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CHAPTER 12

Bioactive Bioceramic Coatings: Part I. Coatings on Non-Metallic Biomaterials

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1. INTRODUCTION
The increasingly longer life expectancy of human beings requires more and more surgical operations for hard-tissue replacement [1]. A national survey (1991–2005) by the
American Academy of Orthopedic Surgeons showed that 160,000 hip prostheses and 266,000 knee prostheses were implanted in the United States in 1998, and these figures increased steadily to 235,000 and 534,000, respectively, in 2005. Transplantation, which utilizes tissues from a human or other species donor, has been almost abandoned nowadays for hard tissue replacements. Man-made materials, including metals and alloys, ceramics, polymers, and composites, have been widely used due to their availability, reproducibility, and reliability to repair or replace the malfunctioning human tissues. The tight and reliable fixation between the implants and surrounding tissues is always favored in restorative surgery, especially in joint-replacement operations. For a total hip replacement, poly(methyl methacrylate) (PMMA) bone cement is normally used to secure hip prostheses. Unfortunately, there have been problems with this type of "mechanical fixation." Severe abrasive wear, which results from the detachment of bone cement, is usually reported clinically. In addition, it is not easy to retrieve the failed implants when a revision surgery is required [2]. To overcome such problems, the so-called "biological fixation," which relies on the in-growth of the bone into the porous surface or textured surface of implants for stabilization, has been developed and clinically used. The porous surface structure can be achieved by plasma spraying metal powders, sintering wire meshes, or sintering metal beads on the implants, and the pore size range can be optimized in the range of 100–350 μm for the best bone in-growth in clinical practices [3, 4]. Although the strategy of using surface porous implants without bone cement (uncemented implants) have proven to be successful, bone in-growth is seldom observed [5]. Therefore, "bioactive fixation" has been advocated in the past several decades since the milestone work by Hench et al. in 1971 [6].

Natural bone is a composite of collagen and mineral nanoparticles made of carbonated hydroxyapatite (CHA). The basic building block of bone material is collagen fibril of ca. 100 nm in diameter, which consists of an assembly of 300 nm long and 1.5 nm thick collagen molecules, deposited by the osteoblasts (bone-forming cells) into the extracellular space and then self-assembled into fibrils. Collagen fibrils are filled and coated with tiny mineral CHA crystals with sizes of ca. 1.5–4.5 nm [7]. Therefore, it is not surprising that the bioactive fixation is defined as the chemical, direct bonding at the molecular level between implants and their surrounding bone tissues through an apatite layer consisting of nano-sized particles [8]. After implantation in the human body for 3–6 months, the interfacial strength achieved by bioactive fixation is comparable to or higher than the strength of the surrounding bone tissue [2]. The key for implants to achieve bioactive fixation when implanted in the human body is their high bioactivity, i.e., the ability to attach to the surrounding bone tissue through chemical bonding in the human body environment [9]. Therefore, over the past 20 or so years, apart from the development of bioactive materials, such as bioactive glasses, ceramics, and glass-ceramics, attention has also been paid to providing bioinert biomaterials with bioactivity through various coating techniques.

The two chapters of this series focus on reviewing bioactive bioceramic coatings on various biomaterial substrates. The in vivo and in vitro evaluation of the bioactivity, mechanisms for apatite formation, and various coating techniques are reviewed. The first chapter deals with several common issues and also coatings on non-metallic biomaterials. Because of the significance and the relatively larger amount of published papers concerning bioactive coatings on metallic biomaterial substrates, the second chapter concentrates on the work of coating metallic biomaterials, with special attention paid to nanostructured titania coatings.

2. BIOACTIVE BONDING AND IN VITRO BIOACTIVITY

2.1. In Vivo Observations of Bioactive Bonding

Host tissues respond naturally to any material that invades the body. There are three types of implant-tissue interactions for biomaterials, as listed in Table 1. In most cases, the human body tends to protect itself spontaneously by forming a fibrous tissue to isolate the invading material. For a material with a controlled level of chemical reactivity,
through certain surface reactions a bioactive interface can be developed that prevents motion and mimics the type of interface that is formed when natural tissues repair themselves. A highly reactive material dissolves or resorbs gradually and is replaced eventually by the newly formed tissues [10]. Biodegradable materials are the most ideal materials to be used as hard tissue substitutes as nothing is left in the human body once the diseased or fractured tissue has been repaired. However, the degradation of the materials surely causes a loss of the implant's mechanical properties. It is not easy to reach a compromise between the degradation (or disappearance) rate and the rate of decrease in mechanical properties during the tissue-regeneration process. Therefore, stable bioactive materials are still attracting considerable attention at the present time.

A cross-sectional scanning electron microscopy (SEM) micrograph published by Fujibayashi et al. [11] clearly shows the typical bonding between bioactive materials and bone (Fig. 1). After implantation for a certain duration, a silicon-rich layer formed on the bioactive glass surface through certain reactions, on which apatite deposition was triggered. The surrounding bone tissue attached to the implant through this apatite layer [11, 12]. The formation of a thin apatite layer, which was characterized by transmission electron microscopy (TEM) to be collagen-free fine nanocrystals having no preferred orientation [13], can be well reproduced in vitro using simulated body fluids (SBF) and hence be well characterized (see the Mechanisms of In Vitro Apatite Deposition section for details). However, the bone apposition process on the thin apatite layer is not yet well clarified due to the complicated bone biology, bone cell mechanotransduction, and cell-surface interactions involved in the new bone development process [14]. Six events have been suggested to be involved in sequence: adsorption of biological molecules in the apatite layer, action of macrophages, attachment of stem cells, differentiation of stem...
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cells, generation of a matrix, and mineralization of the matrix [12]. Many reports have appeared giving observations on proteins adsorption [15, 16] and cells attachment and differentiation [13, 17, 18] on apatite films. However, the latter stage is relatively less well-defined, and the factors controlling the rate still remain unknown [12].

The formation of a thin bone-like apatite layer has been observed without exception on all bioactive materials. For example, after eight weeks of implantation in rats, a thin collagen-free apatite layer was observed on the surfaces of all bioactive glass-ceramics of Ceravital® (Na2O-K2O-MgO-CaO-SiO2-P3O5 glass containing Ca10(PO4)O6 crystals), glass-ceramic A-W (Na2O-K2O-MgO-CaO-SiO2-P2O5 glass containing hydroxyapatite and wollastonite crystals), dense hydroxyapatite (HA), and Bioglass® [13].

2.2. In Vitro Evaluation of Bioactivity

2.2.1. Simulated Body Fluid and Thermodynamics of In Vitro Apatite Formation

In vivo observations have confirmed that a common feature of all bioactive implants is the formation of a carbonate incorporated hydroxyapatite (CHA) layer, which is equivalent in structure and composition to the mineral phase of bone, on their surfaces when implanted [10]. In vivo experiments require sacrificing animals as well as conducting surgical operations and waiting for long durations for revealing effects. To evaluate the bioactivity of biomaterials simply and effectively, Kokubo et al. developed the now widely used SBF, which is a metastable calcium phosphate (Ca-P) solution similar in inorganic ion concentrations to those of human blood plasma, as shown in Table 2 [19–21]. Occasionally, Hank’s balanced salt solution (HBSS) is also used to evaluate in vitro bioactivity.

SBF is a highly supersaturated solution with respect to apatite. It is stable because of the high nucleation energy. Once nucleated, apatite precipitates follow the reaction below:

\[ 10\text{Ca}^{2+} + 6\text{PO}_4^{3-} + 2\text{OH}^- = \text{Ca}_{10} (\text{PO}_4)_6 (\text{OH})_2 \]  

(1)

The driving force for the above reaction can be expressed as:

\[ \Delta G = -RT\ln S \]  

(2)

where \( \Delta G \) is the Gibbs free energy of formation, \( R \) is the gas constant, \( T \) is the absolute temperature, and \( S \) is supersaturation. \( S \) is calculated by

\[ S = (IP/K_{sp})^{1/9} \]  

(3)

\[ IP = (\text{Ca}^{2+})^3(\text{PO}_4^{3-})^3(\text{OH}^-)^3 \]  

(4)

\( K_{sp} \) is the solubility of the apatite. At 37°C, \( K_{sp} = 2.35 \times 10^{-59} \) [23].

The supersaturation corresponding to apatite in SBF is as high as 167 [19]. At the normal body temperature of 36.5°C, the free energy calculated using Eq. (2) is −13.2 KJ/mol. Therefore, once nucleated, apatite grows spontaneously. The control step of apatite deposition in SBF is the nucleation process.

The nucleation rate of apatite is determined by

\[ J = K \exp(-\Delta G^*/kT) \]  

(5)

Table 2. Ion concentrations (mM) of simulated body fluid (SBF) [19], Hank’s balanced salt solution (HBSS) [21, 22] and human blood plasma (HBP).

<table>
<thead>
<tr>
<th></th>
<th>Na⁺</th>
<th>K⁺</th>
<th>Ca²⁺</th>
<th>Mg²⁺</th>
<th>Cl⁻</th>
<th>HCO₃⁻</th>
<th>HPO₄²⁻</th>
<th>SO₄²⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBF</td>
<td>142.0</td>
<td>5.0</td>
<td>2.5</td>
<td>1.5</td>
<td>148.8</td>
<td>4.2</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>HBSS</td>
<td>142.0</td>
<td>5.81</td>
<td>1.26</td>
<td>0.898</td>
<td>146.0</td>
<td>4.17</td>
<td>0.779</td>
<td>0.406</td>
</tr>
<tr>
<td>HBP</td>
<td>142.0</td>
<td>5.0</td>
<td>2.5</td>
<td>1.5</td>
<td>103.0</td>
<td>27.0</td>
<td>1.0</td>
<td>0.5</td>
</tr>
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</table>
where $k$ is the Boltzmann constant, $K$ is a kinetic factor, $\Delta G^*$ is nucleation activation energy. The value of $\Delta G^*$ for a spherical nuclei can be expressed by [23]

$$
\Delta G^* = \frac{16\pi v^2 \gamma^2 f(\theta)}{3k^2T^2(\ln S)^2}
$$

(6)

where $v$ is the molecular volume of apatite ($2.6324 \times 10^{-28}$ m$^3$), $\gamma$ is the interfacial tension between apatite and the solution ($10.4 \text{ mJ/m}^2$), and $f(\theta)$ is a function of the contact angle between the nucleus and substrate. In addition to the solution temperature, the nucleation rate of apatite is determined mainly by the solution supersaturation ($S$) and the contact angle ($\theta$). A higher supersaturation of the solution and a better wetability of the substrate contribute to a higher nucleation rate of apatite. In addition, defects on the substrate surface effectively improve the nucleation rate by reducing the activation energy.

