BONE FORMATION OF HYDROXYAPATITE/STEM CELL BIOCOMPOSITES

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1 Introduction

Hydroxyapatite (HA) ceramics have been used extensively as a substitute in medical and dental applications in forms of granules, discs and coating, because of its compositional similarities to natural human bone and teeth, and its excellent biocompatibility [1-2]. Due to the similarity of the size of nano-sized HA with the HA crystal in natural bone, many works have been done on the synthesis of HA nanoparticles and their applications [3]. In the case of HA nanoparticles, their particle size, morphology, and structure have significant effect on the biological response [4].

Previous studies have demonstrated that 20-40 nm HA particles in the bone play an important role in biomineral formation, which have remarkable physical and chemical features, such as unique mechanical strength, insensitivity to dissolution and flexible structure [5]. Therefore, nanoscale HA powder can be a better candidate in biomedical applications. But, the effects of particles size on the bone formation are not understood clearly.

In this study, we tried to observe the bone formation of the biocomposite mixed with stem cells and hydroxyapatite (HA) powders with different particle size in rabbit long-bone defects and investigated the size effects of hydroxyapatite particles on the formation of new bone.

2 Expreimental procedure

Micronsacle HA powder commercially obtained from Shinyo was used as starting materials in the experiment, which Ca/P ratios of this powder is approximately 1.67. Nanoscale HA powder with a Ca/P ratio of 1.67 was synthesized by an aqueous precipitation method from $Ca(NO_3)_2{\cdot}4H_2O$ and H_3PO_4. Stem cells were obtained from bone marrow of New Zealand white rabbit. Biocomposites with paste type were prepared by mixing of 0.7% agar gel (300μL) and hydroxyapatite powder 1cc with or without 2 x 10^7 stem cells (300μL). Bone implantation for 4 biocomposite samples (m_HAp; micronscale HA powder, n_HAp; nanoscal HA powder, sc/m_HAp; stem cell and micronscale HAp powder, sc/n_HAp; stem cell and nanoscale HA powder) was performed into white rabbits from New Zealand. Thirty two white rabbits (8-10 week-old) were used in this study.

Each biocomposite with paste type was implanted to the bone defect of the eight rabbits, respectively. After an implantation, the morphology and the degree of bone formation were weekly observed by radiographic and microscopic observation from post-operation to eight weeks after. Also, the each microstructure of bone tissue in implanted area after eight weeks was observed by photomicroscope, and compared the formation of new bone with each other.

Bone formation ability in the transplanted area within bone defects was analyzed from the photographs of implanted central area. New bone formation are observed at five area through the photographs magnified to 400 times using a photomicroscope and obtained the ratio of the new bone formation area in a section using an image analyzing program of Image-proplus 5.1

3 Results and discussion

SEM micrograph presents that as-dried HA powder consists of nanoscale crystallites with narrow size distribution (Fig. 1(a)). The powder is sphere-like with a diameter of approximately 70-100 nm. Although this is a typical morphology of particles prepared by a precipitation method [6], it shows low tendency to agglomerate. XRD pattern(Fig. 1(c))

has characteristic peaks consistent HA. In the case of commercial powder, it has a micronscale particle size about 0.5~ 2.0 μm with agglomeration. Its XRD pattern (Fig. 1(d)) has characteristic peaks consistent HA, but HA peaks shows high intensity with narrow width compared to the nanoscale HA powder.

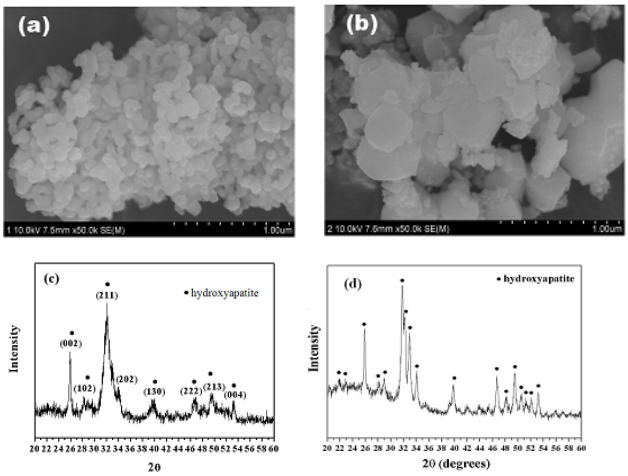

Fig. 1 Microstructure and XRD patterns of (a),(c) nanoscale and (b),(d) microscale HA powders

Fig. 2 shows the microstructure of transplanted n_HAp and sc/m_HAp biocomposites at one week after implantation, which shows the compact microstructure. However some of new HA precipitates were observed on the surface of biocomposite(Fig. 2(a)). This type of new HA precipitate was frequently found in in vitro experiment by the mechanism of dissolution and reprecipitation.. Generally nanoscale HA powder has a higher solubility than that of micronscale HA powder, because the solubility of particle is inversely proportional to the particle radius. In the case of the microstructure in sc/m_HAp biocomposites implanted to bone defect shows the trace of dissolution in HA particles Bonding between HA particles is very weak compared to n-HAp biocomposite (Fig. 2(b))

Fig. 3 shows the radiographs for bone formation of biocomposite in the bone defect. In the case of m_HAp biocomposite, diffuse bone formation was seen at the edge of old bone, but the consolidation just in the lateral position of the defect was appear at eight weeks. The degree of bone formation and consolidation of n_HAp biocomposite is higher than that of m_HAp biocomposite as shown in Fig. 6(b), which indicates that nanoscale HA powder have an advantage compared to the micronscale HA powder.

