Effective Bone Regeneration by Sustained Delivery of BMP-2 via Functional Nanoparticle-Fibrin Gel Complex

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Purpose: For the regeneration of the damaged bone tissues, new approach is to provide a critical initial input to activate regeneration processes by supplying BMP (bone morphogenetic protein), which has been known as a key molecule in bone regeneration, at the defect site for a certain period [1]. Here, in connection with this approach, we present the nanoparticle-hydrogel complex as a new bone defect replacement matrix, which is composed of the heparin-functionalized nanoparticles for the sustained release of the heparin-binding BMP-2 and hydrogel filling the bone defect site and playing a role as a matrix, in which new bone can grow. The role of incorporated heparin are expected to sustain the release of bioactive BMP-2, and also lead to the enhanced bone formation as heparin participates in the bone formation in vivo and in vitro.

Methods: The heparin-functionalized nanoparticle (NP) was prepared by a spontaneous emulsion solvent diffusion method [2]. 4 μg of BMP-2 was first loaded into NP, and the NP with BMP-2 was dispersed in fibrinogen solution. The fibrinogen solution including the BMP-2 loaded NP was placed into a disc-type mold. Fibrin gel formation was initiated by adding thrombin solution, and then incubated. The final disc-type gel was c.a. 2 mm in height and 8 mm in diameter. Calvarial critical size defect (CSD) with 8 mm-diameter was surgically induced in rats, and filled with the prepared implants for each experimental group (fibrin gel, fibrin gel containing NP alone, BMP-2 loaded fibrin gel, and BMP-2 loaded NP-fibrin gel complex). The animals were sacrificed after 4 weeks of surgery, and in vivo evaluation of bone formation was characterized by soft X-ray, histology, immunostaining, and mineral content analysis.

Results/Discussion: The release profile of BMP-2 loaded in the functional nanoparticle (NP)-fibrin gel complex was characterized; BMP-2 directly loaded in fibrin gel was released about 18 % in day 1 with a burst and about 4 % per day up to 9 days, followed by a slower release, whereas BMP-2 loaded into the NP-fibrin gel complex showed a linear release (about 1 % per day) with much lower burst (about 7 % in day 1) (Figure 1). Thus, the sustained release of BMP-2 from the NP-fibrin gel complex was dominated by the specific binding between BMP-2 and heparin, which resulted in the effective bone regeneration compared to the BMP-2 loaded fibrin gel in terms of the radiodensity, the pattern of bone and functional marrow, the degree of osteocalcin expression, and the contents of calcium and phosphate in the regenerated bone area (Figure 2). Even though some sort of bone regeneration was observed by the BMP-2 loaded fibrin gel, the regenerated bone matrix was immature and less mineralized than by the BMP-2 loaded NP-fibrin gel complex. In contrast, the defects filled with fibrin gel alone or the NP-fibrin gel complex without BMP-2 showed only marginal bone regeneration.

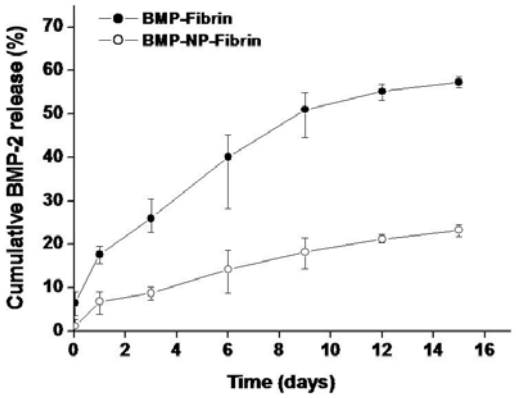

Figure 1. Cumulative release of BMP-2 loaded in fibrin gel alone (●: Fibrin) and the functional nanoparticle-fibrin gel complex (○: NP-Fibrin) (n = 3).

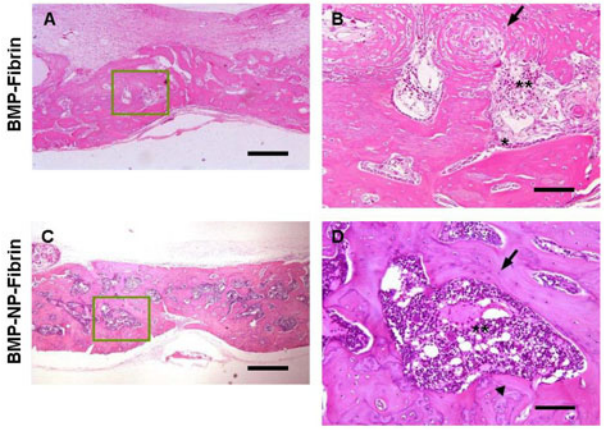

Figure 2. Histological analysis of the bone regeneration effect in the rat calvarial critical size defect model. H&E staining of tissue sections of each defects treated with the BMP-2 loaded fibrin gel (A,B: BMP-Fibrin), or the BMP-2 loaded NP-fibrin gel complex (C,D: BMP-NP-Fibrin).

Conclusions: These results indicate that the remodeling process of new bone developed by BMP-2 was significantly enhanced, and thus the mature and highly-mineralized bone was obtained by utilizing the functional nanoparticle-hydrogel complex. Therefore, the present system is a potential bone defect replacement matrix for clinical application.

References

1. Urist MR. Science 1965;150:893-9.
2. Chung YI, Tae G, Yuk SH. Biomaterials 2006;27:2621.

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