Nanobiomaterials Handbook

Balaji Sitharaman

Nanocomposite Polymer Biomaterials for Tissue Repair of Bone and Cartilage: A Material Science Perspective

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Akhilesh K. Gaharwar, Patrick J. Schexnailder, Gudrun Schmidt
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24.1 Introduction to Polymer Nanocomposite Biomaterials

The design and fabrication of nanocomposite biomaterials for tissue engineering applications requires a fundamental understanding of the interactions between polymers, nanostructures, and cells. Biology offers the best models for strategies on how to rationally design high-performance biomaterials with the properties of materials, such as bone, cartilage, nacre, or silk (Murphy and Mooney 2002; Gao et al. 2003; Tang et al. 2003; Mayer 2005; Lee and Spencer 2008). To translate our fundamental understanding of nature into products that are useful in a clinical setting, the chemical, physical, and biological properties of newly developed bio-nanocomposites need to be optimized to support, regulate, and influence long term cellular activities.

Although we can replicate some physical properties of natural biomaterials, reproducing the complexity and efficiency of natural tissue is challenging. For example, the strength and stiffness of bone...
is related to its highly ordered structure at the nano- and micro-length scales (Weiner and Traub 1992; Weiner and Wagner 1998). Cartilage has unique combinations of nonlinear tensile and compressive properties due to hierarchically arranged collagen fibrils, proteoglycans, and proteins (Mow et al. 1980; Mankin 1982; Buckwalter and Mankin 1997; Cohen et al. 1998). The structural and mechanical properties of such natural tissue can be imitated by engineering bio-nanocomposites made from polymer and nanoparticles (nanospheres, nanotubes, nanoplatelets, etc.) (Engel et al. 2008; Ma 2008; Vaia and Baur 2008; Smith et al. 2009). The nanoparticles often act as physical cross-links to the polymer chains, which reinforce and enhance the mechanical properties of the nanocomposite biomaterial (Kotela et al. 2009; Schexnaider and Schmidt 2009; Smith et al. 2009; Zhang and Webster 2009).

Seen from a material science perspective, several research groups have developed fabrication techniques for generating supramolecular assembled nanocomposites of polymers and nanoparticles (Giannelis 1996; Alexandre and Dubois 2000; Gao et al. 2003; Hule and Pochan 2007). These creative ideas can easily be translated for the development of biomaterials. Many of the studies in this area are based on the observation that small amounts of nanoparticles can dramatically change the physical properties of a polymer matrix, allowing for the engineering of synergistic property combinations that the individual components cannot achieve (Liff et al. 2007; Podsiadlo et al. 2007). For example, fabrication technologies such as electrospinning, layer-by-layer techniques, salt leaching, lyophilization, etc. have been developed to generate structural features that can potentially be used for the design and development of tissue engineering and drug delivery matrices (Doshi and Reneker 1993; Decher 1997; Lee et al. 2005; Ateshian 2007; Kong et al. 2007). In one example, new creative approaches by Liff et al. (2007) were able to overcome the thermodynamic and kinetic barriers of nanoparticle dispersion via solvent exchange. Strong adhesion between silicate and polymer microdomains and the formation of a percolative network induce thermotropic liquid crystalline behavior in addition to mechanical strength (Liff et al. 2007). The physical properties of such polymer materials are ideally suited for tissue repair. Other techniques that could be adapted to biomaterial development include the continuous self-assembly and polymerization of silica, surfactant, and monomers into nacre-like nanolaminated coatings, which was previously achieved by Sellinger et al. (1998). Here, evaporation induced partitioning and self-assembly resulted in the simultaneous organization of thousands of layers at once (Sellinger et al. 1998). Such techniques can further be developed to generate gradient layered scaffolds for diverse tissue engineering applications.

In order to design nanocomposite biomaterials or to develop already existing nanocomposites for the repair of bone and cartilage, we need to consider and compare the structures and properties of the natural tissue, along with the biological influence of cells on the synthetic biomaterial properties. Biomedical research has given us a substantial understanding of the musculoskeletal structure and its function (Weiner and Traub 1992; Rho et al. 1998; Weiner and Wagner 1998; Weiner et al. 1999). Several studies have provided valuable insights into cell–scaffold interactions (Hazan et al. 1993; Stevens and George 2005; Jones 2006; Ma 2008; Stoddart et al. 2009) in addition to physical and chemical material properties. However, nature’s perfection and efficiency are not easily matched, and thus our attempts to repair living tissue with synthetic biomaterials often remain limited.

The scope of this review is to focus on the design, fabrication, and evaluation of polymeric nanocomposite biomaterials that are currently used and that can potentially be used for the tissue engineering of bone, cartilage, and the bone–cartilage interface. We first discuss the properties of the natural tissue to identify the requirements for engineering suitable nanocomposite matrices and scaffolds. Then, we highlight the most recent accomplishments and trends in the field of bio-nanocomposites for the tissue engineering of bone, cartilage, and the bone–cartilage interface and examine the impact of published work on specific tissue engineering applications. Overall, we address some of the most critical challenges that come with the design, fabrication, and evaluation of bio-nanocomposite scaffolds and conclude with a brief outline on future directions.
24.2 Important Structures and Properties

24.2.1 Bone: A Hard Nanocomposite

A better understanding of the relations between the composition, structure, and properties of bone can guide the design of tissue engineered synthetic grafts that ideally remodel and heal bone tissue. Table 24.1 lists the main components of bone, while Figure 24.1 summarizes well-known micrometer and nanometer structural features, and Table 24.2 gives ranges of mechanical properties that are important to know when developing bone replacement tissue.

<table>
<thead>
<tr>
<th>TABLE 24.1</th>
<th>Main Components of Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component</strong></td>
<td><strong>%</strong></td>
</tr>
<tr>
<td>Inorganic</td>
<td></td>
</tr>
<tr>
<td>Hydroxyapatite—((Ca_5(PO_4, CO_3))_3(OH)))</td>
<td>~60</td>
</tr>
<tr>
<td>Carbonate, citrate</td>
<td>~5</td>
</tr>
<tr>
<td>Other minerals, that is, Mg, Na, Cl, F, K+, Sr²⁺, Pb²⁺, Zn²⁺, Cu²⁺, Fe²⁺</td>
<td>~1</td>
</tr>
<tr>
<td>Organic</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>~20</td>
</tr>
<tr>
<td>Water</td>
<td>~9</td>
</tr>
<tr>
<td>Non-collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin, morphogenetic proteins, sialo, and serum protein)</td>
<td>~3</td>
</tr>
<tr>
<td>Other traces: Polysaccharides, lipids, cytokines</td>
<td></td>
</tr>
<tr>
<td>Bone cells: osteoblasts, osteocytes, osteoclasts</td>
<td></td>
</tr>
</tbody>
</table>


From a material science point of view, bone is a natural nanocomposite composed mainly of hydroxyapatite (HAp) nanoparticles, collagen fibrils, and cells (osteoblasts, osteocytes, osteoclasts). The relative proportions and structural orientations of HAp and collagen in combination with the many synergistic property combinations added by the other ingredients influence the biomechanical properties. For example, trace amounts of lipids are important for biomineralization while non-collagenous protein (Heinegard and Oldberg 1989) and collagen (Weiner and Wagner 1998; Reffitt et al. 2003; Fratzl 2008) are involved in the nucleation of HAp. Thus, the combinations and interactions of multiple ingredients during the synthesis and growth of bone determine the properties of the end product. Moreover, the dynamic mechanical and compositional microenvironment of bone marrow influence bone homeostasis (Gurkan and Akkus 2008). In addition, many other components not listed in Table 24.1 indirectly influence bone synthesis. One of these components is silicon, which several studies suggest is essential for the formation of bone by participating in cell metabolism (Carlisle 1970, 1972, 1981; Seaborn and Nielsen 2002).

The biomechanical properties of bone are also determined by the extent of mineralization, the amount of water, and the 3D architecture at each hierarchical level (Aubin and Liu 1996). For example, both cortical and cancellous bone are composed of the same material, but their microstructural arrangement results in significantly different mechanical properties (Table 24.2) (Rho et al. 1998; Weiner and Wagner 1998). Cortical bone is composed of cylindrical-shaped units called osteons, a dense tissue consisting of concentric lamellae, while cancellous bone is a porous structure (Figure 24.1). Moreover, an ordered arrangement of mineralized collagen fibrils impart anisotropic properties to both cortical and cancellous bones (Murugan and Ramakrishna 2005). A variation in mechanical properties is also dependent on the anatomical location of bone (Currey 1988). The yield strength of bone is half and its density is about a quarter as that of steel (Carter and Hayes 1976; Disegi and Eschbach 2000). The complex hierarchical structure, ranging from nanometers to micrometers (Figure 24.1), is mainly responsible for the superior strength while being light weight (Weiner and Traub 1992; Rho et al. 1998; Weiner and Wagner 1998). The basic building blocks of bone are mineralized collagen fibrils that are arranged in an ordered structure and act as a structural framework (Weiner and Wagner 1998; Weiner et al. 1999). In summary, the nanocomposite structure and anisotropic properties with an interconnected porous network are the basis for the high strength architectural framework.

