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Hydroxyapatite-Based Biomaterials for Hard Tissue Applications

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Abstract: Over the past few decades, much effort has been made to improve the mechanical and biological performance of HA, in order to extend its range of applications. As a major inorganic component of human hard tissues, hydroxyapatite bioceramic is regarded as being one of the most biocompatible materials. Numerous in vitro and in vivo studies have confirmed its excellent bioactivity, osteoconductivity and bone forming ability. However, because of its poor mechanical properties, its use in hard tissue applications has been restricted to those areas in which it can be used in the form of small sized powders/granules or in the non-load bearing sites. A number of researchers have focused on improving the mechanical and biological performance of HA, as well as on the formulation of hybrid and composite systems in order to extend its range of applications. In this article, we reviewed our recent works on HA-based biomaterials; i) the strengthening of HA with ceramic oxides, ii) HA-based bioactive coatings on metallic implants, iii) HA-based porous scaffolds and iv) HA-polymer hybrids/composites.

Key words: Hydroxyapatite; Bioceramics; Biomaterials; Medical Implants; Hard Tissue Applications

INTRODUCTION

The fields of biomaterials and tissue engineering have increased significantly over the past few decades, because of the inaccessibility of autografts, as well as the immune response and disease problems associated with allografts. A number of synthetic biomaterials and tissue engineering matrices, such as bioactive ceramics and degradable polymers, which were created to heal the damaged sites and subsequently regenerate new tissues, have proven to be clinically applicable [1-4].

In hard tissue reconstructive surgery, bioactive ceramics such as calcium phosphates (hydroxyapatite and tricalcium phosphate) and bioactive glasses/glass ceramics have shown excellent biocompatibility in vitro and in vivo.

As one of the calcium phosphate compounds found in nature, hydroxyapatite (HA; $Ca_{10}(PO_4)_6(OH)_2$) constitutes the major inorganic component of the hard tissues of vertebrates [2-4]. Therefore, HA is considered to be the most essential bioceramic currently used to replace and regenerate damaged and diseased teeth and bones. HA bioceramic has been proven to bond directly to the bone surface without the formation of fibrous tissues and to possess excellent bioactivity and osteoconductivity. However, because of its poor mechanical properties, such as its low strength and toughness and high elastic modulus, the area of application of HA bioceramic has been restricted, particularly as regards its use in load-bearing sites [2-4].

Over the past few decades, much effort has been made to improve the mechanical and biological performance of HA, in order to extend its range of applications. The present authors have worked on the development of HA-based biomaterials with excellent mechanical and biological properties in various systems. This article reviews most of our recent works on these HA-based biomaterials, in the form of powders/bulk ceramics, coatings on metallic implants, porous bone scaffolds, and biomedical composites with degradable polymers.

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BIOACTIVE AND STRONG CERAMIC COMPOSITES

Because of its poor mechanical properties, such as its low strength and fracture toughness, the application of HA ceramic is limited to non-load bearing sites or areas in which it can be used in the form of powders / granules. A number of reinforcing agents, such as metals and ceramics, have been introduced to improve the mechanical properties of HA. However, it has proven difficult to produce dense composites of HA with these reinforcing agents, due to their reaction with HA, which results in phase degradation and a reduction in the mechanical properties [5-7].

Our research group has focused on the production of HA dense composites with ceramic oxides, particularly with zirconia (ZrO_2) and alumina (Al_2O_3) [8-15]. An emphasis was put on the prevention of the degradation reaction between HA and the reinforcing ceramics. In this section, we discuss the novel approaches to the production of HA-ceramic oxides and their mechanical and biological properties. One methodology is to coat zirconia powders with alumina prior to combining them with the HA matrix. Another one is to incorporate fluorine ions into the HA structure, thereby producing fluorine-substituted hydroxyapatite [FHA, $Ca_{10}(PO_4)_6(OH,F)_2$]. The HA-ceramic oxide composites obtained using these techniques were observed to exhibit improved mechanical properties and favorable biological performance [8-15].

In our first approach, ZrO_2 powders were coated with alumina colloidal particles by utilizing the electrostatic force between the two kinds of powders [8]. When HA was added to this Al_2O_3-coated ZrO_2 powder and sintered, the thermal degradation decreased significantly. As a result, the HA-ceramic oxide composites showed higher mechanical properties (a strength of ~300 MPa and toughness of ~3 $MPam^{1/2}$) when compared to pure HA (a strength of ~100 MPa and toughness of ~1 $MPam^{1/2}$). In vivo tests were carried out on the HA-ceramic oxides prepared in the form of a screw-shaped implant using a rabbit tibia model (Fig. 1(a)) [8]. The results showed that the removal torque at 6 weeks after surgery was significantly higher in the case of the HA-ceramic oxides (~ 50 Ncm) than in the case of the pure HA or Ti implant (~ 20 Ncm) (Fig. 1(b)) [8].

An attempt to further improve the mechanical properties of the composites was made by improving the properties of the reinforcing component, Al_2O_3-ZrO_2. Utilizing the Pechini-type sol-gel process, ultrastructured Al_2O_3-ZrO_2 nanocomposite powders were successfully produced [9]. The HA containing Al_2O_3-ZrO_2 nanocomposite had excellent mechanical properties (~3-4 times higher strength and toughness), while maintaining comparable osteoblastic functional activity to that of pure HA at appropriate Al_2O_3-ZrO_2 contents.

