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Preliminary investigation of bioactivity of nano biocomposite

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Abstract Bioactive biomaterials can form a bone-like apatite layer on their surfaces in the body, which is critical to establishing bone bonding between bioactive materials and living tissue. At present study, the bone-like apatite formation in vitro and vivo on the surface of the nano apatite/polyamide composite was studied, and the bioactive composites implanted into the femora of rabbits were also investigated. The results revealed that the bone-like apatite containing carbonate can form on the surface of the biocomposite both in SBF and dorsal muscle of rabbits, and the composite would form directly combination with the natural bone without fibrous capsule tissue between implant and host bone tissue. All of these indicated that the nano biocomposites have excellent bioactivity and can be used for bone replacement.

1 Introdcution

So-called bioactive biomaterials such as bioglass, hydroxyapatite (HA) and glass-ceramic A-W have been known to spontaneously form a biologically active bone-like apatite layer on their surfaces in the body, and bond to bone through the apatite layer, it is therefore believed that the essential requirement for an artificial materials to bond to living bone is formation of the bone-like apatite layer on its surface [1–3]. It is commonly accepted that when bioactive materials are implanted in the body, they spontaneously bond to bone via an apatite layer deposited on their surface without forming the fibrous tissue around them. Many studies have shown that formation of biological apatite on the surface of artificial bioactive materials is critical to establishing bone bonding between living tissue and biomaterials [4–6].

HA has been attractive in hard tissue repair because of its excellent biocompatibility and osteoconductivity. However, due to the brittleness of HA, it has been restricted in non load-bearing sites for bone repair. In order to enhance its toughness, the emphasis on the development of biomaterials has shifted from monoliths to composites in recent years. By controlling the weight fraction and distribution of the second phase in the composite, the properties of composites can be tailored to meet mechanical and physiological requirements as an implant. In this respect, HA reinforced polyethylene composites have been developed successfully for clinical application. Following this concept of incorporating the bioactive ceramics into biocompatible polymer to increase stiffness and bioactivity, various bioactive ceramics, such as bioglass, HA and tricalcium phosphate in biocompatible polymer matrix like polysulfone and polyhydroxybutyrate have been developed to produce bone substitutes [7–9].

In recent years, we have researched and developed a nano composite of nano apatite (NA) and polyamide (PA) biocomposites for load-bearing orthopedic applications. The Young's modulus and tensile strength of injection molded the composite were reported to be in the range of 3–10 GPa and 40–90 MPa with 65 wt.% apatite, respectively. These results indicate that the mechanical properties of the nano biocomposites are in the regime of cortical bone, making these composites a potential candidate for use in load-bearing applications [10–13]. Although the mechanical properties of these composites have been well documented, the bioactivity of these nano composites is not studied in detail. The aim

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of the present work is, therefore, to investigate the growth of apatite layer on the composites surface both in vitro and vivo, and the combination method between the biomaterials and host bone tissue.

2 Materials and methods

Apatite precipitation in a solid-solution ratio of 1 wt.% was treated hydrothermally in an autoclave at 140°C under 0.3 MPa for 2–4 h. After treatment, the apatite precipitation became NA crystals in a slurry state [14]. NA slurry and DMAC (N, N-dimethyl acetamide) were mixed in a three-neck flask with stirring, the temperature was gradually increased to 100∼120°C. After water was completely evaporated, NA/DMAC slurry was obtained. Composites were prepared by the co-solution method that is mixing NA/DMAC slurry with PA_{66} (19 kDa, purchased from Du Pont Company, USA) DMAC solution. When PA_6 was added, the mixture was stirred and the temperature was gradually increased to 130–160°C and kept for 4 h, then cooled down to room temperature. After fully washed by deionized water and ethanol, the product was dried at 110°C for 48 h. Composite samples were made with an injection mould machine.

