Bone Regeneration with Locally Controlled Application of Granulocyte colony-stimulating factor (G-CSF) in a bone defect of Rabbit Ulna

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Introduction: The capacity for the bone to regenerate is well known. However, the ability to restore form and function without scar formation is limited by injury size and the concept of the critical-sized defect reflects this physiologic phenomenon. Augmentation of bone deficits with various therapies has become common practice, yet, despite the effectiveness of these traditional treatments recognized liabilities have prompted a quest for alternatives.

It is well recognized that re-establishment of vascularity is an early event in fracture healing and appropriate vascularization is emerging as a prerequisite for bone development and regeneration.

Granulocyte colony-stimulating factor (G-CSF) is known to have various functions such as induction of proliferation, survival and differentiation of hematopoietic cells, as well as mobilization of bone marrow cells. We hypothesized that locally applied G-CSF may contribute to the promotion of healing in bone defects via revascularization and mobilization of bone marrow cells.

In this study, we designed a biodegradable cationized gelatin (CG) hydrogel with the ability to control the slow release of G-CSF. The objective of the study was to determine the effect of locally controlled application of G-CSF on the healing of a critical-sized bone defect in rabbit ulna.

Materials and Methods: A segmental bone defect (20 mm) was created at the diaphysis of the rabbit ulna in 24 Japanese white rabbits. Cationized gelatin (CG) hydrogel was used as the drug delivery system for G-CSF. The defects were filled in two groups as follows: Group G0 defects: CG hydrogel only; Group G5 defects: CG hydrogel with G-CSF (5μg). Radiographs of every defect were obtained at 2, 4, and 8 weeks after surgery. Semiquantitative radiographic evaluations were performed using Image J and radiographic healing was graded according to modified Cooks' grading scale. Toluidine blue staining was performed to see endochondral ossification. Histochemical staining for isolectin B4 was performed as an assessment of capillary invasion and immunofluorescent staining for osteocalcin were performed as an osteoblast marker.

Results: Radiographic findings suggested more bone formation occurred in group G5 than in group G0. Bone union was achieved in group G5 at 8 weeks, however no samples obtained bone union in group G0 (Fig.1).

Semiquantitative assessment revealed bone formation was significantly promoted in group G5 as early as 2 weeks (p<0.05). Additionally, radiographic grading also revealed bone formation was significantly promoted in group G5 at 4 and 8 weeks (p<0.05). Toluidine blue staining revealed more cartilaginous matrix was seen in group G5 as early as 2 weeks indicating endochondral ossification was promoted in group G5 (Fig.2).

Discussion: The present study demonstrated the locally controlled application of G-CSF promoted rabbit bone regeneration and suggested G-CSF promoted revascularization and osteoblast mobilization. G-CSF also has the potential for application to other situations requiring both bone repair and neovascularization.