IMPROVING THE MECHANICAL PROPERTIES AND APATITE FORMATION ABILITY OF NANO-HYDROXYAPATITE/POLYAMIDE 66 COMPOSITE FOR USING AS THE INTERVERTEBRAL FUSION CAGE

HAMID REZA JANGHORBAN

Amin Hospital
Ebn-e-Sina St.
Isfahan
Iran
e-mail: hamidrezajanghorban@yahoo.com

Abstract

One of the significant challenges in designing an ideal cage is the mechanical modulus mismatch between the implant and surrounding host tissue which results in stress shielding. In this study, a nano-hydroxyapatite/polyamide 66 (n-HA/PA66) composite with mechanical properties close to spongy bone to be designed and characterized as an intervertebral fusion cage to perform spinal fusion between vertebrae in the lumbar spine. A pure natural nano-hydroxyapatite (n-HA) powder was prepared by annealing bovine bone at 1000ºC followed by 10h mechanical alloying. The nano-hydroxyapaptite/ polyamide 66 and polyamide 66 (PA66) cage were prepared by injection method. X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy dispersive spectrophotometer (EDS) were used to characterize the powder and composites samples. The results showed that addition of 20% nano-hydroxyapaptite results in the improvement of both mechanical and bioactivity properties. The optimum n-HA/PA66 exhibited the compressive strength and elastic modulus of 46.98 ± 2MPa and 1.53 ± 0.3GPa, respectively, which is close

to spongy bone. In addition, observation of the superior apatite formation ability of n-HA/PA66 compared to PA66 indicates that it can be used as a bone replacement material.

1. Introduction

Back pain is very common problem among people around the world [1, 2]. In some specific cases, surgery would be suggested as a healing procedure. Surgery of the spine is a major challenging operation. In large spine defects, when the gaps are huge, a bone graft can stimulate healing [1-4]. In the bone graft, autograft, allograft and synthetic substitute (implants) can replace the degenerative disc disease in the spine to relieve back pain; and it is known as spinal fusion [5, 6]. Disadvantages of autografting and allografting, are limited by the amount of suitable bone and lack of donors, require double operations, increase infection and osteomyelitis, disease transmission and immune response [7, 8]. Hence, synthetic devices have been offered as alternative methods to motivate the bone tissue growth [9]. The intervertebral fusion cage has been designed as a new device to perform the spinal fusion, and in the past 30 decades, it has been produced by metal, polymer, ceramic and composite of these materials [10, 12]. In the previous studies, titanium mesh cage was used successfully and indicated high fusion rates. However, several disadvantages including stress shielding, corrosion, high incidence, subsidence and creating problems in radiography procedure limited its use as an ideal cage [13, 14]. Therefore, use of polymer and ceramic suggested new approaches to make the cage devices. Polyamide (PA) was recognized as a synthetic polymer with excellent biocompatibility due to its chemical networks and active group similarity with proteins and collagen. Polyamide also has excellent mechanical properties resulting from strong hydrogen bonds between the amide groups in PA macromolecules. However, PA is bio inert and does not react or form a bond with bone [15, 16]. Therefore, it is a major drawback of this polymer. Hydroxyapatite (HA) is well known as the main type of calcium phosphate group due to its chemical similarity to mineral portion of bone

tissue. Unique bioactivity, biocompatibility and its excellent osteo-conductive capability make it a suitable ceramic material to be used as hard tissue regeneration [17, 18]. Using composite of polyamide and HA for designing the intervertebral fusion cage with improved mechanical and biological properties is the main aim of this study. Therefore, the mechanical properties and in vitro bioactivity of HA/polyamide composite cage were investigated. In addition, the mechanical properties of n-HA/PA66 cage were compared with traditional polyetheretherketone (peek) cage.

2. Materials and Method

2.1. Preparation of nano-HA powder

In order to prepare the natural nano-HA powder, the femur of bovine was purchased and boiled for 5h in distilled water to remove the visible tissue and marrow's bovine. Then, an electric furnace was used to anneal the bovine's bone at 1000ºC for 3h. The obtained matter was ball milled for 10h in zirconia vial with five 2cm zirconia balls. The ball/powder weight ratio was 10 : 1 and disc rotational speed was set 250rpm.

