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Advances in Biomimetic Apatite Coating on Metal Implants

C.Y. Zhao, H.S. Fan and X.D. Zhang
National Engineering Research Center for Biomaterials, Sichuan University, Sichuan, Chengdu 610064 China

1. Introduction

Artificial implants are generally encapsulated by a fibrous tissue when implanted into bone defects. However, Hench et al. showed that bioglass directly bonded to living bone via a biologically active bone-like apatite layer instead of the formation of surrounding fibrous tissue(Hench et al., 1971). Meanwhile, with the mineral compositional resemblance with the inorganic phase of human bone, calcium phosphate ceramics possessed excellent biocompatibility and osteoconductivity, and it also showed bone-bonding ability via a biologically active bone-like apatite layer(W.P. Cao & Hench, 1996; Hench, 1998). Nowadays, they are both extensively used as hard tissue repair or substitution materials in clinic. However, these materials cannot be used under load-bearing conditions such as femur, tibia and spinal interbody, because they are usually very stiff and brittle, and have low impact resistance and relatively low tensile strength(Rezwan et al., 2006).

Titanium and its alloys are widely used as orthopaedic implants due to their superior mechanical properties and excellent biocompatibility(X.Y. Liu et al., 2004; Ratner, 2001). However, their bioactivity are not as good as that of calcium phosphate ceramics and during implantation they can only form osteointegration at the interface of titanium and bone tissue, instead of bone-bonding(Feng et al., 2002). To overcome these disadvantages, various methods of coating the titanium surface have been developed to combine the mechanical properties of metals with bone-bonding ability of bioactive ceramics, such as ion-beam(Ong et al., 1992) or radiofrequency magnetron sputter deposition(Wolke et al., 1998), sol–gel method (Brendel et al., 1992; Weng & Baptista, 1999) et al, with plasma spraying being the most popular(Y. Cao et al., 1996; J. Chen et al., 1994). However, each of them has its own technical limitations, for example, the inability to coat those complex-shaped implants with internal cavities or porous implants and incorporate biologically active agents. Therefore, an optimal technique for apatite coatings on complex-shaped or porous implants still has to be developed.

One alternative method is the so-called biomimetic apatite coating, which consists of mimicking the bone mineralization process by immersing implants in simulated body fluid (SBF) that mimics the inorganic composition, pH, and temperature of human blood plasma(Abe et al., 1990). As a result of the low temperature conditions of this technique, diverse Ca-P phases such as amorphous calcium phosphate (ACP), octacalcium phosphate (OCP) or carbonated apatite (CA), some of which are stable only at low temperatures, can be

deposited on the metal implants(Barrère et al., 1999, 2001, 2002a, b; Habibovic et al., 2002). Compared with the above mentioned techniques, biomimetic technique might have the following advantages: (1) it is expected to endow the materials with high bioactivity, and the properties of the coating such as phase composition, crystallinity and dissolution can be adjusted by controlling the process parameters to meet specific clinic needs, (2) it is a low-temperature process, free of adverse heat effect on substrates, and even heat-sensitive substrates including polymers can be coated, (3) it can be used to produce biomimetic apatite coating on/or even into porous or complex-shaped implants, (4) it can incorporate biologically active agents or drugs into biomimetic apatite coating through coprecipitation rather than merely absorb on the surface. The degradation of these coatings would result in a gradual release of biologically active agents or drugs rather than in a single rapid burst, (5) it is a simple and cost-effective way(Habibovic et al., 2004a; Wen et al., 1998). Two conditions, however, must be met in order to insure an effective biomimetic apatite deposition: (1) pretreatments of the metal surface, and (2) supersaturation of calcium and phosphate in the solution(Narayanan et al., 2008; Q.Y. Zhang & Leng, 2005). Regarding the pretreatments, surface morphology of metal implants such as surface roughness can affect the nucleation and growth of apatite coating from the simulated body fluid, and the surface chemistry such as hydroxyl groups on the titanium surface is beneficial for the chemical bonding with calcium and phosphate ions (Barrère et al., 2004; Leitão et al., 1997). Regarding the degree of supersaturation in the solution, it influences the calcification ability of metal implants(Barrère et al., 2004). Both factors determine its in vitro and in vivo biological effects. In this chapter, the effects of both factors including pretreatments of the metal surface and the simulated body fluid on biomimetic coating as well as the possibility to incorporate biologically active agents and drugs into the biomimetic apatite coatings are introduced. The in vitro and in vivo biological performances of these biomimetic apatite coatings are also described.

2. Effect of pretreatments on biomimetic apatite coating

During the biomimetic deposition process, the heterogeneous nucleation ability of Ca^{2+} and PO_4^{3-} ions are directly dependent on the activation of metal surface in the pretreatment process. The purpose of the pretreatments is mainly to modify the surface topography, and/or modify the chemical composition or structure of the oxide layer or form a new surface layer. The solvent cleaning to remove the surface contaminants such as oils, greases is not included in this chapter as a pretreatment(Lausmaa, 2001). The main pretreatments are summarized as follows:

2.1 Physical methods to modify the surface topography

The metal surface becomes coarse and porous through special treatments, such as grit blasting or other methods to form porous structure(Barrère et al., 2003a; Habibovic et al., 2002; Ryan et al., 2006). After immersion in supersaturated calcium phosphate solution, the Ca^{2+} and PO_4^{3-} ions adhere to these coarse and/or porous surfaces through mechanical interlocking. Regarding the effects of surface topography on biomimetic apatite coating, previous study showed that the nucleation and morphology of apatite coating could be affected by the surface roughness of the substrate after immersion in Hank's balanced salt solution (HBSS)(Leitão et al., 1997). Furthermore, the adhesion strength of the biomimetic apatite coating was dependent on the mechanical interlock between biomimetic coating and implant surface(Leitão et al., 1997). There were many types of methods for the fabrication of

coarse surface or porous structure. For example, the rough surface on Ti6Al4V plates was obtained via grit blasting by using alumina particles and an average surface roughness of 3.5 μm was required for an optimal apatite coating(Habibovic et al., 2002). The porous implants, such as porous tantalum implants manufactured by chemical vapor infiltration to deposit pure tantalum onto vitreous carbon foams, porous Ti6Al4V implants produced by a positive replica technique, were in favor of apatite deposition(Barrère et al., 2003a; Habibovic et al., 2005). OCP or CA coating was successfully deposited on or into the porous tantalum or porous Ti6Al4V implants by immersion into a highly concentrated simulated body fluid(Barrère et al., 2003a; Habibovic et al., 2005).

2.2 Chemical and electrochemical methods

Chemical and electrochemical treatments of titanium and its alloys, such as acid treatment, alkali or alkali-heat treatment, acid-alkali treatment, hydrogen peroxide (H_2O_2) treatment, anodic (microarc) oxidation, are mainly based on chemical or electrochemical reactions occurring at the interface between titanium and a solution, and a porous sodium titanate gel or titania-based film forms on the substrate(Lausmaa, 2001). After immersion in SBF, a bone-like apatite coating spontaneously deposits on its surface. The mechanism of apatite formation can be interpreted as the electrostatic interaction between Ti-OH functional groups on the film and Ca^{2+}, PO_4^{3-} ions in the simulated body fluid and/ or the matching of crystal structure between the titania and apatite(Kim H.M. et al., 1996; Wang et al., 2000). Chemical methods are substantially biomimetic in nature.

2.2.1 Acid treatment

Acid treatment is often used to remove surface oxide and contamination in order to obtain clean, uniform and rough surface finishes. The mixed acid of 10–30 vol% of HNO_3 and 1–3 vol%of HF in distilled water is the most commonly used and is recommended to be a standard solution as a pre-treatment. The ratio of nitric acid to hydrofluoric acid at 10:1 is preferred to minimize the formation of free hydrogen. The free hydrogen results from the reaction between titanium and hydrofluoric acid and can adsorb on the titanium surface to cause embrittlement of the surface layer(ASTM standard B600, 1997; Lausmaa, 2001). The acid etching of titanium in HCl under inert atmosphere as a pretreatment was used to obtain a uniform initial micro-roughened surface before alkali treatment, which provided an improved condition for a homogenous hydroxycarbonated apatite precipitation after exposition in SBF(Jonášová et al., 2004). Nitric acid passivation was also used as a pretreatment before alkaline treatment to form a microporous surface on NiTi alloy(M.F. Chen et al., 2003). Wen et al. employed a mixture of 100ml HCl(18mass%) and 100ml H_2SO_4 (48 mass%) before alkaline treatment to obtain a microporous surface(Wen et al., 1997, 1998). However, titanium with only acid treatment could not spontaneously induce apatite deposition in SBF. Lately, Lu et al firstly revealed that titanium with nitric acid treatment could induce biomimetic apatite coatings formation in SBF. They confirmed that nitric acid treatment did not increase the oxide thickness on the Ti substrates, but the increase of the nitric acid treatment temperature and duration helped to improve its apatite-forming ability(Lu et al., 2007).

