Introduction:
Bone grafting are often used for large amounts of bone defects, which is one of the challenging for orthopaedic surgeons. Recently, bone tissue engineering has been attracted much attention as a new therapeutic technology which induces bone regeneration by making use of scaffolds, growth factors, cells, or their combination[1]. It is also well recognized that appropriate vascularization is emerging as a prerequisite for bone development and regeneration. Granulocyte colony-stimulating factor (G-CSF) is widely used clinically to elicit hematopoietic cells (HCS) mobilization and known to have various functions such as induction of proliferation, survival and differentiation of HCS, as well as mobilization of bone marrow cells. It has previously reported that CD34 cells mobilized by G-CSF promote rat fracture healing via vasculogenesis and osteogenesis[2]. In this study, we have designed a biodegradable gelatin hydrogel which has ability for the controlled release of G-CSF. The objective of the present study was to determine the effect of local controlled application of G-CSF using a biodegradable hydrogel on the healing of a critical-sized segmental defect in rabbit ulna.

Methods: Gelatin Hydrogels incorporating G-CSF were prepared by chemical cross-linking of aqueous gelatin solution with glutaraldehyde. The gelatin hydrogel sheets were cut into small squares (7 × 30 mm). G-CSF (5 μg/100 μL) were added by drops onto the freeze-dried gelatin hydrogel squares and left for overnight at 4 °C to allow them to impregnate the hydrogels. 24 Japanese white rabbits were used in this study. A segmental bone defect (20 mm) was created at the diaphysis of rabbit ulna. The defects were treated in two groups as follows: Group G0: defects were filled with gelatin hydrogel only, Group G5: defects were filled with gelatin hydrogel with G-CSF (5 μg). Every defect of the radiographs was obtained at 2, 4, and 8 weeks after surgery. Semiquantitative radiographic evaluations were performed using Image J and the radiographic healing was graded according to modified Cooks’ grading scale[2]. 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 (OB) marker. Capillary or OB density was morphometrically evaluated as the average value in 5 randomly selected fields of soft tissue in the peri-fracture site. Capillaries were recognized as tubular structures positive for isolectin B4. OBs were recognized as lining or floating cells positive for OC on new bone surface. All morphometric studies were performed by two examiners blind to treatment.

Results: Radiographic findings suggested that there were more bone formation in group G5, treated with hydrogels incorporated G-CSF (Fig. 1(a)-(c)) than in group G0 (Fig. 1(d)-(f)). Semiquantitative assessment (Fig. 2) and radiographic grading (Fig. 3) also revealed that bone formation was promoted when treated with hydrogels incorporated G-CSF, as early as 2 weeks. Toluidine blue staining revealed that more cartilaginous matrix was seen in group G5 at 2 and 4 weeks and it indicates that endochondral ossification is promoted in group G5. Histochemical staining for isolectin B4 showed that capillary density was significantly increased in group G5 at 2 weeks, although no significant changes were seen at 4 weeks (Fig. 5(a)-(c)). OB density were also increased in group G5 at 2 weeks(Fig. 6(a)-(b)) and these results indicate that OBs were mobilized into the bone defects via vasculogenesis.

Discussion: The present study demonstrates that local controlled application of G-CSF promotes rabbit bone regeneration. The reasons seem that the G-CSF promotes osteoblast mobilization via vasculogenesis. Combined with gelatin hydrogel, local applied G-CSF could be one of attractive therapy for large bone defect.

References:

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

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