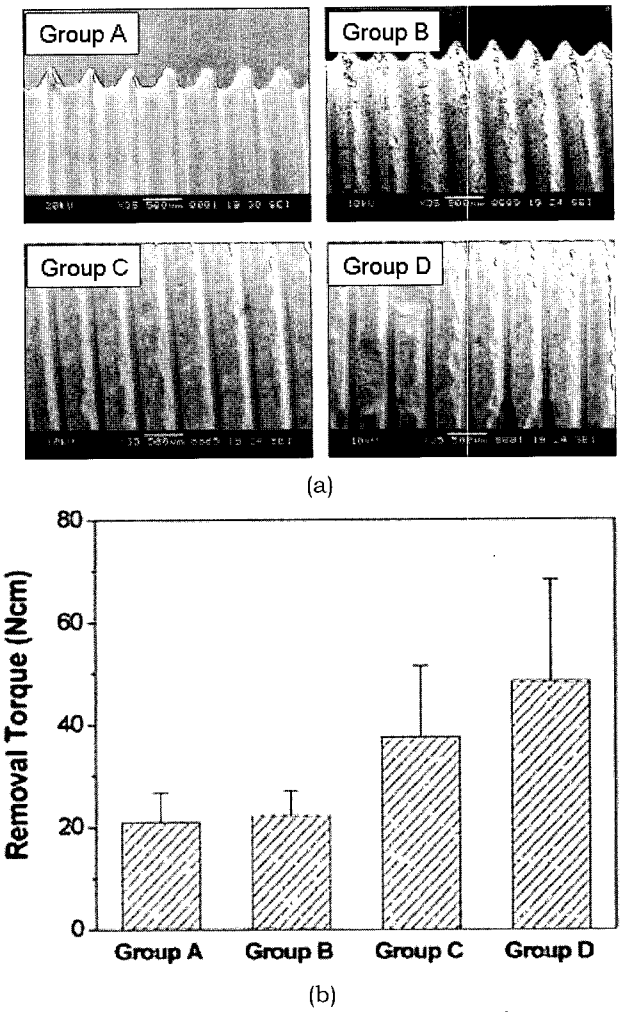

Fig.1. (A) SEM micrographs of the implants prior to surgery (x 35). Group A: cp Ti, Group B: pure HA, Group C: 70% HA + 10% ZrO2 coated with 20%Al2O3, and Group D: 55% HA + 15% ZrO2 coated with 30% Al2O3. (B) Results of the removal torque measured on the implants in a rabbit tibia after healing period of 6 weeks . Ref. [8].

Another methodology used to produce dense HA composites with ceramic oxides has recently been reported in a series of works [10-15]. The introduction of fluorine within the HA lattice and consequent formation of FHA solid solutions was found to be highly effective in suppressing the thermal degradation reaction and maintaining the initial tetra-ZrO_2 / Al_2O_3 and apatite phases. This was attributable to the high thermal stability of the FHA structure, since the substitution of F for OH enhances the crystal symmetry and, thus, the structural stability. The high structural and thermal stability of FHA with respect to ZrO_2 / Al_2O_3 facilitated the production of an almost completely dense composite (> 98 % density) under normal sintering conditions (Fig. 2(a)) [11]. Along with the processing aspects of producing a dense body, the

fluoride ion has specific biological functions in the prevention of dental caries and the enhancement of the crystallization and mineralization of apatite crystals in bone formation and, thus, the treatment of osteoporosis.

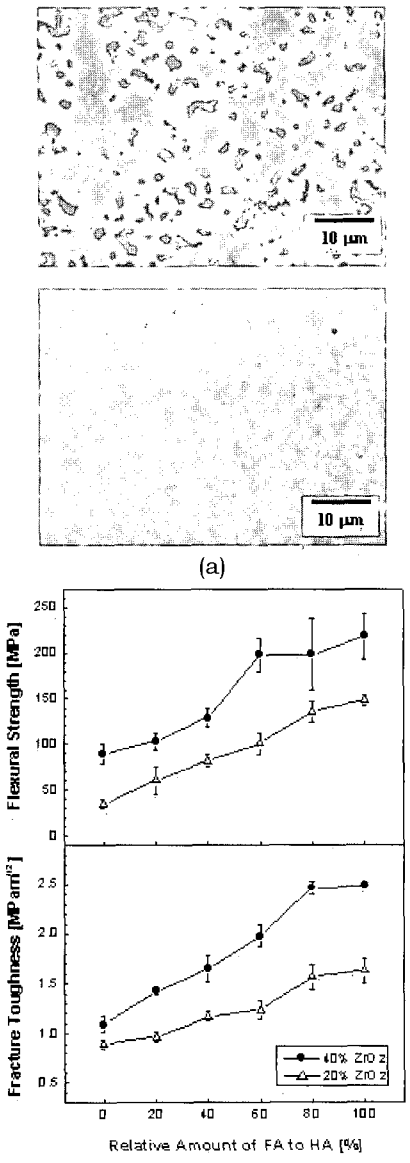

Fig.2. (A) SEM morphologies of the HA-ZrO2 (upper) and FHA-ZrO2 (lower) composite. (B) Strength and fracture toughness changes of the apatite-zirconia composites with fluoridation. Ref. [11].

In various studies, FHA-ZrO_2 / Al_2O_3 composites were shown to have improved mechanical properties, such as increased strength and fracture toughness, with respect to the pure apatites (HA and FHA) or to the HA-composites without fluoridation [Fig. 1], suggesting the effectiveness of the fluoridation of HA, as well as the reinforcing role of ZrO_2 and Al_2O_3 within the FHA composition (Fig. 2(b)) [11]. The biological properties of the FHA-ceramic oxides were investigated by evaluating their in vitro osteoblastic responses. The FHA composites showed similar cell proliferation behaviors to those of the pure apatites (HA or FHA). Moreover, the osteoblastic cells expressed bone-associated phenotypes, such as alkaline phosphatase, osteocalcin and collagen, similarly on the FHA-ceramic oxide composites and pure apatites.

Conclusively, considering both their mechanical performance and cellular properties, the newly developed apatite-ceramic oxide composites are thought to have high potential as load-bearing hard tissue implants.

