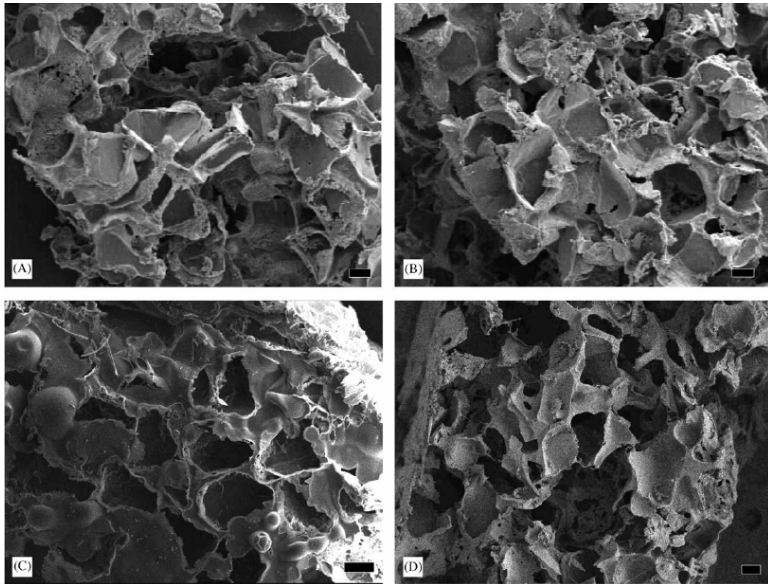


Figure 9. Scanning electron micrographs of (A,C) surfaces and (B,D) cross-sections of the nano-HA/PLGA composite scaffolds fabricated by (A,B) the SC/PL method and (C,D) the GF/PL method. The scale bars indicate 100 μm .

Modified gas-foaming technique using gas foaming salt such as ammonium bicarbonate instead of CO_2 was reported by Nam et al., as shown in Fig. 10.¹⁰⁸ Ammonium bicarbonate was added to a polymer solution to obtain a highly viscous mixture, which could be shaped by hand or using a mold. The solvent was then evaporated and the composite was either vacuum dried or immersed in warm water. Vacuum drying caused the ammonium bicarbonate to sublime while immersion in water resulted in concurrent gas evolution and particle leaching. The latter method was preferred because it did not result in the creation of a nonporous outer skin, as seen in the vacuum-dried scaffolds. Porosities as high as 90% with pore sizes from 200-500 μm were attained using this technique.

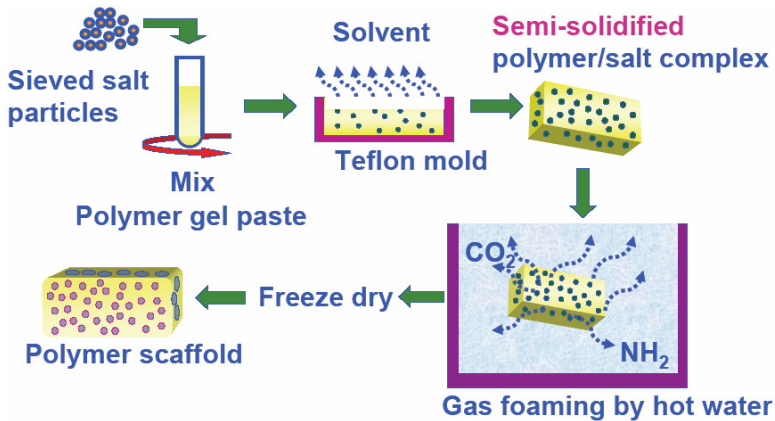


Figure 10. The schematic diagram of gas foaming using ammonium bicarbonate.

5.2.3. Phase Separation and Emulsion Freeze Drying

A homogeneous multi-component system, under certain conditions, becomes thermodynamically unstable and tends to separate into more than one phase in order to lower the system free energy, which is called phase separation. Based on this principle, solid-liquid phase separation (Fig. 11) and liquid-liquid phase separation (Fig. 12) were developed for the fabrication of 3D porous scaffolds.

Solid-liquid phase separation, also called emulsion freeze drying, could be achieved by lowering the temperature to induce solvent crystallization from a polymer suspension (solid phase formation in a liquid phase). After the removal of the solvent crystals (sublimation or solvent exchange), the space originally taken by the solvent crystals becomes pores.¹⁰⁹ As an example, PLGA was first dissolved in chloroform and then distilled water was added. The polymer, organic solvent, and water were mixed by a stirrer to form an emulsion and the emulsion was then cast into a mold and quenched by placing in liquid nitrogen to solidify the mixture and induce solid-liquid phase separation. After quenching, the scaffolds were lyophilized (freeze-dried) at -55°C , resulting in the removal of the dispersed water and polymer solvents. The scaffolds made by this technique had large porosities (91-95%), and small median pore size (13-35 μm) with large pore size distribution

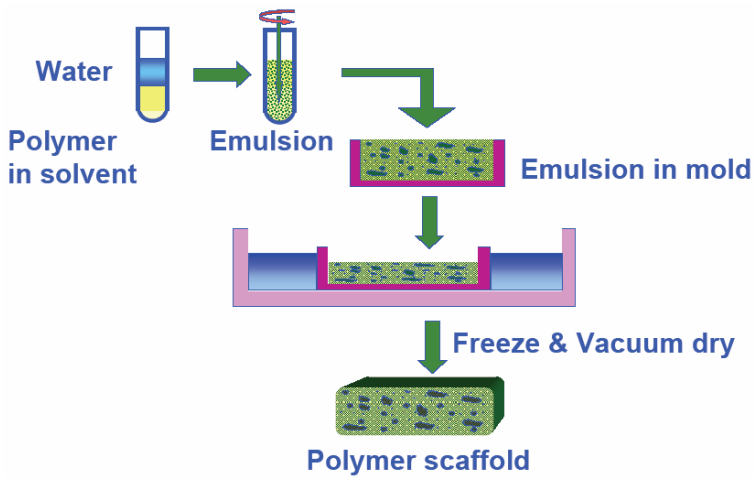


Figure 11. The schematic diagram of solid-liquid phase separation.