There are several Ca-P compounds, shown in Table 3, that are of considerable interest in the biomaterials field. A theoretic analysis of Ca-P precipitation in SBF indicates that apatite precipitation exhibits a higher thermodynamic driving force than does OCP and DCPD in SBF, but OCP precipitation is kinetically favorable in SBF. The nucleation rate of apatite is significantly affected by the pH value. A high pH environment favors apatite nucleation. Precipitation of CHA is more kinetically favorable than that of stoichiometric HA and has the same level of thermodynamic driving force. The precipitation of calcium-deficient HA is also more kinetically favorable, but its thermodynamic driving force is lower than that of stoichiometric HA [23].

Observation with a dynamic light-scattering photometer equipped with a high-power Ar-ion laser revealed Ca-P clusters with an initial hydrodynamic diameter of about 1 nm in the SBF [25]. The cluster remained almost unchanged for a prolonged time up to 7 d at 36.5 °C. A modified SBF, which has totally equal concentrations of ions to those in blood plasma, has also been recommended for in vitro bioactivity evaluation [26].

Juhasz et al. argued recently that human blood serum (HBS) served as well to mimic the in vitro crystallization process on brushite, tricalcium phosphate (TCP) and HA. By using SBF with the addition of HBS, the influence of proteins can be assessed, which hence provides a more accurate in vitro model for the bone-bonding ability of biomaterials [27].

### 2.2.2. Characterization of Apatite Formed In Vitro

The apatite precipitated from SBF is characteristic of bone mineral, i.e., small crystal size, the presence of carbonate ions, ionic substitution by Mg$^{2+}$ and Cl$^-$, and calcium-deficient [28]. A typical apatite coating formed in SBF on a chemically modified commercial pure titanium (CPTi) substrate is shown in Figure 2. Each globule of the coating is actually aggregates of numerous tiny flakes united together. Figure 3 exhibits the corresponding thin-film X-ray diffraction (TF-XRD) pattern of the coating. It gives an abnormally

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Formula</th>
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<tr>
<td>Monocalcium phosphate monohydrate</td>
<td>MCPM</td>
<td>Ca(H$_2$PO$_4$)$_2$·H$_2$O</td>
</tr>
<tr>
<td>Monocalcium phosphate anhydrous</td>
<td>MCPA</td>
<td>Ca(H$_2$PO$_4$)$_2$</td>
</tr>
<tr>
<td>Dicalcium phosphate dihydrate (brushite)</td>
<td>DCPD</td>
<td>CaHPO$_4$·2H$_2$O</td>
</tr>
<tr>
<td>Dicalcium phosphate anhydrous</td>
<td>DCPA</td>
<td>CaHPO$_4$</td>
</tr>
<tr>
<td>Octacalcium phosphate</td>
<td>OCP</td>
<td>Ca$_8$H$_2$(PO$_4$)$_6$·5H$_2$O</td>
</tr>
<tr>
<td>α-Tricalcium phosphate</td>
<td>α-TCP</td>
<td>α-Ca$_5$(PO$_4$)$_3$</td>
</tr>
<tr>
<td>β-Tricalcium phosphate</td>
<td>β-TCP</td>
<td>β-Ca$_5$(PO$_4$)$_3$</td>
</tr>
<tr>
<td>Tetracalcium phosphate</td>
<td>TTCP</td>
<td>Ca$_5$(PO$_4$)$_3$O</td>
</tr>
<tr>
<td>Calcium hydroxyapatite</td>
<td>HA</td>
<td>Ca$_{10}$(PO$_4$)$<em>6$(OH)$</em>$_2$</td>
</tr>
<tr>
<td>Fluorapatite</td>
<td>FAP</td>
<td>Ca$_{10}$(PO$_4$)$_6$F</td>
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Figure 2. A typical apatite coating formed in SBF after 1 d soaking, on the surface of CPTi subjected to treating with 30 mass% H₂O₂ containing 3 mM TaCl₅ at 80°C for 3 d. (a) low magnification and (b) high magnification showing the tiny flakes united together to form large globules.

strong (002) peak, suggesting a preferential growth of the apatite crystal. The XRD peaks corresponding to (211), (112), and (300) planes overlap to give a broad diffraction located around 2θ = 32°. The apatite is poorly crystallized. Figure 4 displays a typical Fourier transform infrared reflection (FT-IR) spectrum of the apatite. The band at 875 cm⁻¹ and the bands between 1418 and 1456 cm⁻¹ correspond to the carbonate group incorporated in the apatite. The two bands at 597 and 555 cm⁻¹ are from the ν₂ mode of O–P–O bending in apatite, whereas the peaks at 1110 and 1000 cm⁻¹ indicate the ν₁ band of P–O stretching mode. The peak at 472 cm⁻¹ is ascribed to the ν₃ mode of O–P–O bending in apatite. The shoulder at 961 cm⁻¹ reflects the ν₁ band of P–O stretching mode in apatite [28, 29].

The electrochemical impedance spectroscopy (EIS) technique was found to serve well to investigate apatite growth on the surface of alkali-treated titanium and its alloys as well as oxide films on the untreated titanium surface. EIS is sensitive to detecting an increase in electrical resistance on the outmost surface when apatite nucleates [30, 31]. At this nucleation stage, apatite cannot be clearly seen even under SEM. Typical EIS spectra of alkali-treated Ti before and after immersion in SBF are shown in Figure 5. For these spectra, by considering the nucleation and growth of apatite on the substrate surface, an equivalent circuit was proposed and curve-fitting for obtained spectra was conducted. It was observed that the electrical resistance of apatite formed in vitro (Rₑᵢ)v increased with the immersion time, indicating the growth (in thickness) of the apatite layer [30, 31].

Nano-indentation was used to determine the mechanical properties of apatite formed on a bioactive composite in SBF [32]. Cross-sections of composite specimens that had been immersed in SBF for various periods of time were ground and polished. A Berkovich diamond indenter was used for the nano-indentation tests, and a series of indentations were made across the specimen surface. Figure 6 exhibits a nano-indentation and a nano-indentation curve of the apatite. The elastic modulus across the apatite layer had little variation, with an average value being around 5.22 GPa. This modulus value did not
Figure 4. FT-IR spectrum of the apatite coating shown in Figure 2.

Figure 5. Bode plot for pretreated Ti before and after immersion in SBF for eight weeks. Reprinted with permission from Ref. [31], C. X. Wang et al., Biomaterials 24, 3069 (2003). © 2003, Elsevier Science Ltd.
vary significantly with regard to the immersion time of the composite in SBF. It was also found that elastic modulus values of apatite formed in vitro on various biomaterials were within a narrow range [32].

2.3. Correlation Between In Vitro Apatite Formation and In Vivo Bone Bonding

It has been found, up until now, that nearly all materials that are able to bond directly to the surrounding bone tissue without being separated by a fibrous capsule induce apatite deposition in SBF at 36.5°C within short periods of time [11, 33, 34]. A comparative study between in vivo bone formation and growth and in vitro apatite formation in SBF was performed using five kinds of Na₂O-CaO-SiO₂ glasses of different compositions [11]. These glasses had various apatite induction times in SBF of 12 h, 3 d, 7 d, 21 d, and longer than 28 d. They were implanted in holes of the femoral condyles of rabbits. The bioactive glasses with the apatite induction times of 12 h and 3 d directly bonded with the surrounding bone tissue, while those with apatite induction times of 7 d, 21 d, and longer than 28 d showed no bone-bonding ability even after 12 weeks of implantation. The formation of an Si-rich layer and bone growth were significantly earlier on the bioactive glass with the apatite induction time of 12 h than those on the glass with the apatite induction time of 3 d. Therefore, the in vivo bioactivity of a material could be shown through its apatite-forming ability in SBF. On the other hand, it was noted that the glasses that induced apatite deposition in SBF with durations longer than 7 d failed to form direct bonding with bone in the in vivo tests. In a separate study [35], it was shown that, although an alkali-treated CPTi induced apatite deposition in SBF within 24 h, it also failed to form a direct bonding with the surrounding bone tissue in vivo, because of the reactive surface layer [36]. Therefore, it cannot be generalized that biomaterials being able to induce in vitro apatite deposition in SBF would also exhibit in vivo bioactivity. Extreme care should be taken when assessing the bioactivity of those biomaterials that induce apatite deposition in SBF but require a significantly long time. An in vivo experiment seems to be inevitable for confirming the bioactivity for such biomaterials.

3. MECHANISMS OF IN VITRO APATITE DEPOSITION

It is now clearly understood that all bioactive biomaterials are characterized of being bonded with surrounding tissues by forming a layer of apatite on their surfaces. In vitro apatite deposition in SBF could indicate in vivo bioactivity. Therefore, understanding the in vitro apatite deposition mechanism and knowing the influencing factors for apatite formation are important for the development of bioactive materials. Apatite deposition on bulk bioactive materials or bioactive coatings can be ascribed to the functioning of surface silica gel, titania gel, a Ca-P layer, other functional groups, or their combinations.
3.1. Silica Gel

Investigations into bioactive glasses have shown that five reaction stages occurred on the material side of the interface during the formation of chemical bonding with surrounding tissue [37]:

1. A rapid exchange of Na\(^+\) or K\(^+\) with H\(^+\) or H\(_2\)O\(^+\) from solution, forming silanols (Si-OH)
2. Loss of soluble silica in the form of Si(OH)\(_n\) to the solution, resulting from the breaking of Si-O-Si bonds and the formation of silanols at the glass-solution interface
3. Polycondensation of silanols to form a hydrated silica gel
4. Formation of an amorphous calcium phosphate (ACP) layer through migration of Ca\(^{2+}\) and PO\(_4^{3-}\) groups to the surface
5. Crystallization of the ACP layer by the incorporation of OH\(^-\), CO\(_3^{2-}\) or F\(^-\) anions from solution to form a bone-like apatite

The cation leaching, silica network formation, and phosphate reprecipitation occurred simultaneously during the dissolution of Bioglass 45S5 in a tris-buffered solution at pH 8 [38]. The presence of silanol groups on the surface was confirmed to be necessary for apatite deposition. The formation of the Si-rich layer as well as the apatite layer around bioactive glass particles when implanted in bone has been confirmed in vivo [11].