### TABLE 24.2 Mechanical Properties of Bone and Cartilage

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Tensile Modulus (MPa)</th>
<th>Tensile Strength (MPa)</th>
<th>Compression Modulus (MPa)</th>
<th>Compressive Strength (MPa)</th>
<th>Fracture Toughness (MPa/m(^{1/2}))</th>
<th>Shear Modulus (MPa)</th>
<th>Density (g/cm(^3))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical bone</td>
<td>5–20 (× 10(^3))</td>
<td>50–150</td>
<td>4–15 (× 10(^3))</td>
<td>170–193</td>
<td>2–12</td>
<td>~300 (× 10(^3))</td>
<td>1.8–2.2</td>
</tr>
<tr>
<td>Cancellous bone</td>
<td>50–500</td>
<td>2–20</td>
<td>15–100</td>
<td>7–10</td>
<td>0.1</td>
<td>1–70</td>
<td>1.5–1.9</td>
</tr>
<tr>
<td>Cartilage</td>
<td>0.5–30</td>
<td>2–50</td>
<td>0.13–1.91</td>
<td>25–65</td>
<td>0.5–3.8</td>
<td>0.8–2.1</td>
<td>~1.12</td>
</tr>
</tbody>
</table>

While it is impossible to imitate the complexity of natural bone formation via bottom-up synthesis or via sophisticated formulation techniques, there are other approaches that might be considered limited, but nevertheless useful. Such approaches aim at developing scaffolds or structural frameworks for supporting natural bone repair in vivo.

### 24.2.2 Cartilage: A Soft Composite

The structural and mechanical properties of cartilage are directly related to the composition and architecture of the extra cellular matrix (ECM) (Zhu et al. 1993; Ulrich-Vinther et al. 2003). Unlike bone, cartilage consists of only one cell type (chondrocytes), which are embedded within the ECM. This ECM consists of collagen fibrils and proteoglycan macromolecules (Table 24.3) that are synergistically able to resist shear deformation (Zhu et al. 1993). According to the literature, the collagen fibrils form a network in which the highly charged and swollen proteoglycans generate a significant prestress. The flexible collagen fibrils elastically resist the tensile forces to provide tensile stiffness, and the prestress provided by the proteoglycans maintains fibril orientation and structural stability within the matrix.

Similar to bone, the hierarchical network structure of cartilage is mainly responsible for its mechanical properties (Figure 24.1) (Heinegard and Oldberg 1989). Based on its biochemical composition and morphology, articular cartilage can be divided into four different zones (Weiss et al. 1968; Temenoff and Mikos 2000). The preferred orientation of the fibrils in these different zones stabilizes the articular cartilage against mechanical loadings.

As articular cartilage is composed of porous 3D networks filled with tissue fluid, the compressive properties are a direct result of its biphasic nature (Mow et al. 1980). Articular cartilage exhibits both creep and stress-relaxation behaviors (Cohen et al. 1998). The unique compressive properties of articular cartilage are due to its nonlinear permeability response, especially at high pressures and strains. Thus, under compression, the nonlinear permeability acts as a protective mechanism and stiffens the cartilage by restricting fluid flow. Additionally, the fluid flow through the ECM determines the viscoelastic properties of cartilage. The aligned collagen fibrils present in the ECM are responsible for nonlinear tensile properties. The mechanical properties of cartilage vary with location due to changes in the organization.

#### TABLE 24.3 Main Components of Cartilage and Their Function

<table>
<thead>
<tr>
<th>Component</th>
<th>Composition and Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular matrix</td>
<td><strong>Collagen</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Proteoglycans</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Non-collagenous proteins</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Tissue fluid</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Cells</strong></td>
</tr>
</tbody>
</table>

of collagen fibers from one zone to another. This makes the tissue engineering of cartilage challenging as the design of such gradient structures and anisotropic properties is not straightforward. For example, articular cartilage shows anisotropic mechanical properties when subjected to tension (Akizuki et al. 1986) and compression (Jurvelin et al. 2003), and the stiffness of cartilage in tension is typically 5–20 times greater than in compression (Akizuki et al. 1986). Replacement scaffolds that fulfill these requirements pose serious material design challenges. In order to restore function, scaffolds for cartilage repair mimic only some of the mechanical properties but often do not mimic the hierarchical structural characteristics of cartilage.

Overall, an understanding of the structure, composition, and mechanical properties of cartilage helps researchers in developing strategies for the repair of natural tissue. Since the basis for the high compressive strength of articular cartilage is the 3D nanofibrous polymeric network filled with tissue fluid, stiff nanocomposite hydrogels might be suitable candidates for cartilage repair.

24.3 The Bone–Cartilage Interface: A Gradient Nanocomposite

While highly vascularized bone undergoes constant remodeling by metabolically active cells, avascular cartilage has limited regeneration potential (Yasui et al. 1982; Buckwalter 1992; Buckwalter et al. 1994; Ulrich-Vinther et al. 2003). The bone–cartilage interface is composed of a mineralized cartilage gradient that contains more calcium than the adjacent bone (Zizak et al. 2003). The sizes and widths of the mineralized HA nanoplatelets in mineralized cartilage and bone are similar. However, an abrupt change in orientation of nanoplatelets happens at the interface. In bone, the mineralized platelets preferentially orient parallel to the interface, while in mineralized cartilage they orient perpendicular to it (Weiner and Traub 1992; Zizak et al. 2003). The change in the orientation of mineralized platelets and the increased amount of calcium at the bone–cartilage interface are responsible for the tight bonding between the two mechanically and structurally different tissues, bone, and cartilage.

When osteochondral injury reaches the subchondral and vascularized bone, a repair response is initiated (Mankin 1982; Brittberg and Winalski 2003). A fibrin clot containing red blood cells, white blood cells, and marrow elements is formed immediately (Mankin 1982; Paletta et al. 1992). This clot is then gradually replaced by fibrous tissue, which is less stiff than native cartilage. Subsequently, the fibrous repair tissue is converted to a hyaline-like chondroidal tissue. The newly formed cartilaginous tissue functions reasonably well if the defect is small. However, for large defects, the newly formed cartilaginous tissue cannot provide enough strength to restore normal function (Brittberg et al. 1994; Peretti et al. 2003).

24.3.1 Properties Required for Tissue Grafts to Work

With our limited capabilities to generate natural tissue and imitate tissue repair, autologous tissue grafts are defined as the gold standard for orthopedic surgeries (Fujishiro et al. 2008). An ideal tissue graft should mimic the structural and functional characteristics of natural tissue and should initiate and support the self-repair of this tissue (Hutmacher 2000; Risbud and Sittiinger 2002; Cancedda et al. 2003; Capito and Spector 2003; Sharma and Elisseeff 2004). Although useful scaffolds can be made of various materials bearing a range of properties, the desired properties can be defined as follows:

The scaffolds should be biocompatible and function similar to that of the ECM to support cell survival, proliferation, and migration (Burg et al. 2000; Jones 2006).

The scaffolds should be adhesive to surrounding tissues in order to adhere at or within the injury site.

The scaffolds should have an appropriate degradation rate that matches the regeneration rate of the defective tissue. The degradation by-product(s) should be nontoxic and not elicit an immune response (Freed et al. 1994; Middleton and Tipton 2000; Agrawal and Ray 2001).
The scaffolds should be able to transmit both chemical and mechanical signals to the cells (Ratner 1996; Parikh 2002; Murugan and Ramakrishna 2005).

The scaffolds should have similar mechanical properties to those of native tissue so that they can function as temporary replacements while new tissue is regenerated (Hutmacher 2000; De Aza et al. 2003; Jahangir et al. 2008).

The scaffolds should be highly porous to support cell penetration, tissue in-growth, and the exchange of nutrients and waste products.

The scaffolds should be easily sterilized and cost effective.