2.2. Preparation of nano-HA/PA66 composite cage

The 20wt% HA-PA66 cage and pure PA66 cage were prepared by injecting the molten liquid of n-HA/PA66 into the steel mold. Figure 1 shows the prepared cages.

Figure 1. (a) The PA66, (b) n-HA/PA66 (without slot between holes), (c) n-HA/PA66 (with slot between holes), (d) n-HA/PA66 (three holes), and (e) it is a hollow elliptical with 27mm in larger outer diameter and 21mm in inner diameter.

2.3. Characterization of powder and composite

Crystallite size and phase transformation of powder were assigned by X-ray diffraction (XRD, X'Pert Philips) with CuKα (λ = 0.154nm) radiation at 40kV and 30mA. The XRD patterns were recorded at the 2Ө range of 20-80° with 0.05° step size and 1s time per step. Williamson-Hall method was used for crystallite size measuring [19].

$$\beta \cos \theta = \frac{0.9\lambda}{D} + 2\varepsilon \sin \theta, \tag{1}$$

where θ is the Bragg diffraction angle, ε is the internal strain, D is the crystallite size, λ is the wavelength of the radiation, β is the diffraction peak width at half maximum intensity, and 0.9 is the Scherrer constant. The bioactivity of the produced cage was investigated by Bohner et al.

[20] protocol and the nano-HA/polyamide and polyamide produced cage was immersed in simulated body fluid (SBF) for 28 days. Scanning electron microscopy (SEM, Philips XL30 at an acceleration voltage of 30kV) and energy dispersive spectrophotometer (EDS) were used to investigate the apatite formation and deposition followed by immersing the cage in SBF. The compressive strength and modulus of n-HA/PA66 and PA66 produced cage were evaluated by universal testing machine ((Hounsfield: H25KS) at a crosshead speed of 1mm/min.

3. Results

The XRD pattern of nano-HA, after it was annealed at 1000°C followed by 10h ball mill, was shown in Figure 2. As can be seen in this pattern, all the peaks correspond to the characteristic peaks of hydroxyapatite phases (JCPDS 01-086-0740). Therefore, in this study, we succeed in inducing the natural HA powder of bovine bone in nano range size that has more chemical and structural similarity to mineralized bone tissue [21]. The crystallite size of hydroxyapatite powder was measured 61 ± 2nm. Nano-crystalline ceramics in comparison to micro particle ceramics have improved properties such as high contact area, high diffusion rates, reduced sintering time or temperature, and high mechanical properties. For example, nano-crystalline HA improves osteoblast cells adhesion, differentiation, proliferation, osteo-integration and Ca containing minerals are deposited on its surface better than micro-crystalline HA [19, 21, 22]. Hence, nano-scale ceramic materials are preferred to use as replacement and regeneration materials. Figure 3 shows the force-extension curve of nano-HA/PA66 (with different designs), peek, PA66, and comparisons of the mechanical properties of different cages including those prepared in this study and spongy and cortical bone are presented in Table 1. As can be seen from Table 1, the presence of the ceramic phase and design of holes have an important effect on the mechanical properties. Based on the obtained results, addition of 20% n-HA in PA66 matrix (without hole) enhanced both