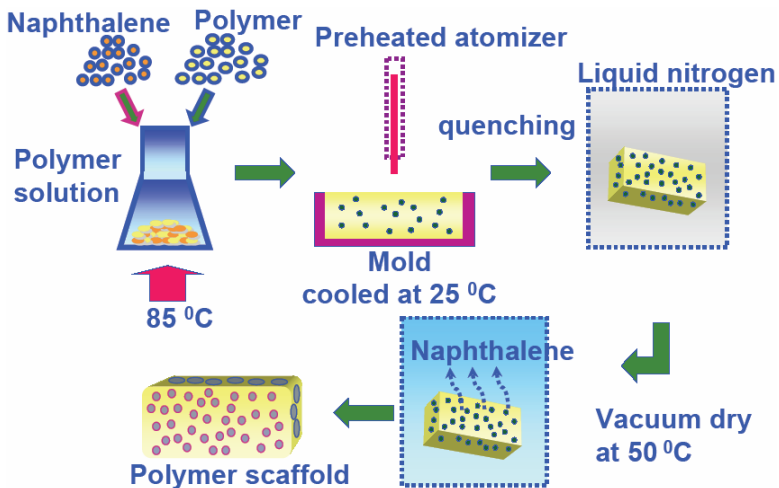


Figure 12. The schematic diagram of liquid-liquid phase separation.

(larger pore diameters greater than 200 μm).¹⁰⁹ Various pore structures can be achieved by varying processing parameters such as water volume fraction in polymer solution, polymer weight percentage, polymer molecular weight and viscosity of the emulsion.

Furthermore, Liu et al. prepared collagen/HA composite scaffolds by solid-liquid phase separation technique.¹¹⁰ HA powder was added into collagen solution, and homogenized by a speed stirrer. The mixture was then poured onto petri dishes, and rapidly transferred into a refrigerator at -30°C to solidify the mixture and induce solid-liquid phase separation. The solidified mixture was maintained at that temperature for 2 h, and then lyophilized for 2 days. Collagen/HA scaffolds were porous with three-dimension interconnected fiber microstructure, pore size were 50-150 μm .

Liquid-liquid phase separation employs thermodynamic principles to create polymer-rich and polymer-poor phases within a polymer solution. The polymer poor phase was then removed, leaving a highly porous polymer network. Both PLA and PLGA scaffolds have been formulated using this technique.¹¹¹⁻¹¹³ The polymers were dissolved in a solvent with a low melting point and that was easy to sublime, such as naphthalene, phenol or 1,4 dioxane. In some cases, small amounts of water were added as a non-solvent to induce phase separation.^{113, 114} The polymer solution was cooled below the melting point of the solvent (polymer poor phase) and then vacuum dried for several days to insure complete solvent sublimation. The cooling parameters for the solution play an important role in determining the morphology of the resultant scaffold. At the temperature just below the critical temperature (or cloud point in the case of polydisperse polymers), the phase separation occurred via a nucleation and growth mechanism. The separation occurred via spinodal decomposition. While the nucleation and growth mechanism results in spheroidal domains, spinodal decomposition causes the formation of interconnected cylinders. Annealing can cause enlargement of domains formed by either mechanism.

Based on these thermodynamic principles, spinodal decomposition is preferred as it increases the number of interconnected pores within the network.¹¹³⁻¹¹⁴ In addition, it has been found that annealing is important to increase pore size. Various parameters that influence the phase diagram of the system, and thus the resulting pore morphology, are polymer concentration, cooling method and time, solvent/nonsolvent ratio, and the presence of surfactants, which can reduce the interfacial

tension between phases and increase pore size and interconnectivity. Various parameters can be changed to tailor pore size and porosity for specific applications is appealing.

This technique can be used to fabricate scaffolds from many types of polymers and polymeric composite materials. However, although this technique is advantageous as it does not require an extra washing/leaching step, the use of organic solvents remains a concern for the inclusion of cells and bioactive molecules. Moreover, the phase diagrams of the polymer-solvent systems must be better characterized before this flexibility can be fully exploited for use in tissue-engineered constructs. The pores formed using phase separation techniques usually have diameters on the order of a few to tens of microns and are often not uniformly distributed.