2.2.2 Alkaline treatment

Kim et al established a simple chemical treatment, i.e. an alkali and heat(AH) treatment process, for spontaneously inducing a uniform bonelike apatite layer on titanium surface in

SBF (H.M. Kim et al., 1996). Treatment of titanium in 5-10M NaOH or KOH solution at 60°C for 24h produced a microporous and graded alkali titanate hydrogel layer(H.M. Kim et al., 1996, 1998,1999; Kokubo et al., 1996). The as-formed gel layer, however, was mechanically unstable. Subsequent heat treatment of the alkali-treated Ti at 600°C for 1h made the hydrogel layer dehydrated and densified to form a cystalline alkali titanate layer. The heat treatment considerably increased the mechanical strength of the surface gel layer to its substrates, but it slightly lowered the bioactivity of the alkali titanate gel layer on NaOH-treated Ti surfaces. That's to say, it would take a little longer time to induce the apatite formation on the titanium surface in SBF(H.M. Kim et al., 1997). The mechanism of apatite formation on AH treated titanium in SBF is as follows: When AH-treated titanium is exposed to SBF, the alkali ions are released from the alkali titanate layer and hydronium ions enter into the surface layer via ions exchange, which result in the formation of negatively charged Ti-OH groups in the surface. At the same time, the released Na^+ ions increase the degree of supersaturation with respect to apatite by increasing pH. Because of the electrostatic interaction, the negatively charged Ti-OH groups combine selectively with the positively charged Ca^{2+} in the fluid to form calcium titanate. Calcium titanate takes the phosphate ions as well as the calcium ions in the fluid to form the apatite nuclei. Once the apatite nuclei are formed, they spontaneously grow by consuming the calcium and phosphate ions from SBF(H.M. Kim et al., 1996; Takadama et al., 2001a, b). The order of calcium and phosphate ion deposition on AH-treated titanium surface is that the precipitation of Ca ions is prior to that of phosphate ions(B.C.Yang et al., 1999).

AH treatment is a simple and economical method. It affects only the top 1 μm of the surface and its effects can extend all over the irregular surface of the implant, which is especially important for porous and porous-coated implants. The AH treatment can provide porous and porous-coated implants with bioactive surface while does not reduce the pore space available for bone ingrowth(Nishiguchi et al., 2001; Takemoto et al., 2005a).

Based on the AH treatment to improve the bioactivity of titanium and its alloys, many researchers have further optimized the treatment process for better bioactivity. Wei et al optimised the bioactivity of alkaline-treated titanium alloy by changing a variety of conditions for the AH treatments of Ti6Al4V alloy, and found that the rate of apatite formation on AH-treated titanium alloy could be significantly accelerated(M. Wei et al., 2002). Uchida et al conjoined the hot water and heat treatments after alkali treatment (Water-AH) to convert the sodium titanate gel into anatase, which significantly improved the apatite-forming ability of the metal in SBF(Uchida et al., 2002). Some researchers have successfully applied these techniques to porous or porous-coated metal implants, and these treated implants all showed apatite-forming ability in SBF(Fujibayashi et al., 2004; Nishiguchi et al., 2001; Takemoto et al., 2005a, 2006). Takemoto et al developed a dilute hydrochloric acid (HCl) treatment between alkali treatment and heat treatment (HCl-AH) for porous titanium implants, which could remove sodium from the alkali-treated porous titanium more effectively than conventional hot water treatment, and the subsequent heat treatment converted titania into anatase. The surface of HCl-AH implants possessed a more complex porous structure than the others, which showed a combination of large and small microporous structures. Both water-AH treated and HCl-AH treated porous titanium showed high apatite-forming ability after immersion in SBF. Island-like apatite deposits could be recognized on the surface of both implants within 1 day. There was larger size of the apatite deposits in the HCl-AH treated implants and higher number of spherulites in the Water-AH group(Fujibayashi et al., 2004; Takemoto et al., 2006).

In addition to titanium and its alloy, the AH treatment was also applied to other metals. Miyazaki et al treated tantalum in a 0.2 or 0.5M NaOH aqueous solution at 60°C for 24 h and the treated tantalum metal induced apatite deposition within 1 week in SBF(Miyazaki et al., 2000, 2001, 2002). High temperature and high pressure were also applied to the alkaline treatment in order to promote the deposition of apatite on the substrate from SBF(De Andrade et al., 2000).

2.2.3 Acid-alkali treatment

The main idea of acid-alkali (AA) treatment is to etch the metal surface with acid solution to acquire a uniform and rough surface, and the subsequent alkali treatment might have two concurrent effects: that is the formation of a microporous surface layer on the acid-etched surface and the formation of more titanium oxide layer on this microporous layer. Both steps are helpful to improve the bioactivity of titanium implants(Wen et al., 1997, 1998).

Wen et al used a mixture of 100 ml 18 wt% HCl and 100 ml 48 wt% H_2SO_4 solutions to etch titanium for 30 min and then treated them in boiling 0.2 M NaOH solution at 140°C for 5 h. Many micrometre-sized acid etched pits or grooves formed on the titanium surface by acid treatment, and large amounts of nanosized fine pits with more titanium oxide layer were produced on the surface by the alkali treatment. Combined acid treatment with alkali treatment, a completely microporous titanium oxide surface on a submicrometre scale was formed. Conformal and adherent apatite coating were rapidly precipitated on the AA-treated surfaces after soaking in supersaturated calcification solution (SCS)(Wen et al., 1997, 1998). When AA treatment was applied to porous titanium, it could induce apatite deposition on its inner pores(Zhao et al., 2010). HNO_3 and NaOH solution were also employed to prepare a bioactive layer on the surface of NiTi alloy, and an apatite layer was spontaneously deposited on the treated titanium surface after soaked in SBF(M.F.Chen et al., 2003).

In order to solve the inhomogeneous and non-uniform apatite deposition of NaOH-treated titanium after exposition in SBF, acid etching of titanium in HCl under inert atmosphere was used as a pretreatment to obtain a uniform micro-roughened surface before alkali treatment, and the acid etching provided an improved condition for homogenous apatite deposition(Jonášová et al., 2004).

Though NaOH-treated titanium could form a bone-like apatite layer on its surface in SBF via the release of Na^+, the inflammation response and cell death would occur if the released Na^+ increased external alkalinity (Silver et al., 2001). Therefore, it would be beneficial to decrease the release of Na^+ into the surrounding tissue. Jonášová et al washed NaOH-treated titanium with distilled water to lower the amount of Na^+ in the surface layer, and they found that the rate of apatite formation was not significantly influenced by a lower amount of Na^+ in the surface layer(Jonášová et al., 2002). Hot water or dilute HCl immersion were also used to partially or almost completely remove Na^+, and this had already been described above(Fujibayashi et al., 2004; Takemoto et al., 2006; Uchida et al., 2002).

2.2.4 Precalcification

The calcium ion implantation process developed by Hanawa et al was reported to form a continuous interface between the surface-modified layer and substrate, which was expected to prevent the interface fracture. Furthermore, the implanted calcium ions formed calcium

titanate in the surface oxide layer, which could possibly accelerate apatite precipitation (Hanawa et al., 1994). However, the calcium ion implantation process required a special apparatus. Therefore, some simple processes for forming a calcium-ion-containing surface layer on titanium were explored. Hanawa et al reported that titanium plates were immersed in the calcium-ion–containing solutions, including calcium nitrate, calcium chloride and calcium oxide solution, at ambient temperature for 7 days, and a surface-modified layer consisting of calcium hydroxide and/or calcium titanate was formed on its surface. The surface-modified layer in which titanium was modified by calcium oxide was thickest. Apatite was deposited on the surface-modified titanium after immersion in HBSS(Hanawa et al., 1997).