compressive strength and modulus from 57.27 to 90.54 and 1.55 to 8.19 and close them to cortical bone with compressive strength and modulus in the range of 130-180MPa and 7-30GPa, respectively. According to Figure 1(b)-(d) and results of Table 1, it can be noted that making holes in the cage walls resulted in reducing the elastic modulus close to spongy bone, which has a compressive strength and elastic modulus in the range of 4-12MPa and 0.1-1.1GPa, respectively.
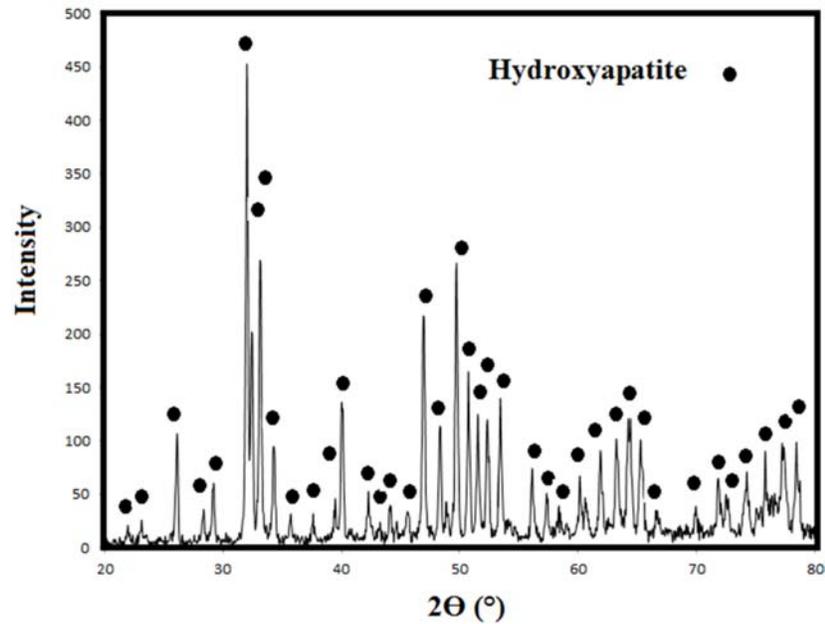
Figure 2. X-ray diffraction pattern of natural nano-HA powder.
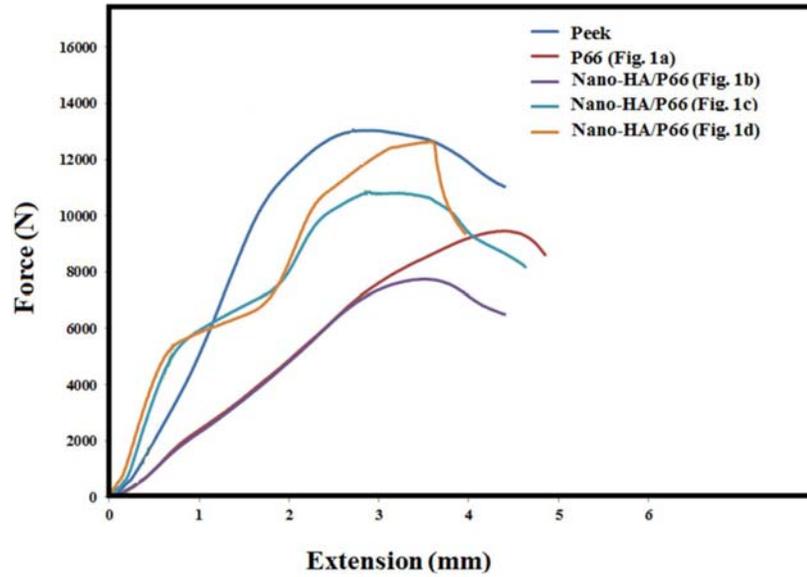
Figure 3. Force-extension curve of nano-HA/PA66 (different design), PA66 and peek cage.

Table 1. Mechanical properties of preparation cage, spongy and cortical bone (mean ± standard deviation)

Samples	Compressive strength (MPa)	Elastic modulus (GPa)
Peek	79 ± 1	3.95 ± 1
PA66 (Figure 1(a))	57.27 ± 1.5	1.55 ± 0.8
n-HA/PA66 (without hole)	90.54 ± 3	8.19 ± 1.2
n-HA/PA66 (Figure 1(b))	46.98 ± 2	1.53 ± 0.3
n-HA/PA66 (Figure 1(c))	65.75 ± 1	5.25 ± 0.42
n-HA/PA66 (Figure 1(d))	76.54 ± 1.53	5.75 ± 0.5
Spongy bone	4-12 [23-25]	0.1-1.1 [23-25]
Cortical bone	130-180 [23-25]	7-30 [23-25]

Some researchers reported the successful fabrication of n-HA/PA66 composite for various clinical applications. Xu et al. [12] synthesized the n-HA/PA66 scaffolds by thermal pressing and injection molding

techniques. The n-HA/PA66 scaffold was implanted into muscle and tibiae of rabbits and the results indicated that at 2 weeks, osteogenesis was detected in porous n-HA/PA66 composite. The study indicated that the scaffolds could facilitate cell adhesion, differentiation and proliferation, and help with fibro vascular and nerve colonization. Also, the compressive strength and modulus of the composite with 64.5wt% n-HA content were 117MPa and 5.6GPa, respectively which is still far away from the mechanical properties of spongy bone and probability of stress shielding phenomenon is existed. Zhang et al. [10] fabricated a novel ternary composite using carbon fibers to reinforce a nano-hydroxyapatite/polyamide 66 composite (HA/PA). They could successfully produce these composites with different carbon fibers content with random orientation in HA/PA66 matrix. The compressive strength and modulus was found to be in the range of 116-212MPa and 2.9-5.8GPa. Despite, these mechanical properties is higher than what needing for cage devises.