Composite film specimens with $4 \times 4 \times 1\ mm^3$ were immersed in simulated body fluid (SBF) for 1, 2, 3 and 4 weeks at 37°C, The SBF was prepared by dissolving the reagent grade NaCl, $NaHCO_3$, KCl, $K_2HPO_4 \cdot 3H_2O$, $MgCl_2 \cdot 6H_2O$, $CaCl_2 \cdot 2H_2O$ and Na_2SO_4 into distilled water, and buffered with Tris (hydroxy-methyl-aminomethane,$NH_2C(CH_2OH)_3$) and hydrochloric acid (HCl) to pH 7.4 at 37°C [15]. After soaking for a given period, the specimens were removed from the solution, washed with ultrapure water, and then dried at room temperature. The adult and healthy rabbits were anaesthethetized with intraabdominal or intravenous injection of 3% sodium pentobarbital. The composite film samples with $4 \times 4 \times 1\ mm^3$ were implanted in dorsal muscle of rabbits. Specimens were harvested at 1 week, 2 weeks, 3 weeks, 4 week after implantation and digested in 10% trapsine. Both the Ca-P deposits on the specimen vitro (SBF) and vivo (intramuscular implantation) were examined using a Scanning Electrical Microscope (SEM) with Energy Dispersive X-ray Spectrometer (EDX), Fourier transform infrared spectrometer (FTIR), X-ray diffractometer (XRD).

Composite cylinders samples with $\Phi 4 \times 8\ mm^3$ implanted into femora of rabbits. Under general anesthesia and sterile conditions, the left femur bone of each rabbit was exposed and two defects (4 mm) were drilled in the middle of the femur. After 3,6 months, the animals were sacrificed by an overdose abdominal injection of pentobarbital sodium and the implants were collected with surrounding tissues and fixed in 4% buffered formaldehyde (pH 7.4). The fixed samples were dehydrated in a series ethanol (70, 80, 90, 95, and 100%) and embedded inmethyl methacrylate. Thin undecalcified sections (20 μm) were made with a diamond saw (KDG95, IsoTis, B.V., The Netherlands). Sections were stained with methylene blue and basic fuchsin for histological observation.

3 Results and discussions

The surface morphology of the NA/PA composite and PA after immersed in SBF for 4 weeks as shown in Fig. 1(a) and (b). It can be seen from these photos that the surface of the composite was covered with sphere-shape apatite precipitation. However, there is little change of the surface morphology and no apatite formed on the surface of pure PA after 4 weeks. The results of apatite formation on the surface of the NA/PA composite and no apatite on pure PA in SBF indicated that the composite has excellent bioactivity while pure PA is bioinert.

The surface morphology of the composite and pure PA implanted in the muscle of rabbits is shown in Fig. 2(a) and (b). The results revealed that a large number of flake apatites are presented on the surface of the composite while no apatite on the pure PA after implanted for 4 weeks. The morphology of apatite formation on composite in vitro is different from that of in vivo, the factors effecting bone-like apatite morphology

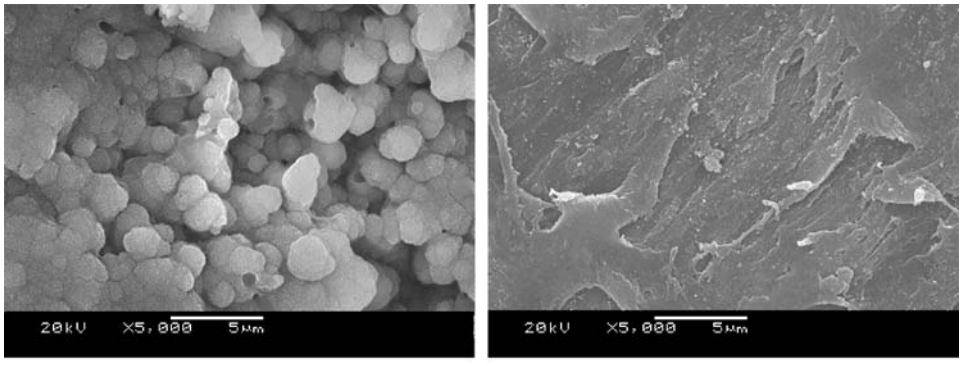

Fig. 1 SEM photographs of NA/PA composite (a) and PA (b) immersed in SBF for 4 weeks.