Sol–gel derived silica induced apatite deposition but dense silica glasses or quartz did not [39]. The silica film derived by a sol–gel dip coating followed by a heat treatment did not induce apatite deposition in SBF either, due probably to the dense structure [40, 41]. These observations emphasize the importance of both the Si-OH groups and the defects on the substrates for apatite deposition. However, larger amounts of Si-OH groups do not necessarily lead to higher apatite nucleation rates [40, 42]. Pores acted as nucleation sites for apatite nucleation, and the CHA nucleation rate increased with increasing pore size and pore volume of a porous gel-silica substrate [42]. Pore sizes larger than 2 nm were found to be necessary to achieve rapid apatite deposition [42]. However, no clear relationship could be found among porosity, Si-OH contents, or synthesis conditions and the in vitro bioactivity for sol-gel derived porous silica films with a wide variety of microstructures [42]. It was shown that the mesoporous material MCM-41 could possess high specific surface (BET specific area of 1134 m\(^2\)/g), high porosity (pore volume 0.984 m\(^3\)/g; peak pore size 2.5 nm), and consist of siloxane and silanol groups, yet they induced no apatite deposition even after two months soaking in SBF [43]. Therefore, it seems that the presence of superficial silanol groups conferring negative charges to the surface and a high porosity are not sufficient to ensure apatite formation. Incorporation of 10% glass, which had a molar composition of 55SiO\(_2\)-41CaO-4P\(_2\)O\(_5\), into the mesoporous material MCM-41 could create high specific surface, high porosity even though the substrate is SBF. The released Ca\(^{2+}\) cations left holes in the substrate that could act as nucleation sites themselves, instead of the initial pores in the material. Ordered mesopores were found to contribute to the apatite deposition ability of mesoporous bioactive glass (M5S) because of the increasing specific surface area and hence the increasing amounts of silanol groups on the surface [44]. The mesoporous bioactive glass with a medium concentration of Si/Ca ratio (80S) was found to possess the best in vitro bioactivity, in contrast to conventional sol–gel derived bioactive glasses which increased in bioactivity with increasing calcium contents [45]. This fact supports the idea that the mesoporous structure do affect readily the in vitro bioactivity.

3.2. Titania Gel

CPTi implants attach to the surrounding bone without being separated by a fibrous tissue, due to its natural oxide layer, which is hydrolyzed to form Ti-OH groups and negatively charged when immersed in human physiological fluid. The Ca\(^{2+}\) ions in the fluid are then attracted onto the surface, which in turn attract the PO\(_4^{3-}\) ions to initiate the deposition of apatite [46, 47]. This postulation of the apatite-forming process is supported by
the fact that the titania gel derived by sol-gel, which is negatively charged in SBF and also full of Ti-\(\text{OH}\) groups, favors apatite deposition in SBF [19]. Apatite formation was also found to be accelerated on an amorphous sodium titanate surface layer that was produced by treating CPTi with an alkali solution [35, 48]. While exposed to SBF, the alkali ions from the alkali titanate layer were released into SBF, which was accompanied by ion exchange with \(\text{H}_3\text{O}^+\) ions and thus resulted in Ti-\(\text{OH}\) functional groups on the surface as well as a pH increase in the surrounding fluid. The Ti-\(\text{OH}\) groups soon combined with the \(\text{Ca}^{2+}\) in the SBF to form a calcium titanate. After a certain period, the calcium titanate took phosphate ions as well as calcium ions from SBF to form apatite nuclei, which then grew spontaneously due to the high supersaturation of the fluid with respect to apatite. The pH increase also gave rise to an increase in the driving force for apatite deposition and hence favored apatite deposition [35, 48]. As proven by TEM and X-ray photoelectron spectroscopy (XPS) examinations, the calcium and phosphate deposition on the alkali- and heat-treated Ti surface was in sequence. Calcium ions can be adsorbed on the surface of the alkali- and heat-treated Ti while phosphate ions cannot without the pre-adsorption of calcium ions. Calcium deposition is thus believed to be the prerequisite for apatite deposition [26, 49]. Phosphate and calcium were detected by XPS on the acid- and alkali-treated titanium surface after soaking in SBF for a time as short as only 2.5 min. The Ca/P ratio for alkali-treated Ti was significantly higher than that of the acid-treated samples [50]. These observations confirmed that the alkali-treated Ti surface favors the adsorption of calcium. It was noticed that the subsequent heat-treatment of an alkali-treated amorphous titania coating, which was derived by vacuum plasma spraying, inhibited the exchange between \(\text{Na}^{+}\) ions in coatings and \(\text{H}_3\text{O}^+\) ions in SBF; hence reducing the Ti-\(\text{OH}\) surface groups, leading to a reduced \textit{in vitro} bioactivity [51].

It should be noted that the apatite-forming process on the sodium titanate surface of alkali- and heat-treated Ti cannot be simply applied to other titania gel surfaces. For some other surface coatings containing mainly titania gel, it is likely that the adsorption of both calcium and phosphate ions occurs simultaneously, which represents a different process for apatite growth on the titania surface. It was found that both calcium and phosphate ions were adsorbed separately and quickly on different sites of a sol-gel derived titania surface and that calcium and phosphate adsorption increased once the corresponding counterion (phosphate or calcium) had been previously adsorbed [52]. The possible reactions leading to the adsorption of calcium and phosphate ions on the titania surface are [53]:

\[
\text{Ti} - \text{OH} + \text{Ca}^{2+} \leftrightarrow \text{Ti} - \text{OH} - \text{Ca}^+ + \text{H}^+ \quad (7)
\]

\[
\text{Ti} - \text{OH} + \text{HPO}_4^{2-} \leftrightarrow \text{Ti} - \text{O} - \text{HPO}_4^+ + \text{OH}^- \quad (8)
\]

The assumption that apatite deposition in SBF can be ascribed to a negatively charged surface is supported directly by the fact that the negatively charged barium titanate (BaTiO\(_3\), BTO) surface, which is ferroelectric and piezoelectric after a poling treatment, showed Ca-P crystal growth, while no Ca-P phase was observed on the positively charged BTO surface [54]. Similarly, the negatively charged HA surface, which was obtained by polarization, induced apatite deposition in 1.5 SBF (a solution with inorganic ion concentrations 1.5 times the SBF), whereas the positively charged or the non-polarized HA surface did not [55]. However, the assumption that the negatively charged surface initiates apatite deposition has been challenged by the finding that the CPTi surface contained more \(\text{HPO}_4^{2-}\) than \(\text{Ca}^{2+}\) ions after soaking in SBF for a certain duration [53]. CPTi subjected to close contact with other surface, such as polytetrafluoroethylene (PTFE), enhanced its apatite-forming ability, due probably to the accumulated titanium ions and the increased local supersaturation of SBF within the closed region [56]. Therefore, as a logical deduction, a porous surface full of cracks is expected to favor apatite deposition. This view is supported by the finding that micro-holes with sizes of around 0.3 mm on the alkali-treated Ti-6Al-V favored apatite deposition both \textit{in vitro} and \textit{in vivo} [57]. However, Peltola et al., on the contrary, found that only surface topographies giving
peak distances of 15–50 nm, observed by atomic force microscopy (AFM), favored apatite deposition [58].

The crystalline structure of titania, which has three common polymorphs of anatase, rutile, and brookite, appears to be more important to initiate apatite nucleation as compared to the increase of supersaturation with respect to apatite in SBF, due to the release of certain ions from the surface layer. The amorphous sol–gel derived titania induced no apatite deposition in SBF [56]. The incorporation of Na\(^+\) and Ca\(^{2+}\) ions in the amorphous titania did not result in apatite deposition either, in spite of the fact that the release of Ca\(^{2+}\) ions as well as the exchange between Na\(^+\) and H\(_3\)O\(^+\) in SBF increased the supersaturation with respect to apatite [60]. On the contrary, the anatase gel containing no such Na\(^+\) and Ca\(^{2+}\) ions induced apatite deposition effectively [60]. Similarly, the amorphous titania gel containing CaO did not induce apatite deposition while the pure anatase gel did [61]. Also, an intermediate hot water soaking to remove sodium from the sodium titanate layer produced by the alkali-treatment of CPTi gave pure anatase upon a subsequent heat treatment, and subsequently enhanced the in vitro bioactivity [62, 63]. Therefore, it can be postulated that the prerequisite for an enhanced ability of titania layer for early apatite deposition is to achieve a crystalline structure of titania.

However, it was noticed that a film of the mixture of anatase and rutile fabricated by micro-arc oxidation (MAO) of CPTi that contained Ca did not induce apatite deposition in 1.5 SBF [64]. Only after a subsequent hydrothermal treatment, which resulted in numerous precipitates assumed to be Ca-P and did not alter the phase composition or the porous surface morphology, could the film induce apatite deposition in 1.5 SBF. The MAO-derived anatase coating containing up to 8 at.% P induced no apatite-formation when subjected to a SBF-soaking for up to 28 d; however, after a subsequent hydrothermal treatment, apatite was induced on the anatase surface after only 9 h of immersion [65]. Similarly, apatite formation was found only after the alkali-treatment (soaking in an NaOH aqueous solution) of an MAO-derived anatase film [66]. These findings emphasize the importance of the surface hydroxyl groups, which have resulted from the hydrothermal treatment, for apatite deposition. However, it is not clear why the crystalline titania film derived by MAO failed to induce apatite deposition.