Surface modification of titanium in CaO solution with hydrothermal treatment in an autoclave was also performed. This process enhanced apatite precipitation on the modified surface in HBSS, which resulted from the effects of high pH, high pressure and high temperature of the CaO solution on titanium surface(Hamada et al., 2002). Heat treatment above 600°C of hydrothermal-modified titanium in CaO solution could enhanced the apatite formation in SBF (Sultana et al., 2009). Hydrothermally deposit Ca ions on porous titanium in calcium hydroxide ($Ca(OH)_2$) solutions as a pre-treatment also endowed porous titanium with apatite-inducing ability(X.B.Chen et al., 2009). In addition, precalcification with boiling saturated $Ca(OH)_2$ solution was used to bioactivate titanium. After precalcification, a uniform calcium phosphate rapidly precipitated onto the surfaces of titanium in SCS(Feng et al., 2002a). Later, heat-treatment in water vapor was carried out prior to precalcification to improve the bond strength of the apatite coating to substrate(Feng et al., 2002b). Precalcification was also applied to AA- or AH-treated titanium by soaking them in Na_2HPO_4 and then saturated $Ca(OH)_2$ solution before immersion in SCS or SBF to speed up the formation of apatite(Liang et al., 2003; Wen et al., 1997).

2.2.5 H_2O_2 treatment

Titania gels were able to induce the precipitation of apatite when soaked in SBF(P. Li et al., 1994). The formation of titania gels on the surface of implant was therefore considered to be one of the potential approaches to improve the bioactivity of implant(P. Li & de Groot K., 1993; P. Li et al., 1994). It was known that biomaterials implanted into the body would cause inflammatory responses, which then resulted in generation of H_2O_2 around the implant(Tengvall et al., 1989a). The interaction between H_2O_2 and titanium implants were thought to be beneficial for the biocompatibility of the titanium(Baker et al., 2009; Tengvall et al., 1989b). Some reports showed that titanium could react with a H_2O_2 solution and formed titania gel(Tengvall & Lundström, 1992; Tengvall et al., 1989b, 1989c). Obviously, it could be an effective and convenient technique as a pretreatment of titanium. Depending on the H_2O_2 concentration and the treatment time, exposure titanium to H_2O_2 solution led to roughening and thickening of its surface oxide(MacDonald et al., 2004; Pan et al., 1996, 1998; Wälivaara et al., 1993). Pan et al. reported that the oxide was composed of a double layer structure with a thin and dense inner oxide and an porous outer layer(Pan et al., 1996, 1998). When titanium was treated in a H_2O_2/0.1M HCl solution at 80°C, an amorphous titania gel layer was formed on its surface. The thickness of the titania gel layers depended almost linearly on the period of time of the chemical treatment. The subsequent heat treatment above 300°C transformed the amorphous gel to crystalline anatase. The presence of rutile and the densification of the gel occurred concurrently when the temperature was raised

above 600°C. The minimum thickness of the titania gel layer reached about 0.2μm and the temperature of heat treatment between 400-500 exhibited excellent apatite-forming ability(Wang et al., 2002).

Titanium treated with a H_2O_2/3mM $TaCl_5$ solution at 80°C also yielded an amorphous titania gel on its surface. The subsequent heat treatment between 300-600°C transformed the amorphous gel to crystalline anatase. The anatase titania layer could induce apatite deposition within 1 day of immersion in SBF, and the thicker titania gels deposited more apatite than that of the thinner one. The nucleation of apatite preferentially took place inside the cracks in the thicker gel layers(Wang et al., 2000).

Although heat treatment after immersion in H_2O_2/0.1M HCl or H_2O_2/3mM $TaCl_5$ solution could induce crystallization of the amorphous titania, it was supposed to cause the loss of the Ti-OH groups that played an important role in initiating the deposition of apatite(H.M. Kim et al., 1997; Wang et al., 2000, 2002). Therefore, a low-temperature mild condition approach to prepare bioactive surface through interactions between titanium and H_2O_2 solutions was of great interest. Ohtsuki et al. reported that titanium treated with H_2O_2/$TaCl_5$ or H_2O_2/$SnCl_2$ solution at 60°C for 24h gave it apatite-forming ability after immersion in SBF. They thought that basic Ti-OH groups in titania hydrogel layers on their surfaces were responsible for apatite nucleation and growth(Ohtsuki et al., 1997). Wu et al soaked titanium in H_2O_2/3mM $TaCl_5$ solution at 80°C for 3 days, on which crystalline titania layers consisting of anatase and rutile were deposited. Those titania layers, regardless of the fraction of anatase and rutile, showed excellent ability to induce deposition of apatite(J.M.Wu et al., 2004).

2.2.6 Electrochemical methods

Electrochemical methods, including electrocrystallization(J.S. Chen et al., 1998; Shirkhanzadeh, 1995), electrophoretic deposition (Zhitomirsky & Gal-Or, 1997)and anodic oxidation(micro-arc oxidation)(Lausmaa, 2001; X.Y. Liu et al., 2004), are based on different chemical reactions occurring at an electrically energized surface (electrode) placed in an electrolyte. In this section, only the anodic oxidation (micro-arc oxidation) is introduced.

Micro-arc oxidation (MAO) is also called anodic spark oxidation or plasma electrolytic oxidation. It is a relatively convenient technique to form ceramic coatings on the surface of metals, such as Ti, Al, Mg and their alloys. The in situ formed ceramic coatings are porous and firmly bond to metal substrate. Furthermore, it is very suitable for modifying metal surfaces with complex geometries, even porous structure(Sun et al., 2008). Since Ishizawa and Ogino first developed anodic titanium oxide coatings containing Ca and P on titanium by using this technique(Ishizawa & Ogino, 1995a, b), the titania-based coatings formed by MAO can be divided into two categories. One is titania-based composite coatings consisting of TiO_2, $CaTiO_3$, $Ca_2P_2O_7$ and $Ca_3(PO_4)_2$ (Han et al., 2003; Huang et al., 2005; Song et al., 2004) or TiO_2 and hydroxyapatite (HA)(M. Kim et al., 2007; Sun et al., 2007), which was produced in electrolytes containing Ca and P at high applied voltage and could induce the formation of apatite on its surface. The other is monophasic TiO_2 coatings, which was produced in electrolytes containing Ca and P at applied voltages lower than 400 V(Ishizawa & Ogino, 1995a, b; D.Q. Wei et al., 2007; Zhu et al., 2001) or in electrolytes containing H_2SO_4, H_3PO_4 or HCl(Das et al., 2007; B.C. Yang et al., 2004). Although most monophasic TiO_2 coatings formed by MAO had no apatite-forming ability, the titanium anodically oxidized in H_2SO_4 solution under the conditions with spark-discharge could induce apatite formation on its surface after immersion in SBF. The increase of the amount of either anatase or rutile by conditioning the

anodic oxidation was helpful to shorten the induction period of apatite formation. However, the titanium anodically oxidized under the condition without spark discharge could not induce apatite formation, even though the anatase was also formed on its surface(B.C. Yang et al., 2004). In view of this, subsequent activation methods such as heat treatment(Das et al., 2007; B.C. Yang et al., 2004), hydrothermal treatment(Huang et al., 2004; Ishizawa & Ogino, 1995a), chemical treatment (D.Q. Wei et al., 2007)and ultraviolet (UV) irradiation(Han et al., 2008) were carried out to improve the bioactivity of the MAO-formed monophasic TiO_2 coatings. Micro-arc oxidation in the aqueous electrolytes containing NaOH was also used to treat porous titanium, and the bioactive thin films were formed on the porous titanium inner-pore walls. The thus-treated porous titanium showed apatite-forming ability in SBF(Sun et al., 2008).

2.3 Surface-induced mineralization (SIM)

Surface-induced mineralization (SIM) is based on the observation that in nature organisms use biopolymers to produce ceramic composites, such as teeth, bones, and shells. The SIM process includes modification of a surface to introduce surface functionalization followed by immersion in aqueous supersaturated calcium phosphate solutions. In short, this technique is based on crystal nucleation and growth onto functionalized interfaces(Bunker et al., 1994; Campbell et al., 1996).

