3.1 Introduction

Advances in chemistry, physics, engineering, and material sciences have enabled the preparation, synthesis, and manufacturing of materials on the nanometer scale, which offers tremendous opportunities to control material properties, to mimic hierarchical structures of biological composites by engineered materials, and to adjust interactions of a material with biological molecules or a biological system (Zhang and Webster 2009). The prefix “nano” in nanomaterials typically defines structures that are smaller than 100 nm in at least one dimension. Due to a very large surface-to-volume ratio, the unique properties of nanomaterials originate from cohesive and/or adhesive interactions at the surface of the material in combination with the materials’ bulk properties. In contrast, the properties of materials at a larger size scale are dominated almost exclusively by their bulk characteristics. The surface of nanoceramics, for example, is more active in terms of dissolution and recrystallization processes and the interaction with organic
molecules as compared to micrometer-sized crystallites. Also, ceramics, which in general suffer from low elasticity, may offer significant ductility before failure when synthesized at the nanoscale (Karch et al. 1987). Nanoscaled ceramics can be sintered at a lower temperature, which reduces processing problems associated with high temperature. Due to strong interactions with organic molecules, nano-sized ceramics can exhibit bioactivity and affect the adhesion, proliferation, and differentiation of cells in direct contact (Webster et al. 1999; LeGeros 2008).

The chapter focuses on providing a summary of ceramic nanostructures that are used as biomaterials. The term “biomaterials” defines natural and synthetic nonviable materials that are intended to interact with biological systems for any diagnostic or biomedical purpose, including the treatment, augmentation, or replacement of any tissue or function of the organism (Hench 1980; Veerapandian and Yun 2009). Ceramic materials by definition are typically obtained from nonmetallic inorganic solids through the application of heat and include crystalline and amorphous materials. Ceramic materials include inorganic oxides, non-oxides, and composites. Certain minerals, especially calcium phosphate minerals, will also be discussed in this chapter as they are the predominant inorganic component of hard tissues. By mass, calcium is the most abundant metal in the human body (Frieden 1972). Due to the physiological properties, calcium minerals are frequently used as biomaterials for orthopedic, maxillofacial, and dental applications.

### 3.2 Ceramic Nanobiomaterials

Several ceramics and minerals have been used as nanobiomaterials in various applications including implant coatings, nanocomposite materials for implants, and cell-carrying scaffolds as well as drug delivery systems and diagnostic devices (Vallet-Regi 2001; Dorozhkin 2010a). In order to be used as a biomaterial, the ceramic nanostructures should exhibit good biocompatibility. A biocompatible material typically can be further classified into three major groups according to the degree of interaction of the material with host tissue (Vallet-Regi 2001). When there is no significant interaction or remodeling, the material is classified as bioinert. This property is favorable when a diagnostic or drug delivery application is envisioned or when a device for permanent replacement of a certain organ or tissue function is designed. Biomaterials that are chemically degraded or metabolized in any way prior to renal or biliary elimination are classified as biodegradable or bioresorbable and preferably used in drug delivery and regenerative applications. Materials with the ability to directly or indirectly influence the development of cells or tissues in contact or close proximity are generally categorized as bioactive. Bioactive materials can be especially beneficial in regenerative applications or to promote hard tissue implant integration. As far as interactions with bony tissue are considered, a bioactive material can either be osteoconductive or osteoinductive (Habibovic and de Groot 2007; Kalita et al. 2007). The latter describes a material’s ability to support osteogenesis, the formation of new bone, even in an ectopic environment in vivo. Osteoconduction describes the process of guided growth of bony tissue along the surface of a bio-material implant away from the initial bone–biomaterial interface.

Compared to other nanobiomaterials, ceramics can combine excellent biocompatibility and bioactivity with mechanical properties appropriate to be used in load-bearing orthopedic applications (Kalita et al. 2007). Looking at the mechanical properties of ceramics more closely, this group of materials is characterized by high stiffness and high resistance to wear and corrosion but low toughness and resilience (Murugan and Ramakrishna 2006). Polymers on the other hand are less stiff but more flexible and resilient. The degradation properties of polymeric materials can be varied over a wide range and chemical surface modifications can be achieved relatively easy (Gunatillake and Adhikari 2003; Drotleff et al. 2004). This makes polymer–ceramic composites very attractive biomaterials as such materials combine the flexibility and resorption properties of a polymer with the mechanical strength and bioactivity of a ceramic biomaterial (Kalita et al. 2007; Dorozhkin 2010b). Metals and alloys are a group of biomaterials with excellent strength and toughness but also significantly higher densities than ceramics. The bioactivity of metals and alloys is typically low, which makes a surface coating with a ceramic nanophase very attractive.
For more than 50 years, specialty bioceramics such as alumina, zirconia, hydroxyapatite, di- and tricalcium phosphates, and bioactive glasses have evolved and found applications in many biomedical applications, particularly as bone substitutes and components of orthopedic, dental, and maxillofacial implants due to the above mentioned advantages (Hench 1998). An overview of important ceramics and minerals that have been used as nanobiomaterials is given in the following paragraphs. The most prominent class is the calcium phosphates, because such minerals constitute the vast majority of inorganic mass in the human body.

### 3.2.1 Calcium Phosphate Minerals

Calcium phosphates are chemically stable low density minerals composed of ions commonly found in physiological environment (Table 3.1) (Kalita et al. 2007; Dorozhkin 2010b). In general, they exhibit excellent biocompatibility, which is likely due to their compositional similarity with bone mineral and is the main reason for the copious *in vivo* applications these materials have been used in. Today, calcium phosphates are probably the most important biomaterials in dentistry and orthopedics. This versatile group of biomaterials exists in different forms and phases depending on temperature, partial pressure of water, and the presence of impurities. Hydroxyapatite/Hydroxylapatite (HA), β-tricalcium phosphate (β-TCP), α-tricalcium phosphate (α-TCP), biphasic calcium phosphate (BCP), monocalcium phosphate monohydrate (MCPM), and unsintered apatite are different calcium phosphate minerals with different chemical and mechanical properties (Kalita et al. 2007; Dorozhkin 2010a). HA, which resembles the mineral phase of bone closest, has strong bioactivity and resists hydrolytic degradation. Tricalcium phosphates, on the other hand, are resorbable minerals that, upon hydrolysis, are transformed into more stable calcium phosphates, such as HA *in vivo*.

In traditional applications, calcium phosphates were used because of their biocompatible chemistry (Dorozhkin 2010a). When it became clear that the unique mechanical properties of bone tissue originated from an evolved interplay between mineral nanocrystals and collagen microfibers (Fratzl et al. 2004), the dimensional component of the minerals also became a design criterion. The apatite crystals, which occur in the form of plates or needles, are about 40–60 nm long, 20 nm wide, and 1.5–5 nm thick (Kalita et al. 2007). The crystals, which constitute two-thirds of the bone mass, are oriented along the long axis of the collagen fibers forming a continuous phase.

Calcium phosphate minerals, besides being well biocompatible, are classified as bioactive materials (LeGeros 2008). In contact with biological tissues such as bone, porous constructs, or coated surfaces have been shown to be osteoconductive. It has also been observed that certain calcium phosphates have osteoinductive properties. In part, the bioactivity of these materials is attributed to the surface roughness.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical Formula</th>
<th>Ca/P Ratio</th>
<th>Crystal Structure</th>
<th>Density [g·cm⁻³]</th>
<th>Solubility −log(Ks) (25°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dicalcium phosphate dihydrate</td>
<td>CaHPO₄·2H₂O</td>
<td>1</td>
<td>Monoclinic, Ia</td>
<td>2.32</td>
<td>6.59</td>
</tr>
<tr>
<td>α-Tricalcium phosphate (α-TCP)</td>
<td>Ca₃(PO₄)₂</td>
<td>1.5</td>
<td>Monoclinic, P2₁/a</td>
<td>2.86</td>
<td>28.9</td>
</tr>
<tr>
<td>β-Tricalcium phosphate (β-TCP)</td>
<td>Ca₅(PO₄)₂</td>
<td>1.5</td>
<td>Pure hexagonal, rhombohedral, R3cH</td>
<td>3.07</td>
<td>25.5</td>
</tr>
<tr>
<td>Hydroxyapatite (HA)</td>
<td>Ca₁₀(PO₄)₆(OH)₂</td>
<td>1.67</td>
<td>Hexagonal, P6₃/m or monoclinic, P2₁/b</td>
<td>3.16</td>
<td>116.8</td>
</tr>
<tr>
<td>Tetracalcium phosphate</td>
<td>Ca₄P₂O₈</td>
<td>2</td>
<td>Monoclinic, P2₁</td>
<td>3.05</td>
<td>38–44</td>
</tr>
</tbody>
</table>

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of nanostructured materials (Dorozhkin 2010b). The osteoinductive properties, however, cannot be directly attributed to the ceramic and are often described as intrinsic (Habibovic and de Groot 2007; LeGeros 2008). The current understanding is that a specific topography comprising interconnected porosities and concavities allows for the absorption, entrapment, and concentration of osteogenic factors such as bone morphogenetic proteins from surrounding body fluids (in vivo) or serum-containing media (in vitro).

3.2.1.1 Hydroxyapatite/Hydroxylapatite

Hard and mineralized tissues such as bone, dentin, and enamel all contain calcium phosphate minerals from the apatite group as the predominant inorganic component (Bigi et al. 1997; LeGeros 2002). These apatite minerals are formed from calcium, phosphorous, oxygen, and one or more channel-filling ion(s), such as chloride, fluoride, or hydroxyl ions. Depending on the exact chemical composition, the apatite’s properties vary, making this group of minerals very flexible. Substitutions in the chemical composition affect the structure and key properties, including solubility, hardness, brittleness, thermal stability, and also optical properties. HA [Ca$_5$(PO$_4$)$_3$(OH)] is an hydroxyl-containing apatite with very specific structure and properties. HA is often described as the main apatite in bone and enamel and due to its natural occurrence a candidate biomaterial (Dorozhkin 2010b). Taking a closer look, bone apatite, enamel apatite, and dentin apatite all slightly differ from the chemical structure of HA; and these differences determine the different properties of these tissues (Wopenka and Pasteris 2005). Via ionic substitution, enamel apatite in contrast to bone apatite has become resistant to dissolution. In comparison to synthetic HA, bone and dentin apatite have also been identified to be less ordered in structure and to contain predominantly carbonate anions instead of hydroxyl ions, which affects the morphology of the apatite. The following approximated formula has been proposed to characterize bone apatite: (Ca,$X$)$_{10}$(PO$_4$,$HPO_4$,$CO_3$)$_{6}$(OH,$Y$)$_2$, with $X$ representing cations (magnesium, sodium, strontium ions) that can substitute for the calcium ions and $Y$ representing anions (chloride or fluoride ions) that can substitute for the hydroxyl group (LeGeros 2002). Pure carbonated HA (cHA), also known as dahlite, is represented by the formula Ca$_{10}$(PO$_4$,$CO_3$)$_{6}$(OH)$_2$.

HA is a bioactive ceramic, and because of its excellent stability above pH 4.3, the ideal calcium phosphate phase for application inside human body when nondegradability is required (Kalita et al. 2007). Obviously, traditional applications of HA powder and particulates are in bone repair and as coatings for metallic prostheses to improve hard tissue integration, but it is also used for controlled drug release. HA possesses a hexagonal structure and has a stoichiometric Ca/P ratio of 1.67 (Table 3.1), which among all pure calcium phosphate phases is closest to mineralized human tissue. Like for most ceramics, the mechanical strength and fracture toughness of pure HA is significantly lower compared to bone (Santos et al. 1996). These properties can be improved by enhanced densification through the use of different sintering techniques. Such strategies include the addition of a low-melting secondary phase, for example glasses, as a binder (Georgiou and Knowles 2001) and the use of nanoscale powders for better densification due to their large surface area. Besides improved sinterability and enhanced densification, nano-HA (nHA) is also expected to have better bioactivity than coarser crystals (Webster et al. 2001a).

A number of techniques, including sol–gel synthesis, solid state reactions, precipitation, hydrothermal reaction, microemulsion synthesis, and mechanochemical routes have been used to fabricate nHA powders (Kalita et al. 2007; Dorozhkin 2010b). A selection of these techniques is described in the “Fabrication of ceramic nanobiomaterials” section. The sol–gel method has recently gained interest for synthesis of calcium phosphates nanoparticles due to its unique advantages. The method is capable of improving chemical homogeneity and offers almost molecular-level control by mixing of the calcium and phosphorus precursors and reducing synthesis temperature in comparison with conventional methods. Nanopowders with different Ca/P ratios have been produced by altering the quantity and the composition of precursors and processing variables. Another common preparation method for nHA powders is chemical precipitation through aqueous solutions of calcium chloride and ammonium hydrogen phosphate. It has been observed that the crystallinity and crystallite size of nHA increases
with temperature and ripening time (Pang and Bao 2003). Particle morphology was found to correlate with crystallinity. Ceramic nanoparticles with regular shape, smooth surface, clear contour, and low water content were obtained by higher crystallinity of HA.