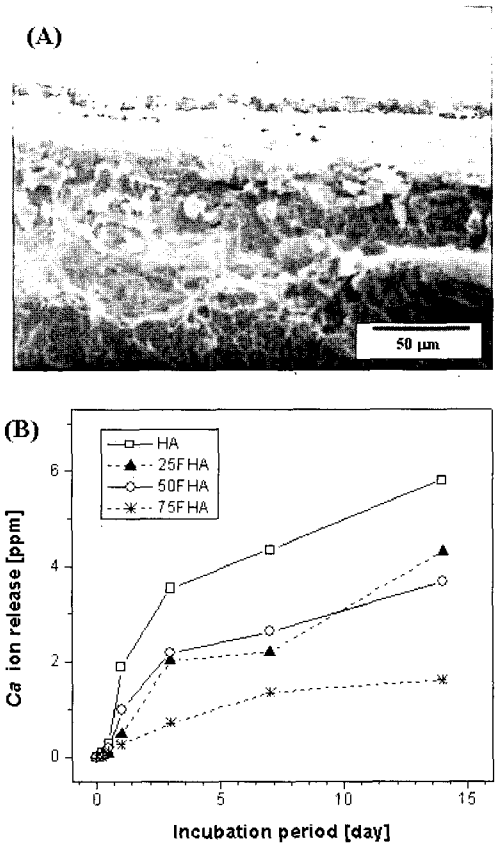

Fig.3. (A) Cross-section view of the HA sol-gel film on Ti. (B) Ca ionic release from the sol-gel derived apatite films with different level of fluoridation (25, 50 and 75%). Ref. [27].

BIOACTIVE COATINGS ON METALLIC LMPLANTS

As described above, although HA bioceramic possesses excellent biological properties, its potential

to be used for hard tissue implants has been limited by its poor mechanical properties. In this respect, HA has been utilized as a coating material when supported on strong substrates, such as metallic implants (Ti and its alloys). Over the past decade, a number of studies have been conducted on the production of HA coatings on Ti-based implants, and these have shown good fixation to the host bone and osseointegration with respect to pure Ti implants [16,17].

Along with HA coatings, the modification of Ti by forming a TiO_2 oxide layer is also considered as a promising methodology to improve the biocompatibility of the metallic implants. Among the methodologies employed to produce the oxide layer, the recently developed anodic oxidation method is an elegant technique, in the sense that it allows the pore morphology and composition to be controlled simultaneously.

In this section, we describe our recent studies on the HA and TiO_2 coating layers on Ti metal implants, and their biological and mechanical performance.

HA Thin Films

Traditionally, HA coatings were deposited by the plasma-spraying method. However, these plasma-sprayed HA coating layers are known to have poor crystallinity and an inhomogeneous structure. Moreover, some in vivo studies on their long term use in load-bearing sites have revealed the degradation of the coating layer and associated inflammatory problems. These were mainly attributed to the high processing temperature and the resultant thick coating layer (~ 50 -200 μm) [16,17].

Recent advances in coating technology have facilitated the production of HA thin films with thicknesses of less than a few micrometers. These HA thin films have been shown to possess high mechanical stability and structural integrity, as well as excellent cellular responses and bone formation [18-22].

In-depth studies on HA films deposited by the sol-gel approach have recently been conducted [21-28]. Thin HA films with thicknesses ranging from hundreds of nanometers to a few micrometers were successfully obtained on Ti after thermal treatment at ~500 °C (Fig. 3(a)) [27]. No delamination or cracks were observed within the film or at the interface. The adhesive strength of these HA thin films was approximately 40 MPa. Osteoblastic cells spread and grew favorably on these HA sol-gel films, and expressed a higher level of ALP activity as compared to that on pure Ti.

The surface morphology and crystallinity of the HA sol-gel films were tailored by adjusting the parameters of the thermal treatment processes, such as the heating rate and holding temperature [25]. The results showed that the tailored physicochemical properties of the films affected the cellular responses significantly: the higher crystallinity of the film improved the cell proliferation and phenotype expression, while the rougher morphology did not affect the cellular responses significantly [25].

Within the HA composition, the authors incorporated fluorine ions to produce fluoridated HA (FHA) films on Ti [26-29]. An FHA coating on a metallic substrate can be potentially useful in areas requiring long-term chemical and mechanical stability, since FHA is known to have a lower bio-resorption rate than HA. Moreover, the biological roles of fluorine can be useful in the case of hard tissue implant coatings, as explained in the above section. From the results, it was found that the fluoridation of the apatite film reduced its solubility (Fig. 3(b)) [27], and that the fluorine ions were released from the FHA films accordingly. A more elegant study of apatite films on Ti was made by designing layered films consisting of outer HA - inner FHA layers [28]. The results showed favorable cellular responses in terms of the cell attachment and phenotype expressions, and this was attributed to the functional roles played by the layered structure, that is, the cellular responses were influenced initially by the HA outer layer and subsequently stimulated by the fluorine ions released from the FHA inner layer over a prolonged period of time.

HA and FHA thin films have also been studied using the electron-beam evaporation technique [20,29]. Unlike the sol-gel approach, which is essentially a wet chemical method, e-beam evaporation is a kind of physical vapor deposition technique operating under conditions of high vacuum. Normally, in the case of e-beam evaporation, the as-deposited films are in the amorphous state, and after thermal treatment (~ over 500 °C), the film phase becomes crystallized. The film thickness is generally less than a few micrometers, and the morphology is dense. Compared to pure HA film, FHA films show lower dissolution rates, as in the case of sol-gel films. In particular, the partially fluoridated apatite films were found to have better adhesive strength than the pure apatite films (either HA or FA), due to the reduced thermal stress generated during the crystallization process. However, the cellular responses of the FHA films were not significantly different form those of the pure HA film. Studies on e-beam evaporated apatite films are now in progress, in order to improve the crystallinity of the apatite film at a lower crystallization temperature [29].