Various functional groups have been introduced onto the surface of Ti and its alloys to functionalize the surface capable of nucleating apatite. Campbell et al. introduced the functionalized end groups including -COOH, $-SO_3H$, $-PO_4H_2$, $-CH_3$, and $-NH_2$ to the surface of Ti to initiate calcium phosphate deposition(Campbell et al., 1996). Mao et al. introduced highly organized arrangement of carboxyl and hydroxyl groups on the surface of hydroxylated titanium with strong (0001) texture through self-assembly of vinyltriethoxysilane. The functionalized substrate showed the ability to induce oriented nucleation and growth of HA (Mao et al., 1998). Majewski et al coated titanium with self-assembled monolayers (SAMs), NH_2-, SH-, and SO_3H-SAMs, respectively. The results showed apatite deposition from SBF and SH-SAM appeared to favor the formation of apatite(Majewski & Allidi, 2006). In another study, various functional groups were introduced onto the surface of commercially pure titanium foils using a SAM technique, and the results suggested that the pre-deposition of HA onto these functionalized SAM surfaces might be an effective and fast way to prepare biomimetic apatite coatings on surgical implants. These results suggested that surface functional groups could play a critical role in inducing Ca/P nucleation(Q. Liu et al., 2002).

3. Effect of simulated body fluid on biomimetic apatite coating

The simulated body fluid plays a significant role in determining the phase composition, crystallization, growth rate of biomimetic apatite coating, which actually are affected by the composition, concentration, pH, flowing state of simulated body fluid and its additives, such as trace elements, proteins and drugs.

3.1 History of the simulated body fluid

Human blood plasma has a Ca/P molar ratio of 2.50(Gamble, 1967). Many researchers try to develop the acellular simulated body fluid (SBF) by mimicking the inorganic ion

concentrations of human blood plasma(Kokubo & Takadama, 2006; Kokubo, 1990a). The historical development of SBF which claims to imitate the human plasma or the extracellular fluid is given in Table 1. In the earlier solutions, Ringer's(Ringer, 1883), Earle's (EBSS, Earle's Balanced Salt Solution)(Earle, 1943) and Hank's (HBSS)(Hanks, 1975; Hanks & Wallace, 1949) solutions were very popular. Later, the pH values of SBF solutions were fixed at 7.4 by using TRIS (tris-hydroxymethyl-aminomethane)-HCl (hydrochloric acid) buffer solution, and the Ca/P molar ratio was raised to 2.50 (Kokubo et al., 1990b). However, the original SBF used by Kokubo et al. (Kokubo et al., 1990b) and Hench et al. (Filgueiras et al., 1993)

	Na^+	K^+	Mg^{2+}	Ca^{2+}	Cl^-	HCO_3^-	HPO_4^-	SO_4^{2-}	Buffer
Human blood plasma (Gamble, 1967)	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	
Ringer(Ringer,1883)	130	4.0		1.4	109.0				
EBSS(Earle, 1943)	143.5	5.37	0.8	1.8	123.5	26.2	1.0	0.8	
HBSS(Hanks,1975; Hanks.&Wallace, 1949)	142.1	5.33	0.9	1.26	146.8	4.2	0.78	0.41	
Original SBF (Kokubo et al., 1990b)	142.0	5.0	1.5	2.5	148.8	4.2	1.0	0	TRIS
c-SBF(Cho et al., 1995; Kokubo, 1991; Ohtsuki et al., 1991)	142.0	5.0	1.5	2.5	147.8	4.2	1.0	0.5	TRIS
Tas-SBF(Tas, 2000)	142.0	5.0	1.5	2.5	125.0	27.0	1.0	0.5	TRIS
Bigi-SBF(Bigi et al., 2000)	141.5	5.0	1.5	2.5	124.5	27.0	1.0	0.5	HEPES
r-SBF(Oyane et al., 2003)	142.0	5.0	1.5	2.5	103.0	27.0	1.0	0.5	HEPES
m-SBF(Oyane et al., 2003)	142.0	5.0	1.5	2.5	103.0	10.0	1.0	0.5	HEPES
i-SBF(Oyane et al., 2003)	142.0	5.0	1.0	1.6	103.0	27.0	1.0	0.5	HEPES
n-SBF(Takadama et al., 2004)	142.0	5.0	1.5	2.5	103.0	4.2	1.0	0.5	TRIS
SBF×5(Barrère et al.,2000a; Habibovic et al., 2002)	714.8		7.5	12.5	723.8	21.0	5.0		
SBFB×5(Habibovic et al., 2002)	704.2		1.5	12.5	711.8	10.5	5.0		
SCS1(Habibovic et al., 2005)	140.4			3.1	142.9		1.86		TRIS
SCS(Wen et al., 1997)	136.8	3.71		3.1	144.5		1.86		TRIS

Table 1. Ion concentrations of SBFs and human blood plasma

lacked the SO_4^{2-} ions which were contained in human blood plasma. In 1991, the corrected SBF papers were published by Kokubo et al(Kokubo, 1991; Ohtsuki C. et al., 1991), and the detailed recipe for preparation of SBF was reported in 1995 by Cho et al due to the difficulty to prepare clear SBF with no precipitation(Cho et al., 1995).

Since the corrected SBF was proposed by Kokubo et al, it still had higher Cl^- ion and lower HCO_3^- ion concentrations than human blood plasma, as could be seen from Table 1. Therefore, a number of slightly different compositions were proposed. Tas et al. used di-sodium hydrogen phosphate in place of di-potassium hydrogen phosphate to raise the HCO_3^- concentration to 27 mM in a TRIS-HCl buffered SBF solution (i.e., Tas-SBF)(Tas, 2000). Bigi et al. increased the content of carbonate ions to 27mM HCO_3^- in a HEPES-NaOH-buffered SBF solution (i.e., Bigi-SBF)(Bigi et al., 2000). Oyane et al. tried to correct this difference. They prepared r-SBF and i-SBF, which had ion concentrations equal to those of blood plasma, and m-SBF, which had ion concentrations equal to those of blood plasma except for the HCO_3^- concentration. However, r-SBF and i-SBF lacked long-term stability due to a strong tendency to precipitate both apatite and calcite from these SBF(Oyane et al., 2003). It was reported that the buffering agent TRIS present in conventional SBF (c- and Tas-SBF) formulations could form soluble complexes with several cations, including Ca^{2+}, which further reduced the concentration of free Ca^{2+} ions available for apatite formation, while HEPES did not show this behavior(Jalota et al., 2007; Serro & Saramago, 2003). Takadama et al. reported a newly improved SBF (n-SBF). They decreased the Cl^- ion concentration equal to those of blood plasma, but maintained the HCO_3^- ion concentration equal to that of the corrected SBF (c-SBF)(Takadama et al., 2004). After comparison with c-SBF, the n-SBF did not differ from c-SBF in stability and reproducibility. In 2003, conventional SBF with the refined recipe was proposed to a standard solution for in vitro measurement of apatite-forming ability of implant materials (Kokubo & Takadama, 2006).

Although SBF mimicked the inorganic composition and the pH of human blood plasma and it was employed by many investigators to produce biomimetic apatite coating on implant surfaces, it would take several days with daily refreshments of SBF solution due to the slow apatite nucleation process(P. Li et al., 1994; P. Li & Ducheyne, 1998; Peltola et al., 1998). In order to shorten the apatite nucleation time, more concentrated SBFs, such as 1.5 SBF(Abe et al., 1990), SBF×5(Barrère et al., 2000a, 2001; Habibovic et al., 2002) or SBF×10(Tas & Bhaduri, 2004), were used. It should be note that SBF×5 was five times more concentrated than c-SBF recipe, but TRIS- or HEPES-free. Instead of activating the metal surface by the above methods, non-bioactive materials could also be coated by SBF×5 immersion, and the induction time of apatite was shortened when compared to incubation in 1.0 SBF(Barrère et al., 2000a, 2001; Habibovic et al., 2002).

