Promoted Bone Regeneration by Nanoparticle-Type Sustained Release System of BMP-2 in Hydrogel

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Introduction

The bone defect replacement matrix, necessary for the bone lost through disease or injury, aims to help new bone tissue grow and replace the damaged parts. The problems associated with the use of autogenous bone include the limit of the amount of bone harvested, the need for a second surgical site, and the possibility of the complications at the donor site [1]. Molecular therapy, a new approach, is to provide a critical initial input to activate regeneration processes by supplying BMP (bone morphogenetic protein), which is known to participate in bone regeneration, at the defect site for a certain period [2]. Here, in connection with this approach, we present the nanoparticle-hydrogel complex as a new bone defect replacement matrix, which is composed of the nanoparticles for the sustained release of BMP and the hydrogel for filling the bone defect site and playing a role as a matrix where new bone can grow. The nanoparticle, which is functionalized with heparin, is composed of biodegradable hydrophobic core (PLGA) with hydrogel surface layer (Pluronic F-127), and key molecule, heparin that is physically incorporated mainly into the Pluronic hydrogel layer [3,4]. The heparin-binding BMP-2 [5,6] was first loaded into the heparin-functionalized nanoparticles, and these BMP-2 loaded nanoparticles were added during fibrin gel formation to make nanoparticle-hydrogel complex. Then, the bone regeneration capacity of this BMP releasing complex was investigated using calvarial critical size defect model.

Experimental

The heparin-functionalized nanoparticles were prepared by a spontaneous emulsion solvent diffusion method [3]. Briefly, PLGA was dissolved in DMSO at room temperature, and then slowly added to deionized water containing Pluronic F-127 and heparin with vigorous stirring. The nanoparticles, which were sterilized by simple filtering with syringe filter, were collected by high-speed centrifugation, and resuspended in PBS. Resuspended nanoparticles was mixed with PBS containing BMP-2, and then incubated at 4 °C overnight with gentle rotation, to load BMP-2 into nanoparticles. Then, BMP-2 loaded nanoparticle suspension was subsequently dispersed in 70 μl of fibrinogen solution. To this, 70 μl of thrombin solution was added and incubated for 30 min at room temperature in a humid atmosphere to make the BMP-2 loaded nanoparticle-fibrin gel complex. Calvarial critical size defect, 8 mm in diameter, was surgically induced in rats, and the disc-type fibrin gels containing nanoparticles alone, BMP-2 alone, and BMP-2 loaded nanoparticles were implanted on the defect site. Fibrin gel itself was also implatend as a control. After 4 weeks, all rats were sacrificed, and the bone regeneration was evaluated by soft X-ray, MT staining, and calcium assay.

Results and discussion

The average values of new bone area and gray level of the initial defect site were quantified from soft X-ray results. In the defect sites filled with bare fibrin gel or the nanoparticle-fibrin gel complex without BMP-2, marginal regeneration near the initial defect boundary was observed. In contrast, the improved bone regeneration was observed in the defects with fibrin gel containing BMP-2, and much more increased regeneration was found for the BMP-2 loaded nanoparticle-fibrin gel complex (**Figure 1**).

The average thickness and the length of the new bone, measured using MT stained sections, showed negligible bone regeneration by bare fibrin gel or the nanoparticle-fibrin gel complex without BMP-2, but effectively covered defect sites were observed by the BMP-2 loaded fibrin gel as well as the BMP-2 loaded nanoparticle-fibrin gel complex (**Figure 2**).

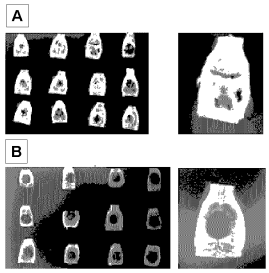

Figure 1. Soft X-ray images of calvarial defects, filled with the BMP-2 loaded nanoparticle-fibrin complex (A), and bare fibrin as a control (B) after 4 weeks.

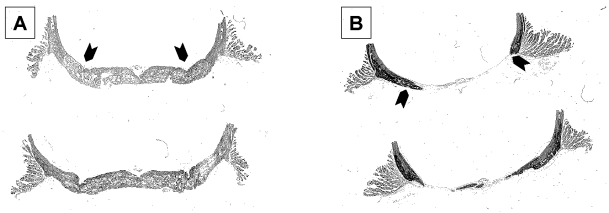

Figure 2. Representative sections of defects, filled with the BMP-2 loaded nanoparticle-fibrin complex (A), and bare fibrin as a control (B) after 4 weeks (arrows: defect margin; MT staining).

The bone marrow area in the defect site sections was also negligible for the case of bare fibrin gel or the nanoparticle-fibrin gel complex without BMP-2. However, the significant bone marrow area was found for the BMP-2 loaded fibrin gel, and more increase in bone marrow was found for the BMP-2 loaded nanoparticle-fibrin gel complex. Also, qualitatively more developed bone tissue, judged from the bone marrow type as well as collagen staining state, were observed for the case of the BMP-2 loaded nanoparticle-fibrin gel complex, compared to the case of the BMP-2 loaded fibrin gel. Calcium contents showed the similar pattern; very low values for bare fibrin gel or the nanoparticle-fibrin gel complex without BMP-2, whereas significantly high value for the BMP-2 loaded fibrin gel, and higher value for the BMP-2 loaded nanoparticle-fibrin gel complex.

Conclusions

The effective bone regeneration was achieved by the BMP-2 loaded nanoparticles in fibrin gel for the rat calvarial critical size defect model. The bone regeneration by the BMP-2 loaded nanoparticles in fibrin gel was clearly distinct from the bone regenartion by bare fibrin gel or the nanoparticle-fibrin gel complex without BMP-2 in all aspects. Even though bone regeneration effect was observed by the BMP-2 loaded fibrin gel, the quality of regenerated bone was significantly higher by the BMP-2 loaded nanoparticles in fibrin gel; the higher gray level in X-ray, the higher Ca content, and the larger bone marrow area as well as qualitatively more developed bone tisseue and bone marrow were found by the BMP-2 loaded nanoparticles in fibrin gel than those by the BMP-2 loaded fibrin gel. Therefore, the present system is a potential bone defect replacement matrix for clinical application.

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