

Effect of basic fibroblast growth factor-loaded biomineral/agarose composite gels on bone regeneration
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Statement of Purpose: Currently, a many kinds of biomaterials have been researched and developed for bone regeneration. We also have created novel biomineral/agarose composite gels as biocompatible and biodegradable bone graft materials¹⁻⁴. These gels have osteoconductive property, but the applications of them alone have limitations on bone augmentation.

For effective tissue regeneration, the role of growth factors is also important⁵. Thus, it is very useful that biomaterials are suitable for carriers of growth factors. In the present study, we selected basic fibroblast growth factor (bFGF), which is one of the growth factors commonly used for tissue regeneration or angiogenesis.

The main purpose of the present study was to evaluate the effect of bFGF-loaded biomineral/agarose composite gels in bone regeneration. We confirmed that these gels were suitable for carriers of bFGF to release. In addition, we implanted bFGF-loaded biomineral/agarose gels in rat cranial bone defects, and analyzed by several means.

Methods: We selected hydroxyapatite (HAP) and calcium carbonate (CaCO₃) as biominerals, and prepared the HAP and CaCO₃/agarose composite gels (HA and Ca gels) by alternate soaking process^{1,2}. In vitro bFGF release study, we designed the following three groups: HA (HAP gel), Ca (Ca gel), Aga (Agarose gel). These gels having 10 mm diameter and 1 mm thickness were respectively immersed into 100 μ l of bFGF aqueous solution (10 μ g/ml, PBS buffer pH 7.4) for 12 h at 4°C. Then, the obtained gels were respectively incubated in 1 ml of PBS for 14 days at 37°C. After the prescribed times, each 100 μ l of the supernatant was collected and the amount of released bFGF from these gels was measured quantitatively by the ELISA method (as shown in Figure 1). In vivo study, we produced surgically 4 mm-diameter full-thickness cranial defects bilaterally in rat skull (Six weeks old male Wister/ST rat), and we used the gels having 4 mm diameter and 0.5 mm thickness. We designed the following four groups by the implanted materials; HA, Ca, Aga, Defect. In HA, Ca, and Aga groups, the bone defects were filled with bFGF-loaded gels at right side of skull and with non bFGF gels (gels only) at left side. In Defect group, the defect at right side was administered bFGF aqueous solution with carrier free and at left side was untreated. We assessed specimens by radiographic analyses using the microfocus-computed tomography (μ -CT) and the Peripheral Quantitative Computed Tomography (pQCT) and histological examination at 2, 4, 8 weeks after implantation.

Results: In vitro bFGF release study, the amount of bFGF retained in HA or Ca gel was larger than that in Aga gel. Moreover, the bFGF release from Aga gel started at burst,

and the release period was short. In contrast, HA or Ca gel showed sustained release kinetics of bFGF, and the release period was longer than that of Aga gel. The results of release study suggested that HA or Ca gel was superior to Aga gel as bFGF release carrier.

In vivo study, μ -CT and the pQCT were used to evaluate the quality of regenerating bone. At all weeks to assess specimens, the content of regenerating bone in HA and Ca was higher than that in Aga. In addition, bFGF-loaded gels were superior to non bFGF-loaded gels in terms of increasing bone formation. In defect, there was no significant difference between single application of bFGF with carrier free and untreated.

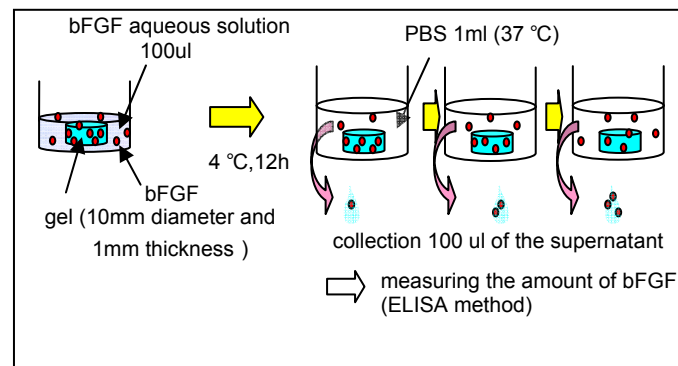


Figure 1. Schema of bFGF release from gels

Conclusions: The result of this study demonstrated that biomineral/agarose composite gels were suitable for sustained release carriers of bFGF, and these gels were useful for early bone regeneration in rat skull. We expect the application of these gels for drug delivery systems.

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