This indicates that this fabrication method currently has limited usefulness in the field of tissue engineering. Controlled phase separation processes, primarily thermally induced phase separation, have also been explored for scaffold fabrication.¹¹⁴

5.2.4. Fiber Meshes/Fiber Bonding

Fibers, produced by textile technology, have been used to make non-woven scaffolds from PGA and PLLA. The lack of structural stability of these nonwoven scaffolds, often resulted in significant deformation due to contractile forces of the cells that have been seeded on the scaffold. This led to the development of a fiber bonding technique to increase the mechanical properties of the scaffolds. This is achieved by dissolving PLLA in methylene chloride and casting over the PGA mesh. The solvent is allowed to evaporate and the construct is then heated above the melting point of PGA. Once the PGA-PLLA construct has cooled, the PLLA is removed by dissolving in methylene chloride again. This treatment results in a mesh of PGA fibers joined at the cross-points.

5.2.5. Melt Molding

This process involves filling a Teflon mould with PLGA powder and gelatine microspheres, of specific diameter, and then heating the mould

above the glass-transition temperature of PLGA while applying pressure to the mixture. This treatment causes the PLGA particles to bond together. Once the mould is removed, the gelatin component is leached out by immersing in water and the scaffold is then dried. Scaffolds produced this way assume the shape of the mould. The melt molding process was modified to incorporate short fibers of HA. A uniform distribution of HA fibers throughout the PLGA scaffold could only be accomplished by using a solvent-casting technique to prepare a composite material of HA fibres, PLGA matrix and gelatine or salt porogen, which was then used in the melt moulding process.

5.2.6. Freeze Drying and Cross-linking

Kim et al. reported that hydroxyapatite (HA) and gelatin composites were fabricated in a foam type via a novel freeze-drying and cross-linking technique. The HA powder was added at up to 30 wt% into the gelatin solution, and the mixtures were freeze-dried and further crosslinked. The pure gelatin foam had a well-developed pore configuration with porosity and pore size of ~90% and 400-500 μm , respectively. With HA addition, the porosity decreased and pore shape became more irregular. The HA particulates, in sizes of about 2-5 μm , were distributed within the gelatin network homogeneously and made the framework surface rougher. All the foams had high water absorption capacities, showing typical hydrogel characteristics, even though the HA addition decreased the degree of water absorption. The HA addition made the foam much stronger and stiffer, i.e., with increasing HA amount the foams sustained higher compressive stress and had higher elastic modulus in both dry and wet states.

5.2.7. Rapid Prototyping Techniques

One of the common shortcomings of the fabrication technologies discussed above is the lack of precise control of the three-dimensional pore architecture of the scaffolds. To tackle this problem, solid free form fabrication (also known as rapid prototyping) techniques are being adopted. The main advantage of these techniques is their ability to

produce complex-shaped objects rapidly from a computer-aided design (CAD) model. One of these rapid prototyping techniques, called 3D printing, was first developed at the Massachusetts Institute of Technology and has been used to process biodegradable polymer scaffolds for tissue engineering applications. This process generates components by ink-jet printing a binder on to sequential powder layers. The operation parameters such as the speed, flow rate, and drop position can be computer controlled to produce complex 3D polymer scaffolds. Biological agents, such as growth factors, can also be incorporated into the scaffolds in the printing process. However, rapid prototyping techniques have inherent shortcomings such as limited material selection and inadequate resolution. The resolution is determined by the size of binder drops, the jet and powder particles, which makes it difficult to design and fabricate scaffolds with fine microstructures. The porosity of the scaffold fabricated with this method is low, and the mechanical properties of the scaffolds have to be significantly improved. Similarly, other rapid prototyping techniques, such as fused deposition modeling (FDM) and stereolithography have also been explored for composite fabrication.

Three-dimensional ink-writing techniques can be divided into two approaches: (1) droplet-based or (2) continuous (filamentary) inks. Three-dimensional periodic structures offer the greatest challenge for designing inks, because they contain self-supporting (or spanning) features. Inks are typically formulated from colloidal, polymeric, or polyelectrolyte building blocks suspended or dissolved in a liquid or heated to create a stable, homogeneous ink with the desired and reproducible rheological (or flow) behavior. The important rheological parameters for a given ink design include its apparent viscosity, yield stress under shear and compression, and viscoelastic properties (i.e. the shear loss and elastic moduli), which are tailored for the specific direct-write technique of interest. Three-dimensional printing (3DP), direct ink-jet printing, and related approaches such as hot-melt printing, involve patterning materials using an ink-jet print head, similar to those used in desktop document printing. These approaches require either low viscosity fluids that must be removed by absorption and evaporation or

wax-based inks that are heated during droplet formation and then solidify upon impact cooling. Cima and Sachs pioneered the concept of using ink-jet printing (3DP) to assemble materials. In 3DP, low viscosity binder droplets are printed onto a powder bed to locally “fuse” material together in a desired pattern. After defining a given two-dimensional layer, an additional powder layer is spread across the bed surface and subsequently patterned. In other ink-jet approaches, three-dimensional structures, such as high-aspect ratio walls or concentrated nanoparticle inks; however, clogging issues still arise when $D/2a \sim 150$, where D is the finest nozzle used (30 μm in diameter) and a is the radius of largest ink particles. These three-dimensional mesoscale structures may find potential application as tissue engineering scaffolds, if constructed from a bioactive ceramic material (e.g. hydroxyapatite), or as structural or functional composites, if the pore space is filled with a second phase.

In addition, the resulting constructs have structural heterogeneity because of the “pixel assembly” nature of the rapid prototyping fabrication process. To overcome this shortcoming, a reverse fabrication technique has been developed to fabricate a negative replica of the scaffold. A polymer/ceramic suspension is cast into such a mold and solidified after the removal of the solvent. The mold is then dissolved away to form the scaffold with the designed three-dimensional pore network. The scaffold is more homogeneous, but the feature resolution is not improved.