The synthesis of carbonate containing HA has become an important target in biomaterials with the objective to more closely mimic hard tissue apatite (Landi et al. 2003). Depending of whether the hydroxyl or the phosphate ions of HA are substituted, one commonly refers to an A-type or B-type carbonation, respectively. In human mineralized tissues, the B-type is the preferred form of cHA. Highly pure B-type cHA nanopowder has been produced with a wet-chemical synthesis from calcium nitrate, ammonium hydrogen phosphate and sodium carbonate. The compressive strength of sintered cHA porous bodies was about twice the strength of analogous HA matrices.

### 3.2.1.2 Other Calcium Phosphates

Calcium phosphates besides HA that have been processed into nano-sized structures include α-TCP, β-TCP, tetracalcium phosphate, dicalcium phosphate dehydrate, dicalcium phosphate anhydrous, and octacalcium phosphate (Table 3.1) (Kalita et al. 2007; Dorozhkin 2010b).

Out of both tricalcium phosphates, β-TCP, also known as β-whitlockite, degrades more slowly and is used as a bioresorbable calcium phosphate ceramic in biomedical applications, particularly in orthopedics. X-ray patterns reveal that β-TCP has a pure hexagonal crystal structure. Nano-sized β-TCP powders have been synthesized utilizing a variety of methods similar to those for the fabrication of HA. The conventional methods include solid-state processes and wet-chemical methods (Bow et al. 2004; Kalita et al. 2007). As for all wet synthesis strategies of calcium phosphates, a calcium source and a phosphate source are used as starting material. For nano-β-TCP, calcium acetate and phosphoric acid are typical sources. During the process, phase transition involving calcium hydrogen phosphate and intermediate amorphous calcium phosphate phases occurs until β-TCP is formed at increased aging times. It has been observed that the incorporation of carbonate favors the formation of the β-TCP phase (Bow et al. 2004).

### 3.2.2 Aluminum Oxides

The most prominent aluminum ceramic is aluminum(III) oxide (Al₂O₃, alumina) (Vallet-Regi 2001; Rahaman et al. 2007). Alumina exists in different modifications, cubic γ-Al₂O₃ and trigonal α-Al₂O₃ (Yang et al. 2009c). γ-Al₂O₃ is often used as a raw material for further processing. Both modifications have different dissolution behaviors. Whereas the γ-form is soluble in strong acid and base, the α-form is insoluble and bioinert. At temperatures above 800°C, the γ-form is transformed into the stable α-form. Traditional applications of alumina are in dentistry, in orthoplasty, and in the treatment of hand and elbow fractures. Similar to nHA, nanophasalumina showed higher bioactivity as compared to grains larger than 100 nm (Webster et al. 2001a). The adhesion of osteoblasts to nano-sized alumina substrates was significantly improved over conventional alumina (Webster et al. 1999). The effects were especially pronounced when serum was present, suggesting a contribution of plasma proteins that absorb to the nanostructured ceramic surfaces (Webster et al. 2001b). Alumina nanopowders can be prepared by plasma spraying of liquid precursors (Karthikeyan et al. 1997) or flame aerosol technology (Pratsinis 1998). With atomic layer deposition, it is possible to deposit uniform alumina nanofilms on zirconia nanoparticles without affecting size distribution and surface area of the particles (Hakim et al. 2005).

Alumoxanes or more precisely carboxylatoalumoxanes ([Al(OR)(OH)]_{x},[O_2CR]_{y}, with 2x + y + z = 3 and R = C1–C13) can be prepared as nano-sized particles by the reaction of pseudo-boehmite ([Al(OH)]_{n}) with carboxylic acids (RCOOH) in an environmentally benign process (Landry et al. 1995; Callender et al. 1997). Depending on the alkyl substituents, the physical properties of the alumoxanes may range from insoluble crystalline powders to powders that readily form solutions or gels in hydrocarbon solvents. Upon thermolysis, the carboxylate-alumoxanes can be converted to alumina. The particle size of the carboxylate-alumoxane can comfortably be selected by the choice of carboxylic acid and by solution pH (Vogelson and Barron 2001). The alkyl residues of the alumoxane nanoparticles can be
used to balance the hydrophilicity of the ceramic in a way that the dispersibility of the nanoparticles in hydrophobic matrices, for example, polymer bulks, is significantly improved. This is a key requisite for the successful fabrication of ceramic-polymer composites (Kim and O’Shaughnessy 2002; Horch et al. 2004). Furthermore, the residues can be chemically modified and reactive moieties introduced to yield hybrid nanoparticles that can be covalently integrated in composite polymer networks.

### 3.2.3 Titanium Oxides

Titanium(IV) oxide, titanium dioxide (TiO$_2$), or titania has eight modifications and is classified as a bioinert material (Vallet-Regi 2001; Rahaman et al. 2007). Titania is the dominant oxide on the surface of passivated titanium and formed upon contact of the pristine metal with air (Gotman 1997). In bioliquids, calcium and phosphate ions are also incorporated into the oxide layers on titanium, forming calcium titanium phosphate and other mineral deposits (Hanawa et al. 1998). The preparation of full-density nanostructured titanium dioxide ceramics is difficult, because of rapid grain growth during sintering (Lee et al. 2003). Titania nanoparticles can be obtained by flame aerosol technology (Pratsinis 1998). Single-crystalline titanium dioxide nanotubes with lengths up to a few hundred nanometers have been synthesized via the hydrolysis of TiF$_4$ at low pH and 60°C (Liu et al. 2002). Positive effects of individual titania nanoparticles on the differentiation of neural stem cells toward neuron have been described (Liu et al. 2010). The biological effects of titanium dioxide nanostructures on implant surfaces have been more widely described. Such coatings are typically applied to hard tissue implants, orthopedic, and dental, to improve integration (Yao and Webster 2006). It has been shown that osteoblast adhesion and activity on nanostructured titanium dioxide surfaces are improved and these effects have been correlated to specific protein adsorption (Webster et al. 1999, 2000b; Colon et al. 2006). The bioactivity of titanium dioxide layers can be further improved through the immobilization of specific peptides (Balasundaram et al. 2008). Positive effects on osteoblast adhesion have also been shown for nanostructured titania-polymer composites (Kay et al. 2002).

### 3.2.4 Zirconium Oxides

Zirconium dioxide (ZrO$_2$), also known as zirconium (IV) oxide or zirconia, is the most prominent oxide of the transition metal zirconium. Zirconia is a white powder with a density of 5.68 g·cm$^{-3}$ that is bioinert and insoluble in water. Zirconium dioxide is available in different modifications: monoclinic, tetragonal, and cubic. Tetragonal zirconium dioxide features the highest mechanical stability and is therefore the preferred modification for biomedical applications. At room temperature, however, the monoclinic modification of zirconium dioxide is prevalent. The transformation from monoclinic to tetragonal takes place at heating to 2370°C; the cubic phase can be obtained at 2690°C and further heating leads to melting. In order to stabilize the tetragonal modification at room temperature, different metallic oxides, for example, magnesium oxide and yttrium (III) oxide (Y$_2$O$_3$), can be introduced (Rahaman et al. 2007), leading to materials like yttria-doped tetragonal zirconia polycrystal (Y-TZP). Doped TZPs have been shown to demonstrate improved strength and fracture toughness (Chevalier and Gremillard 2009). Zirconium dioxide-based ceramics have been used as compounds of implants, for example, prosthetic knee replacements (Rahaman et al. 2007) and for dental restorations (Yang et al. 2009c).

Ultrafine polymer-stabilized nanocrystalline tetragonal zirconium dioxide powders have been synthesized by a microwave-assisted method from an aqueous solution containing Zr(NO$_3$)$_4$, poly(vinyl alcohol), and NaOH (Liang et al. 2002). Nanocrystalline monoclinic zirconium dioxide powders were obtained by forced hydrolysis of inorganic zirconyl salts (Hu et al. 1998). Monoclinic nano-grained zirconium dioxide coatings fabricated by atmospheric plasma spraying have demonstrated promising properties for application as coatings for metallic orthopedic implants (Wang et al. 2010). Zirconia coatings exhibited good bioactivity and biocompatibility, high bonding strength with titanium alloys, and high stability in an aqueous environment. Plasma-sprayed HA coatings, in contrast, are often characterized
by low crystallinity and poor bonding strength on titanium alloys. In cell culture experiments with osteoblasts, zirconium dioxide coatings supported cell attachment and adhesion, and enhanced cell proliferation could be observed. Nano-grained coatings have also been fabricated from yttria-stabilized zirconia (Racek et al. 2006).

Another application of zirconia is to stabilize HA. Composites that contained low amounts of zirconia and were processed at low sintering temperatures to maintain HA crystallinity revealed higher surface roughness, smaller grain size, and increased osteoblast adhesion (Evis et al. 2006).

3.2.5 Glasses

Bioactive glasses are amorphous solids that are classified as ceramic materials in the biomaterial literature (Vallet-Regi 2001). These glass ceramics are described as surface reactive materials and have been shown to exhibit good biocompatibility. A bioactive glass generally consists of formers and modifiers. The forming materials typically are silicon dioxide and phosphorus pentoxide, whereas modifiers include calcium oxide and sodium oxide. Various other substances such as K₂O, MgO, CaF₂, Al₂O₃, B₂O₃, and Fe₂O₃ can be introduced to create materials with specific properties.

Bioglass® is an FDA-approved material that was developed in 1971 by Larry L. Hench and colleagues and is composed of 46.1 mol% SiO₂, 26.9 mol% CaO, 24.4 mol% Na₂O, and 2.5 mol% P₂O₅ (Hench 1997). One aspect of the bioactivity of these amorphous ceramics is the formation of apatite-like crystals on their surfaces out of initially formed silica hydrogel layers upon contact with body fluids. Some reports claim that bioactive glasses show better performance in bone tissue engineering than HA and that the glasses strongly interact with hard tissues (Xynos et al. 2000). Bioactive glasses have gained attention as promising materials for tissue engineering scaffolds, either as fillers or as coatings of polymer structures or as porous materials themselves (Rezwan et al. 2006). SiO₂–CaO–P₂O₅ ternary bioactive glass ceramic nanoparticles (20 nm in diameter) with different compositions have been prepared via a three-step sol–gel method (Hong et al. 2009). Nanoparticles with low phosphorous and high silicon content exhibited enhanced mineralization capability in simulated body fluid and a higher solubility in phosphate buffered saline.

3.2.6 Other Ceramic Nanobiomaterials

Biomaterial literature includes magnetic oxides, graphite, and pyrolytic carbon in the class of bioceramics (Vallet-Regi 2001). Magnetic nanoparticles are typically used for therapeutic drug, gene, and radionuclide delivery as well as for cancer therapy and as contrast enhancing agents (Pankhurst et al. 2003). These materials are discussed elsewhere in this handbook. Carbon nanostructures, such as nanotubes, are a widely investigated group of nanobiomaterials (Webster et al. 2004) and also discussed in a separate chapter of this handbook. Diamond, a different allotrope of carbon, has lately gained increasing interest as nanobiomaterial. Due to superior mechanical and tribological properties, nanostructured diamond coatings on orthopedic implants are of special interest (Yang et al. 2009a).

3.3 Fabrication of Ceramic Nanoparticles

For the production of nano-sized ceramic biomaterials, many methods have been developed and a selection is highlighted in the following paragraphs. These include milling, precipitation, and emulsion processes as well as the application of ultrasound or microwave irradiation. A general classification of the available techniques can also be done according to whether the nanostructures are obtained by disaggregation of larger particles or by controlled crystal growth from corresponding ions. As calcium phosphate materials are the most frequently used and investigated ceramic biomaterials, most methods have been developed for their fabrication (Dorozhkin 2010b). Consequently, most examples given in this chapter also refer to this class of ceramic nanobiomaterials.
3.3.1 Fabrication of Ceramic Nanoparticles by Disaggregation

Technically, most ceramic nanoparticles can be obtained by milling of larger particles. Suitable disaggregation techniques include the application of ultrasound and milling techniques such as vibro milling and ball milling. Bioapatites with chemical compositions similar to HA can be obtained from different biological sources, such as corals, ivory, teeth, and bone (Roy and Linnehan 1974; Dorozhkin 2010a). Nanocrystalline bovine bioapatite, for example, has been obtained as follows (Ruksudjarl et al. 2008): bovine bone samples were harvested, carefully cleaned, and boiled several times in distilled water. The deproteinized material was dried at 200°C and afterwards calcined at 800°C. The product was crushed into small pieces, ball-milled for 24 h, and finally vibro-milled to obtain the nanoscaled product.

Another method that includes a disaggregation step is the controlled hydrolysis of micron-sized ceramics. For the fabrication of nano-sized HA powder, for example, dicalcium phosphate and calcium carbonate were mixed to achieve a Ca/P ratio of 1.67, poured into a solution of NaOH and stirred at 75°C for 1 h (Shih et al. 2004). The hydrolysis reaction was stopped via cooling with ice water. The aggregates were filtered and washed, and the powder was dried at 60°C. The particles were processed through annealing at 600°C, 800°C, or 1000°C for 4 h.