TiO_2 Anodic Oxidation

Recently, significant progress has been made in the field of Ti implants with the introduction of anodic oxidation of the Ti surface [30-32]. The anodic oxidation of Ti is currently considered as one of the most promising strategies in terms of its allowing the Ti surface to be modified both chemically and physically, i.e., enabling the production of a micro-porous and rough morphology, as well as a

biocompatible coating composition. The pore morphology and thickness of the oxide layer could be well controlled by adjusting the applied voltage (Fig. 4(a)) [30]. Moreover, significant levels of Ca and P could be introduced within the coating layer. An in vitro study showed the osteoblastic responses to the anodized Ti obtained at different processing conditions. The results demonstrated that the anodized Ti obtained at a higher applied voltage showed higher ALP activity but lower cell proliferation. This was attributed to the combined effects of both the increased roughness and increased amounts of Ca and P in the oxide layer with increasing voltage. An in vivo study of the anodized Ti using a rabbit tibia model showed that the Ti implants anodized under moderate conditions had significantly higher removal torque values (~ 3 times higher) than the machined Ti implants (Fig. 4(b)) More studies on anodized Ti have been conducted or are currently in progress, in order to generate a bioactive apatite layer on the anodized Ti surface, as described below.

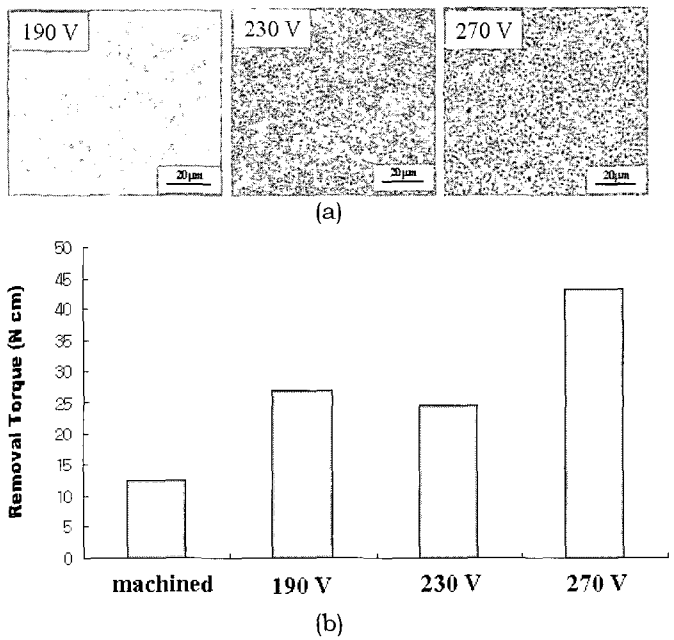

Fig. 4. (A) Surface morphologies of the anodized-Ti at different applying voltages. (B) Removal torque values measured on the machined and anodized-Ti implants in a rabbit tibia after healing period of 4 weeks. Ref. [30].

HA-TiO_2 Coatings

The authors observed that the HA layers directly deposited on a Ti substrate had limited bonding strength, due to the weak bonding capability between the HA ceramic and Ti metal [33]. Therefore, to improve the bonding ability of HA to Ti, a TiO_2 layer was inserted between HA and Ti (Fig. 5(a)) [33]. We observed that the insertion of the TiO_2 layer improved the adhesion strength of the HA film significantly, and this was considered to be due to the high chemical affinity of TiO_2 toward both HA and Ti (Fig. 5(b)) [33]. More importantly, the inserted TiO_2 layer enhanced the corrosion resistance of the Ti substrate. Moreover, the bioactive property of the HA layer was well preserved on the HA/TiO_2 layered films, as confirmed by the in vitro cellular responses.

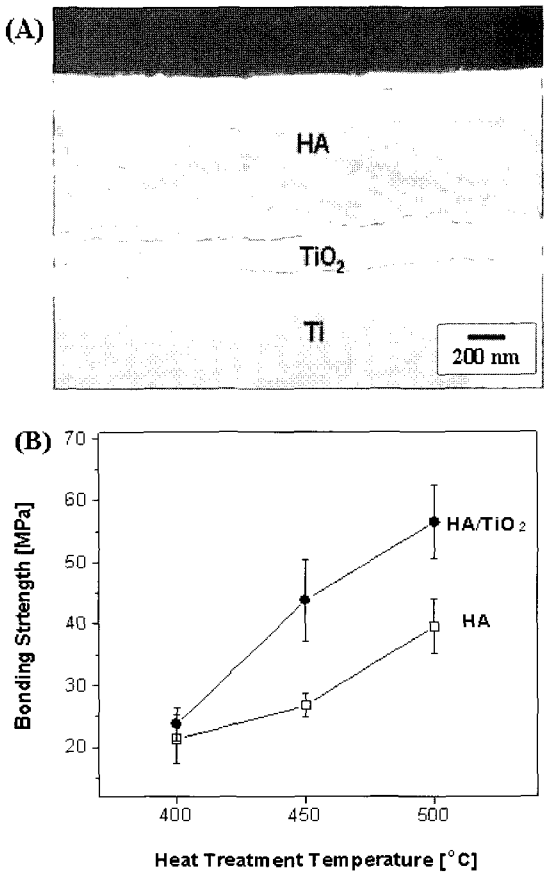

Fig. 5. (A) Cross section view of the HA/TiO2 layered sol-gel film on Ti. (B) Bonding strengths of the sol-gel films on Ti at different heat treatment temperature. Ref. [33].

A similar study was conducted on anodized-Ti, in order to improve its biocompatibility. We aimed to produce an HA bioactive layer on the anodized-Ti without altering the morphological features of the anodized surface [34,35]. The HA / TiO_2 layered coatings were successfully generated either by anodizing the e-beam HA deposited Ti or by the sol-gel HA treatment of the anodized Ti. In both studies, the HA thin layer was observed to cover the anodized oxide film, while maintaining the porous structure of the underlying TiO_2 layer. The in vitro cellular results

showed that the outer HA layer improved the osteoblastic cellular responses, particularly the functional activity of the cells. Some recent studies by other groups have also focused on the incorporation of high levels of Ca and P within the anodized oxide layer, in order to improve the biocompatibility.