To achieve higher resolution for scaffolds with well-controlled interconnected spherical pores, paraffin spheres are fabricated by a dispersion method. These paraffin spheres are then transferred into a three-dimensional mold of the designed shape. The spheres are bonded together through a heat treatment process. A composite suspension is cast into the paraffin assembly in the mold. After removal of the solvent, the paraffin sphere assembly is dissolved away. In this way, an interconnected spherical pore structure is created. Importantly, the features generated have significantly better resolution than those achievable with current rapid prototyping techniques. In addition, investment in expensive equipment is not required, which allows the technology to be easily adapted to a research, as well as an industrial, setting.

5.3. Future Directions in Orthopedics

Simply stated, there are a number of future directions for the use of nanoceramic polymer composites:

- 1) Improve fabrication techniques of polymer/ceramic composites, including resolution and preciseness of three-dimensional structures.
- 2) Improve mechanical properties closer to natural tissue, such as strength and elastic modulus of polymer/ceramic composites.
Improve biocompatibility, particularly long-term cell functions on synthesized biocomposites.
- 3) Study degradation behavior of biodegradable polymer and bioactive ceramic composites, specially the effect of ceramic phase on degradation kinetics and degradation product.
- 4) Incorporate drugs and growth factor into biocomposite scaffolds.
- 5) Modify composites surface to favor cell growth and improve composite-tissue interface.

Bibliography

1. Smith R. (2004) *Bone Health and Osteoporosis: A Report of the Surgeon General*, U.S. Department of Health and Human Services, Public Health Service, Office of the Surgeon General, Rockville, MD, pp. 68-70, 2004.
2. Bren L (2004) *Joint replacement: an inside look*. FDA Consumer, 38(2). http://www.fda.gov/fdac/features/2004/204_joints.html
3. Webster TJ. (2003) Nanophase ceramics as improved bone tissue engineering materials. *American Ceramic Society Bulletin* **82(6)**: 23-28.
4. Rho JY, Kuhn-Spearing L, and Zioupos P. (1998) Mechanical properties and the hierarchical structure of bone. *Medical Engineering & Physics* **20**: 92-102.
5. Kaplan FS, Hayes WC, Keaveny TM, Boskey A, Einhorn TA, and Iannotti J. (1994) Form and function of bone. In: *Orthopaedic Basic Science*, Simon SR, (ed.), American Academy of Orthopaedic Surgeons, Columbus, OH, pp. 127-185.
6. Webster TJ. (2001) Nanophase ceramics: The future orthopedic and dental implant material. In: *Advances in Chemical Engineering*, Vol. 27, Nanostructured Materials, Ying JY (ed.), Academic Press, San Diego, CA, pp. 126-160.
7. Kuhn-Spearing L, Rey C, Kim HM, and Glimcher MJ. (1996) Carbonated apatite nanocrystals of bone. In: *Synthesis and processing of nanocrystalline powder*, Bourell DL (ed.), The Minerals, Metals and Materials Society, Warrendale, PA, USA.

8. Giraud-Guille MM. (1998) Twisted plywood architecture of collagen fibrils in human compact bone osteons. *Calcified tissue international* **42(3)**: 167–180.
9. Bronzino JD. (2000) *The Biomedical Engineering Handbook*, CRC Press, Boca Raton, FL: pp. 274-706.
10. Black J, and Hastings G. (1998) *Handbook of Biomaterial Properties*, Chapman & Hall, New York, NY, pp. 3-21.
11. Fung YC. (1993) *Biomechanics: Mechanical Properties of Living Tissues*, Springer-Verlag, New York, NY, pp. 500-519.
12. Boccaccini AR, and Blaker JJ. (2005) Bioactive composite materials for tissue engineering scaffolds. *Expert Review of Medical Devices*, **2(3)**: 303-317.
13. Guyton AC. (1991) *Textbook of Medical Physiology*, Saunders, Philadelphia, PA, pp. 868-881.
14. Marin BR, and Burr DB (1989) *Structure Function and Adaptation of Compact Bone*, Raven Press, New York, NY, pp. 106-107.
15. Trippel SB. (1998) Potential role of insulin like growth factors in fracture healing. *Clinical Orthopaedics and Related Research* **355S**: S301-313.
16. Stein GS, Lian JB. (1993) Molecular mechanisms mediating proliferation/differentiation interrelationships during progressive development of osteoblast phenotype. *Endocrine Reviews* **14(4)**: 424-442.
17. Wei G. and Ma PX. (2004) Structure and properties of nano-hydroxyapatite/polymer composite scaffolds for bone tissue engineering. *Biomaterials* **25(19)**: 4749-4757.
18. Salgado AJ, Coutinho OP, and Reis RL. (2004) Bone tissue engineering: state of the art and future trends. *Macromolecular Bioscience* **4(8)**: 743-765.
19. Saltzman, WM. (2004) *Tissue Engineering*, Oxford University Press, New York, NY, pp. 6-7.
20. Marra KG, Szem JW, Kumta PN, DiMilla PA, and Weiss LE. (1999) *In vitro* analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering. *Journal of Biomedical Materials Research*, **47(3)**: 324-335.
21. Nather A. (2003) Biology of healing of large deep-frozen cortical bone allografts. In: *Bone Biology and Healing* Phillips GO (ed.), pp. 50-65.
22. Park JB, and Bronzino JD. (2003) *Biomaterials: Principles and Applications*, CRC Press,, Boca Raton, FL, pp. 1-20.
23. McManus AJ, Doremus RH, Siegel RW, and Bizios R. (2005) Evaluation of cytocompatibility and bending modulus of nanoceramic/polymer composites. *Journal of Biomedical Materials Research* **72A(1)**: 98-106.
24. Skalak R and Fox CF. (1988) *Tissue Engineering: Proceedings of a Workshop Held at Granlibakken, Lake Tahoe, CA*, Alan R Liss., New York, pp. 26-29.
25. Mooney DJ, and Mikos AG. (1999) Growing new organs. *Scientific American*, **280(4)**: 60-65.