Apatite deposition was found to be more pronounced on anatase gel than on rutile gel, both of which were prepared by the sol–gel process followed by heating at different temperatures [59]. Titania coating derived by soaking in an anatase slurry was also found to induce more apatite deposition than that derived by soaking in a rutile slurry [67]. However, it was also shown that the low-temperature titania film prepared through simply soaking CPTi at 80°C for 3 d in a 30 mass.% H\(_2\)O\(_2\) solution containing 3 mM TaCl\(_5\), which consisted predominantly of rutile, was equally effective for inducing apatite deposition in SBF, as the lattice mismatch between rutile and apatite was as small as that between anatase and apatite [29]. It was further noted that the pure rutile film derived by various chemical and electrochemical methods exhibited a high ability to induce apatite deposition in SBF [68–72]. Therefore, it may be concluded that the rutile phase has a higher, or at least the same, ability to induce apatite deposition in SBF, in situations where the rutile film contains Ti-OH functional groups.

Titania nanotube arrays can be obtained by anodic oxidation of pure Ti in electrolytes containing fluoride. It has been reported that the unique nanostructure of nanotubes accelerated the apatite-forming procedure in SBF when compared to a compact anodic titania layer. The in vitro bioactivity improved after annealing the amorphous titania nanotube arrays, which crystallized to anatase or a mixture of anatase and rutile, depending on the annealing temperatures [73]. This further confirms the importance of a crystalline structure for titania to induce apatite formation in SBF.

### 3.3. Calcium-Phosphate (Ca-P) Layer

Apatite deposition on bioactive ceramics, such as HA, TCP, and calcium phosphate, containing bioactive glasses can be ascribed to the formation of a surface Ca-P layer. The apatite deposition process on these bioactive bioceramics is relatively simple. The dissolution of Ca\(^{2+}\) ions into the surrounding body fluid increases the supersaturation of
the solution with respect to apatite and at the same time leaves newly formed defects on the surface, which in turn favors apatite nucleation. The bone-like apatite deposited on sintered HA surfaces in SBF comes into being through crystallization of the previously formed Ca-rich, amorphous (or more precisely, nanocrystalline) calcium phosphate (ACP). The zeta potential characterized by electrophoresis indicated that during the exposure to SBF, the HA surface was negatively charged, which interacted with positive calcium ions in the fluid to form ACP [74].

It was found that the ACP incorporated in HA coatings, especially the coatings prepared by plasma spraying, plays an important role in the in vitro bioactivity of these coatings [75–78]. After soaking in SBF, the dissolution of the amorphous phase increased the supersaturation of SBF with respect to apatite and at the same time also increased the surface roughness, which provided nucleation sites because the existence of defects in the structure lowers the nucleation energy [75]. A thermal treatment could increase the crystallinity of plasma-sprayed HA coatings, leading to decreased solubility and hence more stable mechanical properties, but it reduced bioactivity of the coatings [78]. The totally crystallized plasma-sprayed HA coating achieved through heat treatment after plasma spraying did not induce apatite deposition in SBF, although heat treatment did not alter significantly the surface morphology [75, 78]. Therefore, if only the osteoconductive property of Ca-P coating is desired for the initial fixation of implants, having ACP is advantageous. On the other hand, if coating longevity is desired, the crystalline HA coating is preferred [79]. It was also noted that an increased osteoblast-like cell activity appeared on a sintered pure HA surface as compared with the as-received and calcined pure HA samples [18], which suggests that the crystal size, and probably the crystallinity as well, of HA may play an important role in governing cellular response. However, higher crystallinity of the Ca-P coating may not necessarily mean poorer bioactivity. No differences were found in the bone apposition abilities of the implants coated with 62% crystalline HA, 30% crystalline HA or fluorohydroxyapatite (Ca₅(PO₄)₃OHₓₖFₓ₋ₓ, FHA), with all coatings being made by plasma-spraying [80, 81]. In a separate study, HA coatings with different degrees of crystallinity were found to have no significant influence on bone-bonding ability, bone-bonding strength, tensile failure mode, and degradation of the coating [82].

3.4. Other Surface Functional Groups

Surface functional groups that are able to induce apatite deposition include Si-OH [26, 37, 83], Ti-OH [26, 84], Ta-OH [26, 85–87], Nb-OH [26], Zr-OH [26, 88], -COOH [83, 89, 90], -NH₂ [83], and -H₂PO₄ [89, 90]. When biomaterials having these surface functional groups are subjected to immersion in physiological fluid, the Ca²⁺ ions in the fluid are attracted by the functional groups and bind to the surface, which in turn attracts the phosphate ions and hence initiates the apatite nucleation process. Because the physiological fluid is a supersaturated solution with respect to the apatite, the subsequent apatite growth is a spontaneous process. Toworfe et al. utilized a self-assembled monolayer (SAM) technique to create amine (-NH₂), carboxyl (-COOH), and hydroxyl (-OH) functionalized surfaces onto oxidized silicon wafers. The results show that the hydroxyl groups exhibited the most remarkably apatite-inducing ability in SBF [83].

3.5. Effects of SBF-Related Factors

Very few papers can be found that clarify the possible effects of the SBF itself on apatite deposition. After soaking alkali- and heat-treated CPTi in the R-SBF, which has a higher concentration (27.0 mM, exactly equal to that in human plasma) of HCO₃⁻ than the conventional SBF (4.2 mM), OCP, not apatite, nucleated directly from the previously deposited amorphous calcium phosphate layer, due probably to the inhibiting effects of HCO₃⁻ on apatite precipitation. The OCP crystals continuously grew on the treated titanium surfaces rather than transforming into apatite [91]. However, Muller [92] and Jalota et al. [21] achieved apatite on alkali-treated Ti-alloy substrates also using SBF with a HCO₃⁻ concentration of 27.0 mM. Muller et al. found that the HCO₃⁻ content in
SBF influences the composition and structure of the obtained HA. B-type carbonated-HA (CO$_3^{2-}$ replaces PO$_4^{3-}$ in HA) precipitated on chemically-treated Ti substrates when the SBF contained less than 20 mM HCO$_3^{-}$; A-type carbonated-HA (CO$_3^{2-}$ replaces OH$^-$ in HA) formed with higher HCO$_3^{-}$ concentration in SBF [92]. Jalota et al. found that increasing the HCO$_3^{-}$ content in SBF from 4.2 mM to 27.0 mM not only speeded up the apatite-forming procedure on the alkali-treated Ti6Al4V strips, but also modified the morphology of the deposited apatite, which also yielded enhanced cell viability and protein concentrations [21].

The influence of some ions in 5 SBF (a solution with ion concentrations five times the SBF) on the nucleation of biomimetic Ca-P coatings on Ti-6Al-4V has been studied [93, 94]. Sodium chloride increased the ionic strength of the solution and hence delayed the homogeneous Ca-P precipitation in solution, allowing Ca-P to nucleate heterogeneously on the substrates [93]. HCO$_3^{-}$ inhibited apatite formation and hence reduced the apatite crystal size of the coating, allowing a better physical attachment of the coating to Ti-6Al-4V substrates [93]. Mg$^{2+}$ was found to have a stronger inhibitory effect on apatite crystal growth than HCO$_3^{-}$ [94]. An XPS depth profile showed significantly increased contents of Mg$^{2+}$, as well as Ca$^{2+}$, at the titanium/coating interface. A high concentration of Mg$^{2+}$ favored heterogeneous nucleation of tiny Ca-P globules on the substrate and hence enhanced the physical adhesion of coating at the early stage of coating formation [94]. However, it is still not clear whether those ions function similarly in SBF.

As reported in numerous publications, the in vitro bioactivity of biomaterials is generally assessed using SBF in the static condition. It has been suggested that a dynamic process, with continuous exchange of SBF solution with the material, better mimics the actual environment in the human body and hence serves better to evaluate bioactivity [50, 95, 96]. Both static and dynamic processes were used to evaluate the bioactivity of a sol–gel derived glass having the composition of 55%SiO$_2$-41%CaO-4%P$_2$O$_5$ [95]. In the static condition, changes in the Ca$^{2+}$ ion concentration and pH value were significant, while these two parameters remained almost unchanged in the dynamic condition during in vitro tests. Apatite deposition was observed in both conditions. However, the dynamic condition led to an accelerated apatite-forming rate. The morphology of the apatite layers was different: in the static condition, the glass was covered by an incoherent layer of spherical particles of about 1 μm in size; while in the dynamic condition, the glass was fully covered by a layer in which the spherical particles were constituted by hundreds of needle-like crystal aggregates. Similar phenomena were observed on a sol–gel silica-polymer composite [96].

It appears that many factors can influence apatite deposition on biomaterials. These factors include the material itself, pretreatment of the material, material surface morphology (including porosity), and the material’s surface composition and crystallinity. Although great efforts have been made to study the mechanisms of apatite formation on various materials, conflicting results have been reported, and the exact apatite-forming mechanisms still remain ambiguous for a large percentage of biomaterials investigated.

4. BIOACTIVE COATINGS ON NON-METALLIC BIOMATERIAL SUBSTRATES

4.1. Bioceramics

Structural ceramics, such as high purity alumina with fine grains [97, 98], and toughened zirconia ceramics [99] possess high strength, acceptable fracture toughness, high chemical stability, and excellent wear resistance and have already been used for femoral heads of total hip prostheses. Apart from their applications as coatings on metallic or polymer substrates, bioceramics are also used in the forms of particles and bulk shapes in various clinical situations. For bulk ceramics, such as alumina, bioactivity is achieved through either having bioactive coatings or incorporating secondary bioactive phases. The apatite-forming processes on some bioactive glasses, ceramics, and glass-ceramics have been investigated extensively.
4.1.1. Alumina

Thin HA films have been deposited on alumina substrates through radio-frequency sputtering [97, 100] or ion-beam assisted deposition [101], followed by a thermal treatment. Sol–gel dip coating from a solution of \( 1.67\text{Ca(NO}_3\text{)}_2\cdot\text{PO}_4\cdot\text{OH}_{2x}\cdot(x\text{EtO}_3) \) [102–104] or spin coating with a sol consisting mainly of calcium nitrate and phosphoric acid in methanol [105], with a subsequent thermal treatment, also deposited HA layers on alumina. The addition of an appropriate citric acid into the dipping solution resulted in not only a rough or porous morphology but also an improved adhesion strength of the HA coating [102, 104].