Another study was focused on the production of HA-TiO_2 composite films (not layered) directly on Ti implants [36]. The sol-gel technique was used to prepare HA and TiO_2 sols with high chemical and thermal stability. Under controlled processing conditions, phase pure HA and TiO_2 were obtained without producing any byproducts. Compared to pure HA, the HA-TiO_2 composite coatings showed significantly improved adhesion strengths (~55 MPa in the case of HA-20%TiO_2). In particular, the HA-20%TiO_2 composite showed significantly higher osteoblastic responses than the pure HA, suggesting the existence of a synergistic role played by both the HA and TiO_2 phases.

BIOACTIVE CERAMIC SCAFFOLDS

Human bones are constituted of an inorganic hard component (calcium phosphates) and an organic soft part (mainly collageneous fibers). In cortical bone, cylindrical channels of osteons are held together by the tissue framework, whilst in cancellous bone, the framework is highly open-spaced [37]. Many attempts have been made to mimic the interconnected framework structure of bones in terms of their structural and biological characteristics. In these studies, it was found that a minimum pore size of ~100 μm was necessary for bone ingrowth into the channels [38,39].

As a porous bone graft material, HA bioceramic has attracted a great deal of attention due to its excellent bioactivity and osteoconductivity [38,39]. However, there are critical limitations in applying HA to real scaffold systems, because of its poor mechanical properties, such as its low strength and fracture toughness, as mentioned in the previous section.

In this respect, our research group has attempted to overcome the problems posed by HA bioceramic while preserving its excellent bioactivity [40-45]. We introduced a strong ZrO_2 substrate as a porous framework to enhance the load-bearing properties, and then HA was coated on the substrate to provide the scaffold system with excellent bioactivity and osteoconductivity [40]. In this section, we describe the fabrication technique used for the production of the HA coated ZrO_2 porous scaffold and discuss its mechanical and biological performance. Moreover, some novel fabrication methodologies used to endow the biomedical porous scaffold with a more controlled pore configuration are briefly introduced.

HA Coated ZrO_2 Scaffold

In the coating of the HA composition on a ZrO_2 framework, a serious problem is encountered, namely the thermal reaction between ZrO_2 and HA, as mentioned in the previous section [10-13]. This thermal reaction reduces the mechanical properties of the material and degrades its biocompatibility [12,13]. Therefore, prior to coating HA on the ZrO_2 porous framework, it is necessary to suppress the reaction between HA and ZrO_2. To implement this strategy, we inserted fluoridated apatite (FA) as an intermediate layer between the HA and ZrO_2, because the thermal stability of FA is far superior to that of pure HA [40].

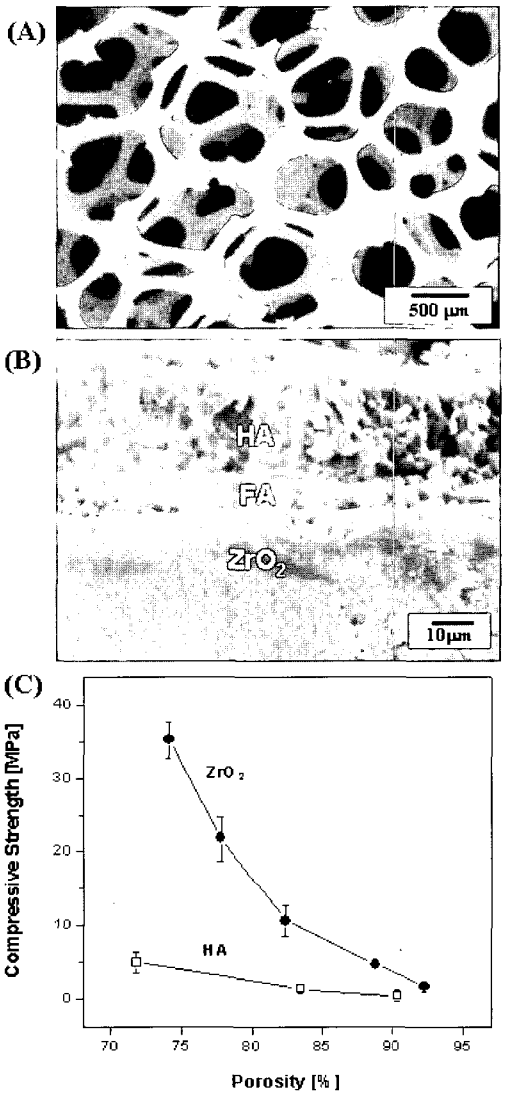

Fig. 6. (A,B) SEM morphologies of the HA coated ZrO2 scaffold with FA intermediate layer. (C) Compressive strengths of the ZrO2 porous scaffolds with different porosities as compared with those of pure HA scaffolds. Ref. [40].

When the FA layer was inserted, there were no chemical reactions between the HA and ZrO_2 substrate. Figure 6(a-b) shows the macro- and micro-morphology of the HA-coated ZrO_2 porous scaffold produced by means of a polymeric foam reticulate technique [40]. The porosity of the scaffold could be adjusted within a wider range (92-74 %) by changing the number of replication cycles. In particular, the ZrO_2 scaffolds with various porosities exhibited excellent compressive strengths (1.6 - 35 MPa over the entire porosity range), which were approximately 7 times higher than those of pure HA with equivalent porosities (Fig. 6(c)) [40]. Moreover, the HA-coated ZrO_2 scaffold allowed the bioactive properties of the HA coating composition to be retained in vitro. In the in vivo experiments carried out using a rabbit calvaria model, the HA-coated scaffolds showed excellent osteoconductive properties. As shown in Fig. 7, after 8 weeks following implantation, the newly formed bone was observed to fill the porous channels almost completely, suggesting that good osseointegration occurred in the HA-coated porous scaffold. In particular, we observed that the scaffold with a higher porosity exhibited higher bone forming ability (Fig. 7).