26. Sachlos E. and Caernuszka JT. (2003) Making tissue engineering scaffolds work: review on the application of solid free form fabrication technology to the production of tissue engineering scaffolds. *European Cells and Materials* **5**: 29-40.
27. Temenoff JS, Steinbis ES, and Mikos AG. (2004) Biodegradable scaffolds. In: *Orthopedic Tissue Engineering: Basic Science and Practice* Goldberg VM and Caplan AI (eds), Marcel Dekker, New York, pp. 77-95.
28. Leong KF, Cheah CM, and Chua CK. (2003) Solid freeform fabrication of three-dimensional scaffolds for engineering replacement tissues and organs. *Biomaterials*, **24(13)**: 2363-2378.
29. Davies JE. (1996) *In vitro* modeling of the bone/implant interface. *The Anatomical Record* **245(2)**: 426-445.
30. Albrektsson T and Johansson C. (2001) Osteoinduction, osteoconduction and osseointegration. *European Spine Journal*, **10(Supply 2)**: S96-101.
31. Kirker-Head CA. (2000) Potential applications and delivery strategies for bone morphogenetic proteins. *Advanced Drug Delivery Reviews*, **43(1)**: 65-92.
32. Silver, FH (1994) *Biomaterials Medical Devices and Tissue Engineering: An Integrated Approach*, Chapman & Hall, New York, pp. 4-29.
33. Park JB and Bronzino JD (2003) *Biomaterials: Principles and Applications*, CRC Press, Boca Raton, FL, pp. 20-77.
34. Reis RL and Cohn D. (2001) *Polymer Based Systems on Tissue Engineering, Replacement and Regeneration*, Kluwer Academic Publishers, Boston, pp. 69-92,130.
35. Porter BD, Oldham JB, He SL, Zobitz ME, Payne RG, An KN, Currier BL, Mikos AG, and Yaszemski MJ. (2000) Mechanical properties of a biodegradable bone regeneration scaffold. *Transactions of the ASME. Journal of Biomechanical Engineering* **122(3)**: 286-288.
36. Agrawal CM, and Ray RB. (2001) Biodegradable polymeric scaffolds for musculoskeletal tissue engineering. *Journal of Biomedical Materials Research*, **55(2)**: 141-150.
37. Thomson RC, Mikos AG, Beahm E, Lemon JC, Satterfield WC, Aufdemorte TB, and Miller MJ. (1999) Guided tissue fabrication from periosteum using preformed biodegradable polymer scaffolds. *Biomaterials* **20(21)**: 2007-2018.
38. Boccaccini AR, and Maquet V. (2003) Bioresorbable and bioactive polymer/bioglass[®] composites with tailored pore structure for tissue engineering applications. *Composites Science and Technology*, **63(16)**: 2417-2429.
39. Liu X. and Ma P.X. (2004) Polymeric scaffolds for bone tissue engineering. *Annals of Biomedical Engineering* **32(3)**: 477-486.
40. Chen VJ, and Ma PX (2004) Nano-fibrous poly(L-lactic acid) scaffolds with interconnected spherical macropores. *Biomaterials* **25(11)**: 2065-2073.
41. Karp, JM, Shoichet MS, and Davies JE. (2003) Bone formation on two-dimensional poly(DL-lactide-co-glycolide) (PLGA) films and three-dimensional