Therefore, n-HA/PA66 (Figure 1(b)) fabricated in this study, with the mechanical properties (compressive strength 46.98 ± 2 and modulus 1.53 ± 0.3GPa) close to spongy bone tissue has significant potential as intervertebral fusion cage in human body.

Figure 4 demonstrates the SEM micrographs of PA66 (a)-(c) and n-HA/PA66 (d)-(h) specimens after 28 days immersing in SBF. Furthermore, the EDS analysis of n-HA/PA66 was used to clarify the chemical compound of precipitated particles presented on the surface of n-HA/PA66 after immersing in SBF for 28 days. As shown in Figure 4(a)-(c), the surface morphology of PA66 after 28 days soaking in SBF does not show the formation of apatite layer. Therefore, based on SBF results, PA66 has no great potential to show the reaction with bone tissue in biological environment and does have the capability of apatite-bone like form. Hence, it was known as a bio-inert polymer.

To determine the effect of n-HA on apatite formation ability of PA66 cage, the n-HA 20wt.% /PA66 was immersed in SBF for 28 days. As can be seen in Figure 4(d)-(h), the precipitates with particles were observed on the surface of n-HA/PA66. The EDS analysis (Figure 4(i)) showed the presence of calcium and phosphor on the surface of this specimen. The average apatite size with a cauliflower-like structure was approximately 2μm. In comparison, the obtained bioactivity results of PA66 and n-HA/PA66 cage obviously showed that the apatite formation ability of n-HA/PA66 cage was much higher than that of PA66 cage. It is directly related to the presence of n-HA in this specimen.
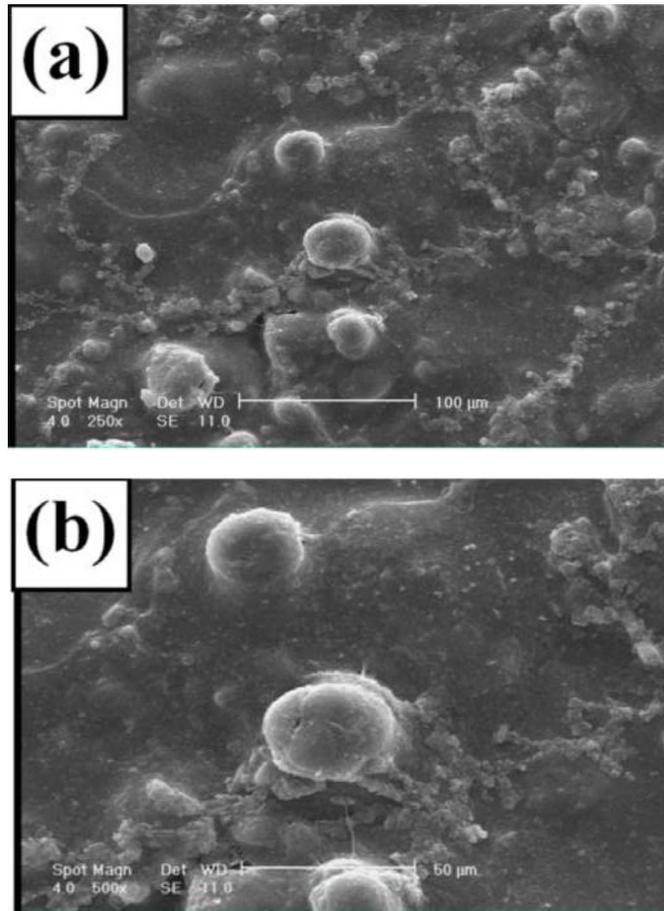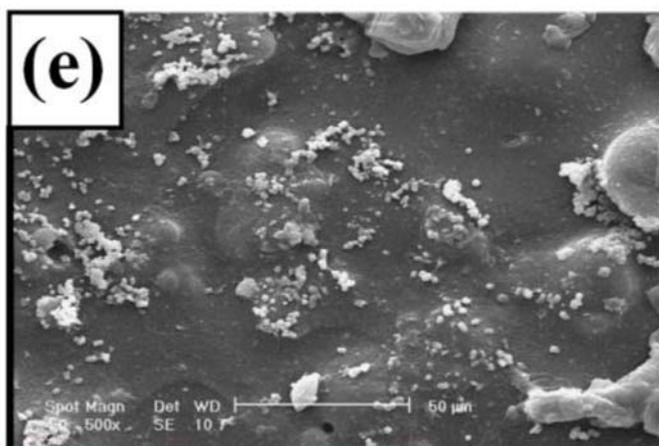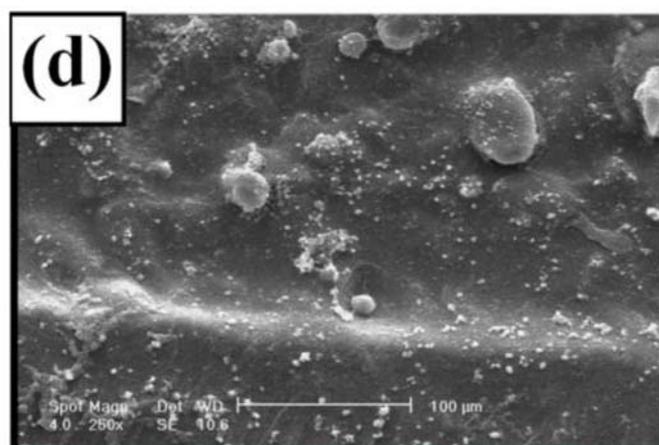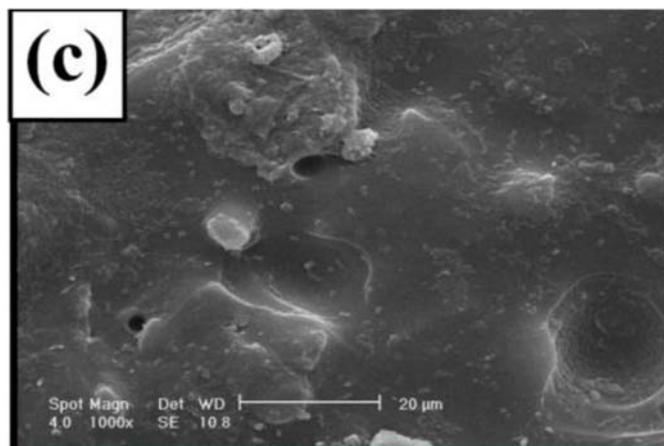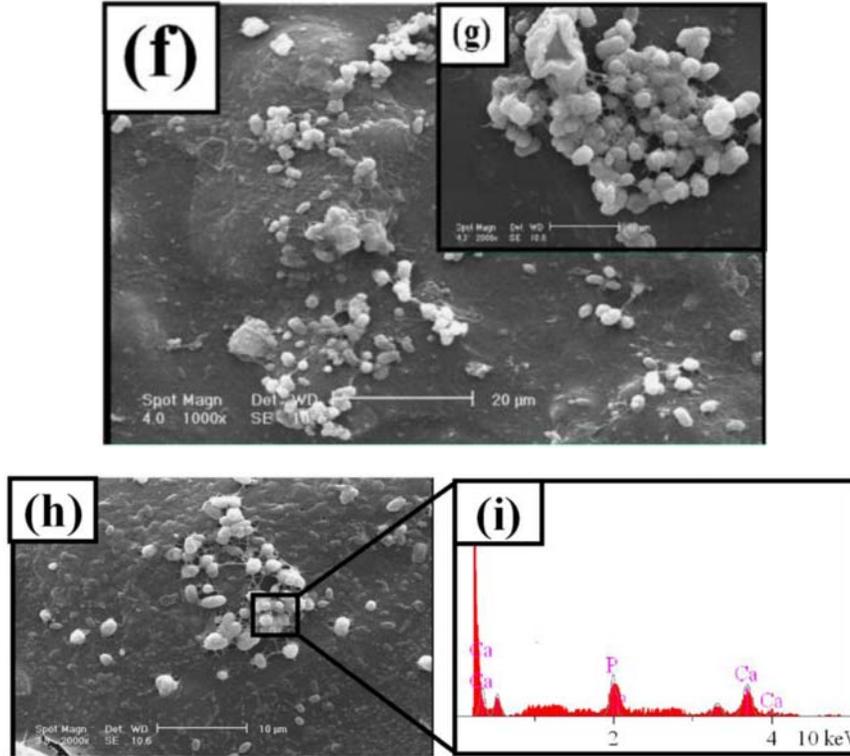
Figure 4. SEM micrograph of (a)-(c) PA66 cage, (d)-(h) n-HA20%/PA66 and (i) EDS analysis of n-HA20%/PA66 after 28 days soaking in SBF.