Through the use of plasma flames, raw ceramic particles can be melted, partially melted or even evaporated. The melted or vaporized material can be quenched or condensed into ultrafine powders by subsequent cooling. Using the radio frequency thermal plasma method, nano-sized (10–100 nm) HA powders have been produced (Xue et al. 2004). Coarse HA particles were pre-heated (1000°C) and entered into a plasma torch. The vaporized material was condensed into ultrafine powders and collected.

3.3.2 Controlled Preparation of Ceramic Nanoparticles

Most processes described in the following section are so-called solution-mediated fabrication processes with the exception of mechanochemical processing. As a common characteristic, nucleation and crystal growth are key steps of these processes. The main parameters that initiate and control these steps differ among fabrication methods. While in precipitation techniques solution properties, for example, solution pH and solvent composition, are changed to induce nucleation, a thermal induction is characteristic for microwave-assisted techniques. In mechanochemical processes, the activation energy for nucleation and growth is delivered by pressure in a mill (Riman et al. 2002).

Controlled precipitation is a straightforward method to prepare nanometer-sized particles from solutions of the constituting ions. Using this method, different ceramic nanoparticles (calcium phosphates, zirconium dioxide, iron oxides, and bioactive glass) have been fabricated. With regard to the different calcium phosphate minerals, the desired Ca/P ratios can be easily adjusted to yield a specific product. Precipitation methods are quite inexpensive and versatile. In addition, key product parameters such as size of the precipitated grains can be controlled. The precipitation can be initiated through different processes, such as pH or temperature adjustment or the addition of a non-solvent. Other protocols involve supersaturated solutions. A classical protocol to obtain nHA starts from individual aqueous solutions of calcium nitrate and diammonium phosphate at pH 11–12 (Wang and Shaw 2009a). Through dropwise addition of the phosphate solution to the calcium solution under stirring, a milky dispersion is obtained. After centrifugation, the slurry is washed and the precipitate sedimented for 6–10 h. The isolated intermediate is dried (90°C), ball-milled, and calcined (300°C) to yield HA nanorods. In comparison to dense HA bodies assembled from micron-sized HA, bodies sintered from the densified nanorods showed enhanced hardness and toughness. For the preparation of bioactive glass nanoceramics, calcium nitrate and tetraethoxysilane were dispersed in a water–ethanol mixture, the pH adjusted with citric acid, dropped into a solution of ammonium dibasic phosphate kept at pH 11, and the precipitated nanoparticles (20–40 nm) were obtained after centrifugation, washing and calcination (Hong et al. 2008, 2009). In order to further reduce the size of particles obtained by a classical precipitation method, a dispersion of the particles can be subjected to a high-intensity ultrasonic field (Jevtic et al. 2009).
**Phase-Controlled Methods:** Progressing from controlled precipitation out of single-phase systems, a variety of methods strive for better control of size and structure of the prepared nanocrystals through the use of multiple-phase systems during precipitation or growth. Using water-in-oil emulsion, for example, small nano- and micron-sized aqueous, constrained reaction environments for nanocrystal synthesis can be prepared (Dorozhkin 2010b). *Emulsion-based methods* are published for organic composite materials and also for monolithic ceramics. Key parameters during emulsion processes are droplet size of the dispersed phase, temperature, pH of aqueous phase, and mechanical stirring. For the synthesis of nano-sized calcium orthophosphate particles, a water-in-oil microemulsion was prepared from an aqueous calcium hydroxide phase and isooctane using sodium dioctylsulfosuccinate as surfactant (Phillips et al. 2003). After the addition of orthophosphoric acid, the pH was adjusted to 10.5 initiating the precipitation. The nanopowder was obtained after filtration, washing, freeze-drying, and calcination.

Through the combination of a microemulsion-controlled crystal growth and a solid template, lightweight hollow porous shells of calcium carbonate have been fabricated (Walsh and Mann 1995). The process started from a supersaturated aqueous calcium carbonate phase to which magnesium chloride was added to initiate crystal growth. This phase was emulsified in tetradecane as oil phase by means of a cationic surfactant. Polystyrene microspheres were covered with a thin film of the resulting micro-emulsion and washed with hexane to remove the oil phase and the surfactant. Finally, the polystyrene templates were removed using acetone-ethanol mixtures yielding hollow microshells with a wall thickness of approximately 125 nm.

A method called *liquid–solid–solution* involves a triphasic system for the controlled growth of HA nanorods (Wang et al. 2006a; Wang and Li 2007). The system was assembled from a liquid phase containing linoleic acid in ethanol, sodium linolate as the solid phase, and an aqueous solution of calcium nitrate (solution phase). Through an ion-exchange process, calcium ions interact with the solid linolate phase. After the addition of sodium phosphate and thermal treatment in an autoclave, calcium phosphate nanostructures start growing. Along with the reaction and ion exchange process, the linoleic acid can be released and absorbed on the *in situ*–generated HA nanorods with the alkyl chains left outside. These hydrophobic nanocrystals will be separated from the aqueous solution spontaneously and can be collected from the vessel (Figure 3.1A).

The *solvothermal method* for the fabrication of HA nanowires is a process that injects aqueous stock solutions of calcium and phosphate ions into cyclohexane and uses a cationic surfactant as stabilizer (Wang et al. 2006b). As two immiscible phases are combined, this process can strictly be regarded as an emulsion-based technique. For the solvothermal process during which the nanowires are formed, the dispersive system is transferred into sealed Teflon containers and subjected to 120°C for 12 h.

Microwave radiation, as applied during hydrothermal *microwave synthesis*, has several advantages over classical chemical processes, such as precipitation, for the fabrication of nanopowders including easy reproducibility, small particle size, narrow particle distribution, high purity, and yield due to fast homogenous nucleation (Siddharthan et al. 2006; Kalita and Verma 2010). Nano-sized HA powders were synthesized from phosphoric acid and calcium hydroxide in a closed-vessel microwave device at 300°C for 30 min. Product characteristics could be controlled by the applied microwave power and Ca/P ratio. At low power and a Ca/P ratio of 1.57, mixed calcium phosphate compounds such as calcium hydroxide, calcium hydrogen phosphate, and HA were yielded, while monophase HA was obtained at higher power and a Ca/P ratio of 1.67 (Han et al. 2006). Bioactive HA nanopowder (5–30 nm) was synthesized from a suspension of calcium nitrate and ethylenediaminetetraacetic acid that was mixed with a sodium hydrogen phosphate solution and adjusted to pH 9 (Kalita and Verma 2010). Following irradiation in a microwave oven, the suspension was filtered and precipitated, and the filtrate was washed and dried in a muffle furnace (200°C, 4 h). Crystalline nHA powder was finally obtained after disaggregation of the dried product.

*Hydrothermal methods* during which the necessary heat energy is introduced in ways other than microwave irradiation have also been described (Riman et al. 2002). For the fabrication of nHA powders,
aqueous solutions of calcium nitrate and diammonium hydrogen phosphate were prepared. The solutions were mixed and different pH values were adjusted for the resulting slurries. The samples were introduced into hydrothermal reactors for 24 h at 50–200°C. The synthesized powders were washed and dried. Depending on the processing parameters stirring and reaction pH, particles with diameters of 20–40 nm as well as nano-sized needles have been fabricated.

Sol–Gel Processes: Compared to classical precipitation techniques, sol–gel combustion is considered advantageous due to its simplicity and shorter fabrication times (Wang and Shaw 2009b). For the fabrication of calcium phosphate nanoparticles, the sol–gel method allows for a molecular-level mixing of the calcium and phosphorous precursors. This way, chemical homogeneity and purity are improved, and the formation temperature of resulting calcium phosphates is decreased in comparison to conventional methods (Han et al. 2004). In a classical sol–gel combustion process to fabricate calcium phosphates, a solution of calcium nitrate and ethyl phosphate was concentrated to form a polymeric gel, which self-combusted into a calcium phosphate precursor powder upon further heating in a hot furnace (Varma et al. 1998). Calcination on heat treatment at 1000°C resulted in β-TCP, HA, or a mixture of the two phases depending on the Ca/P ratio in the gel. Highly pure HA nanopowders were fabricated from a mixture of calcium nitrate and triethyl phosphate in ethanol–water (Wang and Shaw 2009b). The mixture was aged under alkaline conditions and finally gelled by generation of (-Ca-O-P-) oligomers upon heating. A nanopowder with a particle size of approximately 80 nm is obtained via a combustion process after heating the gel with 30°C/min to 350°C for 1 h in a furnace. The process can be traced via TGA measurements and the combustion process was shown to be heating rate dependent. Alternatively, a non-alkoxide sol–gel method, citric acid sol–gel combustion, is available for the preparation of nano-crystalline inorganic powders including HA (Han et al. 2004). The precursors, such as calcium nitrate, citric acid, and diammonium hydrogen phosphate, were dissolved in water and mixed. The solution was acidified and heated under stirring to concentrate the solution and finally form a gel. The gel was dried and calcined to finally yield nHA.

Mechanochemical processing methods have been developed for the fabrication of nano-sized particulates of calcium phosphates, zirconia, and alumina (Yeong et al. 2001; Riman et al. 2002; Dorozhkin 2010b). Typically, lower-energy ball mills or high-energy vibratory and planetary mills are used to introduce the mechanical forces at room temperature. Such processes are relatively straightforward and inexpensive. In early studies that focused on the fabrication of nHA, calcium deficient compositions with low crystallinity were often obtained. Improved routes managed to trigger the formation of single-phase highly crystalline HA from a dry powder mixture of calcium oxide and anhydrous calcium hydrogen phosphate by mechanical activation for more than 20 h. The resulting HA powder exhibits an average particle size of approximately 25 nm (Yeong et al. 2001).
In a process called *mechanical alloying*, a fluorinated HA nanopowder was prepared from a mixture of calcium hydroxide, phosphorous pentoxide, and calcium fluoride (Fathi and Zahrani 2009). The mixture was mechanically alloyed using a high energy planetary ball mill equipped with a zirconia vial and zirconia balls. After 15 h of milling, the final particle size ranged between 35 and 65 nm.

*Wet mechanochemical synthesis*, a special mechanochemical process and often referred to as mecanochemical-hydrothermal synthesis, introduces an aqueous reaction medium to the milling chamber. For the fabrication of nHA powders with this method, aqueous slurries containing calcium hydroxide, calcium or sodium carbonate, and diammonium hydrogen phosphate were prepared and processed in a multi-ring media mill at 25–35°C for several hours (Riman et al. 2002).

### 3.4 Applications of Ceramic Nanobiomaterials

Bioceramics, in general, have tremendously evolved over the last five decades of biomaterials research and have become key or even first choice materials in numerous applications including artificial replacements of hips, knees, teeth, tendons, and ligaments. Bioceramics are also used for the repair of periodontal defects, maxillofacial reconstruction, bone augmentation, bone plates, and in spinal fusion (Dorozhkin 2010a,b). Nanoscale bioceramics, in particular, are emerging, because such materials hold promise to overcome long-standing problems associated with these established materials. As a result of the higher surface area of nanostructured materials, the sintering processes are more effective (Dorozhkin 2010b). It has also been hypothesized that nanostructured ceramics have increased bioactivity due to their higher roughness. With a series of dense HA bodies fabricated from well-defined micron- and nano-sized grains, simultaneous improvements in hardness and toughness have been observed in bodies sintered from nHA grains (Wang and Shaw 2009a).

Depending on whether the ceramic nanostructures alone represent the biomaterial or are part of a larger micro- or macrostructure, one can divide ceramic nanobiomaterials into three main groups: (I) nano-sized monolithic ceramic particles, which are used as particulate nanobiomaterial in form of a powder or dispersion, (II) nano-textured biomaterial, in which ceramic nanoparticles are deposited on the surface of a biomaterial construct of larger dimensions, (III) nanocomposites, in which nanoparticulate ceramics are dispersed in the bulk of another biomaterial.