In a separate study, the composition of the coating layer was modified by adding other calcium phosphates, such as fluoridated apatite, tricalcium phosphate and biphasic calcium phosphates [41-45]. The results showed that varying the coating composition altered the solubility of the scaffold, and consequently the cellular responses. Moreover, the coating layer was able to be endowed with improved bioactivity either by treating it with a sol-gel apatite layer or introducing bioresolvable glasses, thus allowing more bioactive compositions to be produced which show improved osteoblastic cellular activity.

Using a similar approach to that used in our studies, other recent works produced HA coated metallic scaffolds, with the goal of combining the bioactive properties of HA and the mechanical stability of the strong and tough metal substrate.

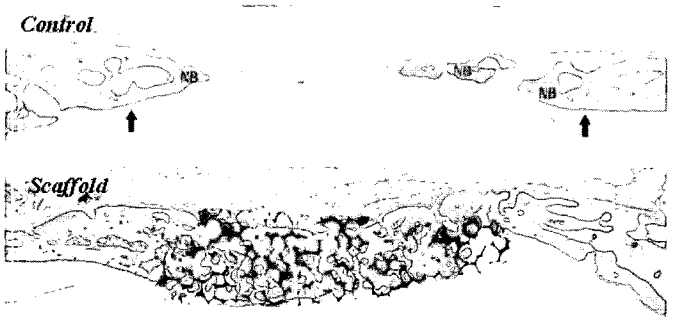

Fig. 7. Histological view of the HA coated ZrO2 porous scaffold. Newly formed bone filled into the porous channels almost completely after implantation for 12 weeks in rabbit calvaria, being in marked contrast to the control

Scaffolds with Defined Pore Configuration

As observed in the scaffolds produced by the polymeric foam reticulate method, the traditional methodologies did not allow for the precise control of the pore configuration, such as the pore shape and size and the porosity. Our research group produced HA-based porous scaffolds with a well-defined pore configuration by adopting various advanced fabrication techniques, such as the extrusion / lamination and rapid prototyping approaches [46-48]. Figure 8 shows HA-coated ZrO_2 scaffolds with three-dimensional macrochannels produced by the extrusion / lamination process [46]. The well-developed frameworks and macrochannels define the remaining HA-ZrO_2 co-extruded feedrods and the spaces replicated from the carbon extruded feedrods, respectively. Therefore, the pore shape and size and the porosity could be precisely controlled by changing the parameters associated with the extruded feedrods.

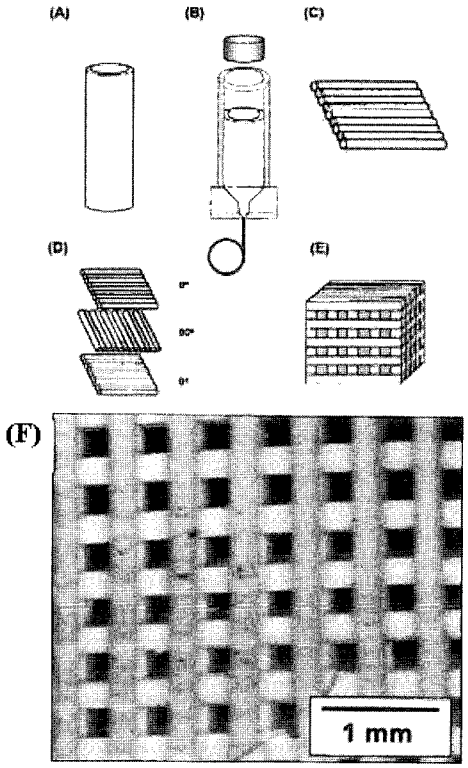

Fig. 8. (A-E) Schematic illustrations showing the processing route used to fabricate the 3-D macrochanneled scaffold by co-extrusion. (F) Macroscopic view of the produced scaffold with defined pore configuration. Ref. [46].

In a more recent study, the computer-aided rapid prototyping (RP) technique was utilized to generate the pore channels directly, without the need for the replication process. The RP technique was based on

the computer-aided design and the direct writing / carving process. This recently developed methodology is expected to provide an efficient means of reconstructing damaged tissues precisely by producing tissue engineering porous scaffolds with a pre-defined matrix shape and pore configuration.

HA-POLYMER HYBRIDS COMPOSITES

A substantial number of ceramic-polymer biological hybrids / composites have been developed, in order to combine their intrinsic properties and to attune their physicochemical and biological properties to the requirements of the hard tissues [49-51]. For hard tissue applications, bioactive ceramics, such as hydroxyapatite, bioactive glasses and glass ceramics have been hybridized with degradable polymers either in synthetic or natural form. The most widely used natural polymer includes collagen, gelatin, glyocosaminoglycan and chitosan. Moreover, degradable synthetic polymers, such as polylactic acid (PLA), polyglycolic acid (PGA), polycaprolactone (PCL) and their copolymers, are currently being studied with a view to developing composites with bioceramics. These hybrid / composite systems are known to possess levels of flexibility, mechanical properties, biological activity and osteoconductivity, which are superior to those of the individual components [49].

Recent studies by our research group have focused on the design and fabrication of these ceramic-polymer hybrids / composites using novel approaches. In this section, we review our recent works on these HA-based biomedical materials hybridized / combined with synthetic and natural polymers.

HA-natural Polymers

For use as hard tissue regeneratives, a variety of systems have been developed to mimic the specifically organized nanoscale structure of bone, which consists of collageneous fibers and mineralized apatite nanocrystals. Among these, hybrids of apatite nanocrystals and natural polymers have received a great deal of attention, in the sense that the hybrid system can provide the compositional benefits and preserve the structural and biological functions of the damaged hard tissues in a more efficient and similar way to the natural system.