24.4 Biomaterials Used for Bone Tissue Engineering

24.4.1 Ceramic, Polymeric, and Composite Biomaterials for Bone Repair

Although the remodeling and restructuring of bones takes place throughout life, surgical intervention is sometimes needed to recover lost function, due to an accident, degradation over time, or certain genetic disorders. In the United States alone, more than 500,000 surgical procedures require bone grafts every year amounting to $2.5 billion annually (Greenwald et al. 2001; Jahangir et al. 2008). Traditional methods for bone repair include autografts and allografts that involve the transplantation of similar tissue from a patient’s own body or from another person’s, respectively (Gazdag et al. 1995; Laurencin et al. 1999). In certain severe cases, for example, knee replacement or hip replacement, total joint replacement is needed. The scarce availability of suitable donors, organ rejection, and surgical complications (Parikh 2002; Fujishiro et al. 2008) are some of the main issues with natural grafts. Hence, synthetic grafts are frequently considered as replacements for natural grafts. Synthetic grafts include metals, polymers, ceramics, and polymer composites that are used as scaffold materials (Hench 1991; Parmakrishna 2005). Although synthetic grafts are fairly successful, the mismatch of structural and mechanical properties, when compared to natural tissue, has prompted researchers to find better substitutes (Laurencin et al. 1999; Hutmacher 2000).

The evolution of bone graft materials is well documented by various researchers (Damien and Parsons 1991; Burg et al. 2000; Hutmacher 2000; Hench and Polak 2002; Murugan and Ramakrishna 2005). Bioceramics used for bone tissue engineering can be classified as bioinert (e.g., alumina, zirconia), bioresorbable (e.g., tricalcium phosphate [TCP]), or bioactive (e.g., HAp bioactive glasses and glass-ceramics) (Larry 1991; El-Ghannam 2005; Yoshikawa and Myoui 2005; Best et al. 2008). Alumina and zirconia are often used in hip and knee implants because of their high fracture toughness, biocompatibility, and inertness (Yoshikawa and Myoui 2005). Bone formation is favorably supported by ceramics containing calcium phosphate (Yuan et al. 1998; De Aza et al. 2003). However, scaffolds made from pure calcium phosphate have poor mechanical properties, for example, they are brittle and have low fracture toughness (Larry 1991). The excellent bioactivity of HAp and bioactive glass makes them useful as bone substitutes or coatings that promote cell adhesion and bone in-growth. One of the fundamental strategies for bone repair is to use osteoconductive and osteoinductive biomaterials. HAp is widely known as an osteoconductive material but not osteoinductive (Yoshikawa and Myoui 2005). Growth factors can provide additional osteoinductive stimuli and enhance bone formation at the defect site (Roberts and Sporn 1996). Although ceramics have desirable characteristics, on their own they exhibit low compressive strength and have unpredictable bioresorption rates (Petite et al. 2000). Some ceramics (TCP, HAp, bioglass) are osteoconductive and support the development of new bone, but have limited applicability due to their brittle nature and the intricacy involved in the fabrication of porous structures. Thus, ceramic scaffolds are used only in non-load-bearing applications such as bone void fillers.

Synthetic and natural polymers have shown promise as bone graft materials due to their plastic and viscoelastic properties, their degradability, and biocompatibility (Ma 2004). Natural polymers that are often used as bone grafts include collagen, silk, fibrin, hyaluronic acid, chitosan, and alginate (Ratner 1996; Peter et al. 1998; Agrawal and Ray 2001). The most common biodegradable polymers for bone
grafts include poly(glycolic acid) (PGA), poly(lactic acid) (PLA), poly(lactic-co-glycolic acid) (PLGA), poly(caprolactone) (PCL), poly(propylene fumarate) (PPF), and poly(ethylene glycol) (PEG) (Engelberg and Kohn 1991; Laurencin et al. 1999; Burg et al. 2000; Agrawal and Ray 2001). More advanced biomaterials for bone repair require an exclusive combination of chemical, physical, and biological properties. Although most of the natural and synthetic polymers are biodegradable and biocompatible, inferior mechanical strength often limits their use in bone repair.

Since biomaterials are in direct contact with the human body, they need to fulfill various requirements that individual polymeric or ceramic components may not fulfill. Organic–inorganic microcomposites often combine the advantages of both inorganic ceramics and organic polymers. For example, HAp, calcium phosphate, and bioglass are attractive inorganic components that have high bioactivity. Calcium phosphate forms hydroxy carbonate apatite in vivo, which is similar to mineralized bone (Hench 1991; Yuan et al. 1998; Jones et al. 2006). Bioactive ceramics may also reinforce a polymer scaffold and thus provide sufficient mechanical strength (Rezwan et al. 2006). Ceramics have been combined with numerous polymers, including natural (collagen, silk, chitosan) as well as synthetic ones (PEG, PLA, PGA, PLGA). However, despite promising outcomes, the main drawback of using polymer microcomposites for bone repair is the lack of uniform dispersion of inorganic particles within the organic polymer matrix. Further, large aggregates and a lack of sufficient interfacial interaction between the inorganic particles with the polymer matrix adversely affect the mechanical properties of the microcomposite.

### 24.4.2 Polymer Nanocomposite Biomaterials for Bone Repair

Nanocomposite biomaterials or bio-nanocomposites offer versatility in designing specific properties due to a better control of interactions between nanoparticles and polymers (Hule and Pochan 2007). Polymer nanocomposite biomaterials possess superior mechanical properties when compared to their macro- and microcomposite counterparts (Winey and Vaia 2007). Some common polymers used for making bio-nanocomposites are dextran, chitosan, hyaluronic acid, polyethylene oxide, PLGA, PLA, and PGA. Many researchers have demonstrated that small amounts of nanoparticles added to these polymers can dramatically change the physical properties of the resulting bio-nanocomposite (Giannelis 1996; Vaia and Wagner 2004; Paul and Robeson 2008). Moreover, various combinations of nanoparticles and polymer matrices can be used to engineer previously unattainable property combinations (Table 24.4). One may choose from a variety of nanoparticles depending on the properties that need to be enhanced. Nanoparticles can be categorized as nanospheres/nanoparticles (HAp, TCP, alumina, titania, silica), nanotubes (carbon nanotubes [CNTs], metallic nanotubes), nanofibers, and nanoplatelets (layered silicates such as Montmorillonite [MMT], Cloisite, and Laponite).

#### 24.4.2.1 Polymer–Hydroxyapatite Nanoparticle Composites

Inorganic nanoparticles such as nano-HAp and calcium phosphate are major constituents of human hard tissue, and thus they are the most common biomaterials studied for bone tissue engineering and repair (Weiner and Traub 1992). HAp is osteoconductive and interacts with the natural tissue without eliciting any inflammatory response. An addition of small amounts of nano-HAp can drastically increase the modulus and the tensile strength of the polymer matrix. Thomas et al. (2007) fabricated such bio-nanocomposite scaffolds from collagen type I and nano-HAp via co-electrospinning. These fibrous scaffolds had well-interconnected pore structures and the fiber diameter increased with nano-HAp concentration. An addition of 10% HAp resulted in a threefold increase in tensile strength and a fourfold increase in modulus. The increase in the mechanical properties was mainly attributed to the strong interaction between collagen and nano-Hap (Thomas et al. 2007). To further improve the mechanical properties, glutaraldehyde was used to chemically cross-link the collagen network. However, glutaraldehyde is not biocompatible (Takigawa and Endo 2006) and might elicit an immune response.

A better biomimetic approach to cross-link nano-HAp with a PLA-co-PEG polymer network was published by Sarvestani et al. (2008). This group reported an increase in the shear modulus of...
PLA-co-PEG–HAp nanocomposites when glutamic acid (Glu), a negatively charged peptide was added as a cross-linker. Glu mimics the osteonectin glycoprotein of bone (a bone connector) and cross-links nano-HAp to the polymer matrix. The addition of the Glu cross-linker resulted in a 100% increase in the shear modulus of the nanocomposite. No significant changes in the mechanical properties were observed with or without the Glu cross-linker when micro-HAp was used. This study confirms that the size of added particles plays an important role in modulating the mechanical properties. A similar study by Wei et al. demonstrated that PLLA/nano-HAp nanocomposites not only have superior mechanical properties but also show improved protein adhesion compared to their microcomposite counterparts (Wei and Ma 2004). These nano- and microcomposites were prepared using thermally induced phase separation. Compared to pure PLLA, the resulting nanocomposites showed an almost twofold increase in compressive moduli with the addition of a 50 wt% of nano-HAp.