- PLGA tissue engineering scaffolds *in vitro*. *Journal of Biomedical Materials Research - Part A* **64**(2): 388-396.
42. Liu X, and Ma PX (2004) Polymeric scaffolds for bone tissue engineering. *Annals of Biomedical Engineering* **32**(3): 477-486.
 43. Gilding DK and Reed AM. (1979) Biodegradable polymers for use in surgery--- polyglycolic/poly(lactic acid) homo- and copolymers. *Polymer* **20**(12): 1459-1464.
 44. Rokkanen P, Bostman O, Vainionpaa S, Vihtonen K, Tormala P, Laiho J, Kilpikari J, and Tamminmaki M. (1985) Biodegradable implants in fracture fixation: early results of treatment of fractures of the ankle. *Lancet* **1**(8443): 1422-1424.
 45. Waris E, Konttinen YT, Ashammakhi N, Suuronen R, and Santavirta S. (2004) Bioabsorbable fixation devices in trauma and bone surgery: current clinical standing. *Expert Review of Medical Devices* **1**(2): 229-240.
 46. Gunatillake PA, and Adhikari R. (2003) Biodegradable synthetic polymers for tissue engineering. *European Cells and Materials* **5**: 1-16.
 47. Kohn J, Abramson S, and Langer R. (2004) Bioresorbable and bioerodible materials. In *Biomaterials Science: an Introduction to Materials in Medicine* Ratner BD (ed.) Elsevier Academic Press, Boston, MA, pp. 115-126.
 48. Cooper SL, Visser SA, Hergenrother RW, and Lamba NMK (2004) Polymers. In: *Biomaterials Science: an Introduction to Materials in Medicine* Ratner BD (ed.) Elsevier Academic Press, Boston, MA, pp. 67-79.
 49. Hasirci V. (2000) Biodegradable biomedical polymers: Review of degradation of and *in vivo* response to polylactides and polyhydroxyalkanoates. In: *Biomaterials and Bioengineering Handbook* Wise DL (ed.), Marcel Dekker, New York, NY, pp. 141-155.
 50. Shih C. (1995) A graphical method for the determination of the mode of hydrolysis of biodegradable polymers. *Pharmaceutical Research* **12**(12): 2036-2060.
 51. Taddei P, Monti P, and Simoni R. (2002) Vibrational and thermal study on the *in vitro* and *in vivo* degradation of a poly(lactic acid)-based bioabsorbable periodontal membrane. *Journal of Materials Science: Materials in Medicine* **13**(5): 469-475.
 52. Park JB and Bronzino JD (2003) *Biomaterials: Principles and Applications*, CRC Press, Boca Raton, FL, pp. 99-103.
 53. Barbucci R. (2002) *Integrated Biomaterials Science*, Kluwer Academic/Plenum Publishers, New York, pp. 189-689.
 54. Yang S, Leong KF, Du Z, and Chua CK. (2001) The design of scaffolds for use in tissue engineering. Part 1. traditional factors. *Tissue Engineering* **7**(6): 679-689.
 55. Ma PX and Langer R. (1995) Degradation, structure and properties of fibrous nonwoven poly(glycolic acid) scaffolds for tissue engineering. *Materials Research Society Symposium - Proceedings, Polymers in Medicine and Pharmacy* **394**: 99-104.
 56. Zhang R and Ma PX. (2000) Degradation behavior of porous poly(α -hydroxy acids)/hydroxyapatite composite scaffolds. *Polymer Preprints*, published by

- Division of Polymer Chemistry Inc. and American Chemical Society, **41(2)**: 1618-1619.
57. Ciapetti G, Ambrosio L, Savarino L, Granchi D, Cenni E, Baldini N, Pagani S, Guizzardi S, Causa F, and Giunti A. (2003) Osteoblast growth and function in porous poly ϵ -caprolactone matrices for bone repair: a preliminary study. *Biomaterials* **24(21)**: 3815-3824.
 58. Pitt CG, Chasalow FI, Hibionada YM, Klimas DM, and Schindler A. (1981) Aliphatic polyesters. I. the degradation of poly(ϵ -caprolactone) *in vivo*. *Journal of Applied Polymer Science* **26(11)**: 3779-378.
 59. Pitt CG, Gratzl MM, Kimmel GL, Surlis J, and Schindler A. (1981) Aliphatic polyesters II. the degradation of poly(DL-lactide), poly(ϵ -caprolactone), and their copolymers *in vivo*. *Biomaterials* **2(4)**: 215-220.
 60. Dunn AS, Campbell PG, and Marra KG. (2001) The Influence of polymer blend composition on the degradation of polymer/hydroxyapatite biomaterial. *Journal of Materials Science: Materials in Medicine* **12(8)**: 673-677.
 61. Lin W. (1999) Comparison of thermal characteristics and degradation properties of ϵ -caprolactone copolymers. *Journal of Biomedical Materials Research*: **47**: 420-423.
 62. Fisher JP, Vehof JWM, Dean D, Van der Waerden JPCM, Holland TA, Mikos AG, and Jansen JA. (2002) Soft and hard tissue response to photo crosslinked poly(propylene fumarate) scaffolds in a rabbit model. *Journal of Biomedical Materials Research* **59(3)**: 547-556.
 63. Temenoff JS and Mikos AG. (2000) Injectable biodegradable materials for orthopedic tissue engineering. *Biomaterials* **21(23)**: 2405-2412.
 64. Rodrigues CVM, Serricella P, Linhares ABR, Guerdes RM, Borojevic R, Rossi, MA, Duarte, MEL, and Farina M. (2003) Characterization of a bovine collagen-hydroxyapatite composite scaffold for bone tissue engineering. *Biomaterials* **24(27)**: 4987-4997.
 65. Dunn MG, Bellincampi LD, Tria AJ, and Zawadsky JP. (1997) Preliminary development of a collagen-PLA composite for ACL reconstruction. *Journal of Applied Polymer Science* **63(11)**: 1423-1428.
 66. Liao SS, Cui FZ, and Zhu Y. (2004) Osteoblasts adherence and migration through three-dimensional porous mineralized collagen based composite: nHAC/PLA. *Journal of Bioactive and Compatible Polymers*, **19(2)**: 117-130.
 67. Ren L, Tsuru K, Hayakawa S, and Osaka A (2002) Novel approach to fabricate porous gelatin-siloxane hybrids for bone tissue engineering. *Biomaterials* **23(24)**: 4765-4773.
 68. Yin Y, Ye F, Cui J, Zhang F, Li X, and Yao K (2003) Preparation and characterization of macroporous chitosan-gelatin/ β -tricalcium phosphate composite scaffolds for bone tissue engineering. *Journal of Biomedical Materials Research - Part A*, **67(3)**: 844-855.

