Evaluation of osteogenic differentiation of rat marrow mesenchymal stromal cells on the biomineral/agarose composite gels

Yoshika Suzawa,[1] Norihiko Mizuta,[1] Takafumi Funaki,[2, 3] Soichi Iwai,[1] Yoshiaki Yura,[1]
Takayoshi Nakano,[2] Hajime Ohgushi,[4] Mitsuru Akashi[2, 3]
[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]Research Institute for Cell Engineering, National Institute of Advanced Industrial Science and Technology, Hyogo, 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] 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, they have biological activities and good cell compatibility. We have already got good results in vivo.[2-4] The purpose of the present study was to examine capacities of proliferation and differentiation of rat mesenchymal stromal cells (MSCs) on these gels used as scaffolding materials for bone regeneration.

Methods: We prepared the HAp and $CaCO_3$/agarose composite gels (HA and $CaCO_3$ 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). Rat bone marrow cells were obtained by flushing out from the bone shaft of the femora of 7 weeks old rats (Fischer 344). The adherent fibroblastic cells "MSCs" released from the dishes after confluence in the primary culture. MSCs were seeded on the bare Agarose (Agarose), HA and $CaCO_3$ gels and then cultured in medium supplemented with ascorbic acid and beta-glycerophosphate. We set additional conditions by presence or absence of dexamethasone (Dex (+) or (Dex (-)). The cultured MSCs on the gels were stained with Live/Dead Viabillity/Citotoxicity Kit and observed fluorescence microscopy on day 1 and 14 to investigate adhesive and proliferative capacities of them. They also stained alkaline phosphatase (ALP) activity staining on day 14, 21, 28. We used SEM to analyze the surface of the gels. Then we measured the quantities of adenosine triphosphate (ATP), ALP activities and the concentrations of osteocalcin (OC) on day 4, 7, 14, 21, 28.

Results: We observed the typical peaks of HA 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. Although we could see very few cells on the Agarose gels, quite a lot of cells were seen on the HA and $CaCO_3$ gels (Figure 1). Additionally, extensions of the cells on the composite gels on day 14 were characteristic findings. SEM images of MSCs on the gels were the same as them. The quantities of ATP (Figure 2), ALP activities, the concentrations of OC and ALP staining properties (Figure 3) of the MSCs on the HA and $CaCO_3$ gels cultured in the presence of Dex were all rising with time. On the other hand, MSCs seeded on the Agarose gels or cultured in the absence of Dex appeared little changes.

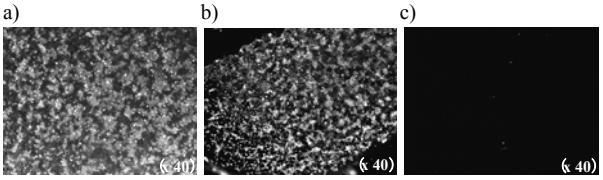

Figure 1. Fluorescence microscope images of MSCs in the surface of the gels on day 1; a)HA gel, b)$CaCO_3$ gel, c)Agarose gel.

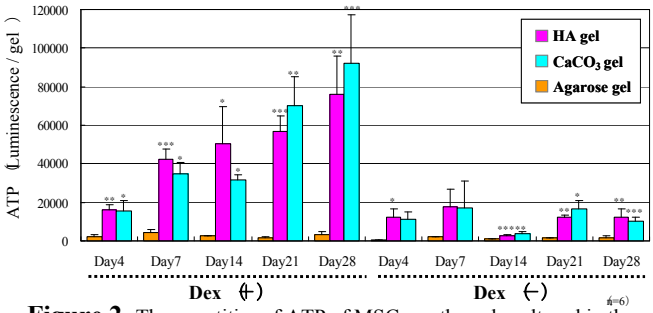

Figure 2. The quantities of ATP of MSCs on the gels cultured in the presence or absence of Dex. (n=6), ∗p<0.05, ∗∗p<0.01, ∗∗∗p<0.001 versus Agarose gels. Results are expressed as mean ± SD.

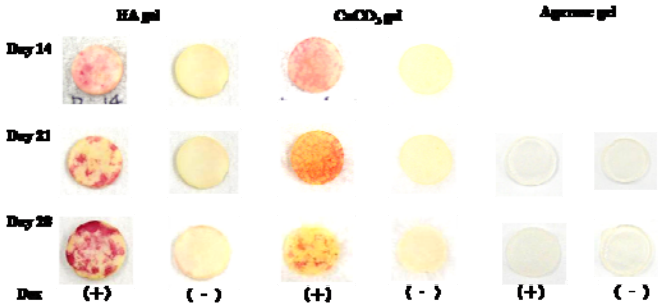

Figure 3. ALP staining of MSCs on the gels cultured in the presence or absence of Dex.

Conclusions: In this study, HA and $CaCO_3$ gels presented better results of several analyses to evaluate cell adhesion, proliferation and differentiation than the Agarose gels. Therefore, it is indicated that these biomineral/agarose composite gels are filler scaffolding materials with affinity for cells and good osteogenic potential.

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. **4.** Suzawa Y. J. Biomed. Mater. Res. Part A. 2009; in press.