### TABLE 24.4 Nanocomposites for Bone Repair

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Polymer</th>
<th>Preparation Method</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano-HAp</td>
<td>Collagen I</td>
<td>Electrospinning</td>
<td>Interconnected pore structure. Glutaraldehyde cross-linked collagen</td>
<td>Thomas et al. (2007)</td>
</tr>
<tr>
<td>Nano-HAp</td>
<td>PLA-co-PEG</td>
<td>Biomimetics</td>
<td>Used glutamic acid to cross-link nano-HAp with the polymer matrix</td>
<td>Sarvestani et al. (2008)</td>
</tr>
<tr>
<td>Nano-HAp</td>
<td>PLLA</td>
<td>Phase separation</td>
<td>High compressive moduli</td>
<td>Wei and Ma (2004)</td>
</tr>
<tr>
<td>Nano-HAp</td>
<td>Collagen</td>
<td>Electrospinning</td>
<td>Increased in vitro mineralization and calcium phosphate activity</td>
<td>Venugopal et al. (2007)</td>
</tr>
<tr>
<td>Nano-HAp</td>
<td>PLLA</td>
<td>Biomimetics</td>
<td>Scaffold consisting of nanospheres loaded with biological factors</td>
<td>Ma and Zhang (1999); Ma (2004); Smith et al. (2009)</td>
</tr>
<tr>
<td>CNTs</td>
<td>Polyurethane</td>
<td>Phase separation</td>
<td>Anisotropic pore structure and nanoscaled surface texture</td>
<td>Jel et al. (2008)</td>
</tr>
<tr>
<td>SWNTs</td>
<td>PPF</td>
<td>Solvent-casting</td>
<td>Improved mechanical strength due to nanofiller</td>
<td>Shi et al. (2006)</td>
</tr>
<tr>
<td>SWNTs</td>
<td>PPF</td>
<td>Leaching</td>
<td>100% interconnectivity, MSCs adhere and proliferate</td>
<td>Shi et al. (2007)</td>
</tr>
<tr>
<td>SWNTs</td>
<td>PPF</td>
<td>Leaching</td>
<td>Promoted bone in-growth and collagen production</td>
<td>Sitharaman et al. (2008)</td>
</tr>
<tr>
<td>CaSiO₃</td>
<td>PCL</td>
<td>Solvent mixing</td>
<td>CaS enhanced apatite formation in vitro</td>
<td>Kotela et al. (2009); Wei et al. (2008)</td>
</tr>
<tr>
<td>Silica/CaSiO₃</td>
<td>Collagen</td>
<td>Sol–gel</td>
<td>Silica improved compressive properties and increased bioactivity</td>
<td>Heinemann et al. (2007, 2009)</td>
</tr>
<tr>
<td>Silica</td>
<td>Chitin</td>
<td>Biomineralization</td>
<td>Chitin acted as template for biomineralization</td>
<td>Ehrlich et al. (2008)</td>
</tr>
<tr>
<td>Silica</td>
<td>Spider silk</td>
<td>Silicification</td>
<td>Controlled structure and morphology of scaffold</td>
<td>Wong Po Foo et al. (2006)</td>
</tr>
<tr>
<td>MMT</td>
<td>PLLA</td>
<td>Solvent mixing</td>
<td>MMT reinforced the network and modified degradation rate</td>
<td>Lee et al. (2003)</td>
</tr>
<tr>
<td>MMT</td>
<td>Gelatin–chitosan</td>
<td>Solvent mixing</td>
<td>Altered degradation rate and enhanced cell adhesion and proliferation</td>
<td>Zhuang et al. (2007)</td>
</tr>
<tr>
<td>MMT</td>
<td>PLA</td>
<td>Leaching</td>
<td>MMT improved compression properties</td>
<td>Ozkoc et al. (2009)</td>
</tr>
<tr>
<td>MMT</td>
<td>PLLA</td>
<td>Solvent mixing</td>
<td>MMT suppressed polymer crystallinity and improved mechanical properties</td>
<td>Krikorian and Pochan (2003)</td>
</tr>
<tr>
<td>Cloisite 20A</td>
<td>EVA/iron oxide</td>
<td>Solvent mixing</td>
<td>Cloisite increased osteoblast proliferation and magnetic field induced</td>
<td>Lewkowitz-Shpuntoff et al. (2009)</td>
</tr>
</tbody>
</table>
One of the important parameters in fabricating bone grafts is the 3D architecture of natural tissue, including its porous structure. While the random incorporation of pores into a synthetic scaffold can adversely affect the mechanical properties, the presence of pores also provides a framework for cell attachment, proliferation, and growth. Karageorgiou and Kaplan (2005) discussed the effect of pore size and porosity with respect to bone regeneration. They concluded that porosity enhances the osseo-integration of the implant and reduces stress shielding. An optimum pore size for bone in-growth was reported to be 200–400 μm (Yang et al. 2001).

A technique to fabricate porous scaffolds with excellent mechanical properties is electrospinning, which allows for making polymer nanofilaments using electrostatic forces (Doshi and Reneker 1993). Venugopala et al. (2007) prepared nanocomposite fiber scaffolds from collagen and nano-HAp using electrospinning. These porous and fibrous scaffolds supported osteoblast adhesion, migration, and proliferation, and a significant increase in mineralization was observed after 10 days of culture. The same group showed that their scaffolds also support the formation of multiple layers of cells (Venugopala et al. 2008).

In another study, Ma et al. reported on the synthesis of biomimetic scaffolds (PLLA-nanoHAp) consisting of a nanofibrous network that is interconnected with a microporous network (Ma and Zhang 1999; Wei and Ma 2004; Ma 2008; Smith et al. 2009). Biomimetic synthesis is a newer approach for the fabrication of uniformly dispersed nano-HAp within a polymer matrix. Ma et al. succeeded in controlling the release kinetics of the biological factors within a bio-mimetic macro- and nano-porous scaffold, which contained microspheres loaded with biological factors. The HAp nanoparticles provided osteoconductivestimuli and the microspheres loaded with regenerative factors provided the necessary osteoinductive environment for optimal bone regeneration (Ma 2004).

Liao et al. (2004) developed fibrous and biomimetic nano-HAp/collagen/PLA composite scaffolds with a hierarchical structure. This group fabricated mineralized collagen fibrils by assembling collagen molecules and nano-HAp, which further self-assembled into fibrillar bundles. These fibrillar bundles were found to be uniformly distributed throughout the polymer matrix. The 3D scaffold promoted in vitro osteoblast adhesion, spreading, and proliferation. Moreover, the hierarchical scaffold structure successfully integrated a 15 mm bone defect in a rat model. Likewise, other researchers were able to fabricate similar biomimetic nanocomposite scaffolds using various polymer matrices from gelatin (Kim et al. 2005) and chitosan (Kong et al. 2005).

### 24.4.2.2 Polymer–Nanotube Nanocomposites

A significant amount of literature describes nanocomposite biomaterials made from polymers and nanotubes. Scaffolds made of such materials should be mechanically robust to withstand in vivo mechanical stresses. Thermally induced phase separation can be used to prepare robust scaffolds from CNTs and polyurethane as reported by Jell et al. (2008). These nanocomposites have an anisotropic porous structure and nanoscale surface texture, and the compressive strength of the scaffold increases with increased CNTs content. However, the reported compressive strength is much lower compared to that of trabecular bone. Interestingly, this nanocomposite showed higher cell proliferation compared to a control (pure polyurethane) sample due to altered surface chemistry/architecture.

A similar study by Shi et al. (2006) showed an increase in the mechanical strength of injectable PPF nanocomposites after incorporating 0.2% of single wall carbon nanotubes (SWNTs). Furthermore, the functionalization of the SWNTs enhances the interaction between nanotubes and the PPF matrix, resulting into a threefold increase in the compressive modulus and a twofold increase in yield strength. The same group fabricated other porous nanocomposite scaffolds (PPF-SWNTs) using a thermal-cross-linking and particulate-leaching technique (Shi et al. 2007). Scaffolds with 100% interconnectivity were fabricated with 75%, 80%, 85%, and 90% controlled porosities. The compressive modulus decreased 100-fold when the porosity was increased from 75% to 90%. In vitro cultures confirmed that mesenchymal stem cells (MSCs) adhere and proliferate on all the scaffolds.

More recently, Sitharaman et al. (2008) studied the in vivo biocompatibility of porous PPF-SWNTs scaffolds in a rabbit model. Implants made of PPF-SWNTs, tested after 4 and 12 weeks, displayed only
mild inflammatory responses. Compared to a PPF control, the PPF-SWNT nanocomposite scaffolds showed increased collagen matrix production and significant bone in-growth after 12 weeks of implantation (Sitharaman et al. 2008).

24.4.2.3 Polymer–Silicate Nanocomposites

Previous research has shown that bioactive materials containing silicon, such as bioactive glass (silica) or silicon-doped calcium phosphate materials, exhibit excellent bioactivity and promote apatite formation in vitro and in vivo (Wu and Chang 2004; Wu et al. 2005, 2006). Moreover, high silicon content implants induce bone formation, stimulate osteogenic proliferation, and activate bone-related gene expression (Xynos et al. 2001; Valerio et al. 2004). Thus, it can be concluded that bioactive materials containing silicon may open new possibilities in the field of bone repair.