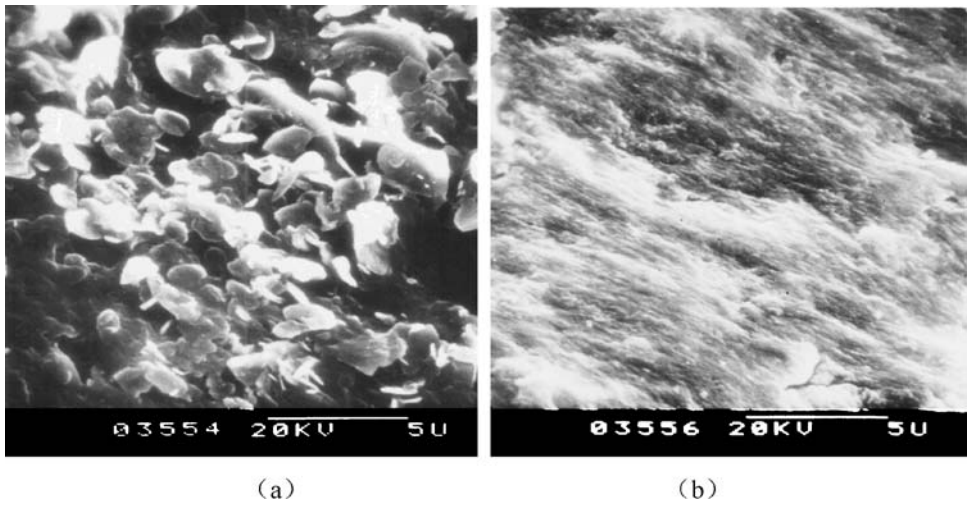

Fig. 2 SEM photographs of NA/PA composite (a) and PA (b) implanted in muscle of rabbit for 4 weeks.

on surface of the composite in vivo are not clear yet. The results of bone like apatite formation in vivo indicated that the composite is bioactive while pure PA is not.

The EDX results revealed that the Ca/P ratio of the apatite on the composite surface is 1.57 after immersed SBF and 1.62 after implanted in muscle of rabbits for 4 weeks (Ca/P ratio of stoichiometric hydroxyapatite is 1.67). These indicated that apatite formation on the composite surface is poorly crystallized nonstoichiometric apatite not only in SBF but also in the body of the rabbits. The variations of Ca/P ratio of the apatite deposits on the surface of the composite within 4 weeks is shown in Fig. 4 the results revealed that the Ca/P ratio slightly decrease in the first week, perhaps only a little Ca-P deposited on the surface of the composite during this stage; the Ca/P ratio of the composite surface was very low after the second week, which revealed that Ca-P deposits have occurred on the surface of the composite; continuous increasing the Ca/P of the composite surface from the third week to the fourth week indicated that the apatite increasingly deposited on the surface of the composite and the precipitation growth in size and thickness increasingly.

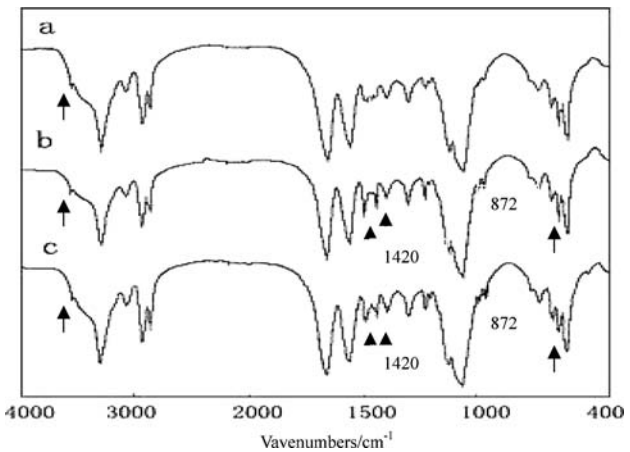

Fig. 3 IR spectra of NA/PA composite (a), composite immersed in SBF (b) and implanted in muscle of rabbit (c) for 4 weeks.