The authors recently produced HA nanocomposites with collage-based polymers, and evaluated their mechanical and biological properties [52]. In the first approach, we produced collagen-HA nanocomposites using the biomimetic coprecipitation approach. In particular, fluorine ions were incorporated into the apatite structure to produce FHA-collagen nanocomposites. The FHA nanocrystallines were observed to be precipitated within the assembled collagen fibrous structure (Fig. 9(a)) [52]. Of special interest is the fact that the fluoridated nanocomposite improved the chemical stability of the apatite-collagen significantly, suggesting the long-term use of the FHA-collagen as a hard tissue regenerative. Moreover, the effective role of fluorine was manifested in the osteoblastic cellular responses, such as the stimulation of the cell proliferation and ALP synthesis (Fig. 9(b)) [52]. This is because the fluorine that is released from the nanocomposite into the culturing medium can affect the cellular responses significantly, with only small changes in its concentration (~ micro molar level) [53].

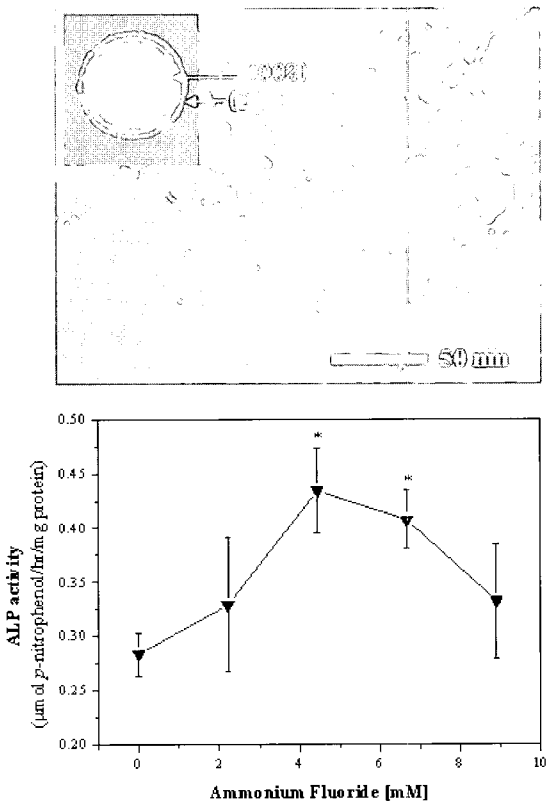

Fig. 9. (A) TEM image and diffraction ring pattern (inset) of the FHA-collagen nanocomposite obtained by a biomimetic approach. (B) ALP activity of the osteoblastic cells on the apatite-collagen nanocomposites with different level of fluoridation. Ref. [52].

We further produced a biomimetic nanocomposite in a porous scaffold for use as a hard tissue engineering matrix [54,55]. In this case, collagen-based gelatin was used and formulated with Ca and P precursors, in order to generate an apatite precipitated gelatin macroporous scaffold (Fig. 10(a)). In a test using a laboratory designed 3-D culturing system, we

observed that the osteoblastic cells attached and proliferated more on the biomimetic than on the conventional gelatin-HA composites [54]. Moreover, the cells on the nanocomposites produced ALP and osteocalcin at a significantly higher level than those on the conventional ones. The improved cellular responses on the nanocomposites are considered to result from the increased ionic release and serum protein adsorption on the nanocomposites, which is derived from the different structural and morphological characteristics. These findings suggest that these biomimetically synthesized apatite-collagen based polymer nanocomposites are potentially useful in the hard tissue regeneration and engineering fields.

Moreover, the HA-gelatin nanocomposite scaffolds were shown to be able to entrap and deliver the antibiotic drug, tetracycline, in a controllable manner. Compared to pure gelatin, the gelatin-HA nanocomposites had lower drug releases, due to their lower water uptake and degradation. In particular, the nanocomposite scaffolds released drugs in proportion to the initial drug loading amount, suggesting that they have the capacity to deliver drugs in a controlled manner (Fig. 10(b)) [55].

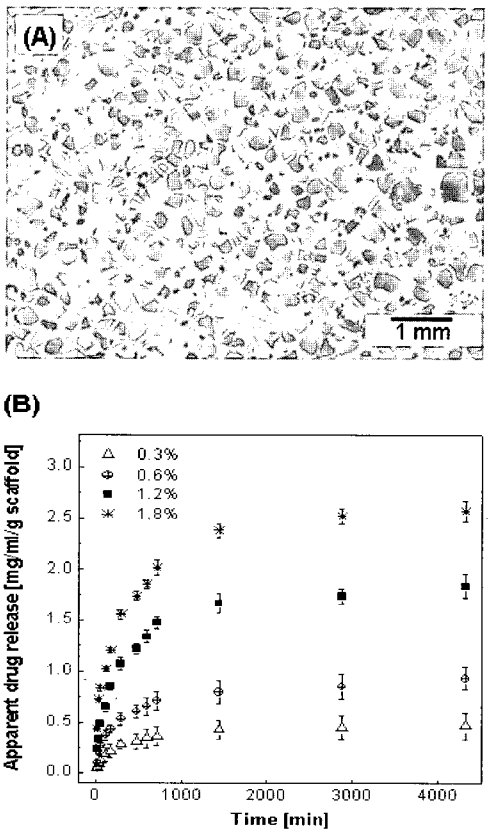

Fig. 10. Macroscopic view of the HA-gelatin nanocomposite scaffold obtained by a biomimetic approach. (B) Tetracycline antibiotic drug release profile from the nanocomposite scaffolds with different initial drug loadings (0.3-1.8%). Ref. [55].