Kotela et al. (2009) proposed to develop nanocomposites from polycaprolactone and wollastonite nanoparticles for bone repair. Wollastonite is a calcium silicate (CaSiO$_3$) with bioactive properties. The addition of small amounts (0.5%–1%) of wollastonite significantly improved Young’s modulus, the tensile strength, and the fracture toughness of the polymer nanocomposite. The bioactivity of the nanocomposite was verified by submerging it in simulated body fluid. Apatite nucleation was observed on the wollastonite surfaces after 7 days. Similar results were obtained by Wei et al. (2008) on PCL-calcium silicate nanocomposites.

In a different approach, Heinemann and coworkers attempted to mimic the natural processes of biosilicification to fabricate silica–collagen hybrid xerogels under ambient conditions (Heinemann et al. 2007, 2009). This group used the sol–gel technique to fabricate monolithic composite materials by varying the ratio of organic (collagen) and inorganic (silica) components. As a result, the compressive properties of silica were substantially improved by the addition of collagen. To further enhance the bioactivity of the nanocomposite material, they incorporated a third phase (calcium phosphate cement). The addition of calcium phosphate accelerated the formation of bone apatite layers when tested in a simulated body fluid. The feasibility of using silica-based biomaterials for bone repair was shown by the differentiation of human monocytes into osteoclast-like cells.

Similar to the Heinemann study, Ehrlich et al. (2008) explored silica–chitin based natural bionanocomposites fabricated from living glass sponges. They showed that the chitinous organic matrix provides a template for the biodirected deposition of the mineral phase (silica) and that the resulting biocompatible structures could be used for the tissue engineering of both bone and cartilage replacements.

Highly repetitive amino acid sequences present in fibrous proteins, such as collagen and silk, can be explored to obtain self-assembled nanocomposites (Meinel et al. 2005; Wang et al. 2006; Wong Po Foo et al. 2006). Foo et al. fabricated biomimetic nanocomposites consisting of silica nanoparticles using fusion (chimeric) proteins (Wong Po Foo et al. 2006). Films and fibers were fabricated from spider silk protein, and silica nanoparticles were deposited on this polymer using silification reactions. The morphology and structure of the silica nanoparticles can be governed by controlling the processing conditions. The same group reported the chemical attachment of cell binding domains (Sofia et al. 2001) and growth factors (bone morphogenetic protein-2 [BMP-2]) (Karageorgiou et al. 2004) onto silk-based biomaterials to induce an osteogenic differentiation of human bone marrow stromal cells.

24.4.2.4 Polymer–Clay Nanocomposites

During the last few decades, nanocomposites from polymer and clay silicate have been the subject of intense fundamental studies. The knowledge gained on how to best tailor structure–property relationships can be applied to developing new functional biomaterials (Vaia and Wagner 2004; Sinha Ray and Bousmina 2005). For example, the strong specific interactions between silicate nanoparticle surfaces and polymer chains, combined with new fabrication techniques, allow the assembly of supramolecular structures over many length scales (Giannelis 1996; Alexandre and Dubois 2000; Hule and Pochan 2007). Besides covalent bonding, physical cross-linking via hydrogen bonding, Van der Waals, and ionic interactions are often responsible for the mechanical strength of materials and their extensibility.
(Zilg et al. 1999). While small amounts of silicate nanoparticles can dramatically change the physical properties of polymers (Giannelis 1996; LeBaron et al. 1999; Vaia and Wagner 2004; Sinha Ray and Bousmina 2005), higher amounts of silicate can lead to ultrastrong materials with hierarchical structures and properties that may approach the theoretical calculated maximum (Liff et al. 2007; Podsiadlo et al. 2007). Other physically cross-linked polymer nanocomposites exhibit structure similar to nacre and display directional dependent mechanical properties (Dundigalla et al. 2005; Gaharwale et al. 2011b).

Here, the silicate not only enhances the mechanical properties of the nanocomposite but also facilitates cell adhesion, spreading, and proliferation (Gaharwale et al. 2011a,b; Jin et al. 2009).

The addition of silicates to thermoresponsive polymer hydrogels can be used to tune phase transitions and control dissolution properties that are important for the sustained release of biomolecules (Wu and Schmidt 2009). The examples above show that, with further materials development and formulation, much of the fundamental research published can be translated for applications that focus on the repair of bone. The following studies show several more attempts in this direction.

Besides mechanical strength, the degradation rate is another important property of a scaffold that is to be used for bone repair. While the degradation rates of polymers can be easily modified, the degradation of natural clays such as Montmorillonite remains a challenge. Nevertheless, MMT can reinforce polymers significantly as shown by Lee et al. (2003), who modified the degradation rate of exfoliated PLLA-MMT nanocomposites by varying the amounts of MMT in a PLLA matrix. After the MMT-PLLA scaffold was immersed in water, the modulus of the scaffold decreased due to the degradation of the PLLA matrix (Lee et al. 2005). The authors, however, did not elaborate on what happened with the MMT once the polymer degraded.

In another study, Zhuang et al. (2007) showed that the intercalated structure of MMT–gelatin–chitosan has a lower degradation rate when compared to a gelatin–chitosan scaffold and that the degradation rate can be altered by changing the MMT concentration. Enhanced cell adhesion and proliferation on the MMT–gelatin–chitosan nanocomposite film was observed.

Ozkoc et al. (2009) fabricated porous PLA–MMT nanocomposites using microcompounding and polymer/particle leaching. The addition of MMT improved the compression properties of the nanocomposites to be close to those of cancellous bone. The hydrophilicity of the nanocomposite surfaces directly affected cell adhesion. For example, an addition of 3% MMT reduced the water contact angle from 60.7° to 31.4°. This is due to a decrease in the interfacial tension between polymer and water, making the PLA surface more hydrophilic.

Similarly, Krikorian et al. reported significant improvement in mechanical properties of PLLA due to the addition of MMT (Krikorian and Pochan 2003). Higher amounts of MMT and fully exfoliated structures gave rise to stiffer materials compared to microphase separated or intercalated composites. Exfoliated nanoplatelets suppressed polymer crystallization due to enhanced surface interactions. Moreover, an increase in silicate concentration and exfoliation resulted in stiffer and transparent PLLA-MMT nanocomposites. As mentioned before, Lee et al. (2003) tailored the mechanical properties of such nanocomposites by incorporating different amounts of MMT in a PLLA matrix. Highly porous (~92% porosity) scaffolds were fabricated via the salt leaching/gas foaming technique. An increase in MMT concentration also decreased the glass transition temperature. The decrease in crystallinity accelerated the degradation of PLLA-MMT nanocomposites. The tensile strength of the scaffold was modulated between 40 and 60 MPa, which fits the requirements for soft and hard scaffold applications.

More recently, Lewkowitz-Shpuntoff et al. (2009) reported that the addition of 10% cloisite clay to ethylene vinyl acetate dramatically increased osteoblast proliferation on the nanocomposite surface. Magnetic nanocomposites that were obtained by the physical adsorption of iron oxide nanoparticles on the cloisite surface showed enhanced proliferation and alignment of MC3T3 preosteoblast cells in a static magnetic field. The alignment of the cells in the magnetic field was attributed to an increase in the internal field near the cells. Previous research has shown that a magnetic field can stimulate osteogenesis and upregulate the transcription of BMP-2 and BMP-4 (Fitzsimmons et al. 1994; Bodamyali et al. 1998). Thus, external magnetic fields may influence tissue formation and cellular organization of cells.

The following studies show several more attempts in this direction.
Overall, these experimental results show that polymer nanocomposites have the potential to be used for the repair and regeneration of bone.

24.5 Biomaterials Used for Cartilage Tissue Engineering

The extracellular matrix of articular cartilage is principally composed of hierarchically arranged collagen fibrils, proteoglycans, and non-collagenous proteins on the nanometer scale (Figure 24.1 and Table 24.3) (Mow et al. 1980; Mankin 1982; Buckwalter and Mankin 1997; Cohen et al. 1998). The complex interactions between individual macromolecules synergistically provide an advanced mechanism for load-support and low-frictional properties (Akizuki et al. 1986; Heinegard and Oldberg 1989; Zhu et al. 1993; Jurvelin et al. 2003). If biomaterial scaffolds are considered for cartilage tissue repair, they need to satisfy some of the requirements mentioned before.