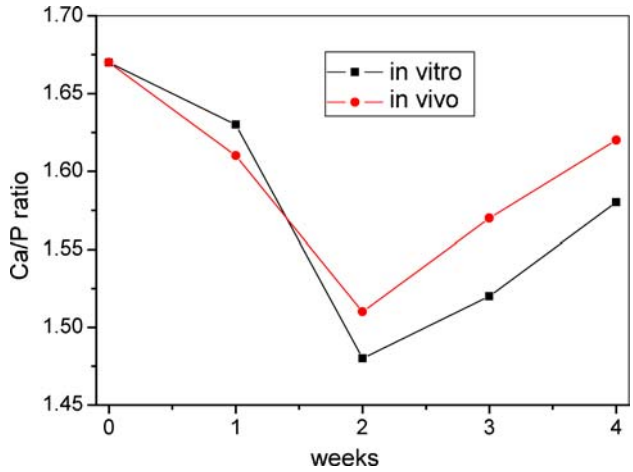

Fig. 4 Variations of Ca/P ratio on the surface of composite in vitro and in vivo with time.

IR spectra of the composite surface after immersion in SBF and implantation in muscle of rabbit for up to 4 weeks are shown in Fig. 3. The spectra further confirmed the results from SEM and EDX analysis that a continuous bone-like apatite layer had grown on the composite surface. The spectrum of HA in the composite comprised of the v3 bands of phosphate group indicated by strong broad peaks around 1089 and 1031 cm^{-1}. The peaks at 602 and 568 cm^{-1} were due to the v3 vibration of PO_4^{3-} and the shoulder at 980 cm^{-1} was the v1 band of PO_4^{3-}. Similarly, the peaks at 3640 and 631 cm^{-1} were due to vibrational bands of OH^- group (long arrow) [16]. The spectrum of the surface of the composite both in vitro and in vivo after 4 weeks show that three new small peaks compared with the HA in the composite, which belongs to the vibrational bands of CO_3^{2-} groups, they were v2 band at the 872 cm^{-1}, v3 bands at 1458 cm^{-1} and 1420 cm^{-1} (short arrow) [17]. The results show that

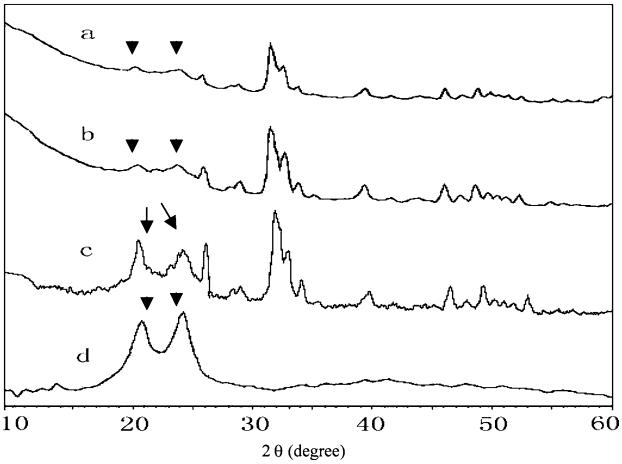

Fig. 5 XRD spectra of n-HA/PA composite intramuscularly implanted in rabbit (a) and immersed in R-SBF for 4 weeks (b), composite (c), pure PA (d).

some carbonated-containing HA layer form on the surface of the composite both in vitro (SBF) and in vivo (muscle of rabbit).

XRD spectra of the composite surface before and after immersed in SBF and implanted in muscle of rabbit for 4 weeks are shown in Fig. 5. Comparing the composite to those of immersion in SBF and implantation in vivo, there is little change of the main structure of composite. However, some peaks have become widen slightly, these indicated that the crystallinity of the apatite layer formation on the composite surface is lower than that of HA in the composite, and the two crystallized peak at $2\theta = 20$ and $2\theta = 23.9$ (arrow) of PA decreased and almost disappear, which may be covered with the apatite deposits layer on the composite surface. Since XRD analysis does not show the existence of any phase other than HA and PA, it can be said that all carbonate peaks found in the IR spectrum of apatite are attributed from the precipitated apatite. Therefore, XRD analysis is accordance with IR and EDX, all the evidence indicate that the carbonate apatite formation on the composite surface.