#### 3.4.1 Monolithic Ceramic Nanoparticles

**3.4.1.1 Delivery of Therapeutic Agents**

Particulate nanoceramics have applications in diagnostics and the delivery of therapeutic agents. Depending on the desired application, ceramic nanoparticles bear advantages over nanostructures made from other materials. Basic properties of the ceramic nanoparticles including size distribution, shape, morphology, and porosity can be directly controlled by the fabrication process (Sahoo and Labhasetwar 2003; Koo et al. 2005). Additionally, the nano-sized ceramic particles are rigid, chemically and physically inert, interact with organic molecules, and are susceptible to specific surface modification. Compared to other materials, ceramic nanoparticles show no pH-induced swelling or change in porosity, are resistant to microbial growth, are stable in physiological aqueous systems, and have the ability to entrap and stabilize drugs. There are some biodegradable ceramics, but most are bioinert, which can be considered critical for delivery applications due to possible accumulation of the particles in the body (Kriven et al. 2004; Medina et al. 2007). Nanoparticulate formulations in general are of special interest for delivery applications because of their submicron size, which is considered a prerequisite for targeting strategies and allows such particles to access almost all areas of the body. Especially for passive drug targeting applications, where access to specific organs or tissues is controlled by particle size, the ability to precisely control nanoparticle size during fabrication and application is essential.
Active drug targeting, which aims at delivering highly potent drugs to specific sites or cell types in order to minimize side effects, requires modification of the nanoparticle surfaces with site-specific structures, such as integrin or other receptor ligands and antibodies (Roy et al. 2003; Torchilin 2006). In combination with the stabilizing effects that some ceramic materials have on sensitive therapeutic molecules, ceramic nanoparticles may open new therapeutic possibilities. To exemplify the spectrum of options ceramic nanoparticles offer for drug delivery strategies, selected applications are described below. The ability to entrap and stabilize proteins has been shown with enzymes (Jain et al. 1998). With 80% efficacy, a peroxidase was encapsulated in mono-disperse hydrated silica nanoparticles by a reverse micelle preparation technique. The enzyme did practically not leach out of the particles over more than a month and remained active showing normal substrate conversion kinetics. It is assumed that substrate can diffuse into the ceramic particle to be converted by the entrapped enzyme. This ability to effectively entrap enzymes and maintain enzyme activity is attractive for therapeutic in vivo application, because the risk of allergic or proteolytic reactions of these enzymes is drastically reduced due to their practically zero leachability. Advancing the outlined strategy, a water-insoluble photoactivating anticancer drug was formulated in silica-based ceramic nanoparticles, passively targeted to the region of therapeutic interest and subsequently, locally activated by irradiation with light. Thereby, the drug remained within the formulation, but the photochemically generated cytotoxic singlet oxygen diffused through the pores of the ceramic nano-matrix and into the tumor tissue (Roy et al. 2003). Aquasomes are self-assembled nanoparticulate delivery vehicles composed of a ceramic core for stability, typically composed of a calcium phosphate or diamond, grafted with a hydrophilic oligomeric polyhydroxy coating, for example, oligosaccharides, to which the therapeutic substances are adsorbed (Cherian et al. 2000; Goyal et al. 2008; Umashankar et al. 2010). Aquasomes are of special interest with regard to peptides and protein delivery, because the hydrophilic glassy coating stabilizes their structure and activity through a water replacement effect (Umashankar et al. 2010). For an insulin delivery formulation, for example, the aquasome formulation was able to reduce the percentage blood glucose more effectively and prolonged compared to a standard plain insulin solution (Cherian et al. 2000). Further applications include the oral delivery of enzymes, the use as hemoglobin-loaded oxygen carriers, and antigen delivery vehicles. In the latter application, benefit and safety of these novel systems remain to be finally determined (Goyal et al. 2008). There is evidence, however, that aquasome formulations have shown better adjuvanticity and effective antigen presentation, because the structural integrity of the proteins is preserved. Applications that involve biodegradable nanoceramics include gene delivery (Link 2000; Kriven et al. 2004; Ladewig et al. 2010). The ceramic nanoparticles used in such studies are comprised of layered double hydroxides (LDHs), the so-called anionic clays, which consist of cationic brucite (MgOH$_2$)-like layers and exchangeable interlayer anions. By anion exchange, DNA and functional anionic biomolecules can be encapsulated in the inorganic particles. With regard to gene delivery, the interactions of DNA with the LDH nanostructures, similar to other non-viral vectors, neutralize the charges of the DNA and facilitate internalization into cells. Once internalized by endocytosis, the slightly acidic pH in the lysosomes dissolves hydroxide layers of the LDH, and interlayer DNA is replaced by other anions in the cell electrolyte and finally released into the cytosol. A recent study showed that DNA-LDH complexes yield considerable transfection efficiencies when used on adherent cell lines (Ladewig et al. 2010). The transfection of cells in suspension culture, however, was unsuccessful. Transmission electron microscopy (TEM) investigations revealed that, in contrast to smaller anions, plasmid DNA did not become intercalated in the LDHs but was wrapped around the nanoparticles.

### 3.4.1.2 Diagnostics

For diagnostic application, magnetic iron oxide-based nanoparticles are widely investigated and discussed elsewhere in this handbook. One major application is their use as contrast agents for magnetic resonance imaging (Weissleder et al. 1990; Gupta and Gupta 2005). Magnetic nanoparticles have also
been combined with fluorescent moieties (Corr et al. 2008) and quantum dots to obtain magnetic luminescent nanocomposites (Hong et al. 2004).

### 3.4.1.3 Other Applications of Ceramic Nanoparticles

Calcium phosphate nanoparticles, especially HA and β-TCP, are part of bone and dental cement formulations or of powder mixtures that are processed into tissue engineering scaffolds (Chris Arts et al. 2006; Xu et al. 2008; Dorozhkin 2010b). Due to the favorable interactions of these calcium phosphates with drugs and proteins (Habraken et al. 2007), such matrices can serve as drug delivery systems for a variety of remedies such as antibiotics, antitumor, and anti-inflammatory drugs or growth factors.

### 3.4.2 Ceramic Nanocoatings

Orthopedic and joint implants have tremendously improved the quality of life for countless individuals. In order to achieve clinical success, implanted materials must form a stable interface with surrounding tissue as well as being compatible with the mechanical properties of natural tissue (Campbell 2003). To date, metals—especially titanium—are the biomaterials of choice for such applications due to their mechanical properties, but the interfacial bonding between the metallic surface and the surrounding bone is poor. Poor interfacial bonding leads to the formation of a non-adherent, fibrous tissue layer, and upon further loosening and movement at the implant-tissue interface, the implant will finally fail. A promising approach to address this problem has been the use of ceramic coatings applied to implant surfaces. Nanoceramic coatings can be achieved by a variety of methods. In the following section, a selection will be discussed with the focus on nanostructured HA coatings (Paital and Dahotre 2009).

#### 3.4.2.1 Coating Methods for Nanostructured HA

HA is probably one of the most applied coatings on implant and prosthesis surfaces, because it resembles the natural inorganic component of bone. Due to its low toughness and brittleness, HA is unsuitable as a load-bearing substrate itself. Coatings of HA on load-bearing substrates, however, especially if they are thin enough, can improve osseointegration and reduce fibrous capsule formation. HA coatings are broadly used in dentistry and orthopedic applications to speed up formation of bone around the device and stabilize the implant in its position (Campbell 2003). As it has been shown for other nano-sized materials, nHA allows for increased osteoblast adhesion and differentiation relative to micrometer-structured surfaces (Catledge et al. 2002). Surface coatings deposited by these processes need to meet the guidelines set by the U.S. Food and Drug Administration (FDA) and the International Organization for Standardization (ISO) (Table 3.2) (Paital and Dahotre 2009). A variety of surface coating processes are known for HA, such as plasma spray deposition, ion beam–assisted deposition (IBAD), electrophoretic deposition (EPD), chemical vapor deposition (CVD), microarc oxidation, magnetron sputtering, sol–gel-derived coatings, and biomineralization (Paital and Dahotre 2009). A selection of these methods is outlined in the following section.

### TABLE 3.2 Specifications for HA Coatings by the U.S. Food and Drug Administration (FDA)

<table>
<thead>
<tr>
<th>Property</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crystallinity</td>
<td>≥62%</td>
</tr>
<tr>
<td>Phase purity</td>
<td>≥95%</td>
</tr>
<tr>
<td>Ca/P atomic ratio</td>
<td>1.67–1.76</td>
</tr>
<tr>
<td>Tensile strength</td>
<td>&gt;50.8 MPa</td>
</tr>
<tr>
<td>Shear strength</td>
<td>&gt;22 MPa</td>
</tr>
</tbody>
</table>

3.4.2.1.1 Plasma Spraying

The most widely commercially used technique for surface coating of implants with HA is plasma spraying (Tang et al. 2010). Due to the extremely high temperature in the plasma flame, almost any coating feedstock material melts and can be coated to a substrate with this method. A typical setup for plasma spraying is given in Figure 3.2A. A plasma gun comprises a chamber with an electrode as cathode and a nozzle as anode. When a plasma-forming gas flows through the chamber, current power is applied to the cathode arching the nozzle (anode) and thereby stripping the gas molecules of their electrons to form a plasma plume. The plasma-forming gas usually consists of argon, helium, nitrogen, or hydrogen. Among these, argon can be easily ionized and provides a stable arc at a low operating voltage (Paital and Dahotre 2009). As the unstable plasma ions recombine back to the gaseous state, a huge amount of thermal energy is released, providing temperatures exceeding 30,000 K in the hottest areas of the plasma plume.

**FIGURE 3.2** (A) Plasma spraying: Argon gas is ionized forming a plasma plume in an electric arc between the cathode in the center of the plasma gun and the anodic nozzle. Upon recombination of the plasma ions to the gaseous state, a tremendous amount of energy is released. Into the hot gaseous plume HA powder is fed and propelled toward the substrate. (B) IBAD: The coating material (target) is located in an electron-beam-heated vaporizer. Vaporized target material is deposited on the substrate. Simultaneously, substrate and coating experience bombardment with a high energy ion beam. The process takes place under vacuum conditions. (C) Magnetron sputtering deposition: The argon gas is ionized by an electrical field. The target resides on a series of magnets. The shape of the magnetic field entails circulation of primary and secondary ions close to the target surface due to the Lorentz force and prolongs their flight path. Therefore, argon gas is ionized with high efficiency forming plasma rods. Plasma ions impact into the cathodic target sputtering target molecules that are deposited on the substrate. (D) FACVD: An organic precursor solution is atomized in a nozzle and carried into an oxidizing gas where combustion and pyrolysis take place. By the organic solvent, a secondary flame is formed that contributes to substrate heating and allows for diffusion and good coating adherence to the substrate.
plume. Into the hot gas plume, HA powder is injected, melted, and propelled toward the substrate to be coated, which remains cool. Depending on the particle size of the fed HA powder, HA is coated in a melted or softened particulate form onto the substrate surface. Different physical conditions may coexist especially in larger particles, with an evaporating layer of phosphorous pentoxide and the formation of calcium oxide at the outer layer of the particle resulting in an enrichment of calcium in the coating. In the same particle, a molten layer beneath may solidify as an amorphous coating when deposited due to rapid cooling on the substrate. Furthermore, α-TCP and tetracalcium phosphate (TP) may form, while at lower temperatures, HA remains intact (Dyshlovenko et al. 2004). Therefore, crystalline HA coatings fabricated by plasma spraying contain varying amounts of amorphous HA and impurities of calcium oxide, TCP, and TP (Tang et al. 2010).

Plasma spraying is considered the most efficient and economical method for surface coating with HA for orthopedics and dental implants, but it suffers from low adherence to the substrate, low fracture toughness, and considerable thick coating films of several hundred micrometer. A high thickness of a coating (>100μm) bears the risk of fatigue failure (Paital and Dahotre 2009).

In order to improve adherence of the plasma-sprayed coatings to the substrate, a mixture of a titanium alloy and HA was deposited on the surface of a titanium alloy implant (Khor et al. 2000). Another recommendation is to pre-coat the substrate with a titanium layer to roughen the surface and to use high plasma power and suitable gas mixtures to ensure melting of HA particles in addition to a subsequent heat treatment at 700°C for 1 h to increase the crystallinity and improve the in vivo performance (Tsui et al. 1998).

While low fracture toughness is an inherent shortcoming of HA, a reduction in coating thickness is suitable to positively affect mechanical stability of the coating-implant interface. Therefore, alternative methods focus on thinner coatings (Paital and Dahotre 2009).

3.4.2.1.2 Ion Beam–Assisted Deposition

With this technique, a target material is evaporated usually employing an electron beam or resistive heating and deposited onto the substrate. A reactive ion beam that focuses on the substrate enables surface chemical reaction with the substrate materials and within the coated film (Figure 3.2B). Bond strength between coating and substrate is enhanced by atomic bonding, densification of the coating, and lower thermal stresses as compared to plasma spraying (Bai et al. 2009). Therefore, IBAD is suitable to improve adherence of HA to the substrate surface (Paital and Dahotre 2009).

IBAD coating results in coating thickness in the nanometer range. Coatings show improved mechanical properties meaning good adherence and low tendency for delamination (Rabiei et al. 2006). Crystallinity and phase composition of calcium phosphate are controlled by energy input. This is realized by either heat treatment of the substrate during the coating process (Rabiei et al. 2006; Bai et al. 2009) or by energy input of the ion beam (Hamdi and Ide-Ektessabi 2003). Additionally, the presence of water vapor is discussed to improve phase purity (Bai et al. 2009).

Layering in the structure of the coating can be controlled by ion beam current, control of substrate temperature during coating and post-heat treatment. It has been shown that by manipulating the substrate temperature during the deposition process to avoid time-consuming post-heat treatment, nanocrystalline calcium phosphate forms the layer closest to the substrate surface, and crystallinity decreased with increasing distance to the substrate interface (Bai et al. 2009). This was considered advantageous, because the amorphous layer may dissolve in contact to bone and provide ions for osseointegration while the remaining nanocrystalline layer allows for optimal interaction with adhering osteoblasts.