The importance of developing biomaterials for cartilage tissue engineering is warranted by the more than 600,000 joints, including knees and hips, that are replaced annually in the United States (see website: http://www.aaos.org). The failure of articular cartilage usually results from trauma, arthritis, or sports injuries. Articular cartilage has a very limited capability to repair and regenerate due to the absence of blood vessels and nerves in the tissue (Cohen et al. 1998; Hunziker 2002). If cartilage defects are smaller than 2–4 mm, they can be healed by a continuous passive motion of the joint (O’Driscoll 1998) or by techniques such as subchondral drilling, abrasion, microfracture, and the administration of bioactive agents such as growth factors and cytokines (O’Driscoll et al. 1986; O’Driscoll 1998). However, for large cartilage defects, the replacement of the whole knee or hip with artificial components (metal, ceramic, composite, etc.) is employed (Hunziker 2002). These artificial components do not fully restore joint function, and surgeries are generally not recommended for young people due to the limited life span of the artificial components (O’Driscol 1998). Other commonly used strategies for cartilage repair include autografts and allografts (Mankin 1982; O’Driscol 1998; Hunziker 2002). Disadvantages associated with autografts include lack of donor sites, complications from surgical procedures, and an increased risk of inflammation at the donor sites. Additionally, allografts are associated with increased immune response and disease transmission (Parikh 2002; Fujishiro et al. 2008).

Tissue engineering approaches have shown promise to regenerate damaged articular cartilage and to help regain normal body functions (Temenoff and Mikos 2000; Risbud and Sittinger 2002; Capito and Spector 2003). Engineered polymeric scaffolds need to provide necessary support for cells to proliferate and mechanical strength to keep the new tissue in place. The scaffold materials that have been investigated for cartilage repair can be classified into two major categories: (1) polymers (natural and synthetic) and (2) microcomposites. Although polymers have attractive chemical and biological properties, they often do not have sufficient mechanical strength. Therefore, polymers are often combined with ceramic nanoparticles to reinforce the structural and mechanical strength via strong polymer–nanoparticle interactions (Hule and Pochan 2007; Winey and Vaia 2007). Even the nanotopography of specific fibrous biomaterials has been shown to be important to chondrogenesis (Savaiano and Webster 2004; Park et al. 2005; Moutos et al. 2007; Stoddart et al. 2009).

24.5.1 Available Biomaterials for Cartilage Repair

There is a large body of literature, and some comprehensive reviews cover the numerous biomaterials that have been considered for cartilage repair (Temenoff and Mikos 2000; Risbud and Sittinger 2002; Capito and Spector 2003; Bonzani et al. 2006). Since the tissue engineering of cartilage does not require the inclusion of extensive vascularization, tissue engineered cartilage was one of the first products to be tested in human trials (Khademhossein et al. 2009).

The most commonly used biomaterials are natural and synthetic polymers (Hutmacher 2000; Capito and Spector 2003; Sharma and Elisseff 2004; Cheung et al. 2007), many of which have been optimized to
not only provide 3D support to cells but also to maintain their differentiated phenotype and to encourage cell migration and proliferation (Temenoff and Mikos 2000; Hunziker 2002; Kloxin et al. 2009). Nevertheless, many of the polymer scaffolds do not have sufficient mechanical strength to continuously support the formation of cartilage tissue.

Natural polymers such as collagen, hyaluronic acid (HA), alginate, silk fibroin, and chitosan have been extensively studied (Hutmacher 2000; Temenoff and Mikos 2000; Risbud and Sittinger 2002; Capito and Spector 2003). A key advantage of using natural polymers is their ability to interact with cells and cellular enzymes, and to be remodeled and/or degraded when space for growing tissue is needed. Although synthetic polymers such as PLA, PGA, PLGA, PCL, and polycarbonate (Hutmacher 2000; Capito and Spector 2003) are not bioactive when compared to natural polymers, they do provide more control over physical and chemical properties. If these properties are not satisfactory for a specific application, the addition of ceramic micro- and nanoparticles may enhance mechanical strength, degradation rate, bioactivity, and surface chemistry (Kokubo et al. 2003; Mano et al. 2004). In the following section, we outline current trends in the development of nanocomposite materials for cartilage repair (Table 24.5).

### 24.5.2 Polymer Nanocomposite Biomaterials for Cartilage Repair

#### 24.5.2.1 Polymer–Hydroxyapatite Nanoparticle Composites

Although polymer hydroxyapatite nanoparticle composites have been most extensively explored for bone repair, several studies also attempt to use these materials for cartilage regeneration. For example, Li et al. (2009) studied the feasibility of using PLGA/nano-HAp nanocomposite for cartilage repair in a rat model. A nanofibrous mesh was made from PLGA using electrospinning and nano-HAp was deposited on the fiber surface using a sol–gel technique. MSCs were seeded on the nanofibrous scaffold and the interconnected network allowed uniform cell in-growth throughout the scaffold. After 12 weeks of implantation with MSC seeded scaffolds, the defects were perfectly integrated with the surrounding tissue and had a smooth surface morphology. Although, the nanocomposite scaffolds were not fully degraded after 12 weeks of implantation, they facilitated the formation of cartilage tissue.

Most recently, in 2009, Spadaccio et al. (2009) developed PLLA/nanoHAp nanocomposites for cartilage regeneration by utilizing fibrous scaffolds combined with molecular signaling mechanisms.

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Polymer</th>
<th>Preparation Method</th>
<th>Remarks</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano-HAp</td>
<td>PLLA</td>
<td>Electrospinning</td>
<td>Intense in vitro deposition of proteoglycans and GAGs.</td>
<td>Spadaccio et al. (2009)</td>
</tr>
<tr>
<td>Nano-HAp</td>
<td>PVA</td>
<td>Solvent mixing</td>
<td>Increased storage modulus and elastic properties.</td>
<td>Pan et al. (2008)</td>
</tr>
<tr>
<td>Au</td>
<td>Collagen II</td>
<td>Solvent mixing</td>
<td>Promoted chondrocyte proliferation.</td>
<td>Hsu et al. (2007)</td>
</tr>
<tr>
<td>Titania</td>
<td>PLGA</td>
<td>Solvent casting and NaOH treated</td>
<td>Nanostructure roughness promoted chondrocyte attachment.</td>
<td>Kay et al. (2002)</td>
</tr>
<tr>
<td>CNTs</td>
<td>PCU</td>
<td>Solvent casting</td>
<td>Nano-surface roughness and electrical stimulation promoted chondrogenesis.</td>
<td>Khang et al. (2008)</td>
</tr>
<tr>
<td>CNTs</td>
<td>Collagen I</td>
<td>Solvent mixing</td>
<td>Despite high CNTs concentration high cell viability and proliferation were observed.</td>
<td>MacDonald et al. (2005)</td>
</tr>
</tbody>
</table>
The fibrous scaffold architecture facilitates initial cell attachment and the subsequent migration of human-MSC. Nano-HAp plays an important role in the differentiation of stem cells into chondrocyte-like cells that produce proteoglycan. After 14 days of culturing human-MSCs within a PLLA/nanoHAp scaffold in chondrogenic media, significantly higher amounts of chondrogenic transcription factors such as SOX9 were expressed (these activate type II collagen and aggrecan production) (Bosnakovski et al. 2006) compared to a PLLA scaffold control (Spadaccio et al. 2009). Moreover, neo-ECM was observed around the cells, as the scaffold stained positive for Toluidine Blue and Safranin O. Overall, the nanocomposite scaffolds show more deposition of proteoglycans and glycosaminoglycans (GAGs) when compared to the neat polymer scaffolds (Spadaccio et al. 2009).

Besides PLGA, polymers, such as poly (vinyl alcohol) (PVA), have been tested for cartilage repair. When nano-HAp is combined with a PVA hydrogel, the mechanical properties of the gel improve and the nanocomposite becomes bioactive (Pan et al. 2008). The storage modulus of the hydrogel also increased with the increasing frequency of deformation, which is very similar to that of articular cartilage. The overall elastic properties of hydrogel increased with the increasing nano-HAp concentration (less than 6%) due to the cross-linking of PVA and nano-HAp. However, at higher concentrations (greater than 6%), nanoparticles aggregate and weaken the composite network structure. As PVA alone is neither biodegradable nor bioactive, long-term implantation remains a problem.

### 24.5.2.2 Polymer–Metal Nanoparticle Composites

The unique optical, electronic, and biological properties of metallic nanoparticles, such as gold (Au), allow the development of many biomedical applications (especially in cancer research). Recently, some researchers are trying to use metallic gold in cartilage repair. For example, Hsu et al. (2007) demonstrated that the incorporation of Au-nanoparticles within type II collagen hydrogels increases the mechanical properties and the antioxidative effects of the resulting nanocomposite. Interactions between the positively charged Au nanoparticles and the negatively charged collagen fibrils (below the isoelectric point), allow the Au nanoparticles to adhere to the collagen fibrils at low concentrations (0.1% Au). At higher concentrations (more than 0.2% Au) Au aggregates are formed and a decrease in the dynamic storage modulus is observed. Below a 10 ppm concentration, Au nanoparticles show very good biocompatibility and do not modify gene expression if internalized. The authors claim that the nanocomposites containing 0.1% Au nanoparticles promote chondrocyte proliferation and may activate certain genes that are responsible for sensing surface roughness (filopodia and lamellipodia were observed) (Hsu et al. 2007).