69. Zhao F, Yin Y, Lu WW, Leong JC, Zhang W, Zhang J, Zhang M, and Yao K (2002) Preparation and histological evaluation of biomimetic three-dimensional hydroxyapatite/chitosan-gelatin network composite scaffolds. *Biomaterials* **23(15)**: 3227-3234.
70. Lawson AC and Czernuszka JT. (1998) Collagen-calcium phosphate composites. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine* **212(H6)**: 413-425.
71. Tadic D, and Epple M. (2004) A thorough physicochemical characterisation of 14 calcium phosphate-based bone substitution materials in comparison to natural bone. *Biomaterials* **25(6)**: 987-994.
72. Ramay HRR and Zhang M. (2004) Biphasic calcium phosphate nanocomposite porous scaffolds for load-bearing bone tissue engineering. *Biomaterials* **25(21)**: 5171-5180.
73. LeGeros RZ Lin S, Rohanizadeh R Mijares D, and Legeros JP. (2003) Biphasic calcium phosphate bioceramics: preparation, properties and applications. *Journal of Materials Science: Materials in Medicine* **14(3)**: 201-209.
74. Hench LL and Polak JM. (2002) Third-generation biomedical materials. *Science* **295(5557)**: 1014-1017.
75. Ducheyne P and de Groot K. (1981) *In vivo* surface activity of a hydroxyapatite alveolar bone substitute. *Journal of Biomedical Materials Research* **15(3)**: 441-445.
76. Rabiei A and Thomas B. (2005) Processing and development of nano-scale HA coating for biomedical application. *Materials Research Society Symposium Proceeding* **845**: 193-199.
77. Hutmacher DW. (2000) Scaffolds in tissue engineering bone and cartilage. *Biomaterials* **21(24)**: 2529-2543.
78. Thomson RC, Yaszemski MJ, Powers JM, and Mikos AG. (1998) Hydroxyapatite fiber reinforced poly(α -hydroxy ester) foams for bone regeneration. *Biomaterials* **19(21)**: 1935-1943.
79. Boccaccini AR, Roether JA, Hench LL, Maquet V, and Jerome R. (2002) A composite approach to tissue engineering. *Ceramic Engineering and Science Proceedings* **23(4)**: 805-816.
80. Marra KG, Szem JW, Kumta PN, DiMilla PA, and Weiss LE. (1999) *In vitro* analysis of biodegradable polymer blend/hydroxyapatite composites for bone tissue engineering. *Journal of Biomedical Materials Research* **47(3)**: 324-335.
81. Kalita S, Finley J, Bose S, Hosick H, and Bandyopadhyay A. (2002) Development of porous polymer-ceramic composites as bone grafts. *Materials Research Society Symposium Proceedings* **726**: 91-96.
82. Blaker JJ, Gough JE, Maquet V, Notingher I, and Boccaccini AR. (2003) *In vitro* evaluation of novel bioactive composites based on bioglass[®]-filled polylactide foams for bone tissue engineering scaffolds. *Journal of Biomedical Materials Research-Part A* **67(4)**: 1401-1411.

83. Ma PX, Zhang R, Xiao G, and Franceschi R. (2001) Engineering new bone tissue *in vitro* on highly porous poly(α -hydroxyl acids)/hydroxyapatite composite scaffolds. *Journal of Biomedical Materials Research* **54(2)**: 284-293.
84. Kasuga T, Ota Y, Nogami M, and Abe Y. (2001) Preparation and Mechanical Properties of Poly(lactic Acid Composites Containing Hydroxyapatite fibers. *Biomaterials*: **22(1)**: 19-23.
85. Navarro M, Ginebra MP, Planell JA, Zepetelli S, and Ambrosio L. (2004) Development and cell response of a new biodegradable composite scaffold for guided bone regeneration. *Journal of Materials Science: Materials in Medicine* **15(4)**: 419-422.
86. Khan YM, Katti DS, and Laurencin CT. (2004) Novel polymer-synthesized ceramic composite-based system for bone repair: an *in vitro* evaluation. *Journal of Biomedical Materials Research - Part A* **69(4)**: 728-737.
87. Rho JY, Kuhn-Spearing L, and Zioupos P (1998) Mechanical properties and the hierarchical structure of bone. *Medical Engineering & Physics* **20(2)**: 92-102.
88. Khan, Y.M., Katti, D.S., and Laurencin, C.T. (2004) Novel polymer-synthesized ceramic composite-based system for bone repair: An *in vitro* evaluation. *Journal of Biomedical Materials Research: A* **69**: 728-737.
89. Kikuchi M, Cho SB, Suetsugu Y, and Tanaka J. (1997) *In vitro* tests and *in vivo* tests developed TCP/CPLA composites. *Bioceramics* **10**: 407-410.
90. Du C, Cui FZ, Zhu XD, and de Groot K. (1999) Three-dimensional nano-HAP/collagen matrix loading with osteogenic cells in organ culture. *Journal of Biomedical Materials Research* **44(4)**: 407-415.
91. Webster TJ, Siegel RW, and Bizios R. (2001) Nanoceramic surface roughness enhances osteoblast and osteoclast functions for improved orthopaedic/dental implant efficacy. *Scripta Materialia* **44(8-9)**: 1639-1642.
92. Webster TJ, Ergun C, Doremus RH, Siegel RW, and Bizios R. (2000) Specific proteins mediate enhanced osteoblast adhesion on nanophase ceramics. *Journal of Biomedical Materials Research* **51(3)**: 475-483.
93. Webster TJ, Siegel RW, and Bizios R. (1999) Osteoblast adhesion on nanophase ceramics. *Biomaterials* **20(13)**: 1221-1227.
94. Dulgar Tulloch AJ, Bizios R, and Siegel RW. (2003) Nanophase alumina/poly(L-lactic acid) composite scaffolds for biomedical applications. *Materials Research Society Symposium Proceedings* **740**: 161-166.
95. Webster TJ, Siegel RW, and Bizios R. (2001) Nanoceramic surface roughness enhances osteoblast and osteoclast functions for improved orthopaedic/dental implant efficacy. *Scripta Materialia* **44(8-9)**: 1639-1642.
96. Yamasaki H and Sakai H. (1992) Osteogenic response to porous hydroxyapatite ceramics under the skin of dogs. *Biomaterials* **13(5)**: 308-312.
97. Yuan H, Kurashina K, de Bruijin JD, Li Y, de Groot K, and Zhang X. (1999) A preliminary study on osteoinduction of two kinds of calcium phosphate ceramics. *Biomaterials* **20(19)**: 1799-1806.