3.4.2.1.3 Electrophoretic Deposition

An electric field is applied to a conductive substrate in a nonaqueous suspension of charged particles. Depending on the charge of the particles, the substrate acts as anode or cathode, on which the oppositely charged particles are deposited, and the process is called anodic or cathodic electrophoretic deposition, respectively (Paital and Dahotre 2009). Advantages of this method are the ability to coat irregular
surfaces homogeneously in variable thickness and that the coatings adhere strongly. Complex compositions and layered coatings can be easily realized with this comparatively simple setup. HA coatings prepared by EPD typically undergo a sintering step subsequent to EPD. HA coatings densify with increasing sintering temperatures, which may improve the strength of the coating, but on the other hand decrease the osteoconductive potential of the coating that is known to correlate with microporosity (Wang et al. 2002a).

3.4.2.1.4 Magnetron Sputtering Deposition

Magnetron sputtering deposition is a physical vapor deposition method to fabricate coatings in a vacuum environment similar to ion beam sputtering (Figure 3.2C) (Paital and Dahotre 2009). Sputtering involves bombardment of a target with high-energy particles to eject atoms or molecules from the target surface. In order to preserve sufficient ion energy for bombardment and to allow for deposition of the ejected molecules on a substrate, vacuum is required. A specially shaped magnetic field is applied to the sputtering target to enhance the effectiveness of the high-energy particle bombardment. HA films generated by magnetron sputtering are comparably thin and tightly bound on the substrates. Crystallinity depends on the substrate temperature during the deposition process or thereafter. In a study that describes the fabrication of HA-coated titanium alloys, it was found that heat treatment (>300°C) either during the sputtering process or as posttreatment was necessary to create crystalline HA layers on the surface (Nelea et al. 2003).

3.4.2.1.5 Sol–Gel-Derived Coatings

Similar to sol–gel processes described for nanoparticle fabrication, sol–gel-derived coatings rely on the formation of a sol phase consisting of appropriate calcium and phosphate containing precursor solutions. Upon sol aging, ultrafine HA particles precipitate in vitro and start to gel the suspension. The aged sol is homogeneously subjected to a substrate, for example, by dip coating (Nguyen et al. 2004). The sol layer on the substrate finally forms the gel upon drying and becomes annealed onto the substrate. For example, an HA sol was prepared by mixing triethyl phosphate and calcium nitrate in an ethanol–water mixture (Li et al. 2005). The solution was stirred for 1 h and subsequently aged at ambient conditions for 5 days. Substrates were subsequently spin coated, dried at 80°C for 2 h, and finally heat treated at 550°C for 2 h. Sol aging is accelerated by the addition of ammonium hydroxide that works as an acceptor for protons released during HA formation keeping polymerization in progress during aging (Kim et al. 2005). The resulting coatings are thin and show good adherence on metallic surfaces. This technique allows for the incorporation of a variety of organic and inorganic compounds (Ganguli 1993). Processes normally deal with substrate temperatures between 200°C and 600°C for sintering and are comparatively simple, economic, and effective. Depending on the concentration of the suspended particles in the sol and its viscosity, porous structures of a substrate may be preserved or only partly covered (Nguyen et al. 2004; Li et al. 2005).

3.4.2.1.6 Chemical Vapor Deposition (CVD)

This method uses a nozzle to atomize a solution of precursor salts that react in the gaseous phase under energy input that is provided by heat, light, or plasma (Choy 2003). Depending on gas temperature, either intermediate species are decomposed forming homogeneous solid products that are deposited onto the substrate or are adsorbed onto the heated substrate and may react heterogeneously with the components of the substrate and form deposits. Higher temperatures that favor homogeneous deposits result in only low adherence to substrates and provide a method for nanoparticle generation. Heterogeneous deposits, on the other hand, diffuse on the substrate surface and form crystallization centers on the substrate. For dense coatings, reaction conditions are tailored to favor heterogeneous reactions, whereas a combination of homogeneous and heterogeneous reactions results in porous coatings. CVD has been described as a non-line-of-sight deposition method that can be used for the deposition of homogenous coatings on complex structure surfaces.
In flame-assisted CVD (FACVD), a subtype of CVD, precursors are dissolved in an organic solvent (Choy 2003). The solution is atomized through a nozzle and carried into a flame by an oxidizing gas. The precursors undergo combustion and pyrolysis in the flame and form the deposit. In addition, a secondary flame is formed by the burning organic solvent that heats the substrate during the coating process and promotes diffusion processes within the forming coating (Figure 3.2D) (Trommer et al. 2007). Substrate temperature was maintained at 500°C during the process. Calcium acetate and ammonium phosphate in combination with nitric acid served as precursors and ethanol as organic solvent. While acetate and ammonium ions are decomposed in the flame, calcium ions are free to react with phosphate. The formation of desired coating components can be controlled through the initial ratio of calcium to phosphate precursors. With this process, for example, HA has been deposited on stainless steel.

### 3.4.2.1.7 Biomineralization

Biomineralization is a coating process that is induced by soaking a substrate in simulated body fluid (SBF) at physiological temperature and pH. In contrast to many other methods, it is possible to homogeneously coat porous materials with this technique (Habibovic et al. 2002). Calcium phosphate coatings precipitate on substrates by time without the involvement of any cellular activity (Kokubo 1998; Li and Ducheyne 1998). Density and crystallinity of calcium phosphate coatings, which determine the rate of degradation, can be controlled by pH, volume and ionic strength of SBF (Qu and Wei 2008a,b). The original process takes 7–28 days to coat a substrate with a reasonable thickness (5–30 μm) (Li and Ducheyne 1998; Yu et al. 2009). Habibovic et al. introduced a method for accelerated HA mineralization by increasing the ionic concentration of SBF through a transient decrease in pH caused by carbon dioxide addition (Barrere et al. 2002; Habibovic et al. 2002). Upon degassing of the solution, the pH slowly increased and induced rapid precipitation of HA providing coatings within 24 h. The process involved two steps. At first, a thin amorphous layer of calcium phosphate containing multiple nucleation seeds is introduced. In a second step, HA crystals are grown from the crystallization seeds.

Within the process of precipitation, bioactive molecules, such as growth factors, can be co-precipitated within calcium phosphate crystals onto the surface of metallic implant materials (Yu et al. 2009). In a study that used bovine serum albumin (BSA) as a model protein, BSA incorporation into octocalcium phosphate crystals was shown to be highly efficient, if substrate surface area relative to SBF volume was increased.

### 3.4.2.2 Nanoceramic Coatings Other Than HA

A major challenge for prostheses at articulate surfaces is the necessity for low friction and high wear resistance. It has been shown that wear resistance of ceramics, such as zirconia (Kumar et al. 1991) and diamond coatings, is considerably better than that of metallic surfaces. Especially diamond coatings on metal surfaces have been intensely investigated during the last decade, because such coatings showed low friction and high wear resistance in addition to chemical resistance, high fracture toughness, and bonding strength (Drory et al. 1991; Catledge et al. 2002; Yang et al. 2009a).

#### 3.4.2.2.1 Nanostructured Diamond Coating

Nanostructured diamond coatings are usually generated by CVD or variations of this technique (Catledge et al. 2002), such as microwave-assisted CVD (Yang et al. 2009a,b). In an example of the latter method, substrates were pre-coated with a dispersion of diamond nanopowder in methanol. The pre-coated substrate was treated with a mixture of methane, hydrogen, and argon gas at high pressure and 800°C for 2 h in order to generate adhesion to the substrate. Diamond, a crystal of tetrahedrally bonded carbon atoms (sp3), is believed to develop from C2 dimers that result from collision of acetylene with argon. Hydrogen gas acts as inhibitor for secondary nucleation, and leads to crystal growth and allows for grain size control (Gruen 1999). Surface chemistry and contact angle can be further modified by the
addition of oxygen/helium or pure hydrogen plasma during sample cooling (Clem et al. 2008). Figure 3.1B depicts the surface structure of a nanocrystalline diamond film deposited by microwave plasma-enhanced CVD (Williams et al. 2007).

Cell adhesion to nanocrystalline diamond coatings was found to depend on surface chemistry and surface topography, because both parameters likely influence the adsorption of proteins to such surfaces (Yang et al. 2009a,b). A higher number of osteoblasts adhered to nanocrystalline diamond consisting of small crystallites that formed aggregates with mean grain sizes of 30–100 nm compared to submicron crystalline diamond with grain sizes of 100–600 nm. Cells were also more spread and showed higher proliferation and mineralization on nanocrystalline diamond (Yang et al. 2009b). In this context, a correlation between contact angles and surface roughness was considered a relevant factor. Surface roughness, a parameter defined as ratio between geometrical area determined by AFM and projected area, is lower for nano- than for submicron-crystalline diamond. As adsorption and bioactivity of adhesion proteins such as fibronectin and vitronectin are lower on substrates with higher contact angles, the authors discuss this correlation as a factor that contributes to improved osteoblast adhesion (Yang et al. 2008). Moreover, submicron-sized diamond crystals may offer a limited number of adhesion points for osteoblasts. In another study, reduced osteoblast adhesion was shown on surfaces with adhesion points in distances larger than 73 nm (Arnold et al. 2004).

3.4.2.2 Alumina Coatings

Alumina offers excellent biocompatibility, strength, and fracture resistance and has therefore been used as prosthesis material for several decades (Vallet-Regi 2001). In addition, alumina shows very high wear resistance at articulating surfaces. As a bioinert material, alumina shows almost no interaction with tissues; especially smooth surfaces do not osseointegrate (Griss et al. 1975). Pores in the micrometer range, however, have been shown to improve integration (Schreiner et al. 2002), and alumina of grain sizes smaller than 100 nm showed increased osteoblast proliferation and differentiation compared to conventional alumina (Webster et al. 2000a).

In another study, the challenge of cementless implant design is addressed (Karlsson et al. 2003; Briggs et al. 2004). HA is known to be bioactive, but the material itself and the bonding to metallic surfaces are weak. Alumina coatings can be deposited onto metallic implants by electron beam evaporation at 300°C. The resulting layer on the implant provides high-bonding mechanical strength. By subsequent anodization, a nanoporous layer of alumina has been formed and is tested in vitro and in vivo with promising outcome.

3.4.2.3 Titania Coatings

Titanium implants have been shown to be susceptible to modification after treatment with H₂O₂/HCl, leading to titania gel formation on the surface that is transformed to anatase (bioactive crystalline titania) by heat treatment at 400°C for 1 h (Wang et al. 2001). This nanostructured titania layer was stable in contact with buffer and allowed for biomimORIZATION within 2 days. It is hypothesized that both the crystal structure and free negative charges promoted biomimORIZATION.

3.4.2.3 Fabrication of Topography

With the objective to generate hierarchical structures and to investigate relevant topographical structures for cells and tissue regeneration, a plethora of studies have been performed. Since there is a cell-specific reaction to topography, these studies also intended to specifically guide osteoblasts to the surface. Some of these studies, especially those involving nano-sized ceramics and micrometer scale patterns, will be highlighted in the following.

In order to guide cell alignment on the surface of an implant, topographical features have been created on silicon wafer model surfaces by photolithography and subsequent coating with nanoceramics. In order to learn about the cooperation between micro- and nanoscale features, the generated micrometer scale structures are tested for effects on cells. Tan and Saltzman, for example, chemically introduced
carboxyl groups onto the surface of microstructured silicon wafers with the objective to accelerated HA synthesis by biominerlization (Tan and Saltzman 2004). MG63 cells alignment was directed along the ridges (4μm height, 10μm spacing) with and without HA coating. In a similar approach, Lu and Leng investigated the effect of microgrooves of different width (8 and 24μm) on osteoblasts and myoblasts (Lu and Leng 2009). Smooth HA layers were generated on structured wafers by magnetron sputtering and both cell types were found to align along 8μm grooves, but only myoblasts showed alignment along 24μm grooves. Nanocrystalline diamond was coated on microstructured silicon wafers using a microwave plasma enhanced CVD technique (Grausova et al. 2009). Surface hydrophilicity was enhanced by oxygen-containing plasma. Osteoblasts adhered, proliferated, and differentiated better on nanocrystal-line diamond surfaces as compared to polystyrene controls.

Beside these techniques for silicon wafer surface modification, techniques exist that can introduce topographical features onto metal surfaces. Laser ablation is a technique that allows for defined removal of materials from surfaces in order to create surface topography (Peruzzi et al. 2004). Defined topography can be realized on a point-to-point basis with a moving beam or through a template, the latter with higher ablation velocities (Norton et al. 2006). Template-assisted electrohydrodynamic atomization spraying can also be used to generate microstructured surfaces (Li et al. 2008a,b). A nanosized HA dispersion in ethanol was syringed through a small needle and deposited onto a wafer on a grounded metal plate. High voltage between the needle and the grounded plate generated a cone-like jet spraying the suspension onto the substrate. To create a surface pattern, a gold template was placed on top of the titanium substrate. In this study, 15μm wide lines were generated on titanium substrates. Heating of the substrate to 80°C, the boiling point of ethanol, resulted in fast drying and narrow lines (Li et al. 2008a).