The surface modification of nanocomposite scaffolds can promote chondrocyte adhesion and proliferation. Surface properties such as surface area, roughness, and charge can be easily altered by changing the type and amount of nanoparticles. Research done by Kay et al. (2002) demonstrated that nanostructure roughness promotes chondrocyte adhesion in PLGA–titania nanocomposites. The PLGA scaffold was first treated with a NaOH solution to develop nanostructured features. Then different sizes of titania particles (micron- or nano-sized grains) were added. Increased chondrocyte adhesion was observed on the nanostructured PLGA films (NaOH treated) when compared to conventional PLGA films. The study also showed that 1.5–2 times more chondrocytes attached to the nanocomposite (PLGA/titania) surfaces compared to the microcomposite PLGA/titania surfaces. The same group demonstrated that PLGA–titania nanocomposites can alter long-term chondrocyte responses (Savaiano and Webster 2004). Although the total number of cells seeded on the nanocomposite films remained constant, after 21 days of culture the total amount of intracellular proteins (alkaline phosphatase and chondrocyte expressed protein-68 [CEP-68]) almost doubled when compared to the control. These results suggest that titania-containing polymer nanocomposites may have potential for studying chondrocyte function, which is important in cartilage repair.

### 24.5.2.3 Polymer–Nanotube Nanocomposites for Cartilage Repair

CNTs have long been considered for hard and soft tissue engineering as their unique mechanical (flexural and fatigue strength, high strength-to-weight ratio) and electrical properties are desired (MacGinitie
et al. 1994; Harrison and Atala 2007). The structural arrangements of CNTs on a nanometer length scale mimic the ECM network and provide better biocompatibility when compared to other nanoscale materials. The natural ECM has a nanotopography that allows cells to attach and interact via adhesion proteins. Thus, it is expected that nanotopography significantly influences cell orientation, morphology, and cytoskeleton arrangements.

In a related study, Khan et al. (2008) provided direct evidence of the influence of surface roughness and electrical stimulation on chondrocyte function. These authors observed an increase in chondrocyte density when cells were grown on CNTs–polycarbonate urethane. Compared to the individual components, the nanocomposite displays significantly higher surface roughness and hydrophilicity. The electrical conductivity of CNTs was used to increase the cell density on the nanocomposite surface via electrical stimulation. Previous studies already indicated that electric fields influence cartilage growth, remodeling, and biosynthesis (MacGinitie et al. 1994; Wang et al. 2004) but the long-term effect of CNTs and electrical stimulation on chondrocyte activity is not yet known. Another approach to mimic natural ECM is to use collagen as a matrix, as collagen is a major component of ECM. Thus, collagen-CNT nanocomposites should provide a suitable environment for cell growth. MacDonald et al. (2005) suggested that collagen-CNT scaffolds might be used for cartilage repair. In their work, CNTs were physically entrapped within a collagen matrix with no evidence of chemical interaction between polymer and CNT. Although the resulting nanocomposites had a high amount of SWNTs and delayed gelation characteristics, cell viability and cell proliferation were high (MacDonald et al. 2005).

### 24.6 Osteochondral Tissue Engineering

Osteochondral defects often occur from repetitive trauma to the joints (Buckwalter et al. 1994). Such injuries lead to loss of bone tissue and the formation of cystic lesions, which may cause the collapse of the remaining cartilage tissue (Buckwalter 1992; Ulrich-Vinthner et al. 2003). Osteochondral defects can also result from genetic or metabolic causes, which alter the structural and mechanical properties of the joints. Most often, such defects are repaired by mosaicplasty, in which damaged cartilage is replaced by healthy cartilage that is removed from non-weight-bearing regions of the body (Hangody et al. 2008). In 1994, Brittberg et al. (1994) demonstrated for the first time that the implantation of in vitro cultured human chondrocytes led to the formation of hyaline-like articular cartilage. Such therapies, however, cannot be used for repairing large defects as graft sites are limited and donor site morbidity is a problem. For repairing large defects, osteochondral scaffolds are needed (Sharma and Eliseeff 2004; Mikos et al. 2006; Martin et al. 2007; Grayson et al. 2008; Keeney and Pandit 2009). These scaffolds promote the regeneration of articular cartilage and subchondral bone while maintaining mechanical stability. Osteochondral scaffolds have several design issues, which are not satisfactorily addressed. Because bone and cartilage have different physical, chemical, and biological properties, an osteochondral scaffold should mimic the structural and mechanical properties of both tissues. This can be done by fabricating layered scaffolds for bone and cartilage (Martin et al. 2007; O’Shea and Miao 2008; Keeney and Pandit 2009). To integrate the bone and cartilage regions within one scaffold, suturing, cell-mediated ECM formation, and the use of glues have been applied (Schafer et al. 2000; Wang et al. 2007; Moroni and Eliseeff 2008). The cartilage layer is seeded with chondrocytes or MSCs, and the bone layer remains acellular or is seeded with osteocytes, MSCs, peristomal cells, or bone marrow. If this approach is used for cell-based therapies, the age of the donor and host directly affects the proliferation and the chondrocytic/osteocytic potential of the implanted cells (Morihara et al. 2002).

#### 24.6.1 Biomaterial Scaffolds Used for Osteochondral Repair

A number of synthetic and natural biomaterials have been investigated for osteochondral repair (Yaylaoglu et al. 1999; Martin et al. 2007; Grayson et al. 2008; Tampieri et al. 2008). Usually, the natural materials provide enhanced biological interaction with the host tissue and can accelerate the healing
Polymer microcomposite scaffolds can combine properties such as gradient structure, composition, bioactivity, and mechanical properties (Martin et al. 2007; Hangody et al. 2008; Keeney and Pandit 2009). Porous osteochondral scaffolds with a gradient in structure, composition, porosity, and mechanical properties can also be fabricated by a 3D printing process (Sherwood et al. 2002). This printing process allows the generation of scaffolds with relevant biological and anatomical features.

Scaffolds with predictable architecture and porosity usually have predictable mechanical properties (Schek et al. 2004). One way to enhance the mechanical properties of a scaffold for osteochondral repair is to combine polymers with nanoparticles to form nanocomposites (Table 24.6).

### 24.6.2 Polymer Nanocomposites for Osteochondral Repair

The concentration and structural orientation of HAp nanoplatelets varies significantly within the bone–cartilage interface. The change in HAp concentration across the interface leads to differences in mechanical properties that need to be considered when developing gradient scaffolds.

A functionally graded nanocomposite scaffold from biodegradable PCL and nanoparticle TCP (Erisken et al. 2008) can be made by a new hybrid twin-screw extrusion/electrospinning process, which was reported by Erisken et al. (2008). The resulting nonwoven, highly interconnected nanofibrous scaffold

<table>
<thead>
<tr>
<th>Bone Region</th>
<th>Cartilage Region</th>
<th>Preparation Method</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL/TCP</td>
<td>PCL</td>
<td>Twin-screw extrusion/electrospinning</td>
<td>Highly interconnected nanofibrous mesh with spatial gradations in composition and porosity. Supported differentiation of preosteoblast to osteoblast cells.</td>
</tr>
<tr>
<td>Collagen/nano-HAp</td>
<td>Collagen</td>
<td>Biomimetic</td>
<td>Closely mimics the structural and compositional properties of native tissue. Supported osteogenic and chondrogenic differentiation based on changes in protein expression.</td>
</tr>
<tr>
<td>MSCs ECM/nano-HAp</td>
<td>MSCs ECM/Nano-HAp</td>
<td>Alternate soaking process</td>
<td>Fabrication method is faster than biometric process. Crystal size of HAp controlled by number of cycles or ion concentrations. In vivo study indicated the osteoinductive nature of scaffold.</td>
</tr>
<tr>
<td>Collagen/nano-HAp</td>
<td>Collagen/hyaluronic acid</td>
<td>Synchronous biomineralization/freeze drying</td>
<td>Bilayered composite was chemically cross-linked to eliminate the risk of delamination.</td>
</tr>
<tr>
<td>Collagen/nano-HAp (30/70)</td>
<td>Collagen/nano-HAp (60/40)</td>
<td>Biomimetic/freeze drying</td>
<td>Supported osteogenic and chondrogenic differentiation in vitro.</td>
</tr>
</tbody>
</table>

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**Table 24.6** Nanocomposites for Bone–Cartilage Repair