4. Discussion

Bone can be considered as nano-composite materials. 35% of bone is organic ground substances and collagen fibers and 65% of bone is inorganic (hydroxyapatite). Hydroxyapatite is oriented in the direction of the collagen fibers and collagen is known to nucleate apatite crystals [26]. Despite, the natural bone have the capability of growing and being self-repairing, if the damage gaps to be bigger than several mm, a bone graft can stimulate healing [26, 27].

The cage devises made in the past (polymer, metals and ceramics) could not bond to bone due to their mechanical mismatch (stress shielding phenomenon) or their inert nature [1, 2]. In this study, we

fabricated the n-HA/PA66 cage with special design to improved its mechanical properties and apatite formation ability by addition of 20% n-HA in PA66 matrix. The results indicated that n-HA had good interaction with the PA matrix, which is as a results of electrostatic forces and hydrogen bonds existing between the ions and groups in the composite material. These included interactions between the Ca^{2+} and $PO4^{3-}$ charged groups of HA and the –C–O and –NH– groups in PA66. Additionally, coordination bonds were formed between the –COO group of PA66 and Ca^{2+} group of n-HA [6, 10]. Therefore, the improved mechanical properties and apatite formation ability was observed in this composites.

Biomaterials elastic modulus is one of the most important mechanical properties for hard tissue applications. If biomaterials show much higher or lower elastic modulus compared to the native bone tissue, the stress shielding phenomena may occur [21]. As a result, using materials with elastic modulus close to that of human bone is desirable. In the present study, the obtained composite (n-HA/PA66) exhibited a compressive strength of 46.98-90.54MPa and a modulus of 1.53-8.19GPa, with different designing. It is obvious that the addition of n-HA and changing of designing have a significant effects on the mechanical properties of the n-HA/PA66 composite. Furthermore, the compressive strength and modulus of n-HA/PA66 (Figure 1(b)) was more close to mechanical properties of spongy bone in comparison to peek and other prepared composite in currently study.

Biocompatibility and bioactivity of cage devises are really important. The imperative demand of cage since it works in contact with leaving cells in vitro and with tissue, it must be tolerated in the body and it must not produce an adverse immune response [28]. Previous studies shown that hydroxyapatite/polyamide composite have biological safety, good biocompatibility, and osteoinduction and osseointegration in *in vitro* and *in vivo* studies, and have been shown to be a prospective application in

the clinical setting [1, 2, 10]. Xiong et al. [6] shown the capability of the n-HA/PA66 scaffold composite for bone defect repair. In their study, a retrospective review was carried out for 21 patients who suffer from bone tumor. The radiologic analysis showed that porous n-HA/PA66 composite had been completely incorporated with the host bone about 1.5 years later.

Furthermore n-HA is known as an excellent bioactive material and according to the literature, when it is immersed in the simulated body fluid (SBF), apatite-like calcium phosphates are formed on its surface [29]. Also, its rapid rate of surface reactions, which leads to fast tissue bonding, is another advantage of hydroxyapatite [30]. Therefore, when the n-HA/PA66 cage is used as an implant in the human body, it induces the strong bond to bone and enhances the bone tissue in growth; also stimulates the osteoblast proliferation and differentiation *in vitro*. Fabrication of n-HA/PA66 cage with improved compressive strength, elastic modulus and apatite formation ability can open a bright horizon to treat the problem of degenerative disc disease.

5. Conclusion

This study is the first report of successful design of n-HA/PA66 nanocomposite as an intervertebral fusion cage with improved mechanical and apatite formation ability. Based on the obtained results, addition of 20% nano HA to polyamide matrix results in improved compressive strength and elastic modules and close to spongy bone, respectively, compared to PA66 and peek cage. Moreover, the improvement in apatite formation ability and bioactivity of n-HA/PA66 was compared to PA66, and was proved.

Acknowledgements

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