In template-assisted ion beam sputtering, a grid is used in front of the substrate in order to generate patterns. Grain size on the patterned surface can be controlled by the deposition rate. Puckett et al. (2008), for example, generated linear micron-sized features on titanium substrates. Ion beam-sputtered parts of the surface were covered with a nanostructured layer of anatase titanium dioxide, while the untreated surface consisted of rutile titanium dioxide. Other approaches to generate micro- and nanostructured titania include acid and oxidative etching (Vetrone et al. 2009) and (sand-) blasting (Zinger et al. 2005).

Bacterial adhesion was compared on different nanostructured titania surfaces (Puckett et al. 2010). Nano-rough surfaces of anatase that were generated by electron beam evaporation showed lower bacterial adhesion as compared to nanotubular and nanotextured amorphous titania surfaces fabricated by anodization.

3.4.2.4 Biological Effects of Nanocoatings

For most nanostructured surfaces, an improved interaction with osteoblasts has been observed (Webster et al. 1999). It is discussed that nanostructured surfaces in comparison to microstructured ones positively affect the adhesion of osteoblasts, while fibroblast adhesion is reduced. This way, improved osseo-integration is mediated and fibrous encapsulation of implants is suppressed (Webster et al. 2000b). The role of serum proteins in improved osteoblast adhesion and function has been demonstrated (Webster et al. 2000a,b). Among adhesion-mediating serum proteins, fibronectin and vitronectin are the most prominent ones. In order to determine the impact of these adhesion proteins, osteoblast adhesion to vitronectin and fibronectin pre-coated nanoparticles was investigated (Webster et al. 2000b). On alumina nanoparticles, vitronectin and fibronectin pre-coating led to increased osteoblast adhesion to small nanoparticles compared to 167nm particles, suggesting that both proteins are involved in the size-dependent effects. When the amount of adsorbed proteins to alumina was determined, significant size-dependent differences were only found for vitronectin. Similar effects have also been found on HA particles. An improved availability of vitronectin adhesion sites for osteoblasts by conformational differences after adsorption to small nanoparticles was discussed to explain the phenomenon. Nuffer and
Siegalsofoundincreasedadhesionofosteoblasts,butedecreasedfibroblastadhesiontosmall spherical silica nanoparticles (20 nm) compared to 100 nm particles (Nuffer and Siegel 2010). As possible explanation, different conformations of pre-adsorbed fibronectin on small nanoparticles compared to 100 nm particles were discussed. In another study, it was reported that β-sheet structure of the protein was lost on the 100 nm particles as compared to fibronectin in solution and fibronectin pre-adsorbed to small nanoparticles (Ballard et al. 2005). No particle size-dependent effect, however, was found for the conformation of pre-adsorbed vitronectin on spherical silica particles of different diameter. In another approach, it was shown that fibronectin adsorption correlated with surface nanoroughness and surface energy (Khang et al. 2007). In this study, surface nanoroughness was controlled by the amount of carbon nanotubes dispersed in a polymer, and the contribution of surface nanostructure and surface chemistry on protein adsorption was determined. The authors found that 30% of fibronectin adsorption depended on the nanostructure, whereas 70% were controlled by surface chemistry. Moreover, cell adhesion was shown to correlate with surface energy and wettability. Studies on self-assembled monolayers revealed that albumin, the most abundant protein in serum, irreversibly adsorbs to hydrophobic surfaces while displacement with vitronectin and fibronectin is possible for hydrophilic surfaces (Arima and Iwata 2007).

Taken together, protein interactions with nanostructured biomaterials influence cell adhesion and proliferation and have positive effects on osteoblast functions. These favorable effects are partially mediated by the amount and conformation of adsorbed proteins.

### 3.4.3 Nanocomposites

Nanocomposites are solid dispersions of a nanoparticulate phase within another biomaterial. As bulk materials, ceramics, synthetic and natural polymers, and structural proteins, such as collagen, have been used. Nanocomposites comprising ceramic nanoparticles are typically inspired by the hierarchical nanoscale structures of mineralized tissues and designed to mechanically reinforce a material that is more elastic but less hard than a ceramic (Šupová 2009). Due to the high surface area of ceramic nanoparticles, effective interactions between the particulate filler and the bulk material can be achieved.

Ceramic/ceramic nanocomposites are structurally divided into nano/nano-type and nano/micro-type or nano/macro-type composites (Niihara 1991; Komarneni 1992; Sternitzke 1997; Choi and Awaji 2005). Nano/nano-type composites are obtained by sintering mixtures of two or more nanoparticulate ceramics. Correspondingly, nano/micro- or nano/macro-type composites are sintered from mixtures of the nanoparticulate ceramic and larger particles of the bulk material. These types of nanocomposites can be further classified as inter(granular)-type, intra(granular)-type, and intra(granular)/inter(granular)-type composites. In the intra(granular)-type, the nanoparticulate phase is dispersed mainly within the matrix grains, while in the inter(granular)-type, the nanoparticles are predominantly located at the grain boundaries.

Ceramic/inorganic nanocomposites represent the most researched type of nanocomposite biomaterials for biomedical applications toward the regeneration of bone (Murugan and Ramakrishna 2005; Christenson et al. 2007). Especially nHA/collagen composites have been investigated in a plethora of scientific studies, because this composition most closely resembles the mineralized matrix of bone (Cui et al. 2007). A large number of studies have also been published on composites of nHA with other natural or synthetic polymers or combinations of other nano-sized ceramic components with polymers. Table 3.3 highlights selected in vivo studies investigating different ceramic nanocomposites. Most nanocomposites are composed of an organic bulk component, either a natural or a synthetic polymer. The predominant application is in bone regeneration. Ectopic bone formation has been observed for composites with nHA that were seeded with adipose tissue-derived stromal cells (Lin et al. 2007) or loaded with bone morphogenetic protein-2 (BMP-2) (Sotome et al. 2004).
<table>
<thead>
<tr>
<th>Nano-Sized Ceramic</th>
<th>Bulk</th>
<th>Application</th>
<th>Implantation Study</th>
<th>Duration (Weeks)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biocompatibility testing</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>Collagen</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Subcutaneous (back)</td>
<td>Kikuchi et al. (2004a)</td>
</tr>
<tr>
<td>HA</td>
<td>PLGA</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Intramuscular</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>HA</td>
<td>Poly(propylene fumarate)</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Intramuscular</td>
<td>Jayabal et al. (2010)</td>
</tr>
<tr>
<td>HA</td>
<td>Chitosan/ carboxymethyl cellulose</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Intramuscular</td>
<td>Jiang et al. (2009)</td>
</tr>
<tr>
<td>HA</td>
<td>Amino acid (nanoconjugates)</td>
<td>Bone regeneration</td>
<td>Mouse</td>
<td>Subcutaneous</td>
<td>Babister et al. (2009)</td>
</tr>
<tr>
<td>Hybrid alumoxane</td>
<td>Poly(propylene fumarate)</td>
<td>Bone regeneration</td>
<td>Goat</td>
<td>Subcutaneous (back)</td>
<td>Mistry et al. (2010)</td>
</tr>
<tr>
<td>Ce-TZP</td>
<td>Alumina</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Intramuscular</td>
<td>Tanaka et al. (2002)</td>
</tr>
<tr>
<td>Alumina</td>
<td>Zirconia</td>
<td>Endoprosthesis</td>
<td>Rat</td>
<td>Stifle</td>
<td>Roualdes et al. (2010)</td>
</tr>
<tr>
<td><strong>Ectopic implantation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>Collagen/alginate (BMP-2 absorbed)</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Intramuscular</td>
<td>Sotome et al. (2004)</td>
</tr>
<tr>
<td>HA fibers</td>
<td>β-TCP (with stromal cells)</td>
<td>Bone regeneration</td>
<td>Mouse</td>
<td>Subcutaneous</td>
<td>Lin et al. (2007)</td>
</tr>
<tr>
<td><strong>Orthotopic implantation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA</td>
<td>Collagen</td>
<td>Bone regeneration</td>
<td>Beagle</td>
<td>Tibia</td>
<td>Kikuchi et al. (2001)</td>
</tr>
<tr>
<td>HA</td>
<td>Collagen, cross-linked</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Tibia</td>
<td>Kikuchi et al. (2004b)</td>
</tr>
<tr>
<td>HA</td>
<td>Collagen</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Tibia</td>
<td>Kikuchi et al. (2004a)</td>
</tr>
<tr>
<td>HA</td>
<td>Collagen</td>
<td>Bone regeneration</td>
<td>Beagle</td>
<td>Tibia</td>
<td>Kikuchi et al. (2004a)</td>
</tr>
<tr>
<td>HA</td>
<td>Collagen</td>
<td>Bone regeneration</td>
<td>Beagle</td>
<td>Tibia</td>
<td>Itoh et al. (2005)</td>
</tr>
<tr>
<td>HA</td>
<td>Collagen/alginate (BMP-2 absorbed)</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Femur</td>
<td>Sotome et al. (2004)</td>
</tr>
<tr>
<td>HA</td>
<td>Chitosan</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Fibula</td>
<td>Kong et al. (2007)</td>
</tr>
<tr>
<td>HA</td>
<td>PLGA</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Forelimbs</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td>HA</td>
<td>PLGA</td>
<td>Cartilage regeneration</td>
<td>Rat</td>
<td>Stifle</td>
<td>Li et al. (2009)</td>
</tr>
<tr>
<td>HA</td>
<td>Poly(propylene fumarate)</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Femur</td>
<td>Jayabal et al. (2010)</td>
</tr>
<tr>
<td>HA</td>
<td>Polyamide</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Mandible</td>
<td>Wang et al. (2007)</td>
</tr>
</tbody>
</table>
3.4.3.1 Fabrication of Ceramic Nanocomposites

As a consequence of the chemical and structural diversity of the ceramic nanocomposites available, it is impossible to provide a comprehensive overview on all utilized fabrication techniques. The following section strives to highlight important techniques in reference to the techniques described for the individual ceramic nanoparticles and to illustrate how different bulk materials can be introduced.

For the fabrication of ceramic/ceramic nanocomposites, mechanochemical processes have been described. For the synthesis of calcium phosphate/titanium particles, commercially available powders of calcium dihydrogen phosphate and titania were mixed and treated in a planetary mill for 15 h (Silva et al. 2007). To avoid excessive heating, the milling was performed in 60 min steps with 10 min pauses. The resulting particles ranged between 20–60 nm in diameter. An intra-type nano-zirconia/alumina composite has been fabricated from alumina powder and zirconium alkoxide (Chevalier et al. 2005). The alumina powder together with a small amount of zirconia is suspended in ethanol, and a zirconium alkoxide solution is added dropwise. The dispersion was dried under stirring at 70°C and the resulting powder was heat-treated at 850°C for 2 h, milled, and finally sintered at 1600°C for 2 h. The resulting intra/inter-type composites contained nano-sized zirconia particles located in between micron-sized alumina grains (Figure 3.1C).

A wet-mechanochemical synthesis route has been described for ceramic/polymer nanocomposites (Wang et al. 2002b). A calcium hydroxide dispersion containing silk fibroin and ammonium polyacrylic acid was prepared. After the addition of phosphoric acid, the mixture was stirred for 1 h and transferred to a multi-ring mill where it was treated for 3 h at 1250 rpm. The milled composite was dried in vacuum, and the dispersed HA nanorods were found to measure 20–30 nm in length and 8–10 nm in width. The resulting ceramic/ceramic nanocomposite was fabricated by a hydrothermal method (Pushpakanth et al. 2008). In order to improve the load sharing and stress distribution of nHA, in situ nanostructured high-strength HA–titanium dioxide was fabricated by microwave-assisted co-precipitation. Minerals were dissolved from cancellous bone with acids and an aqueous solution of titanyl dichloride was added. The pH was then adjusted to 10 and the resulting slurry was placed into a microwave oven and irradiated for 5 min. The precipitate was centrifuged, washed, and dried in a vacuum oven at 100°C to obtain composite nanorods of good chemical and structural uniformity.

Derived from classical controlled precipitation protocols, co-precipitation techniques have become popular for the synthesis of ceramic/polymer nanocomposites. Consequently, parameters that influence the morphology of inorganic/organic composites fabricated via such processes include properties of the co-precipitated polymer, choice of solvent, pH, precipitation time, and temperature (Rusu et al. 2005). For the fabrication of nHA/chitosan composites, for example, an aqueous chitosan solution was prepared with acetic acid and calcium chloride and sodium dihydrogen phosphate were added. The pH was adjusted to 11 and after 24 h the resulting gelatinous dispersion was filtered, washed, and dried to give a rigid material. In a similar process, a nHA/collagen composite was fabricated (Zhang et al. 2003). Collagen, sodium dihydrogen phosphate, and calcium chloride were dissolved in an acidic aqueous

TABLE 3.3 (continued) Selected In Vivo Studies with Ceramic Nanocomposites

<table>
<thead>
<tr>
<th>Nano-Sized Ceramic</th>
<th>Bulk</th>
<th>Application</th>
<th>Animal</th>
<th>Site</th>
<th>Duration (Weeks)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA fibers</td>
<td>β-TCP (with stromal cells)</td>
<td>Bone regeneration</td>
<td>Rat</td>
<td>Cranium</td>
<td>24</td>
<td>Lin et al. (2007)</td>
</tr>
<tr>
<td>HA</td>
<td>Alumina-coated zirconia</td>
<td>Dental implant</td>
<td>Rabbit</td>
<td>Tibia</td>
<td>6</td>
<td>Kong et al. (2002)</td>
</tr>
<tr>
<td>TCP</td>
<td>PLGA</td>
<td>Bone regeneration</td>
<td>Rabbit</td>
<td>Cranium</td>
<td>4</td>
<td>Schneider et al. (2009)</td>
</tr>
</tbody>
</table>
solution. Via pH adjustment to 7, co-precipitation is initiated and the composite was obtained by centrifugation, washed and lyophilized. Within the composite, self-assembled collagen nanofibrils were found on which nHA crystals grew along the longitudinal axes of the fibrils.