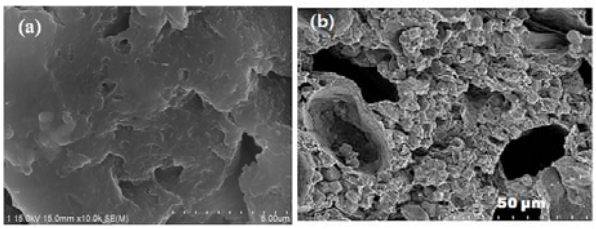

Fig. 2 Microstructure of transplanted (a) n_HAp and (b) sc/m_HAp biocomposite into the bone defects at one week after operation

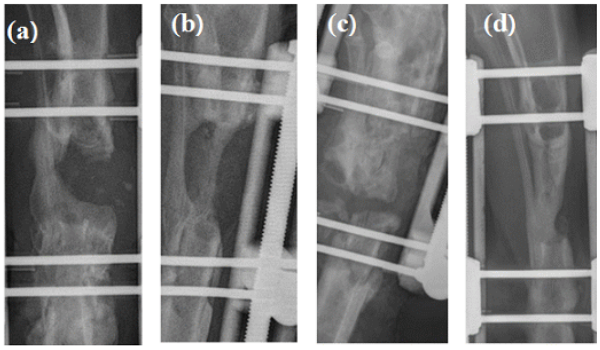

Fig. 3 Radiographs for bone formation of (a) m_HAp biocomposite, (b) n_HAp biocomposite, (c) sc/m_HAp biocomposite and (d) sc/n_HAp biocomposite by implantation into the bone defect at eight weeks after operation

By the previous study, bioactivity of nanoscale HA particles is higher than that of micronscale HA particles because nanoparticles may promote the adhesion, proliferation and synthesis of alkaline phosphatase of osteoblasts and lead to more rapid repair of hard tissue injury [7]. Recent research suggested that better osteoconductivity would be achieved if synthetic materials could resemble bone minerals in composition, size, and morphology [8]. Therefore, nanoscale biomaterials may also have other special properties due to small particle size and enormous specific surface area. For example, the nano-sized ceramic materials have shown significant increase in protein adsorption and osteoblast adhesion [9].

Better new bone formation and consolidation was found in the HAp specimens mixed with stem cells, as shown in Fig. 3 (c) and (d). HAp biocomposites added stem cells have higher bone formation ability than those of specimens without stem cells, and then most of bone defects are filled with new bone in these specimens at eight weeks after operation. Especially a complete bone consolidation is shown in the case of sc/n_HAp biocomposite. Many previous reports showed excellent bone formation by stem cells on scaffolds with coculture [10]. Also, it is well known that bone marrow consists of stem cells called mesenchymal stem cells and stromal stem cells, and mesenchymal stem cells can differentiate into osteoblasts, chondrocytes, adipocytes, and muscle cells, and can also differentiate into osteoblastic cells after culturing in osteoinductive medium [11]. Based on these results, it is suggested that the implanted stem cells with hydroxyapatite powders could survive, and differentiate into osteoblasts, or release various kinds of cytokines for bone formation in the presence of an immunosuppressive agent [12].

Bone formation ability in the transimplanted area within bone defects is shown in Fig. 4 by analyzing from the photographs of implanted central area. The ratio of the new bone formation area for a section was calculated using an image analyzing program of Image-proplus 5.1. As shown the results of radiographs for bone formation described in Fig. 3, bone formation ability of nanoscale HA biocomposites is higher than that of micronscale HA biocomposites. Also, biocomposites with stem cells show two times higher bone formation ability than those of biocomposites without stem cells, as shown in Fig. 4.

The significance of cells for bone formation in hydroxyapatite implants has been demonstrated previously [10-12]. Bone forming capacities of ectopically implanted hydroxyapatite scaffolds shows the significant difference according to the addition of stem cells, which indicates the crucial role of the cells in bone formation. In this study, sc/n_HAp biocomposite has better bone repair properties than that of others. So, it is important to add the stem cells and use the nanoscale HA particles in hydroxyapatite-based scafford to enhance the bone formation and repair ability of the biocomposite.

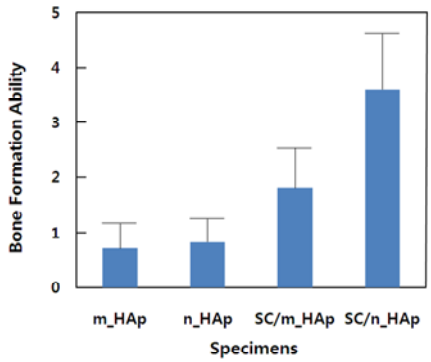

Fig. 4 Bone formation ability in the transimplanted area within bone defects compacted with (a) m_HAp biocomposite, (b) n_HAp biocomposite, (c) sc/m_HAp biocomposite and (d) sc/n_HAp biocomposite at eight weeks after operation

4 Summary and Conclusion

Bone formation and repair characteristics were studied in the biocomposites composed of hydroxyapatite (HA) powders and stem cells in rabbit long-bone defects and also investigated the size effects of hydroxyapatite particles on the formation of new bone. Nanoscale hydroxyapatite added biocomposites show the higher bone formation ability than that of micronscale HA biocomposites. Added stem cells into the biocomposite induced the homogeneous bone formation and enhanced the bone formation ability. Conclusively Nanoscale HA powder with stem cell is adequate biocomposite for new bone formation of the bone defects and it could provide a powerful scaffold for bone tissue engineering.

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