3.2 Effects of ion concentration and type in simulated body fluid on biomimetic apatite coatings

Biomimetic apatite coatings based on heterogeneous nucleation of apatite have been successfully obtained after immersion the pretreated metal implants in SBF. However, it would take several days with daily refreshing of SBF solution due to the metastability of SBF at physiological conditions (P. Li et al., 1994; P. Li & Ducheyne, 1998; Peltola et al., 1998). In order to accelerate the coating process, one approach was to pretreat metal implants with more effective methods to enhance the deposition of apatite as described above. Another possibility for shortening the coating process was by concentrating the SBF

solution. A 5 times more concentrated SBF (so-called SBF×5) was developed by decreasing pH with carbon dioxide gas. The subsequent release of carbon dioxide resulting in a pH increase and thus increasing supersaturation of SBF×5 solution. This process allowed the deposition of a uniform apatite coating within 24 h without refreshing the metastable solution. The coating was dense and composed of globules with amorphous carbonated apatite(Barrère et al., 2000a).

The formation and properties of biomimetic apatite coatings on titanium were strongly related to the ionic strength, carbonate and magnesium contents in the SBF×5 solution.

NaCl controlled the ionic strength of the solution, and thereby controlled CO_2 release, i.e. pH profile. Low ionic strength in SBF×5 solution led to the earlier precipitation in the solution resulting in later and thinner formation of apatite coating on Ti6Al4V, while high ionic strength delayed precipitation in the solution and favored apatite heterogeneous nucleation on Ti6Al4V(Barrère et al., 2002a).

HCO_3^- content increased the pH of the solution due to its buffering capacity and influenced the release rate of dissolved CO_2. Thus, HCO_3^- content strongly affected the supersaturation and apatite structure. Furthermore, HCO_3^- favored the attachment of apatite on Ti6Al4V by decreasing apatite crystal size resulting in a better physical attachment of apatite coating on Ti6Al4V substrate(Barrère et al., 2002a).

The formation and attachment of apatite coating was strongly related to Mg^{2+} content. Mg^{2+} inhibited precipitation in the solution and favored the formation of apatite coating due to its relatively high concentration at the coating/substrate interface. It had a stronger inhibitory effect on apatite crystal growth than HCO_3^-. Mg^{2+} content in SBF×5 solution also changed the phase composition and crystallinity of the coating(Barrère et al., 2002b).

A two-step method was developed to deposit CA or OCP coating on titanium implants. The implants were first soaked in a SBF×5 solution for 24 h to seed the metal surface with calcium phosphate nuclei. Then, the implants were soaked for another 24 h in a SBFB×5 solution to induce the fast growth of CA coating. The CA coating consisted of well-formed crystals 1–3μm in size uniformly precipitated on the surface of implant. The thickness of coating was approximately 30μm, the crystallinity was around 75% and Ca/P ratio was 1.67. A homogeneous CA coatings in thickness was deposited on the entire surface of porous implants(Barrère et al., 2003c; Habibovic et al., 2002).

By changing the SBFB×5 into SCS 1 and prolonging the immersion time to 48h, OCP coating with a 55 μm in thickness homogeneously was deposited on the surface of the metal. Large crystals 30-60 μm in size perpendicularly grew onto the metal surface. The crystallinity of the coating was around 100% and its Ca/P ratio 1.33(Barrère et al., 2001). However, the thickness of the OCP coating was not the same throughout the porous implant. It was thicker at the exterior of the porous implant than that of the interior(Barrère et al., 2003a; Habibovic et al., 2005).

3.3 Effects of pH of simulated body fluid on biomimetic apatite coatings

Barrère et al. systematically investigated the interdependence of ionic strength, pH, carbonate concentration in SBF×5 solution, and their influences on the formation of the resultant apatite(Barrère et al., 2002a). Later, the influence of Mg^{2+} on pH was investigated(Barrère et al., 2002b). However, the pH in these studies was indirectly controlled by ionic strength, carbonate concentration or Mg^{2+} of SBF×5 solution. Li et al reported that Ca/P molar ratios and chemical compositions of the calcium phosphate

precipitates were affected by the pH of the SBF, and an apatite with Ca/P molar ratio close to the HA was obtained if the pH of the solution was continuously adjusted to 7.26 during calcium phosphate precipitated from SBF(J.G. Li, et al., 1997). Chou et al used two-step immersion process to investigate the influence of solution pH of initial SBF×5 solution on micro-structural evolution and final apatite structure. They first immersed the argon plasma etched polystyrene culture dishes into SBF×5 solution with different pH (5.8 or 6.5), then immersed into the identical Mg^{2+} and HCO_3^--free SBF×5 (pH=6.0). The results showed that the pH of the initial SBF×5 solution influenced the final structure of the crystalline apatite. Precursor spheres formed with high initial pH of SBF×5 (pH 6.5) transformed into larger, single crystals plates, while precursor spheres formed with low initial pH of SBF×5 (pH 5.8) developed minute, polycrystalline plate-like structures over predominantly spherical precursor substrate(Chou et al., 2004).

3.4 Effects of flowing state of simulated body fluid on biomimetic apatite coatings

The immersion process in simulated body fluid required daily refreshment of SBF to maintain ions concentration and a constant pH for apatite growth. However, most of soaking processes were maintained under static conditions without fluid flow, which might lead to local precipitation or uneven coatings (Habibovic et al, 2004a). Body fluid of human was a dynamic circulating system, so the flowing SBF was better in mimicking the living body fluid than static one. Habibovic et al soaked the implants into more concentrated body fluids with stirring at a speed of 250 rounds per minute to make the SBF homogeneous(Habibovic et al., 2002). Siriphannon et al investigated the formation of HA in simulated body fluid under static and flowing systems, and found that compared with static systems, the formation of HA layer under flowing SBF differed in the formation rates, formation behavior, and microstructure(Siriphannon et al., 2002). Papadopoulou et al investigated the surface changes of dental ceramics coated with bioactive glass after exposure in a simulated body fluid under static and dynamic conditions. The CA layer formed on the surface of material in static environment was more dense and compact than that formed under dynamic conditions(Papadopoulou et al., 2003). Deng et al investigated the influence of dynamic flow rate on bone-like apatite formation in porous calcium phosphate ceramic in revised simulated body fluid (r-SBF). They reported that the crystal shape of bone-like apatite changed with the flow rate(Deng et al., 2005).

3.5 Effects of trace elements in simulated body fluid on biomimetic apatite coatings

Bone mineral contains calcium, phosphate,carbonate and other inorganic compounds such as sodium, fluoride, chloride, magnesium, strontium, zinc, copper and iron in varying quantities. These elements can affect bone mineral characteristics, such as crystallinity, degradation behavior and mechanical properties(Becker et al., 1968; L.Yang et al., 2010). Many researchers studied the possibility to incorporate different ions into the apatite by a biomimetic method.

Oliveira et al incorporated different amounts of Sr into nano-apatite coatings by adding $SrCl_2$ in SBF solution with higher concentrations of Ca^{2+} and HPO_4^{2-} than that of human blood plasma. The presence of Sr ions in solution inhibited the apatite formation and resulted in the decrease of coating thickness, and it incorporated in the apatite layer by replacing Ca in the apatite lattice(Oliveira et al., 2007). Bracci et al employed a modified calcium phosphate calcifying solution by replacing part of Ca^{2+} ions with Sr^{2+} and Mn^{2+} ions

to investigate the influences of Sr^{2+} and Mn^{2+} ions on the chemical, structural and morphology of coatings deposited on metallic substrates. A Sr-containing hydroxyapatite was deposited on metallic substrates in a few hours, but the presence of Sr^{2+} inhibited apatite precipitation, reduced the dimensions of the spherical aggregates and decreased the degree of crystallinity of apatite. Mn^{2+} ions completely hindered the precipitation of apatite and yielded an amorphous phosphate relatively rich in Mn(Bracci et al., 2009). In addition, cobalt, copper, zinc, strontium and fluoride with varying concentrations were incorporated in the calcium phosphate films by using a biomimetic approach consisting of precalcification and calcification steps. The additives affected morphology and composition of calcium phosphate films to different extent by a dose dependent manner(Patntirapong et al., 2009; L.Yang et al., 2010).