In all examples mentioned above, the nanoceramic component of the different composites was prepared during composite fabrication. An immense amount of studies investigates ceramic nanocomposites that are fabricated from a ceramic nanopowder that is somehow dispersed into the bulk component and processed further. Ceramic/ceramic nanocomposites, for example, were fabricated from different nanosized ceramics by mixing and sintering to improve hardness and fracture toughness in dental applications (Nevarez-Rascon et al. 2010). Nano-sized ceramics (Al₂O₃, MgO, Al₂O₃ whiskers, and Y₂O₃–ZrO₂) were dispersed in ethanol and intensely mixed. To the dried mixture magnesium oxide was added to inhibit grain growth of the alumina powder during sintering. The powder was uniaxially pressed at 50 MPa into disks, and the disks were placed into alumina crucibles with zirconia and alumina bed powders and sintered (1500°C, 2h) (Figure 3.1D). Further routes to obtain ceramic/ceramic nanocomposites include conventional powder processing, sol–gel processing, and polymer processing (Sternitzke 1997; Chevalier and Gremillard 2009).

Following the classical powder-processing protocol, the raw powders—ultrafine particles of the matrix-forming ceramic and the nanoparticulate ceramic—are mixed and micro-milled. Wet-mixing is preferred using ultrasound, ball mills, or attrition mills. After drying, the powder mixture is densified, for example, by hot-pressing above 1500°C in a controlled atmosphere. During sol–gel processing, a sol is initially prepared from the nanoparticulate component dispersed in a solution or slurry of matrix precursors. Upon hydrothermal processing, the sol is transferred into a gel, which is turned into an ultrafine powder after drying and calcination. The powder is finally densified by hot-pressing to yield a solid composite with defined density and mechanical properties. As mentioned before, dispersion problems and agglomeration of the ultra-fine powder are critical challenges of these processes.

In approaches toward the fabrication of ceramic/polymer nanocomposites from nano-sized ceramic particles, an effective and homogeneous dispersion of the nano-sized ceramic component is also of critical importance, especially when the bulk materials are hydrophobic. A study that incorporated degradable calcium phosphate particles in a degradable hydrogel matrix showed that a co-precipitation technique for composite fabrication resulted in a much higher degree of dispersion of the ceramic crystals inside the resulting gels compared to a physical mixing strategy (Leeuwenburgh et al. 2007). The physical mixing of ceramic nanoparticles with a solution of the bulk polymer, however, is one of the most popular methods to fabricate ceramic/polymer nanocomposites. In a study that fabricated nHA/poly(lactide-co-glycolide) (PLGA) composite nanospheres, HA, which was synthesized by a homogeneous precipitation method in an ultrasonic field from calcium nitrate and ammonium dihydrogen phosphate, was mixed and dispersed within a solution of the polymer in acetone (Jevtic et al. 2009). Polymer precipitation was initiated through the dropwise addition of the non-solvent ethanol, while the reaction vessel was kept in an ultrasonic field and under temperature control. The particulate composites were stabilized through the addition of a polymeric stabilizer, centrifuged, and air-dried. The morphology of the composite particles was highly regular.

Another strategy to improve the dispersion of ceramic nanoparticles within a polymer matrix includes the use of surface-modified nanoparticles. An alumoxane/biodegradable cross-linked polymer nanocomposite was fabricated using surface-modified nanoparticles (Horch et al. 2004). The hybrid alumoxane nanoparticles modified with a long carbon chain and a reactive double bond were dispersed in the pre-polymer mixture and chemically integrated upon cross-copolymerization. The hybrid nanocomposite showed improved dispersion compared to unmodified nanocomposites and significantly improved mechanical properties.

With regard to tissue engineering application, the composite materials often need to be processed into macroporous constructs. Many techniques are available to achieve such structures. Tissue engineering scaffolds from the above mentioned alumoxane/polymer nanocomposites were prepared by a classical salt leaching technique (Mistry et al. 2009). Modified alumoxane nanoparticles were dispersed in pre-polymer mixture and sieved salt particles were added. The dispersion was packed in a mold and photo-cross-copolymerized. The cross-linked blocks were submerged in distilled water to wash out the
salt and yield macroporous nanocomposite scaffolds. Besides leaching techniques, thermally induced phase separation is a fairly common method for the fabrication of porous scaffolds for tissue engineering (Liu and Webster 2007). This method typically employs a solvent/non-solvent mixture or a poor solvent to process the matrix material. At ambient or elevated temperature, the matrix polymer is soluble in the mixture. Once the temperature is decreased, the system starts to phase separate into a polymer-solvent phase and a non-solvent phase. The metastable partially phase-separated system is rapidly frozen and both solvent and non-solvent are removed by lyophilization generating a porous matrix. Utilizing this technique, bioactive glass/poly(l-lactide) (PLLA) nanocomposite scaffolds were fabricated (Hong et al. 2009). Bioactive glass powder was homogeneously dispersed in dioxane in an ultrasonic field, and PLLA was added to the solution. After lyophilization for 1 week, porous composite scaffolds were obtained. Ceramic/polymer nanocomposites were also processed into nanofiber meshes by electrospinning (Pham et al. 2006; Nie and Wang 2007).

Another important strategy to generate bioactive, nanostructured calcium phosphate within tissue engineering scaffolds is biomineralization (Bonzani et al. 2006; Kretlow and Mikos 2007; LeGeros 2008; Ma 2008; Palmer et al. 2008). Biomineralization strategies, typically involving the immersion of a polymeric construct in SBF, can be applied to both hydrogel systems and macroporous solids. It has been demonstrated that certain functional groups can promote and regulate crystal growth on the substrate surface (Kretlow and Mikos 2007). Carboxylic acid and hydroxy group that have been generated on poly(hydroxyl esters) surfaces by controlled hydrolysis, for example, have been shown to regulate calcium binding to the polymer surface and heterogeneous mineral growth (Murphy and Mooney 2002). The composition of the mineral grown in SBF solutions was a carbonate apatite similar to vertebrate bone mineral. Crystal size and morphology were predominantly controlled by the mineralization media and not by polymer surface characteristics. A template-driven mineralization technique has also been demonstrated for a poly(2-hydroxyethyl methacrylate) hydrogel scaffold (Song et al. 2003). By a gradual increase in pH controlled by thermal decomposition of urea, carboxylic acid groups were exposed on the surface by ester hydrolysis. These anionic groups promoted high-affinity nucleation and growth of calcium phosphate on the surface along with extensive calcification and the formation of robust surface mineral layers. Biomimetic mineralization processes have also been employed to deposit calcium phosphates on electrospun bioactive glass nanofibers (Figure 3.1E) (Xia et al. 2007) and silica on electrospun polymer fiber meshes (Patel et al. 2009). There are also reports on the fabrication of nano-sized ceramic/ceramic composites by mineralization. Anionic functional groups were chemically introduced in single-walled carbon nanotubes (SWNTs), and HA was shown to nucleate and crystallize on the nanotube surface (Zhao et al. 2005).

### 3.5 Characterization of Ceramic Nanobiomaterials

The interactions between a nanobiomaterial and biological fluids, cells, or complete biological systems are complex and dependent on many parameters. Especially for nano-sized substrates, the bulk properties of the material become less significant, and commonly known property-response relations have most often to be renewed (Jones and Grainger 2009). As a consequence of the high surface-to-volume ratio, substrate properties are strongly determined by surface state and morphological parameters in contrast to micro- and macro-dimensioned substrates. For some ceramic materials that are considered well biocompatible in the micro- and macroscale, certain cytotoxic effects have been reported for murine fibroblasts and macrophages (Yamamoto et al. 2004). The observed cytotoxic effects of titanium dioxide, aluminum oxide, zirconium dioxide, silicon nitride, and silicon carbide nanoparticles are suggested to be based neither on chemical properties nor on size, but on the total volume of particles and their shape. This so-called mechanical toxicity is supported by findings that spiked nanoparticles bear a higher cytotoxicity than spherical nanoparticles. Such effects were more pronounced in macrophages than in fibroblasts, likely as a result of the phagocytic process. As another example, the toxicity mechanism and long-time health effects of certain silica-based materials such as crocidolite asbestos, although
not fully understood yet, depend on properties including respirability or ability to enter the lung, durability due to insolubility and lack of clearance by macrophages, fibrous geometry, aspect ratio, and surface properties associated with the generation of reactive oxygen or nitrogen species (Hillegass et al. 2010). Due to the high surface area of nanomaterials, surface contaminations, which may significantly alter the original surface chemistry and pattern and furthermore the biological response, are another challenge (Grainger and Castner 2008). Surface science provides many tools that can be used for the characterization of surfaces and interfacial conditions of nanobiomaterials. However, significant effort is devoted toward the development of new methods and the adaption of known methods to meet the special requirements of nanoscale analytics. In the next paragraphs, a selection of important morphological, surface, and bulk characterization techniques is given and their applications in ceramic nanotechnology are highlighted.

### 3.5.1 Particle Size and Morphology

**Transmission electron microscope** (TEM) is often employed to determine size and morphology of ceramic nanostructures (Roy et al. 2003; Bhattarai et al. 2007; Wang and Shaw 2009b). Usually, TEM samples are prepared from dried nanoparticle suspensions. Alternatively, an electron transparent lamella is cut out of the ceramic sample by ion milling (for details on the milling process, refer to section Bulk Characterization), lifted out of the milling trench, and introduced to TEM optics (Kooi et al. 2003). High resolution TEM (HRTEM) is able to image the crystallographic structure of a sample at resolutions in the sub-Ångström range (O’Keefe et al. 2005). This setup was utilized to explore the mineralization state of collagen fibrils with nanocrystals from HA (Zhang et al. 2003) or basal-plane stacking faults in ceramic titanium silicon carbide crystals (Kooi et al. 2003). In a study that compared nHA powders synthesized by an ethanol-based and a water-based sol–gel technique (Kalita et al. 2007), HRTEM analysis revealed that particle size of powders synthesized via the ethanol-based and the water-based method was 20–50 and 5–10 nm in diameter, respectively.

**Scanning electron microscopy** (SEM) can be used to image size and morphology of specimens, which are impassable for electrons. With this microscopic technique, different material properties can be assessed depending on the recorded signal, such as secondary electrons, backscattered electrons, Auger electrons, and X-ray bremsstrahlung. Secondary electrons, which are emitted from the uppermost nanometers of the sample surface, are used to detect surface topography and to visualize surface structures. From the electron micrograph, the dimension of nanostructures can be derived (Zhang et al. 2003; Dulgar-Tulloch et al. 2009). For PLGA/HA composite nanospheres, for example, effects of the fabrication process on nanosphere morphology have been comparatively assessed by SEM image analysis (Jevtic et al. 2009).

### 3.5.2 Surface Characterization

**Atomic force microscopy** (AFM) is a versatile tool in surface characterization and provides image information on surface structures and patterns as well as data on surface functionalization and reactivity. In the classical operating mode, the cantilever-mounted tip scans the sample surface registering differences in height in order to create a topographical image of the sample surface. With this method, nanometer and micron-scale diamond film surface topographies that showed significant effects on osteoblast functions were imaged (Yang et al. 2009b). To date, variants of the classical AFM method are available, in which the probing tip is modified. Lateral force microscopy (LFM) (Liu et al. 1994; Crossley et al. 1999), chemical force microscopy (CFM) (Frisbie et al. 1994), or friction force microscopy (FFM) (Overney et al. 1992) are such techniques that are used to explore the chemical quality of a surface in AFM contact mode. In order to detect tip-to-surface interactions that depend on the chemical quality of the surface, certain functional groups as well as entire molecules including proteins are covalently bound to the tip. A stronger interaction is represented by a stronger torsion of the tip-bearing cantilever. AFM can also
provide information toward the explanation of biological effects observed on nanostructured ceramic surfaces, because it can be used to qualitatively determine protein structures that became adsorbed to the surface (Liu and Webster 2007). AFM was utilized to examine the HA structure at the interface to citrate, in which the carboxylic acid groups have chemical similarities to the residues of osteocalcin that interact with bone minerals (Jiang et al. 2008). In another example of how AFM is utilized, the mineral deposits on dip-coated PLGA and PLGA/collagen nanofibers as well as the nanotexture of the fibers have been visualized by AFM (Ngiam et al. 2009). The most common application for AFM is to determine surface roughness as shown for dense HA and β-TCP substrates (dos Santos et al. 2008).