The mechanism of bone-like apatite formation on the surface of the composite is one of two process or the two process together: a little solution of HA from the composite at the beginning of soaking composite entered into SBF, then Ca^{2+} and PO_4^{3-} ions are concentrated on the surface area of the composite, resulting in the formation of the apatite layer, or HA crystals on the composite surface acted as crystal seed or nucleation point, inducing the apatite formation on the composite surface. The apatites grew up to form the flake and/or sphere with (excessive saturation concentration) Ca^{2+} and PO_4^{3-} ions in SBF.

Histological analysis of PA and NA/PA composite specimens implanted in the femur of the rabbits are shown in Fig. 6. The result (b) indicated that the composite would form directly combination with the natural bone, which is known as bone-bonding. Combination of bone-bonding can ensure the implant integrate with natural bone through biochemical reaction at the interface between materials and bone. The fibrous capsule around the PA implant is presented at the interface of the implant and natural bone tissue shown in (a), this combination is not fastness which would cause loose of the implant in the bone and bone absorption, ultimately, bring about the failure of the implant. The results showed that the composite has excellent bioactivity, and would combine with bone through bone-bonding.

Bioactive materials are known to bond to living bone in the body via formation of an apatite layer on their surfaces, the essential requirement for an artificial material to bond to living bone is the formation of a biologically active bone-like apatite layer on its surface in the body [18–19]. When they are implanted into bone, apatite formed at the surface of materials and newly formed bone tissue fill up the gap between the surrounding bone and the apatite layer, tight

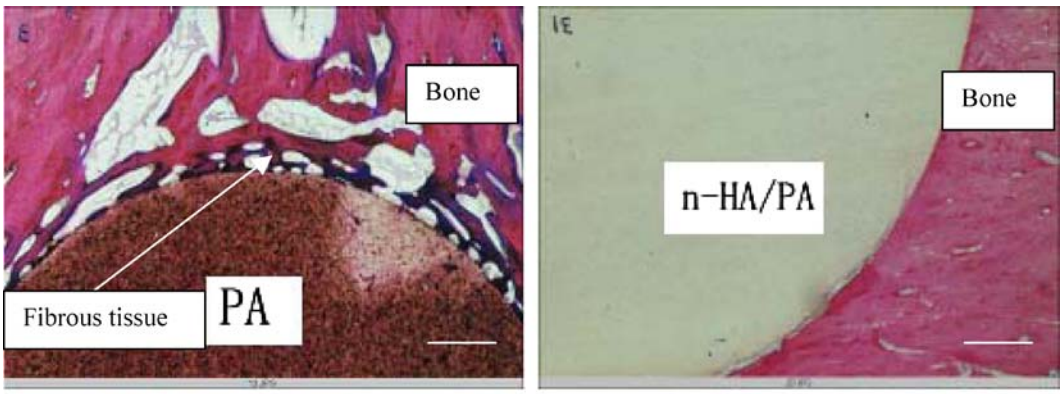

Fig. 6 Photographs of histological analysis of PA and NA/PA composite bone implantation, fibrous capsule (arrow) around PA implant (a), composite directly combined with bone (b), bar = 200 μm.

chemical bonding then formed between the bony apatite and the surface apatite.

In a word, bone-like apaite layer wiht poorly crystallized nonstoichiometric apatite and contains carbonate formed on the surface of the nano-composite immersed in SBF and implanted in vivo, and direct bone-bonding formed with the host bone tissue. All the results showed that the composites have excellent bioactivity and osteoconductivity.

4 Conclusions

Bone-like apatite can form on the surface of the nano composite of apatite and polyamide both in vitro and vivo. The apatite formation is nonstoichiometric apatite (Ca/P ratio low than 1.67) and contains carbonate. The composite can form directly combination with the natural bone without fibrous capsule tissue. The nano biocomposites have excellent bioactivity and can be used for bone replacement.

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