Porous \( \beta\text{-TCP} \) films with a thickness of \( \sim 50 \mu m \) were formed on alumina ceramics using a spray-pyrolysis technique [106]. Improved cellular responses with respect to cell proliferation and differentiation were found on the coated specimen as compared with the uncoated alumina.

Full-density medical-grade \( \alpha\text{-alumina} \) was coated with a bioactive FHA glass-ceramic layer (50 \( \mu m \) thick) through a suspension deposition and sintering process, with an intermediate layer of a SiO\text{2-CaO} glass (400 \( \mu m \)) to aid the adhesion and also to impede the coating/alumina interfacial reaction [107, 108]. Bone-like apatite precipitated on the coating, but didn’t cover the whole coating, after 1 month of soaking in SBF [108]. An in vitro study using human osteoblast-like cells showed that the coatings exhibited cell attachment and proliferation similar to bulk FHA glass-ceramic and obviously better than alumina [107]. Enhanced protein adsorption that favored osteoblast-like cell adhesion and spreading and reduced the macrophage complement-mediated local inflammatory reaction as well, were observed on the FHA glass-ceramic coatings as compared with the alumina [108].

4.1.2. Hydroxyapatite

Soaking HA in SBF leads to the formation of bone-like apatite on its surface through the following surface structural changes in sequence: formation of a Ca-rich amorphous or nano-crystalline Ca-P, formation of a Ca-poor crystalline calcium phosphate, crystallization into bone-like apatite. The exposure time for the formation of the Ca-P was delayed on HA sintered at 1200°C, as compared to the one sintered at 800°C, because a higher sintering temperature resulted in reduced surface hydroxyl groups and surface negativity [74]. Stoichiometric HA is generally considered to possess a limited in vitro reactivity [55, 109]. A composite of HA and 5% sol-gel glass exhibited higher in vitro bioactivity than pure HA [109]. The small amount of the bioactive glass was enough to induce a positive response in just a few hours in SBF, which led to the formation of a new apatite-like layer that reached a thickness of 3 \( \mu m \) after about 3 d. The incorporation of silicon into the HA structure provides another way to improve the bioactivity [110]. An XPS investigation revealed the substitution of PO\text{4}^- groups with SiO\text{4}^- groups. An increase of the silicon content up to 1.6 wt% led to the polymerization of the silicate species on the surface. The formation of apatite on the surface of silicon-substituted HA was strongly enhanced as compared to pure HA. The samples containing monomeric silicate species showed higher in vitro bioactivity than that of silicon-rich sample containing polymeric silicate species [110].

Under the influence of an electric field of DC 1 kV/cm at 300°C, HA can be polarized due to proton movement [55]. After soaking in 1.5 SBF for 1 d, apatite deposition took place on the negatively charged surface (N-surface), whereas no crystal growth was observed on the positively charged surface (P-surface) even after a 3 d immersion in 1.5 SBF. The apatite growth rate on the N-surface was also greater than that on the non-polarized HA surface. Similarly, the negatively charged surface of barium titanate (BaTiO\text{3}, BTO), which is ferroelectric and piezoelectric after poling treatment, showed Ca-P crystal growth, while no Ca-P phase was detected on the positively charged BTO surfaces [54].
4.1.3. Glass-Ceramics

Bioactive glass-ceramics were prepared through tape casting 45S5 Bioglass followed by sintering at 800, 900, or 1000°C for 3 h, which produced a crystalline material with a major phase of Na₂Ca₂Si₃O₉ [111]. Samples sintered at 1000°C induced apatite deposition in SBF after 20 to 24 h, while the apatite induction time for samples sintered at 900°C was only 2 h. The difference in bioactive response was attributed to the specific surface area of sintered 45S5 Bioglass® [111].

The bioactive glass-ceramic A-W, which was prepared through heat treatment of a MgO-CaO-SiO₂-P₂O₅ glass to precipitate crystalline apatite and wollastonite, showed high in vitro bioactivity. However, the addition of a small amount of Al₂O₃ to this glass-ceramic to improve its bending strength resulted in the elimination of its apatite deposition ability. After being treated with 0.1 M HCl, apatite deposition occurred due to the formation of hydrated silica on the surface of the alumina-containing glass-ceramic A-W [112].

The glass-ceramic 60CaO · 30P₂O₅ · 7Na₂O · TiO₂ formed apatite after 20 d soaking in SBF [113]. After a hydrothermal treatment in water at 140°C for 1 h, this glass-ceramic was completely covered with bone-like apatite after 10 d soaking in SBF, due mainly to the hydrated titania groups resulting from the hydrothermal treatment.

4.1.4. Other Bioceramics

A wide variety of bioinert surfaces, such as Teflon, silicon wafers, gold, and quartz, can be converted to activated surfaces for biomolecule adsorption by coating them with a thin sol–gel titania film, which can bind strongly with proteins, peptides, and polysaccharides. Before applying the sol–gel titania coating, the bioinert surfaces should be oxygenated or contain free hydroxyl groups [114].

It has been shown that both the 80SiO₂-20CaO and the 80SiO₂-20CaO-3P₂O₅ (in mol%) gel glasses induced apatite deposition in SBF [115]. The addition of P₂O₅ in the gel glass led to a quicker growth of apatite crystals [115]. Substitution of M₂O₃ (M = Ga, Al, In, La, Y) for CaO in the binary CaO-SiO₂ glass composition progressively reduced the ability to form a Ca-P layer on the surfaces exposed to SBF [116].

The trace amounts of Fe₂O₃ in the β-wollastonite (CaSiO₃) matrix of ferromagnetic glass-ceramics, which were designed as thermoseeds for hyperthermia treatment of bone tumors and consisted of magnetite (Fe₃O₄) precipitation in the β-wollastonite glass matrix, were found to inhibit the apatite deposition in SBF [117]. The addition of 3 wt% Na₂O and the combination of B₂O₃ and P₂O₅ into the glass-ceramics improved in vitro bioactivity by inducing apatite deposition in SBF within 10 to 30 d, because for Na₂O, the exchange of Na⁺ with the H₃O⁺ in the SBF increased the pH value of the surrounding fluid, and the combination of B₂O₃ and P₂O₅ may have accelerated apatite formation through the formation of hydrated silica on the surfaces.

A study revealed that the akermanite ceramic (Ca₂MgSi₂O₇) possessed an apatite-formation ability comparable to the wollastonite ceramic after soaking in SBF for 20 d. In addition, the Ca, Si, and Mg ions dissolved from akermanite at certain ranges of concentration stimulated significantly osteoblast and cell proliferation, which suggests additionally that the ceramic is a suitable candidate as bone substitutes [118].

DCPD plays an important role in the biological mineralization process as well as the setting of a variety of calcium phosphate cements for orthopedic and dental uses. It was shown that a layer of apatite could be coated on the surface of gel-grown DCPD single crystals through a hydrothermal-electrochemical deposition method, which used heated SBF in an autoclave as an electrolyte [119]. The apatite formed was confirmed through SEM, XRD, and FT-IR analyses. Under physiological conditions, DCPD gradually transforms into apatite.

Phospholipids have been found to initiate calcium phosphate deposition in cartilage, bone, healing fracture callus, and certain calcifying bacteria. To study the possibility of using Ca-P complexed phospholipid (Ca-PL-PO₄) coatings on solid surfaces for enhancing bone-implant interactions, Ca-PL-PO₄ complexes were prepared using
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CaCl₂, (NH₄)₂HPO₄, and various phospholipids, such as phosphatidylcholine (PC), phosphatidylserine (PS), and phosphatidylinositol (PI) [120]. After coating the complexes on glass substrates, samples with coatings were subjected to soaking in SBF, and cell culture was also carried out. Results showed that surfaces coated with Ca-PS-PO₄ exhibited the highest amount of calcium deposition and enhanced osteoblast differentiation [120]. The Ca-PS-PO₄-coated CPTi surfaces exhibited enhanced protein synthesis and alkaline phosphatase (ALP) activity as compared to other phospholipids-coated or uncoated surfaces [121].

HA was also deposited on carbon fabric through a sonoelectrodeposition approach. An increased current density achieved a much denser and uniform structure with smaller HA crystal sizes [122].

4.2. Biomedical Polymers

Polymers have a long history for their applications in medicine. Synthetic polymers such as poly(methyl methacrylate) (PMMA) and ultrahigh molecular weight polyethylene (UHMWPE) and poly(glycolic acid) (PLA) are successfully used in orthopedics and surgery. In recent years, natural polymers such as collagen and chitosan have gained increasing attention. Major efforts have been made to use polymers for hard tissue replacement/regeneration. For achieving bone-bonding, investigations have been conducted to make polymer surfaces bioactive (osteconductive). On the other hand, bone at the microstructural level is a natural composite consisting of bone apatite about 50 nm × 25 nm × 3 nm in size and collagen [123]. Efforts have thus been made to produce bone substitutes with an analogous structure to that of natural bone through either industrialized plastics processing technologies or biomimetic processes. These materials should have the ability to induce in vitro apatite deposition.

Several approaches to provide biomedical polymers with the ability to induce apatite-formation ability in SBF will be discussed in the next several paragraphs. However, it has also been reported that certain polymers possess an inherent ability to induce apatite deposition without additional treatments. For example, when soaking poly(2-hydroxyethylmethacrylate) (PHEMA)-based hydrogels in SBF at 37°C for several weeks, apatite deposition was significant on all the hydrogels. The nucleation of apatite was ascribed to the strong tendency of Ca²⁺ ions to chelate to oxygen atoms in the unit structure of PHEMA, which was supported by the fact that the degree of calcification for the hydrogels lowered with decreasing oxygen atoms as a result of certain copolymerizations [124]. A type of silk protein, sericin, also possessed the ability to induce apatite nucleation in a 1.5 SBF when having a β-sheet structure that forms polypeptides with ordered and concentrated carboxyl groups [125].