**X-ray photoelectron spectroscopy (XPS) or electron spectroscopy for chemical analysis (ESCA)** allows for the qualitative and quantitative determination of the surface elemental composition including its chemical state. In XPS, the sample is irradiated with a focused X-ray beam in ultra-high vacuum (UHV) causing a photoelectric effect. Surface electrons are knocked out of their orbitals and their kinetic energies are measured with an electron energy analyzer. The final spectrum is achieved by plotting the electron counts against the calculated electron binding energies. From these spectra, the chemical surface composition can be identified, because each element is represented by a characteristic set of binding energy peaks. Analysis of signal intensity then provides quantitative information. With a sampling depth of less than 10 nm (depending on the angle between analyzer and sample), XPS acquires surface data that is practically unimpaired by signals from the bulk phase. Consequently, surface contaminations can be easily detected in XPS spectra (Chen et al. 2008). With regard to ceramic nanobiomaterials, the formation of HA layers on bioactive titanium in SBF was investigated using XPS (Takadama et al. 2001).

**Secondary ion mass spectrometry (SIMS)** has a sampling depth of 1–2 nm, which is even lower than that of XPS. However, SIMS is a destructive method, during which molecules are sputtered from the sample surface by a focused primary ion beam in UHV. One distinguishes between dynamic SIMS, which enables compositional analysis from the top layer into deeper layers due to the utilization of a high ion dose beam, and static SIMS (McPhail 2006). Static SIMS utilizes low ion doses to ensure that only the uppermost sample layer is analyzed. Usually, static SIMS is coupled with a time-of-flight analyzer (ToF-SIMS) and is a versatile method to determine the elemental and chemical composition of a surface. It has also been utilized to identify and characterize surface-adsorbed proteins (Tidwell et al. 2001). In a specific example, the interface between strontium-containing HA and cancellous as well as cortical bone was monitored by ToF-SIMS over six months in a rabbit model (Ni et al. 2006). The study revealed that higher concentrations of calcium, phosphor, sodium, and oxygen were found on the interface with cancellous bone indicating different dissolution rates of the ceramic due to the type of adjacent bone.

**Energy-dispersive X-ray spectroscopy** (EDX or EDS) is another technique that allows for elemental analysis of a surface. The sample surface is irradiated by a SEM electron beam and emitted X-rays are detected. The energy spectra of the emitted X-rays can be correlated to specific elements. This method has been used to characterize nanostructured ceramic coatings due to their elemental composition (Wang et al. 2009) and to analyze minerals deposited during biomineralization of titanium implants (Serro and Saramago 2003).

**Auger electron spectroscopy (AES)** is another technique in which the sample surface is excited by a SEM electron beam. Electron gaps created in lower orbital shells are filled with electrons from a higher orbital, and the corresponding transition energy is emitted. The peculiarity of the so-called Auger effect is that the emitted energy is absorbed by another electron in the same atom. This excited Auger electron is then emitted from the atom and traceable in UHV. The electron energy patterns are unique for each element providing information about the elemental surface composition. With regard to ceramic materials, it should be considered that most ceramics are isolators and sample charging may occur when exposed to high energy beams. In order to perform AES analysis with the high spatial resolution of a few nanometers, a conductive layer has to be introduced to the reverse side of a thinned sample (Yu and Jin 2001). AES was applied to determine the surface composition of a titanium dioxide coating on a titanium alloy substrate before and after base treatment (Zhao et al. 2006).
Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy is a powerful tool for surface characterization because one can detect changes in chemical structure or chemical environment as expressed in frequency shifts and changes in relative band intensities. The functional principle of ATR-FTIR is based on so-called evanescent waves. Whenever a light beam hits a phase boundary at an angle of incidence that is larger than the critical angle, total reflection occurs. A certain small amount of the beam energy, however, passes the phase boundary as an evanescent wave, which penetrates the adjacent phase over a distance depending on the refractive index of the material and the wavelength of the reflected beam. The phenomena of total reflection in combination with the formation of evanescent waves occur when the light-conducting phase is of higher optical density than the adjacent phase. Therefore, the crystals utilized in ATR-FTIR spectroscopy are selected to have a higher refractive index than the sample material. In an ATR-FTIR analysis, the attenuation of the irradiated intensity is determined. This loss in energy in the IR-wavelength range is characteristic for the quality and state of the specific atom groups in the sample. In a specific example, the formation of an apatite coating on alginate/chitosan microparticles was confirmed by ATR-FTIR spectroscopy through characteristic signals of phosphate and carbonate groups (Lee et al. 2009). When ATR-FTIR is utilized for surface analysis, it has to be considered that the sampling depth of this method is about 1000 nm and not exclusively limited to the surface layer (Liu and Webster 2007).

3.5.3 Bulk Characterization

Thermogravimetric analysis (TGA) acquires changes in sample mass depending on temperature and time. Measured weight loss, gain, and/or fluctuations at distinct temperatures can be characteristic for a specimen and its composition. Alterations, usually mass loss, in the thermogravimetric curve of a substance are attributed to impurities, chemical modification, degradation, oxidation, or pyrolysis. Magnetic iron oxide nanoparticles stabilized with modified chitosan were examined utilizing TGA to estimate the amount of bound chitosan from the percentage weight loss by pyrolysis of the organic component (Bhattarai et al. 2007). With regard to ceramic materials, TGA is mainly utilized to test for thermal stability of the sample (Kalita and Verma 2010).

Focused ion beam (FIB) tomography is a technique of great potential to visualize the inner chemical composition and structure of a material (Möbus and Inkson 2007; Uchic et al. 2007; Munroe 2009). In a so-called milling process, the ion beam ablates nanometer layers of a sample. In a single-beam FIB device, the ion beam is used to both mill the sample and sputter ions from the newly exposed surfaces for analysis. This requires movement of the sample in between the milling and the sputtering step. In combination with SIMS (FIB-SIMS), the elemental composition of a sample can be analyzed layer by layer. With such a setup, a spatial resolution of approximately 50 nm in lateral direction, 5 nm in depth, and a few micrometers in sectional direction can be achieved (Tomiyasu et al. 1998). Dual-beam devices, which use individual beams for milling and detection and therefore do not require the sample to be moved during analysis, provide the highest spatial resolution. In such dual-beam devices, a SEM image of the freshly exposed surface can be recorded in a frequency of roughly 25 slices per hour. From these images, a three-dimensional reconstruction of the sample can be done, which allows for a quantification of pore and grain volumes. A resolution of up to 6 nm in lateral direction, 7 nm in depth, and 17 nm in sectional direction has been achieved (Holzer et al. 2004), and sample volumes larger than 1000 μm³ can be analyzed (Uchic et al. 2007). Recently, rod-shaped nanoparticles of HA were produced by a hydrothermal synthesis technique and investigated for their infiltration into dentinal tubules of etched human molars (Earl et al. 2009). Information on the depth of infiltration was obtained from sections of dentine prepared using FIB milling (FIB-SEM).

X-ray diffraction (XRD) is a non-destructive technique to determine the crystallographic orientation and structure of a sample. It is based on the scattering of an X-ray beam due to the molecular quality of the target. There are two different modes of data acquisition: small angle X-ray scattering (SAXS) and wide angle X-ray scattering (WAXS) (Cancedda et al. 2007). According to Bragg’s law,
larger plane spacings in a crystal lattice entail smaller scattering angles. SAXS is used to determine size, shape, and orientation of mesoscopic structures that are relatively large. WAXS is used to determine the distance of molecule or atom layers arranged in an orderly pattern such as in crystalline solids. This way, WAXS provides information about the quality of crystal unit cells that means the crystal inner structure. The simultaneous collection of SAXS and WAXS information with a small area microbeam entails acquisition of highly resolved data, because each volume is described with both acquisition modes at the same time (Guagliardi et al. 2009). Quantitative XRD has been shown to be a more accurate tool than wet chemistry to identify the Ca/P ratio of calcium phosphate apatites that strongly affects the chemical and biological properties of these materials (Raynaud et al. 2001, 2002; Han et al. 2006). Apatite nanostructures have also been identified using this technique in in situ–generated nanocomposites with collagen (Lin et al. 2004) (Figure 3.1F). In another application example, biomimetic collagen/nanoapatite composite scaffolds for tissue engineering were prepared by a precipitation method and analyzed (Liu 2008). The crystalline phase was identified as nHA of lower crystallinity than that of a rabbit ulna. It is discussed that crystal growth and final crystallinity are influenced by the orientation of the collagen fibers. With regard to the identification of optimal sintering temperatures and conditions, XRD can also be used to study phase evolution/ transformation in calcium phosphate and titanium dioxide samples (Kalita et al. 2007, 2008). In another study, the in situ transformation of anhydrous dicalcium phosphate cement into HA was monitored over 24 h by XRD (Hsu et al. 2009).

X-ray computed tomography (CT or μCT) has been applied in mesoscale physics to image porous media and to derive mechanical properties of the specimen from this data (Sakellariou et al. 2004). In addition, pore size and structure of ceramic bodies can be investigated (Ritman 2004; Cancedda et al. 2007). Due to the mesoscale resolution of this technique, a visualization of nanostructures is practically not possible. However, certain applications of μCT with nanocomposites have been described. For a composite of nano-sized calcium phosphate crystals and an injectable hydrogel matrix prepared by precipitation of the mineral in presence of the hydrogel precursors, μCT analysis demonstrated the absence of aggregates in the micrometer range indicating a high degree of dispersion (Leeuwenburgh et al. 2007).

### 3.5.4 Cytocompatibility, Biocompatibility, and Toxicity

The prominent class of calcium phosphate nanobiomaterials is most likely the least debated with regard to biocompatibility and environmental safety because of the physiological chemistry of these minerals and the high abundance of such nanomaterials in organisms. Reliable and predictive models to determine the acute and chronic biosafety of nanomaterials in general, however, have not yet been developed to a satisfactory level. A common scientific consensus on how to responsibly address urgent questions regarding potential health and environment risks of nanomaterials has not yet been agreed on (Webster 2008; Grainger 2009). In vitro cell cultures provide fairly straightforward and cost-effective methods to screen the toxicity of nanomaterials in early stages of product development. Such in vitro methods include cell culture assays for cytotoxicity (altered metabolism, decreased growth, lytic or apoptotic cell death), cell stress, proliferation, genotoxicity, altered gene expression, and assays for cell-based production of reactive oxygen species (Jones and Grainger 2009; Hillegass et al. 2010). Following such screening test, which cannot imitate a complementary in vivo system, small mammalian models can help to assess possible toxicities and biodistribution of nanomaterials in humans (Fischer and Chan 2007). Furthermore, quick, cheap, and facile models, such as the zebrafish, have been described to conservatively assess toxicity of nanomaterials (Fako and Furgeson 2009). Using this assay, nanocrystalline zinc oxide, nanocrystalline titanium dioxide, and nanocrystalline alumina were tested. Only nanocrystalline zinc oxide showed visible signs of toxicity indicated by a delayed hatching rate and development of zebrafish embryos and larvae as well as tissue damage and decreased survival.
3.6 Conclusion and Perspective

Ceramics have grown to an important class of biomaterials, especially for the fabrication of orthopedic and dental implants and in strategies aiming at the regeneration of bone and teeth (Vallet-Regi 2001, 2006, 2008; Salinas and Vallet-Regi 2007; Chevalier and Gremillard 2009; Dorozhkin 2010a). With ceramic materials being available as nano-sized structures, the opportunities to utilize this class of materials toward a desired mechanical or biological effect have significantly broadened. In the form of nano-grained coatings, ceramic nanobiomaterials improve integration of metal or metal alloy implants with the surrounding hard tissue and have potential to significantly prolong implant lifetimes. In ceramic nanocomposites, the unique chemical composition, mechanical properties, and specific interactions with proteins that are attributed to the bioactive properties of ceramic nanostructures can be embedded in a composite biomaterial with improved bulk properties determined by the bulk material. In both applications, nano-grained ceramic coatings and ceramic nanocomposites, the bulk properties of the resulting biomaterial or medical device are determined by a non-ceramic material in most cases. The most prominent nanoceramic to date is nHA, but a key interest toward the fabrication and use of nanocrystalline cHA can be identified. Another strategy to generate nanocrystalline calcium phosphate that closely resembles bioapatite involves controlled mineralization processes and holds promise to generate coatings and composites with improved biocompatibility and bioactivity. Such developments have to be accompanied by ongoing analytical efforts to better understand the chemistry and structure of biological apatites and how these parameters control the apatites’ physical and biological properties. Rising interest is also devoted to nanocrystalline diamond. Exciting mechanical improvement of articular prostheses by nano-diamond coatings combining ultra-low friction and biomimetic nanostructure has been described. In bone regeneration strategies, it will be necessary to optimize structure, bioactivity, and remodelability, as well as degradative properties of ceramic nanocomposite scaffolds. At the same time, scientific proof of nanoceramic safety and compatibility is a vital prerequisite for patient compliance and a successful regulatory process.

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