3.6 Effects of protein addition in simulated body fluid on biomimetic apatite coatings

With the plasma-spraying technique, it is impossible for biologically active molecules such as osteogenetic agents and growth factors to incorporate the HA coating during spray process due to the extremely high temperatures(>10,000°C). However, the development of biomimetic coating techniques make it possible, which involves soaking the implants in supersaturated calcium phosphate solutions at physiological temperatures(Abe et al., 1990). By this technique, biologically active molecules can be coprecipitated with apatite crystals onto metal implants rather than being merely adsorbed on its surface(Y. Liu et al., 2001, 2003, 2006; Wen et al., 1999). Therefore, the biomimetic apatite coating can be used as the carriers for biologically active molecules. After implantation in vivo, the degradation of these biomimetic coatings would lead to the gradual exposure and release of incorporated molecules from these coatings rather than in a single rapid burst, which also renders the biomimetic coatings of great potential value as a drug-carrier system in orthopedics(Y. Liu et al., 2006).

Liu et al first biomimetically precoated titanium alloy implants with a thin and dense layer of calcium phosphate, and then incubated in a supersaturated solution of calcium phosphate with bovine serum albumin (BSA) at various concentrations under physiological conditions for 48 h. BSA successfully coprecipitated with the Ca^{2+} and PO_4^{3-} ions on the surface of Ti alloy implants, and the release of this protein from the biomimetic coatings took place gradually over the span of several days. BSA influenced the properties of biomimetic coating in a concentration-dependent manner. With the increase of BSA concentration, the coatings became denser and thinner, and showed smaller crystal size and lower crystallinity. Crystal geometry changed more curve, and crystal structure of the coating transformed from an OCP to a CA(Y. Liu et al., 2001). Wen et al pretreated titanium by acid etching, boiling diluted alkali incubation, precalcification, and immersed it in SCS with or without containing BSA. Their results also showed that the incorporation of BSA significantly modified the morphology, composition, and crystallinity of the apatite coating(Wen et al., 1999). The release rate of Ca^{2+} ions from these BSA-containing apatite layers was slower than from non-protein-containing ones within the bathing medium, which indicated that BSA bonded strongly to Ca^{2+} ions within the crystal lattice. BSA incorporated into the crystal lattice enhanced the mechanical strength of coating in a concentration-dependent manner within the bathing medium (Y. Liu et al., 2003).

Fibronectin is known to promote cell adhesion, which plays a special role in the process of osteointegration due to its ability to attach osteoblasts to the extracellular matrix components, in spite of its low concentration (~0.2 mg/ml) in biological fluids(Tamada &

Ikada, 1993). Furthermore, it contains calcium-sensitive heparin binding sites, which should interfere with the apatite deposition. When fibronectin was dissolved in HBSS, the influence of fibronectin on apatite deposition was found to be concentration-dependent. Low concentration of fibronectin (0.01 mg/ml) did not significantly affect apatite precipitation, but when increased to 0.05 mg/ml, it strongly inhibited the apatite nucleation(do Serro et al., 2000).

In a study by Liu et al. Recombinant Human Bone Morphogenetic Protein -2(rh-BMP-2) was incorporated in a dose-dependent manner into biomimetic apatite coatings on titanium implants. The incorporated BMP-2 underwent gradual release (over a period of weeks) into the surrounding tissue wherein it retained its biological activity. Its biological performance will be described later(Y. Liu et al., 2006). Uchida et al immobilized laminin on titanium by immersion the AH -treated titanium in a calcium phosphate solution containing laminin at 25°C for 1 day(Uchida et al., 2004).

3.7 Effects of drugs in simulated body fluid on biomimetic apatite coatings

Bone infections still represent a challenging problem for orthopaedic implant surgery, which result from the poor access to the bone-infected site by systemically administered antibiotics. Local therapy is therefore desired and can be achieved by using a suitable carrier for a controlled drug delivery(Radin et al., 1997). Polymethylmethacrylate (PMMA) cement is a standard antibiotic carrier used in clinic, and PMMA beads loaded with antibiotics is frequently used for the treatment of infections(Buchholz et al., 1984; Garvin et al., 1988; Henry et al., 1991; Josefsson et al., 1990). However, the need for a second operation to remove the non-absorbable PMMA beads and the possibility of thermal damage to the antibiotics caused by the exothermic polymerization reaction of the cement is still a problem(Radin et al., 1997). Furthermore, the non-absorbable drug carrier like PMMA, and the biodegradable drug carriers, such as poly(lactide/glycolide) copolymer and poly(propylene glycol-fumerate)/methyl methacrylate composite are non-bioactive(Gerhart et al., 1993; Garvin & Feschuk, 2005; Henry et al., 1991). Therefore, combining osteoconductive properties of bioactive materials with a local and sustained release of antibiotic can be used to not only enhance early osteointegration of implants, but also prevent post-surgical infections. However, like proteins and biologically active agents, the antibiotic can not be incorporated during the preparation of plasma-sprayed HA coating or ceramics due to the extremely high processing temperature. The adsorption of these antibiotic drugs on the surface of these bioactive materials limits their loading and release characteristics.

Loading antibiotic by using a biomimetic method attracted much attention. Campbell and coworkers incorporated an antibiotic chlorhexidine into apatite coatings by using a surface induced mineralization approach. After treating with silane-coupling molecules and sulfonation, the substrates were immersed into various chlorhexidine solutions between mineralization cycles. The release test showed an initial rapid antibiotic release followed by a period of slower sustained release. The apatite containing chlorhexidine showed good anti-microbial efficacy(Campbell et al., 2000).

In a study by Stigter et al., the metal implants were first immersed into a SBF×5 at 37°C for 24 h to obtain a thin ACP coating for inducing the subsequent precipitation. The ACP-coated implants were then immersed in a supersaturated calcium phosphate (SCP) solution containing various concentrations of tobramycin at 37°C for 48 h, and different quantities of tobramycin was co-precipitated with a CA coating on the surface of

implants. With the increase of the amount of incorporated tobramycin, the thickness of coating decreased, but it did not change the morphology of the coating. The dissolution of coating showed a fast initial dissolution of the coating followed by a plateau at both pH 7.3 and at pH 5, initial dissolution rate and at total release of calcium at pH 7.3 were slower and lower than that at pH 5. The release rate of tobramycin was gradual and faster at pH 7.3 than at pH 5. Tobramycin released from the biomimetic apatite coating could inhibit growth of Staphylococcus aureus bacteria in vitro(Stigter et al., 2002). Later, different antibiotics including acidic antibiotics with almost similar chemical structure such as cephalothin, cefamandol, amoxicillin and carbenicillin and basic antibiotics such as vancomycin, gentamicin and tobramycin were incorporated into the CA coatings, and their release and efficacy against bacteria growth were investigated in vitro. With the increase of concentrations of antibiotics in SCP solution, more antibiotic incorporated into the CA coating. The incorporation efficiency of antibiotic was strongly related to their chemical structure. Antibiotics containing carboxylic groups were better incorporated than that lacking these groups, but slower released from the CA coating, which probably resulted from the binding or chelating between carboxylic groups in their chemical structure and calcium. All antibiotics that were released from the CA coating showed inhibition of growth of Staphylococcus aureus bacteria(Stigter et al., 2004). In another study, antibiotics cephradine containing carboxylic groups in simulated body fluid was also found to be beneficial for the apatite coprecipitation. However, the coprecipitation did not take place between apatite and a traditional Chinese medicine salviae miltlorrhizae (SM). The authors speculated that Chinese medicine SM was probably more absorbed on the surface of the Ti, when calcium and phosphate ions precipitated(Z. Wu et al., 2008).

4. Biological performance of biomimetic apatie coatings

The purpose of pretreatments and the biomimetic apatite coating process was to obtain satisfactory biological performance. The biomimetic apatite coating formed in vitro and in vivo determined its biological performance.

4.1 Effects of biomimetic apatite coatings on in vitro behavior of osteoblasts and osteoclasts

Leeuwenburgh et al investigated the resorption behavior of three different biomimetic calcium phosphate coatings (ACP, CA and OCP) by using osteoclast-enriched mouse bone-marrow cell cultures for 7 days. No release of particles and morphologic changes could be observed for all biomimetic coatings after preincubation for 7 days in α-minimal essential medium(α-MEM). However, both CA and OCP coatings degraded in the presence of cells. Osteoclasts degraded the CA coatings by normal osteoclastic resorption, but the resorption pattern of the OCP coatings differed from that of CA coatings. It seemed that ACP coating was too thin to detect resorption lacunae, if there were any. The nature of the apatite coatings such as crystal size and chemical composition influenced the cell-mediated degradation(Leeuwenburgh et al., 2001).