98. Liu H, Slamovich EB, and Webster TJ. (2005) Increased osteoblast functions on nanophase titania dispersed in poly-lactic-co-glycolic acid composites. *Nanotechnology* **16(7)**: S601-S608.
99. Webster TJ, Schadler LS, Siegel RW, and Bizios R. (2001) Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. *Tissue Engineering* **7(3)**: 291-302.
100. Qin XY, Kim JG, and Lee JS. (1999) Synthesis and magnetic properties of nanostructured γ -Ni-Fe alloys. *Nanostructured Materials* **11(2)**: 259-270.
101. Li P, Miser DE, Rabiei S, Yadav RT, and Hajaligol MR. (2003) The removal of carbon monoxide by iron oxide nanoparticles. *Applied Catalysis B: Environmental* **43(2)**: 151-162.
102. Surowiak Z, Osinska, K, and Czekaj D. (2001) Structure and physical properties of nano-structured $\text{Pb}(\text{Zr}_{0.5}\text{Ti}_{0.5})\text{O}_3$ piezoceramics. *Proceedings of SPIE-The International Society for Optical Engineering* **4413**: 163-168.
103. Suh SW, Shin JY, Kim J, Kim J, Beak CH, Kim DI, Kim H, Jeon SS, and Choo IW. (2002) Effect of different particles on cell proliferation in polymer scaffolds using a solvent-casting and particulate leaching technique. *ASAIO Journal* **48(5)**: 460-464.
104. Shastri V P, Martin I, and Langer R. (2000) Macroporous polymer foams by hydrocarbon templating. *Proceedings of the National Academy of Sciences USA* **97**: 1970-1975.
105. Wu L, Zhang H, Zhang J, and Ding J. (2005) Fabrication of three-dimensional porous scaffolds of complicated shape for tissue engineering. I. Compression molding based on flexible-rigid combined mold. *Tissue Engineering* **11(7-8)**: 1105-1114.
106. Harris LD, Kim BS, and Mooney DJ. (1998) Open pore biodegradable matrices formed with gas foaming. *Journal of Biomedical Materials Research* **42**: 396-402.
107. Kim SS, Park MS, Jeon O, Choi CY, and Kim BS. (2006) Poly(lactide-co-lycolide)/hydroxyapatite composite scaffolds for bone tissue engineering. *Biomaterials* **27(8)**: 1399-1409.
108. Nam Y S, Yoon J J, and Park T G. (2000) A novel fabrication method of macroporous biodegradable polymer scaffolds using gas foaming salt as a porogen additive. *Journal of Biomedical Materials Research: Part B Applied Biomaterials* **53**: 1-7.
109. Whang K, Thomas C H, Healy K E, and Nuber G. (1995) Novel method to fabricate bioabsorbable scaffolds. *Polymer* **36(4)**: 837-842.
110. Liu L, Zhang, L, Ren B, Wang F, and Zhang Q. (2003) Preparation and characterization of collagen-hydroxyapatite composite used for bone tissue engineering scaffold. *Artificial Cells, Blood Substitutes, and Biotechnology* **31(4)**: 435-448.

111. Lo H, Kadiyala S, Guggino SE, and Leong KW. (1996) Poly(L-lactic acid) foams with cell seeding and controlled-release capacity. *Journal of Biomedical Materials Research* **30**: 475-484.
112. Lo H, Ponticciello MS, and Leong KW. (1995) Fabrication of controlled release biodegradable foams by phase separation. *Tissue Engineering* **1**: 15-28.
113. Schugens C, Maquet V, Grandfils C, Jerome R, and Teyssie P. (1996) Polylactide macroporous biodegradable implants for cell transplantation II. Preparation of polylactide foams for liquid-liquid phase separation. *Journal of Biomedical Materials Research* **30**: 449-461.
114. Nam YS, and Park TG. (1996) Porous biodegradable polymeric scaffolds prepared by thermally induced phase separation. *Journal of Biomedical Materials Research* **47**: 17.