HA-synthetic Polymers

The use of HA-synthetic polymer composites has been proposed to improve the mechanical and biological properties of hard tissue regenerative matrices [56-63]. From the bioceramic viewpoint, combining HA with synthetic polymers can enhance its flexibility and moldability, and extend its field of application in drug delivery systems, since HA is highly brittle and lacking in shape flexibility and size availability. From the polymer aspect, although degradable polymers are promising as a temporary tissue replacement and drug carrier, several issues need to be resolved before they can be used in hard tissue applications, including the acidic environment and inflammatory problems driven by the degradation of the polymers and the abrupt reduction in the mechanical stability that this gives rise to. Conclusively, the composite approach to bioceramic / degradable polymers can produce materials which possess a high level of flexibility and appropriate mechanical properties, biological activity and osteoconductivity, along with drug delivery potential.

Our research group recently produced a series of HA-polymer composites and assessed their mechanical and biological properties and drug delivering capacity [60-64]. The HA-polycaprolactone composites showed significant improvements in cell proliferation and ALP activity as compared to pure polycaprolactone, confirming the stimulation of the cell viability and functional activity by the HA component (Fig. 11) [60]. This was mainly attributed to the excellent bioactivity of HA, as well as the enhanced hydrophilicity. In practice, the water absorption and weight loss of the HA-added composites were increased. However, the mechanical properties, particularly the strength, were not enhanced significantly, and this was attributed to the large size of the HA agglomerates, caused by the mixing and homogenization problems of HA powders within the hydrophobic polymer solution. In this respect, a study is currently under way to resolve these problems, in order to obtain the desired mechanical improvement.

To prevent the degradation of the composites, we used phosphate-based glass as a ceramic component [63]. The composition of the glass was varied, in order to control the degradation rate. The degradation of the composites, as well as the antibiotic release rate, could be controlled by adjusting the glass composition. In particular, the calcium and phosphate ions released from the glass component stimulated the osteoblastic cellular responses significantly.

Within the HA-polycaprolactone composites, we incorporated fluorine ions through the fluoridation of HA, in an attempt to take advantage of the biological benefit afforded by the fluorine [64]. The FHA-polycaprolactone composites released fluorine ions in a controlled manner, depending on the level of fluoridation (from 5 to 75 %). The attachment and proliferation of the osteoblastic cells were not significantly affected by the fluoridation. However, the

beneficial effect of fluoridation was manifested in the gene expression and production of the osteoblast phenotypes, especially ALP and osteocalcin. It was confirmed that the fluoridation of HA in the apatite-PCL composites stimulated the osteoblastic cellular activities at the mRNA level, as well as the intracellular protein synthesis. Considering the similar degree of material degradation that was observed, these different cellular activities observed between the HA- and FHA-composites with PCL were attributed to the varying amounts of fluorine ion releases. This effective role of the fluorine was also observed in the FHA-collagen nanocomposites, as explained in the previous section.

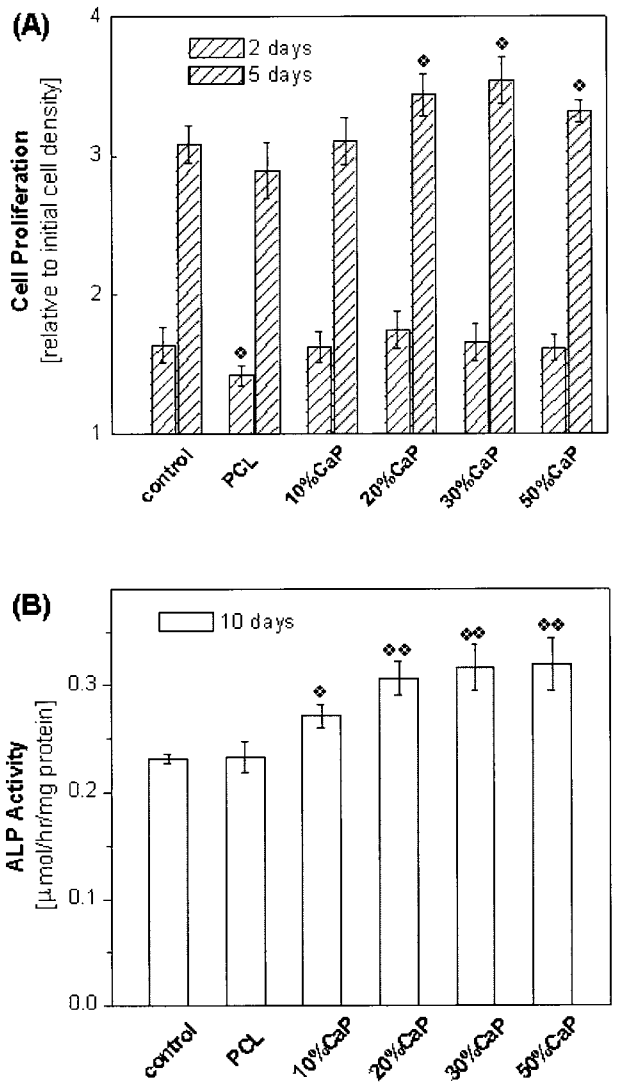

Fig. 11. Osteoblastic cellular responses to the HA-TCP added polycaprolactone biomedical membranes: (A) cell proliferation and (B) ALP activity. Ref. [60].

CLOSING REMARKS

As a major inorganic component of human hard tissues, hydroxyapatite bioceramic is regarded as being one of the most biocompatible materials. Numerous in vitro and in vivo studies have confirmed its excellent bioactivity, osteoconductivity and bone forming ability. However, because of its poor mechanical properties, its use in hard tissue applications has been restricted to those ares in which it can be used in the form of small sized powders/granules or in the non-load bearing sites. A number of researchers have focused on improving the mechanical and biological performance of HA, as well as on the formulation of hybrid and composite systems in order to extend its range of applications. In this article, we reviewed our recent works on HA-based biomaterials which include 1) the strengthening of HA with ceramic oxides, 2) HA-based bioactive coatings on metallic implants, 3) HA-based porous scaffolds and 4) HA-polymer hybrids/composites. As knowledge of the fabrication techniques and biological performance of HA-based biomaterials improves, their potential for applications in hard tissue surgery is expected to increase greatly.

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