The biomimetic apatite on the surface of AH-treated titanium through immersion in SBF could promote differentiation of bone marrow stromal cells along osteogenic lineage(Nishio et al., 2000). Jalota et al showed that, compared with the neat and NaOH-treated titanium foams, biomimetically apatite coating on the surface of titanium foams formed in 1.5×Tas-

SBF exhibited the highest protein production and rat osteoblasts attachment (Jalota et al., 2007).

Trace elements in the biomimetic coating also influenced the cell behavior. Mg-containing apatite, Sr-containing apatite and an amorphous phosphate relatively rich in Mn coating promoted human osteoblast-like MG-63 cells differentiation and mineralization due to the presence of the ions, and the differentiation and mineralization followed the order: Mg^{2+} < Sr^{2+}<Mn^{2+}. Mg^{2+} and Sr^{2+} apatite coatings promoted proliferation and expression of collagen type I while the relatively high content of Mn^{2+} in the phosphate had a significant beneficial effect on osteocalcin production(Bracci et al., 2009).

Yang et al investigated the effects of inorganic additives (copper, zinc, strontium, fluoride and carbonate) to calcium phosphate coating on in vitro behavior of osteoblasts and osteoclasts by a medium-throughput system based on deposition of calcium phosphate films in multi-well tissue culture plates. The proliferation and differentiation of MC3T3-E1 osteoblasts on these films depended on the inorganic additives and concentration tested. In general, copper and zinc ions inhibited osteoblast proliferation, but had no effect or mild inhibitory on osteoblast differentiation. The effect of strontium on osteoblast proliferation was concentration-dependent, whereas both films containing fluoride and carbonate augmented osteoblast proliferation. Compared with the control films without additives, strontium, fluoride and carbonate ions clearly decreased osteoblast differentiation. The resorptive activity of primary rabbit osteoclasts cultured on calcium phosphate films containing additives significantly decreased and it was concentration-dependent as compared to the control, independent of the element incorporated. The elements in the tested concentrations showed no cytotoxic effect(L.Yang et al., 2010). In another study by Patntirapong et al, calcium phosphate film with Co^{2+} incorporation increased both osteoclast differentiation and resorptive function(Patntirapong et al., 2009).

4.2 Bone tissue engineering on apatite-coated titanium discs

Bone tissue engineering has already been proven to be feasible in porous scaffold by many research groups, and the in vitro bone tissue engineering constructs can provide implants with better fixation(Burg et al., 2000; Hutmacher, 2000; Rezwan et al., 2006; Rose & Oreffo, 2002). Dekker et al first showed that tissue engineering technology was effective on flat surfaces. They seeded both primary and subcultured rat bone marrow cells on biomimetic amorphous calcium phosphate-coated titanium plates and cultured in the presence or absence of dexamethasone for 7 days, then subcutaneously implanted in nude mice for 4 weeks. De novo bone formation was detected on the calcium phosphate-coated plates with primary or subcultured cells, which had been continuously cultured in medium with dexamethasone(Dekker et al., 1998).

In another study by Dekker et al, subcultured rat bone marrow cells were seeded on the amorphous CA and crystalline OCP-coated discs for their use in bone tissue engineering. After 1 week of culture, the cells covered the entire surface of all substrates with a continuous multi-layer. The crystalline OCP-coated discs were higher in the amount of cells while the amorphous CA-coated discs exhibited a visually higher in the amount of mineralized extracellular matrix. After subcutaneously implanted in nude mice for 4 week, clear de novo bone formation was observed on all discs with cultured cells. Compared to the amorphous CA-coated discs, the newly formed bone on the crystalline OCP-coated discs was more organized and showed a significantly higher volume and the percentage of bone contact(Dekker et al., 2005).

4.3 Effects of biomimetic apatite coatings on osteoinduction of implants

Yuan et al. reported that OCP-coated porous tantalum implants induced bone formation after implantation in the dorsal muscles of adult dogs for 3 months, while the uncoated one did not(Yuan, 2001).

In the goat study by Barrère et al. porous Ta and dense Ti alloy (The alloy had a dense surface, but it had a center hole with a diameter of 2.5 mm, with one side open and the other side closed) with OCP coating were implanted in the dorsal muscles of goats at 12 and 24 weeks. Both OCP-coated implants induced ectopic bone formation, and the newly formed bone was observed either in the inner pores of porous Ta or in the inner cavity of the dense Ti alloy, but not on flat surface of dense Ti alloy. The formed bone was in direct contact with the implants without the intervention of fibrous tissue. On the other hand, uncoated implants did not show any ectopic bone formation. This study indicated that both the presence of a Ca-P coating and the architecture of the implant were important factors for inducing ectopic bone formation(Barrère et al., 2003a). A similar study by Habibovic et al. showed that OCP-coated porous Ti alloy implants could also induce ectopic bone formation after implanted intramuscularly for 6 and 12 weeks in goats(Habibovic et al., 2005).

Another goat study by Habibovic et al. investigated the influence of OCP coating on osteoinductive performance of different porous materials. Their results showed that the OCP coating could improve the osteoinductive potential of different kinds of orthopedic implants(Habibovic et al., 2004b).

In a study by Liu et al. rh-BMP-2 was incorporated into OCP coating on Ti alloy implants, and subsequently implanted in a rat model to investigate protein release and osteoinduction. The incorporated BMP-2 which retained its biological activity was gradually released from the coating and induced the formation of bone tissue not only upon the implant surface but also within its immediate surroundings(Y. Liu et al., 2006).

Apart from coating implants with apatite in vitro, the bioactive implants which could induce bone-like apatite in vivo also had the ability to induce ectopic bone formation. Fujibayashi et al first reported that the non-soluble plasma-sprayed porous titanium metal that contained no calcium or phosphorus could induce ectopic bone formation when treated by water-AH treatments to form an appropriate microstructure(Fujibayashi et al., 2004). The water-AH treated porous titanium showed an in vitro apatite-forming ability after soaked in the SBF within a 7-day period(Fujibayashi et al., 2004). Though the in vitro apatite-forming ability of the samples could not reflect completely its in vivo behavior, it was widely believed that bone-like apatite layer formation on the pore surface in the early stages was a key factor for bone induction by non-CaP biomaterials and CaP-based porous ceramics(Habibovic & de Groot, 2007; X.D. Zhang et al., 2000). Takemoto et al. had partially confirmed the existence of bone-like apatite on the porous bioactive titanium by SEM-EDX, which were implanted in the dorsal muscles of beagle dogs(Takemoto et al., 2006). Later, our group found that porous titanium with a series of surface treatments, such as AA treatment(Zhao et al. 2010b), H_2O_2 treatment and $H_2O_2/TaCl_5$ treatment(unpublished data), could induce ectopic formation after implantation in the dorsal muscles of dogs for 3 or 5 months. Porous titanium with those treatments all showed in vitro apatite-forming ability after immersion in SBF for only one day(Zhao et al. 2010b).

Although the exact mechanism of osteoinduction by biomaterials was still not well understood, some previous studies reported that osteoinductive biomaterials showed better performance than non-osteoinductive one at orthotopic sites(Habibovic & de Groot, 2007; Habibovic et al., 2005, 2006). Therefore, the osteoinductive porous metals with good

biomechanical compatibility were attractive in clinical application under load-bearing conditions.

4.4 Effects of biomimetic apatite coatings on osteointegration or osteogenecity of implants

In a study by Barrère et al, uncoated and bone-like carbonated apatite (BCA)-coated dense titanium alloy (Ti6Al4V) and porous Ta cylinders were implanted in the femoral diaphysis of adult female goats in a press-fit manner for 6, 12, and 24 weeks. Bone contact was always found significantly higher for BCA-coated dense Ti6Al4V and porous Ta cylinders than the corresponding uncoated one, which indicated that BCA coating enhanced the bone integration as compared to the uncoated implants and was highly beneficial for the long-term fixation of metal prostheses in load-bearing applications(Barrère et al., 2003c).