4.2.1. Surface Seeding with Ca-P Phases

Many biological materials, including bone that is discussed above, are composites with particles reinforcing a polymer matrix. In the biological environment, the particles are grown in situ within the polymer matrix and under the control of the matrix. Calvert and Mann reviewed some synthetic and biological composites formed by in situ precipitation [126]. According to the matrix-mediated processes, they classified biological composites into four categories: Type I (matrix-inert), Type II (nucleation), Type III (amorphous) and Type IV (oriented) biocomposites. They also discussed the principles governing the growth of particles when constrained by a polymer matrix. It appears possible to produce various types of precipitates within a polymer matrix. However, through an extensive literature survey, it seems that the majority of in situ precipitation investigations have ended with products that have precipitates either on polymer surfaces or into a limited depth of the bulk polymer if the polymers are soaked in the (normally ion-saturated) solutions. There are good examples of Ca-P particles precipitating only in areas near the surface of collagen membranes.

A biomimetic process was used to coat biomedical polymers with apatite [79, 127–131]. This biomimetic process included the following two steps: (1) seeding the organic substrates with apatite nuclei by bringing them in contact with particles of CaO-SiO₂-based
bioactive glasses (termed “G glass”) soaked in SBF, and (2) coating the organic substrates with apatite by a subsequent soaking in 1.5 SBF, in which apatite nuclei grew on the substrate in situ to cover the whole surface. This biomimetic method was also used to coat apatite on biomedical hydrogels [132]. When using this method to coat apatite on titanium substrate, the G glass could be replaced by a sodium silicate solution at the seeding stage [133].

Apatite deposition on polystyrene could be accelerated by soaking the polymer substrate firstly at 37°C in 5 SBF, followed by soaking in a second 5 SBF solution which was devoid of crystal growth inhibitors of Mg$^{2+}$ and HCO$_3^-$ [134]. The pH value of the first soaking solution affected the final apatite structure. A high pH value led to larger single crystal plates while a low pH value resulted in minute, polycrystallite plate-like structures. The surface of silica gel beads or plates could also be coated with an apatite layer through alternative soaking in a CaCl$_2$/tris-HCl aqueous solution and a Na$_2$HPO$_4$ aqueous solution [135].

Calcium alginate fibers were produced by extruding an aqueous sodium alginate solution through nozzles into an aqueous calcium chloride solution first and then through a calcium chloride methanol solution [136]. After soaking the fibers in an aqueous saturated calcium hydroxide solution for 5 d and then in SBF for 7 d, apatite was deposited on the surface of the fibers with certain alginate subunits, due to the release of the calcium ions from the fibers, which left free carboxyl groups and increased the supersaturation with respect to apatite in SBF as well. Du et al. produced various types of calcium phosphate/collagen composites through the mineralization of the type I collagen matrix, which was realized by soaking collagen in a PO$_4^{3-}$-containing solution and then in a Ca$^{2+}$-containing solution [137, 138]. The mineral contributed up to 60–70% of the weight of the composites, and the strength and Young’s modulus of the composites exceeded the lower bound of bone. The interconnected porous structure of the composites provided a large surface area for cell attachment and sufficient space for nutrient transportation. New bone matrix was synthesized at the bone-implant interface when the composite was in contact with bone fragments [137].

4.2.2. Surface Functional Groups of Si-OH, COOH, Ti-OH, and so on

Silanol groups were produced on silicones through a sol–gel process [139]. Although the silanol-modified silicones did not induce apatite formation in SBF within 21 d, a bone-like apatite layer formed on their surfaces in 1.5 SBF within 7 d. Silanol groups were also introduced to the polyethylene (PE) surface through the vapor-phase photografting of vinyltrimethoxysilane (VTMS) followed by hydrolysis [140]. The photografting process formed methoxy-silyl groups on the PE surface, which were transformed into silanol groups subsequently by hydrolysis in a 1.0 M HCl solution. The surface-modified PE was covered with a dense and homogeneous bone-like apatite layer after soaking in 1.5 SBF for 7 d. Modification of alginate with silanol groups, through reacting alginate with 3-aminopropyltrimethoxysilane (APES) that gave silanol groups after hydrolysis, and calcium ions, through soaking in 1 mole/L CaCl$_2$ solution at 36.5°C for 24 h, could induce apatite deposition in SBF or 1.5 SBF both on its surface and within its structures [141].

Polymers previously subjected to a glow discharge treatment in oxygen gas to produce polar groups on the surfaces were soaked in sodium silicate solutions to activate the surfaces [142]. After soaking in 1.5 SBF for a certain time, bone-like apatite thoroughly covered the polymer surface. Silicate oligomers with structures such as dimer, linear trimer and cyclic tetramer contributed most to the apatite nucleation. EVOH was treated with a silane coupling agent and calcium silicate solutions. A smooth and uniform bone-like apatite layer was formed on both the EVOH plate and the EVOH-knitted fibers in SBF within 2 d [143]. When the silane coupling agent-treated EVOH was soaked in a titania sol, Ti-OH groups were introduced on its surface [144]. A subsequent treatment with an HCl solution crystallized the amorphous titania to anatase. Apatite was then formed on the anatase-coated copolymer substrates after soaking in SBF for 2 d.
A Ca-P layer was coated on the surface of a soft tissue (tendon) through alternative soaking in a CaCl₂ solution and a Na₂HPO₄ solution [145]. The Ca²⁺ ions were suggested to interact with the tendon surface, most probably with the carboxyl (COOH) functional groups of collagen, and subsequently form nucleation centers for the Ca-P crystals. Similarly, alternately dipping poly(L-lactic acid) (PLLA) substrates, which were previously treated with oxygen plasma to produce oxygen-containing functional groups on the surface, in aqueous CaCl₂ solution and a K₂HPO₄ solution deposited apatite precursors on the surface. A dense and uniform surface apatite layer formed after immersion for 24 h in SBF [146]. Apatite deposition was observed on both raw silk cloth and sericin film in 1.5 SBF after 7 d immersion, attributable to the catalytic effect of sericin, which contained approximately 20 mol% of acidic amino acids that resulted in a surface structure abundant in carboxyl groups [147]. Pre-soaking in a 1 M CaCl₂ solution accelerated apatite deposition on silk fiber. After hydrolysis in water at 37°C for 7 d to produce carboxyl groups on the surface, PLLA fibers also induced apatite deposition in 1.5 SBF at 37°C and pH 7.3 [148]. Carboxyl groups were also introduced on the surface of a chitin non-woven fabric [149] and gels of chitin and gellan gum [150]. After immersion in a saturated Ca(OH)₂ aqueous solution under nitrogen gas, apatite began to precipitate on the polymer surface when soaked in SBF.

The biomimetic deposition of apatite on polymers has also been extended to making organic–inorganic hybrids as bone substitutes. Low temperature formation of HA/poly (alkyloxybenzoate)phosphazene composites made by the formation of HA through reacting Ca(PO₄)₂ with CaHPO₄·2H₂O in the presence of poly(ethyloxybenzoate) phosphazene (PN-EOB) and poly(propyloxabenzoate)phosphazene (PN-POB) was reported [151]. The formation of HA through the following acid–base reaction caused the pH of the solution to increase:

\[
2\text{CaHPO}_4 \cdot 2\text{H}_2\text{O} + 2\text{Ca}_3(\text{PO}_4)_2 \text{H}_2\text{O} \rightarrow \text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2 + 4\text{H}_2\text{O}
\] (9)

In the presence of PN-EOB or PN-POB, the generation of alkaline conditions promoted partial hydrolysis of the alkyl ester side-groups, resulting in the formation of free carboxylate groups on the polymer. Calcium ions in the solution cross-linked these carboxylate groups, leading to the formation of a calcium cross-linked polyphosphazene surface layer, which in turn initiated the deposition of apatite on the surface. A similar in situ hybridization procedure was used to prepare an HA-reinforced chitosan nanocomposite [152].

Apatite could be deposited on silicone in SBF within 7 d after anatase particles had been introduced on its surface [153]. Soaking silicone in tetraisopropyl titanate (TITP) at 30°C for various periods and then, in sequence, in water at 25°C, in a hydrochloride solution at 25°C, in an ammonia solution at 25°C or hot water at 80°C for 24 d, anatase precipitated on the silicone surface. The amount of the apatite deposited on silicone increased with an increasing amount of the precipitated anatase.

Osteopontin is a prominent, highly acidic phosphoglycoprotein of mineralized bone and dentin. It is believed to bind to extracellular matrix such as type I collagen to mediate adhesion of cells to mineralize tissues. Osteopontin, which was purified from bovine milk, was cross-linked or adsorbed to agarose beads [154]. It was then found that the cross-linked osteopontin induced apatite deposition while the adsorbed osteopontin failed to induce apatite formation.

Highly porous PTFE membranes, which are used in facial reconstructive surgery, can be modified to possess bioactivity through the introduction of ionic groups onto its surface. After introducing a monolayer of monoacryloxyethyl phosphate on the surface, which did not alter the surface morphology but improved the hydrophilicity of the surface, apatite-like coatings were obtained on PTFE after soaking in SBF [155].

### 4.2.3. Electroless Plating

An electroless plating technique has been used to coat an HA layer on high molecular weight polyethylene (HMWPE), starch/ethylene vinyl alcohol (SEVA) blends and
starch/cellulose acetate (SCA) blends [156]. The bath solution consisted mainly of calcium chloride, sodium hypophosphate, and other additions. PdCl$_2$ was used as a catalyst and worked as a channel for ionic exchange between the substrate and the solution. After immersing the polymers in the acidic bath at 60°C or in the basic bath at 80°C for 1 h, bone-like apatite with \textit{in vitro} bioactivity was deposited on the polymers auto-catalytically.

