In vivo evaluation of the biomineral/agarose composite gels as scaffolding materials

Yoshika Suzawa,[1] Takafumi Funaki,[2, 3] Soichi Iwai,[1] Yoshiaki Yura,[1] Junji Watanabe,[2, 4]
Takayoshi Nakano,[2] Mitsuru Akashi[2, 3, 4]
[1]Graduate School of Dentistry, Osaka University, Osaka, Japan
[2]Graduate School of Engineering, Osaka University, Osaka, Japan, [3]BioMedical Technology Hybrid, Ltd., Kagoshima, Japan
[4]21st Century COE Program "Center for Integrated Cell and Tissue Regulation", Osaka University, Japan

Introduction: In the fields of dental and oral surgery, bone grafting is required for osseous defects caused by removal lesions and cleft palate or preparation for implant placement. A patient's own bone is often used in the case. However, we would like to reduce the burden on patients by avoid collecting their own bone as possible. Thus, we have created novel biomineral/agarose composite gels[1-3] as scaffolding materials play a role as alternative biocompatible and biodegradable bone grafting filler materials for autogenous bone. We selected hydroxyapatite (HAp) and calcium carbonate ($CaCO_3$) as biominerals because they are common in nature and have biological activities and good cell compatibility. In the present study, we used these gels for animal model and analyzed by several means, with the purpose of evaluating their abilities as scaffolding materials for bone regeneration.

Methods: We prepared the HAp and $CaCO_3$/agarose composite gels (HA and Ca gels) by an alternate soaking process.[1, 2] We characterized the minerals by their X-ray diffraction (XRD) patterns and observed the morphology of them using a scanning electron microscope (SEM).We used the gels for the treatment of surgically produced bone defects in rat skull model (n=5). We designed 5 groups; HA (HA gel) -D (disk type), HA -H (homogenized type), Ca (Ca gel) -D, Ca -H and Defect (no filling materials). We assessed specimens by radiographic analyses using the microfocus-computed tomography (μ-CT) and histological examination 0, 2, 4, 8 weeks after implantation. We also performed radiological analyses using the Dual Energy X-ray Absorptiometry (DEXA) and the Peripheral Quantitative Computed Tomography (pQCT).

Results: We observed the typical peaks of HAp and $CaCO_3$ (calcite and vaterite) of the gels studied with XRD. We confirmed numerous HAp and $CaCO_3$ particles on the surface of the gels by the SEM views. The results of the μ-CT analysis show Figure 1. Each number represents the percentages of the opacity based on the native bone of the same area. We set the only radiolucent circular contours in the defect could be seen at 0 week. Then they indicated changes over time of images of new bone formation and the radiopacity was increased. At 4 weeks in the HA and Ca gels, there were new bone bridgings between the native bone and the implant sites. At 8 weeks, the Defect clearly still showed more radiolucent images than the HA and Ca gels. The results of the DEXA analysis denoted the same tendency of the pQCT analysis. Figure 2. shows the latter results as Bone Mineral Contents (BMC). HA and Ca gels indicated increasing BMC values with time and they were higher than the Defect after 8 weeks. A comparison of application forms revealed that HA gel, Disk type seemed to be consistently higher than the Homogenized type. The histological images of the HA and Ca gels after 4 weeks, new bone bridge formations derived from the bony borders observed directed towards the center of the defect, with the presence of osteoid tissue, osteoblasts and blood vessels lined around them.

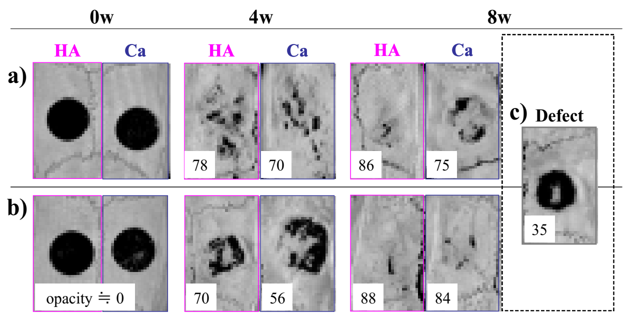

Figure 1. μ-CT images of the skull of a) Disk type, b) Homogenized type of HA and Ca gels after 0, 4, 8weeks and c) Defect after 8 weeks.

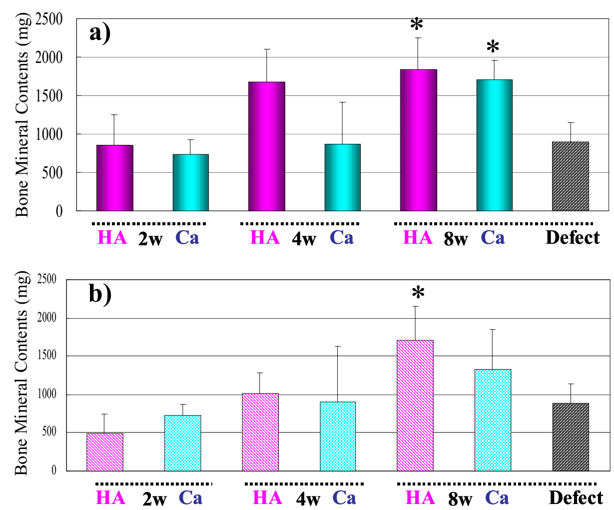

Figure 2. BMC by the pQCT analysis. a) Disk type and b) Homogenized type of HA and Ca gels after 2, 4, 8 weeks and Defect after 8 weeks. (n=5) ✻, p<0.01 versus Defect. Results are expressed as mean ± SD.

Conclusions: In this study, HA and Ca gels presented better results of several radiographic and radiological analyses than the Defect. Therefore, it is indicated that these gels are filler scaffolding materials with good osteogenic potential. In addition, we expect that these gels can respond to any cases as shape-independent materials because they showed the effects of bone regeneration regardless of their application forms.

References: 1. Taguchi T. Chem. Lett. 1998; 27: 711. **2.** Tabata M. J. Biomed. Mater. Res. Part B. 2005; 75B:378-386. **3.** Watanabe J. J. Biomed. Mater. Res. Part A. 2007; 83A:845-852.