In another study, Barrère et al compared the osteogenic potentials of BCA-coated, OCP-coated, and bare porous tantalum cylinders in a gap of 1 mm created in the femoral condyle of a goat at 12 weeks. After 12 weeks, bone did not fill the gap in any of the porous implants, but OCP-coated porous cylinders exhibited bone formation in the center of the implant compared to the two other groups. This study suggested that the nature of the Ca-P coating, via its microstructure, dissolution rate, and specific interactions with body fluid, might influence the osteogenecity of the Ca-P biomaterial(Barrère et al., 2003a). Similar to the previously described study, Habibovic et al. found that the application of OCP coating on porous Ti6Al4V implants could improve its performance in bone healing process in femoral defects of goats(Habibovic et al., 2005). In a study, AA- or AH-pretreated porous titanium with biomimetic apatite coatings were hemi-transcortically implanted into the femurs of dogs for 2 months, and they showed excellent osteointegration with host bone(Zhao et al., 2010a).

Yan et al investigated the effects of AH treatment, and bone-like apatite-formed on titanium after such treatment on the bone-bonding ability of Ti implants by implanted into the tibial metaphyses of mature rabbits. Both treated implants exhibited significantly higher failure loads compared with untreated Ti implants at all time periods and directly bonded to bone tissue during the early post-implantation period. Scanning electron microscopy-energy dispersive X-ray microanalysis (SEM-EMPA) showed a uniform calcium- and phosphorus-rich layer was detected at the interface between the treated implants and bone, which indicated that Ti implants with AH treatment could induce bone-like apatite deposition in vivo, and therefore accelerated the bone-bonding behavior of implants and enhanced the strength of bone-implant bonding(Yan et al., 1997a, 1997b). Titanium alloys with AH treatment showed a similar enhancement of the bonding strength(Nishiguchi et al., 1999a). However, heat treatment after alkali treatment was an essential step for good bone-bonding ability. The unstable reactive surface layer of alkali-treated titanium would result in no bone-bonding ability(Nishiguchi et al., 1999b). AH-treated titanium cylindrical mesh cage was successfully used to repair a segmental rabbit femur defect, and it enhanced the bone repairing process and achieved faster repair of long bone segmental defects(Fujibayashi et al., 2003). It could also provide porous titanium coating implants with earlier stable fixation(Nishiguchi et al., 2001).

Water-AH-treated Ti could achieve earlier fixation than AH-treated one because of the formation of anatase, but sodium removal decreased the bonding strength between the implants and bones due to the loss of the surface graded structure of the bioactive layer(Fujibayashi et al., 2001). On the other hand, Water-AH-treated porous titanium

enhanced bone ingrowth and apposition(Takemoto et al., 2005b). In addition, AH-treated tantalum implants also could bond to bone(Kato et al., 2000).
Hydrogen peroxide solution containing tantalum chloride ($H_2O_2/TaCl_5$) treatment was also used to provide titanium with the apatite-forming ability in SBF(Ohtsuki et al., 1997). $H_2O_2/TaCl_5$ -treated titanium implants showed higher bonding strength with living bone than untreated one after implantation in rabbit tibia, which was attributed to high potential of osteoconductive properties and/or direct bonding to living bone(Kaneko et al., 2001). It was reported that bonding phenomena between implants and living bone was initiated by the formation of a bone-like apatite layer on the surface of implants(Neo et al., 1993). Titanium fiber mesh treated by the same method enhanced bone growth and achieved faster tight bonding with bone than untreated titanium fiber mesh(T. Kim et al., 2003).

4.5 In vitro and in vivo degradation of biomimetic apatite coating

When biomimetic apatite-coated metal was implanted in vivo, they reacted dynamically towards the surrounding body fluids and showed a series of different biological behavior such as enhancing bone integration, inducing ectopic bone formation and combining with cultured bone marrow cells to inducing bone formation, which was closely related to the degradation behavior of the coating (Barrère et al., 2003a, 2003c; Dekker et al., 1998, 2005; Habibovic et al., 2005).
In a simulated physiological solution CA and OCP coatings showed different dissolution rates. CA dissolved faster than OCP at pH = 7.3 while CA dissolved slower than OCP at pH = 5.0(Barrère et al., 2000b). When the coated plates were soaked in α-MEM for 1, 2, and 4 weeks and were implanted subcutaneously in Wistar rats for similar periods. A carbonate apatite formed onto CA and OCP coatings via a dissolution-precipitation process both in vitro and in vivo, and organic compounds incorporated the carbonate apatite coating in vivo. However, both coatings dissolved overtime in vitro, whereas in vivo CA calcified and OCP partially dissolved after 1 week. Specific incorporations of organic compounds, different surface microstructure, different thermodynamic stability, or a combination of all these factors could contribute to the different degradation behavior of OCP and CA coatings(Barrère et al., 2003b).
In the study of femoral diaphysis of goats by Barrère et al, CA coating completely dissolved in the medullar cavity after 6 weeks of implantation. On the other hand, the coating thickness decreased with time and it was still present even after 24 weeks of implantation in the cortical region. The coating only remained on the implants when it was integrated in the newly formed bone. The in vivo degradation of CA coating was related to mechanical forces, dissolution, cellular activity, or combinations of those effects(Barrère et al., 2003c).
Intramuscular implantation of OCP-coated Ti6Al4V cylinders and porous tantalum cylinders in the goat showed that, after 12 and 24 weeks, the OCP coating had dissolved extensively and remained in only some places after 12 weeks of implantation. The remaining OCP coating on porous tantalum cylinders was detected as an integrated layer in the newly formed bone. After 12 weeks of gap-healing implantation in the femoral condyle of goat, the CA coating on porous tantalum cylinders had almost completely disappeared while the OCP coating partially remained after 12 weeks of implantation. In a bony environment, physic-chemistry of the Ca-P coating determined the osteoclastic activity. The osteoclastic activity of CA coating was supposed to by higher in vivo than that of OCP coatings(Barrère et al., 2003a). In a in vitro study by Leeuwenburgh et al. CA coatings were

resorbed by osteoclasts in a normal osteoclastic resorption manner while OCP coatings were degraded not by classical pit formation(Leeuwenburgh et al., 2001).
In another study by Habibovic et al, OCP-coated porous Ti6Al4V implants was implanted in the back muscle and femur of goats for 6 and 12 weeks. The in vivo dissolution behavior of the OCP coating was similar to that on porous tantalum cylinders. After 6 weeks of intramuscular implantation, the OCP coating had extensively dissolved. In the remaining OCP coating areas, signs of its resorption by multinucleated cells could be observed. After 12 weeks of implantation, the coating was further degraded and could only occasionally be detected. The remaining OCP coating was often observed to incorporate into the newly formed bone(Habibovic et al., 2005).

5. Conclusions

Biomimetic coating process allows the deposition of an apatite layer on the complex-shaped implant or within the porous implant at low temperature. The thus-treated implants show excellent bioactivity and can bond to living bone directly. The properties of the biomimetic coatings can be adjusted by controlling the process parameters to meet specific clinic needs. The biomimetic apatite coating also can be used as a carrier of biologically active molecules, such as osteogenetic agents and growth factors, or drugs. Furthermore, it is simple and cost-effective. It offers the most promising alternative to plasma spraying and other coating methods. However, the biomimetic apatite coatings are still unsatisfactory and remain under investigation. The lower bond strengths between biomimetic-deposited apatite coating and its underlying substrate have limited their applications for clinical use. The in vivo cirumstances are far more complex than that of in vitro biomimetic process. Therefore, the mechanism of biomineralization is needed to be further investigated and combine the biomimetic process to develop implants with better performance. On the other hand, the pretreatments on metals that can induce bone-like apatite deposition in vivo provide another promising process for better biological performance. The pretreatments that can induce faster bone-like apatite deposition in vivo and earlier fixation with bone tissue are needed to be developed.

6. References

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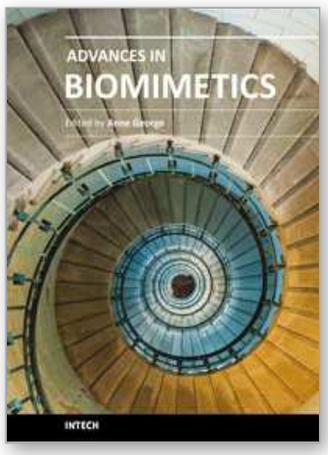
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Phone: +86-21-62489820
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