### 4.3. Biomedical Composites

#### 4.3.1. Ceramic/Ceramic Composites

Zirconia-toughened alumina (ZTA) composites have been developed to replace alumina ceramics in orthopedic applications requiring high fracture resistance. In ZTA composites, unstabilized or stabilized zirconia particles were embedded homogeneously throughout the alumina matrix, which enhances the flexural strength, fracture toughness, and fatigue resistance due to the stress-induced phase transformation that tetragonal zirconia undergoes into the more stable monoclinic phase. However, these composites are bioinert. A Y-PSZ/Al$_2$O$_3$ composite, in which the reinforcement is partially stabilized zirconia containing 2–4% Y$_2$O$_3$, was first subjected to incubation in a sodium silicate solution at 37°C for 7 d to deposit surface functional group of Si-OH on the composite, which initiated apatite nucleation and growth in the subsequent immersing in the 1.5 SBF at 37°C for another 6 d [157]. The use of sodium silicate solution in place of generally utilized bioactive glass as a nucleant was suggested to be effective for the formation of a carbonated hydroxyapatite layer on surface of the ZTA composites [157]. Applying a similar biomimetic method, by soaking Mg-PSZ/Al$_2$O$_3$ composite in SBF containing either a bed of wollastonite (CaSiO$_3$) ceramics or bioactive glass for 7 d and then in 1.4 SBF for 14 d, led to the formation of a 15 to 30 μm thick apatite. It is found that using wollastonite as nucleants, the deposited apatite layer was thicker than that using bioactive glass [158]. A dense and homogeneous bone-like apatite layer 20 μm in thickness was also achieved simply by soaking the Mg-PSZ/Al$_2$O$_3$ composite in SBF for 7 d followed by soaking in 1.4 SBF for 14 d, which provided bioactivity for the ZTA composite [159].

Ceramic/ceramic composites consisting of constituents with distinct bone cell responses are of interest as a new kind of bone replacement material. Zinc shows a simulating effect on osteoblastic cell proliferation and bone formation; therefore, ZnO has been added to a bovine bone-derived HA. The results indicate that the addition of 5 wt% ZnO increases the microhardness of the sintered composite but negligibly affects the compressive strength [160]. CaCO$_3$/HA composites were also prepared by infiltrating porous HA scaffolds with calcium carbonate slip, followed by sintering. The resultant composite possessed enhanced elastic and shear moduli compared to pure HA, falling into the range of human bone [161]. Slosarczyk et al. fabricated a carbon fiber-reinforced HA composite by hot pressing the two components. Pre-coating of the carbon fiber with a sol–gel calcium phosphate layer improved the bonding of the reinforcement and the matrix; thus achieving a homogeneous composite with ideal mechanical properties [162].

The addition of 10 mol% strontium in the HA ceramic was reported to improve not only the \textit{in vitro} bioactivity in SBF, but also the cellular attachment, proliferation, and differentiation [163].

TCP coating has been achieved on a C/C composite through a sonoelectrodeposition approach. A subsequent soaking of the deposited TCP coating in a mixed solution of NaF, K$_2$HPO$_4$ and KH$_2$PO$_4$ changed the composition to a mixture of HA and F-rich apatite and also increased the crystallization degree of the coating [164].

#### 4.3.2. Ceramic/Metal Composites

Another composite developed for load-bearing implants is HA/Ti, which combines the bioactivity of HA and the load-bearing ability of Ti. The composite was produced by hot pressing a mixture of 50 vol% HA, 40 vol% Ti and 10 vol% bioactive glass [165, 166].
The bioactive glass was added to aid sintering and to enhance the bioactivity of the composite. During hot pressing, the following reaction occurred:

\[ \text{Ti} + \text{Ca}_{10} (\text{PO}_4)_2 (\text{OH})_2 \rightarrow \text{Ti}_2 \text{O} + \text{CaTiO}_3 + \text{CaO} + \text{Ti}_x \text{P}_y \]  \hspace{1cm} (10)

The composite thus had additional CaO, Ti2O, CaTiO3, and TiP-like phases. In vitro bioactivity tests showed that the HA/Ti composite induced apatite deposition in SBF within 2 h. An apatite layer covered the entire surface after 24 h soaking in SBF. It was suggested that apatite deposition was initiated through the interaction of Ti2O with SBF to form Ti-OH groups and a negatively charged surface [165]. The dissolution of the CaO phase provided favorable conditions for apatite formation by forming open pores on the surface and by increasing the degree of supersaturation of SBF with respect to apatite [166].

4.3.3. Ceramic/Polymer Composites

Ceramic/polymer composites, also called organic–inorganic hybrids, combine the high strength and wear resistance of ceramics and high toughness and flexibility of polymers to overcome their individual disadvantages [167–174]. Apart from biological properties, if mechanical properties similar to those of surrounding tissue are obtained, such biomaterials can minimize the stress-shielding problem among the materials and the surrounding tissue [167, 175–177]. Therefore, ceramic/polymer composites have been extensively studied and their bioactivity evaluated. Wang provided a comprehensive review of bioactive composite materials for tissue replacements [167].

Table 4 lists some organic–inorganic hybrids and their in vitro bioactivity. The apatite-forming ability of the hybrids has been ascribed mainly to the bioactive reinforcements, such as HA, TCP, and Bioglass, and also silica, titania, and CaO. The apatite induction time decreases with increasing contents of the reinforcements [178, 179, 191, 193–195, 197]. The addition of TiO2 in the hybrid of SiO2-CaO-P2O5/PDMS increased readily its apatite-forming ability, due probably also to the increasing specific surface area of the hybrid as a result of the addition of TiO2 [198]. Enhanced in vitro bioactivity can also be obtained by modifying the matrix. For example, after treating the HA/chitin composite with an alkali solution, more D-glucopyranose was introduced in the chitin, which dissolved into SBF, leading to the increase in hydroxyl concentration in the vicinity of the composite surface and hence favored apatite deposition [183]. It was also noted that SBF-soaking can significantly affect the mechanical properties of the hybrids. The storage modulus of the HA/PHB composite increased initially with immersion time in SBF, due to apatite

<table>
<thead>
<tr>
<th>Matrix(^a)</th>
<th>Reinforcements</th>
<th>Apatite inducer</th>
<th>Shortest Apatite induction time in SBF</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEEK</td>
<td>HA</td>
<td>HA</td>
<td>3 d</td>
<td>[178]</td>
</tr>
<tr>
<td>PHB</td>
<td>HA</td>
<td>HA</td>
<td>1 d</td>
<td>[179, 181, 182]</td>
</tr>
<tr>
<td>Chitin</td>
<td>HA</td>
<td>HA, D-glucopyranose</td>
<td>2 d</td>
<td>[183]</td>
</tr>
<tr>
<td>PHB-PHV</td>
<td>HA, TCP</td>
<td>HA, TCP</td>
<td>7 d</td>
<td>[184]</td>
</tr>
<tr>
<td>PHB, PHV</td>
<td>HA</td>
<td>HA, TCP</td>
<td>1 d</td>
<td>[185, 186]</td>
</tr>
<tr>
<td>PE</td>
<td>Bioglass(^a)</td>
<td>Bioglass(^a)</td>
<td>3 d</td>
<td>[187]</td>
</tr>
<tr>
<td>PE</td>
<td>HA</td>
<td>HA</td>
<td>7 d</td>
<td>[188]</td>
</tr>
<tr>
<td>POMO</td>
<td>CaO, TiO2</td>
<td>CaO, TiO2</td>
<td>1 d</td>
<td>[189, 190]</td>
</tr>
<tr>
<td>POMO</td>
<td>CaO, SiO2</td>
<td>CaO, SiO2</td>
<td>12 h</td>
<td>[191]</td>
</tr>
<tr>
<td>Aerogel</td>
<td>CaSiO3</td>
<td>CaSiO3</td>
<td>25 d</td>
<td>[192]</td>
</tr>
<tr>
<td>POMO</td>
<td>TiO2</td>
<td>TiO2</td>
<td>1 d</td>
<td>[193]</td>
</tr>
<tr>
<td>POMO</td>
<td>CaO, SiO2, TiO2</td>
<td>CaO</td>
<td>12 h</td>
<td>[194]</td>
</tr>
<tr>
<td>PCL</td>
<td>SiO2</td>
<td>SiO2</td>
<td>3 h</td>
<td>[96, 195, 196]</td>
</tr>
<tr>
<td>ORMOSIL</td>
<td>Silica</td>
<td>Ca</td>
<td>3 d</td>
<td>[197]</td>
</tr>
</tbody>
</table>

\(^a\)PEEK: Polyetheretherketone; PHB: Polyhydroxybutyrate; PHBV: Polyhydroxyvalerate; PE: polyethylene; PTMO: Poly(hexamethylene oxide); PDMS: Polydimethylsiloxane; PCL: Poly(ε-caprolactone); ORMOSIL: Organically modified silicate.
formation on the surface, and decreased after prolonged immersion in SBF, because of the degradation of the PHB in SBF [179].

A nanocomposite consisting of a bioactive glass nanofiber (58SiO₂-38CaO-4P₂O₅) and type I collagen matrix has been reported [180]. The nanofiber was prepared by electrospinning the bioactive glass sol, which combined with self-assembled collagen sol in the aqueous solution, and then cross-linked to produce the nanocomposite. Such nanocomposites induced the rapid formation of bone-like apatite on the surface when incubated in SBF. Similarly, the type I collagen matrix dispersed with bioactive glass (CaO-P₂O₅-SiO₂) and silica micro-particles possessed in vitro apatite-forming ability. Interestingly, the silica particles and collagen hydrogel alone induced no apatite deposition, which suggests a possible synergistic effect between collagen and silica on the apatite-forming ability [199].

Eglin et al. [96] used three types of tests to assess in vitro apatite forming ability of silica/PCL composites: soaking in a static SBF at 37°C, soaking in a dynamic (circulating) SBF with a flow rate of 0.2 ml/min at 37°C, and alternate soaking in 0.2 M CaCl₂ at 37°C for 60 s and 0.12 M Na₂HPO₄ at 37°C for 60 s. The results obtained indicated that even though the dynamic condition best mimics the in vivo condition, both the static apatite-forming ability test and alternative soaking process are useful in providing simple methods for gaining quantitative information on the apatite-forming ability of biomaterials.