- **Bone Region**: PCL/TCP, Collagen/nano-HAp, MSCs ECM/nano-HAp, Collagen/nano-HAp (30/70), Collagen/nano-HAp (60/40)
- **Cartilage Region**: PCL, Collagen, MSCs ECM/Nano-HAp, Collagen/hyaluronic acid, Collagen/nano-HAp (60/40)
- **Preparation Method**: Twin-screw extrusion/electrospinning, Biomimetic, Alternate soaking process, Synchronous biomineralization/freeze drying, Biomimetic/freeze drying
- **Remarks**: Highly interconnected nanofibrous mesh with spatial gradations in composition and porosity. Supported differentiation of preosteoblast to osteoblast cells. Closely mimics the structural and compositional properties of native tissue. Supported osteogenic and chondrogenic differentiation based on changes in protein expression. Fabrication method is faster than biometric process. Crystal size of HAp controlled by number of cycles or ion concentrations. In vivo study indicated the osteoinductive nature of scaffold. Bilayered composite was chemically cross-linked to eliminate the risk of delamination. Supported osteogenic and chondrogenic differentiation in vitro.
- **References**: Erisken et al. (2008), Dawson et al. (2008), Matsusaki et al. (2009), Gelinsky et al. (2008), Tampieri et al. (2003)
has spatial gradients in composition and porosity and the tensile strength can be tailored to increase from bottom to top. When the nanocomposite scaffold was seeded with preosteoblastic (MC3T3-E1) cells, most of the cells differentiated into osteoblast cells within 4 weeks of culture. Moreover, the newly formed ECM showed collagen I and mineral deposits indicating the activity of bone cells. The formation of bone-like structures increased the modulus of the cell seeded nanocomposite scaffold to almost double its starting value (Erisken et al. 2008).

Dawson et al. (2008) and Sachlos et al. (2006) fabricated improved nanocomposite scaffolds that mimic the structure and composition of bone and cartilage. The bi-layered scaffolds consist of both bone and cartilage regions. The bone region of the scaffold was composed of nano-sized HA crystals that were precipitated on a fibrous network of collagen (ColHAp). The presence of microchannels in the bone region supported the perfusion of nutrient-rich media (Sachlos et al. 2006; Dawson et al. 2008). The bone region was found to support the osteogenic differentiation of human bone marrow stromal cells (HBMSCs) (Dawson et al. 2008). After 28 days of in vitro culture, the scaffold showed high alkaline phosphatase (ALP) activity and significant cell penetration, and integration throughout the scaffold. The implanted (in mice) ColHAp scaffold showed complete integration with the surrounding tissue after only 4 weeks (Dawson et al. 2008). The cartilage region of the scaffold was composed of collagen type I (Col) with predefined internal channels. Solid freeform fabrication and critical point drying techniques were used to fabricate the collagen scaffold with complex internal morphology (Sachlos et al. 2003; Dawson et al. 2008). The in vitro culture of the scaffold in chondrogenic media showed the formation of a dense proteoglycan and collagen II rich matrix (Dawson et al. 2008). The scaffold supported the chondrogenic differentiation of HBMSCs that was evident from the protein expression of SOX9 and the mRNA expression of collagens IX and XI, aggrecan, and proteoglycans (Dawson et al. 2008). Furthermore, the incorporation of microchannels into the scaffold enhanced chondrogenesis. Overall, the collagen-based scaffolds (Col and ColHAp) not only supported the growth and differentiation of stem cells, but the study also provided strong clues for improving chondrogenesis and osteogenesis.

Matsusaki et al. (2009) used an alternate soaking process to deposit HAp crystals on a tissue-engineered scaffold composed of MSCs and the ECM produced by the cells. The HAp deposited by this process had low crystallinity and was biodegradable. Compared to a biomimetic process, the alternate soaking process was almost a hundred times faster. The crystal size of HAp particles can be increased by increasing the number of cycles or the concentration of ions. However, exposure to higher calcium or phosphorus concentrations (more than 100 mM) during the process led to DNA damage and cell death. Moreover, the in vivo studies demonstrate that the engineered scaffold was osteoinductive.

One of the major problems associated with fabricating layered osteochondral grafts is the delamination of the bone and cartilage regions. In order to avoid delamination, Gelinsky et al. (2008) fabricated monolithic scaffolds composed of two layers that were fused together by a unified cross-linking process. The bone layer consisted of mineralized composite (collagen I/HAp nanocomposite) and the cartilage layer consisted of non-mineralized composite (collagen I/hyaluronic acid [HA]). The mineralized composite can be synthesized by using the synchronous biomineralization of collagen (Bradt et al. 1999; Meyer et al. 2009). In this process, the assembly of collagen fibrils was initiated and the growing fibrils acted as a template for HAp crystallization. The non-mineralized composite was formed by adding HAp during the assembly of collagen fibrils. To fabricate the monolithic scaffold, the freeze-dried bi-layered composite was chemically cross-linked. The unified cross-linked scaffold eliminated the risk of the delamination of mineralized and non-mineralized layers.

As the mechanical properties of cartilage are very different from those of bone, one has to design scaffolds that reduce the mismatch of the properties at the interface. In order to do so, Tampieri et al. (2008) fabricated a tri-layered scaffold, where the bony layer was composed of biomineralized collagen (70% HAp and 30% collagen I), the cartilage layer was composed of hyaluronic acid–collagen composite, and the intermediate layer was composed of low-density biomineralized collagen (40% HAp and
60% collagen I). The biomineralized collagen was fabricated by nucleating hydroxyapatite nanocrystals on self-assembled collagen fibers (Tampieri et al. 2003). The HAp nanocrystals grew on the collagen fibers with their c-axis oriented along the fiber axis. An integrated monolithic composite was fabricated by freeze drying the stacked tri-layered scaffold (Tampieri et al. 2008). Although the flexural strength of the biomineralized layer decreased with increasing porosity, the elastic modulus was in the range of trabecular bone. Moreover, a pulling test did not result in delamination, indicating strong adhesion between the layers. In chondrogenic media, MSCs differentiated into chondrocytes and formed cartilaginous tissue within the hyaluronic acid–collagen layer. After 8 weeks, the nanocomposite scaffold showed bone tissue in the bone region and connective tissue in the cartilage region.

24.7 Conclusions

The literature reviewed here has shown that the synergistic combinations of chemical, physical, and biological properties of nanocomposite biomaterials used for bone and cartilage tissue engineering have become more sophisticated. In a similar way, the fabrication technologies and engineering approaches have advanced. Nevertheless, significant challenges persist when “giving life” to these scaffolds. This suggests that both the material science perspective and the biology that figures out how cells interact with the scaffolds and with each other and how genes and proteins influence these interactions are needed for biomaterials design (Khademhosseini et al. 2009).

Acknowledgments

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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Au</td>
<td>gold</td>
</tr>
<tr>
<td>BMP</td>
<td>bone morphogenetic protein</td>
</tr>
<tr>
<td>CNT</td>
<td>carbon nanotube</td>
</tr>
<tr>
<td>ECM</td>
<td>extra cellular matrix</td>
</tr>
<tr>
<td>GAG</td>
<td>glycosaminoglycans</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamic acid</td>
</tr>
<tr>
<td>HA</td>
<td>hyaluronic acid</td>
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<tr>
<td>Hap</td>
<td>hydroxyapatite</td>
</tr>
<tr>
<td>HBMSC</td>
<td>human bone marrow stromal cells</td>
</tr>
<tr>
<td>MMT</td>
<td>mmtmorillonite</td>
</tr>
<tr>
<td>MSC</td>
<td>mesenchymal stem cell</td>
</tr>
<tr>
<td>PCL</td>
<td>poly(caprolactone)</td>
</tr>
<tr>
<td>PEG</td>
<td>poly(ethylene glycol)</td>
</tr>
<tr>
<td>PEO</td>
<td>poly(ethylene oxide)</td>
</tr>
<tr>
<td>PGA</td>
<td>poly(glycolic acid)</td>
</tr>
<tr>
<td>PLA</td>
<td>poly(lactic acid)</td>
</tr>
<tr>
<td>PLGA</td>
<td>poly(lactic-co-glycolic acid)</td>
</tr>
<tr>
<td>PLLA</td>
<td>poly(l-lactic acid)</td>
</tr>
<tr>
<td>PPF</td>
<td>poly(propylene fumarate)</td>
</tr>
<tr>
<td>PVA</td>
<td>poly (vinyl alcohol)</td>
</tr>
<tr>
<td>SWNT</td>
<td>single wall carbon nanotubes</td>
</tr>
<tr>
<td>TCP</td>
<td>tricalcium phosphate</td>
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</tbody>
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References


Ratner, B. D. and S. J. Bryant. 2004. Biomaterials: Where we have been and where we are going. Annual Review of Biomedical Engineering 6 (1):41–75.