4.4. Scaffolds for Tissue Engineering

Tissue engineering requires suitable biodegradable scaffolds in order to produce biological substitutes for reconstructive surgery. In the case of bone tissue engineering, scaffolds are used either to induce the formation of bone from the surrounding tissue or to act as a carrier or template for bone cells and/or other agents such as growth factors. Materials for tissue engineering scaffolds must be biocompatible and bioactive and have a macroporous structure. Bioceramics such as HA [200–203] and β-TCP [204, 205], polymers [206, 207], and organic–inorganic hybrids [181, 200, 208, 211] having porous structures have been made for bone tissue engineering. Scaffolds made of biodegradable polymers are very weak in strength. Porous ceramic materials have a high risk of breaking from normal handling during operations. Composite scaffolds based on polymers and containing bioactive materials such as HA or Bioglass appear to be more suitable for bone tissue engineering [212].

4.4.1. Ceramics

Two deposition methods, suspension and thermal decomposition, were used to deposit HA on porous alumina [187]. Reticulated alumina, identified as a strong ceramic with interconnecting pores, was used as the substrate. The coatings were found to be formed uniformly on inner pore surfaces of the alumina. In vitro bioactivity was assessed by immersing the HA-coated alumina in SBF for up to 21 d. It was shown that the in vitro bioactivity of coated samples was affected by both the crystallinity and specific surface area of the HA coating. Well-crystallized HA coatings achieved by heat treatment at high temperatures led to reduced bioactivity. Their stability in SBF is thought to be associated with the high driving force required for the formation of apatite on their surfaces.

Powder mixtures of HA and phosphate-based glass (CaO · Na₂O · P₂O₅) were dip-coated onto a porous ZrO₂ structure and heat-treated at above 800°C for 2 h in air, which resulted in a composite coating consisting of HA, TCP, DCP, and residual glass [200]. The adhesion strength of the composite coating increased with increasing glass addition and decreasing CaO content in glass. The differentiation of cells was improved after such a coating was applied [200]. In another study, different types of calcium phosphates, i.e., HA, FA, TCP, and their composites of HA+FA, HA+TCP, were deposited onto the porous ZrO₂ using a powder slurry method [214]. The intermediate layer of FA effectively suppressed the reaction between the Ca-P layer and the ZrO₂ substrate. All coatings indicated favorable and comparable cell viability. The ALP activity of the cells on pure HA and HA composite coatings was expressed at higher levels than those on pure FA
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and pure TCP coatings. The ALP levels shown on all coatings were higher than that on the pure ZrO₂ scaffold [214].

Porous CaSiO₃ scaffolds were obtained by sintering a ceramic slip-coated polymer foam at 1350°C. The obtained scaffolds, which were degradable in Ringer’s solution, had a well-interconnected porous structure with pore sizes ranging from several micrometers to more than 100 μm and porosities of 88.5 ± 2.8%. After soaking in SBF for 7 d, HA was formed on the surface of the scaffolds. Osteoblast-like cells were found to be able to seed into the CaSiO₃ scaffolds, and the proliferation rate and ALP activity of the cells in the scaffolds improved as compared to the controls [215].

4.4.2. Polymers

The biomimetic apatite deposition process has been used to coat polymer scaffolds with a layer of bone-like apatite.

Polyactive® is a series of segmented block copolymers consisting of alternating hydrophilic polyethylene oxide (PEO) and hydrophobic polybutylene terephthalate (PBT) segments. Polyactive® 1000/70/30 is a member of the copolymer family with a PBT content of 30% in weight and a molecular weight of PEO of 1000 g/mol. It could be used as a bone defect filler for bone tissue engineering due to its bone-bonding ability and an intermediate degradation rate. To enhance the bioactivity, a continuous biomimetic ACP or bone-like apatite coating was produced on Polyactive® 1000/70/30, in the form of either plates or scaffolds, through 1 d soaking in a 5 SBF solution, which was enabled by bubbling CO₂ gas [206].

To make the scaffolds osteoconductive, an accelerated bone-like apatite deposition was achieved on the surfaces of biodegradable scaffolds of poly(L-lactic acid) (PLLA) and poly(glycolic acid) (PGA) by soaking them in 5 SBF for only 1 d [212]. An accelerated apatite formation could be very useful for biodegradable polymers, which are used for bone tissue engineering, as some of these polymers degrade in vitro too fast to be coated with an apatite layer in a normal biomimetic process. The apatite formed in 5 SBF was similar in morphology and composition to that formed in the classical biomimetic process employing SBF. Current work concentrates on the accelerated deposition of an apatite/collagen composite coating on PLLA scaffolds and a dynamic system for thoroughly coating surfaces of inner pores of the scaffolds.

Carbonxylate groups were introduced on the surface of poly(ε-caprolactone) (PCL) plates and scaffolds by treatment with an aqueous NaOH solution [216]. When subsequently soaked in a Ca-P solution, the carbonxylate groups formed apatite nuclei, which induced a dense and uniform bone-like apatite layer on the PCL surface after incubation for 24 h in SBF.

Slurry-dipping and electrophoretic deposition techniques were developed to achieve Bioglass® coatings on a poly(DL-lactide) foam [209]. Stable and homogeneous Bioglass® coatings as well as infiltration of Bioglass® particles throughout the porous network were obtained after the process was optimized. The coated foam exhibited in vitro bioactivity as it induced apatite deposition in SBF after 7 d.

4.4.3. Organic–Inorganic Hybrids

Many organic scaffolds for tissue engineering applications encounter problems of releasing acidic degradation products which lead to inflammatory responses and poor cell adhesion due to their hydrophobicity. The incorporation of certain bioactive inorganic components is effective in controlling these degradation behaviors because of the buffering effect of such alkaline inorganic phases. Composite scaffolds containing bioactive particles can be produced using a variety of techniques. These bioactive particles are incorporated in the scaffold and can induce apatite deposition in vitro and in vivo.

A poly(lactic acid)/vaterite composite sponge was prepared through a conventional particle-leaching technique using sucrose as the sacrificial phase [208, 217, 218]. After soaking the composite sponge in SBF for 3 d, apatite particles were found covering
the sponge. In a separate study, a large number of apatite particles were also found on the pore surface of a composite scaffold consisting of poorly crystallized HA and PLA, after soaking in SBF for 7 d [181]. The carboxyl (COOH) groups that resulted from hydrolyzation of PLA are believed to initiate apatite nucleation on the surfaces of the above-mentioned hybrids.

Spherical Ca-P particles were prepared from highly crystalline feedstock HA particles by plasma spraying the HA particles onto the surface of ice blocks or into water [219]. The Ca-P particles obtained were highly bioactive due to the presence of ACP in these particles. Bioactive and biodegradable composite scaffolds produced by incorporating plasma-sprayed Ca-P particles into a degradable PLA polymer were subjected to in vitro bioactivity tests. The scaffold exhibited high bioactivity through the formation of apatite in the SBF within a short period of 24 h [219].

The incorporation of calcium carbonate in vaterite crystalline form in macroporous poly(DL-lactide) foam achieved a organic–inorganic hybrid scaffold with the ability to induce apatite deposition after soaking in SBF for 7 d. The hybrid was prepared by dip-coating the organic foam in a slurry of vaterite in methanol or ethanol. The release of Ca\(^{2+}\) ions as a result of the decomposition of vaterite in the hybrid was suggested to be responsible for the apatite deposition on the surface [220].

Hirata et al. developed a new biodegradable scaffold. Carbonate apatite (CHA) was mixed with neutralized collagen gel, which was then lyophilized into sponges in a porous HA frame ring. After complexing with a bone morphogenic protein (rh-BMP2), the scaffold was implanted beneath the periosteum craniio of rats, and significant new bone was observed after 4 weeks [221]. Also, in vitro cell culture experiments revealed that the addition of wollastonite into a PHB-PHV matrix stimulated osteoblasts to proliferate and differentiate. The hybrids containing Si and Ca as ionic products of wollastonite were hence more suitable for bone implant and tissue engineering scaffolds [222].

5. CONCLUDING REMARKS

The prerequisite for biomaterials to form a chemical bond to living bone tissue is their bioactivity; that is, the ability to form a bone-like apatite layer under physiological fluid when implanted in vivo. The Kokubo’s SBF is generally used to evaluate the bioactivity in vitro. The in vitro apatite deposition of biomaterials is initiated by the surface functional groups of Si-OH, Ti-OH, COOH, and so on. Other factors contributing to the in vitro bioactivity include the negatively charged surface, surface micropores, large specific surface area, certain surface defects, and certain crystal structures. Incorporation of elements such as Ca, P, K and Na in the surface layer also benefits the apatite deposition through either directly dissolving the Ca and P ions into the surrounding solution or indirectly exchanging K and Na ions with the H\(^{+}\) ions in the surrounding solution to increase the supersaturation with respect to apatite. For apatite deposition on titania gel, a crystalline titania, either in an anatase or rutile structure, is necessary for apatite-forming within a short time. However, too many factors influence the apatite-forming ability of biomaterials, and some contradictory results have been reported. There is still much work to do to clarify the exact apatite deposition procedure both in vitro and in vivo.

Although nearly all the biomaterials capable of forming bone bonding in vivo induce apatite deposition in SBF within a short time, not all the biomaterials capable of inducing apatite deposition in SBF are able to develop a direct bone bonding when implanted in vivo. Thus, although the SBF is simple and helpful to select a bioactive material or coating, an in vivo animal evaluation seems to be necessary to ensure the bioactivity before bringing the biomaterials to a clinical application.

Various coating techniques have been applied to provide biomaterials of ceramics, polymers, and composites with bioactivity. Some coating techniques on polymers have been utilized successfully to prepare the organic–inorganic hybrids analogous to natural bone.
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REFERENCES

23. X. Lu and Y. Leng, Biomaterials 26, 1097 (2005).
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P.J. Li and P.Ducheyne, 47. 48.
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