Ectopic Bone Formation by Combinational Technology of Gene Therapy and Tissue Engineering

Hossein Hosseinkhani[1], Mohsen Hosseinkhani[2], Ali Khademhosseini[3,4], Hisatoshi Kobayashi[5,6], Tony Azzam[7], Avi Domb[7]

[1]International Center for Young Scientists (ICYS), National Institute for Materials Science (NIMS), Tsukuba, Japan
[2]Department of Cardiovascular Medicine, Graduate School of Medicine, Kyoto University Hospital, Kyoto, Japan
[3]Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, USA
[4]Center for Biomedical Engineering, Brigham and Women's Hospital, Harvard Medical School, Cambridge, USA
[5]Biomaterials Center, National Institute for Materials Science (NIMS), Tsukuba, Japan
[6]Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, Tokyo, Japan
[7]Department of Medicinal Chemistry, The Hebrew University-Hadassah Medical School, Jerusalem, Israel

Statement of Purpose: Bone regeneration by gene transfer into MSC has been reported [1]. These reports have mainly used a retrovirus, or adenovirus vector carrying human BMP-2, -4, or -7 as the therapeutic gene. However, considering the immunological and safety issues of viral vectors, necessity in the development of non-viral vector systems has been increasingly magnified. The objective of this study is to enhance *in vivo* ectopic bone formation by combinational technology of 3-dimensional (3D) tissue engineered scaffolds and non-viral gene carrier.

Methods: As the mesenchymal stem cells (MSC) scaffold, collagen sponge reinforced by incorporation of poly(glycolic acid) (PGA) fibers was used. As a material of non-viral vector, spermine was chemically introduced to dextran to obtain a cationized dextran capable for polyion complexation of plasmid DNA. A complex of the cationized dextran and plasmid DNA of BMP-2 was impregnated into the scaffold. MCS were seeded into scaffold and cultured by a static (tissue culture plate) method and bioreactor perfusion culture. Different culture systems, such as static and perfusion culture methods, were used to evaluate the effect of the culture system on the transfection efficiency of cationized dextran-plasmid DNA encoding BMP-2 complex on MSC. The osteoinduction activity of the PGA-reinforced collagen sponges seeded with PBS, MSC, naked plasmid DNA of BMP-2, cationized dextran-plasmid DNA of BMP-2 complex, and transfected MSC with static were studied following the implantation into the back subcutis of rats in terms of histological and biochemical examinations.

Results/Discussion: Figure 1 shows soft x-ray photographs of rat subcutis 1, 2, 3, and 4 weeks after implantation of scaffolds. Apparently, the edge of implants and the subcutaneous tissue became radiopaque strongly on the x-ray film when MSC transfected by cationized dextran impregnated into saccfold, whereas no radiopaque area was found at the scaffolds and skin when implanted with PBS, naked plasmid DNA-BMP-2, and MSC. The radiopaque area was larger for MSC transfected by cationized dextran than naked plasmid DNA. Homogeneous bone formation was histologically observed throughout the sponges seeded with naked plasmid DNA and cationized dextran-plasmid DNA of BMP-2 complex, although the extent of bone formation was higher for cationized dextran-plasmid DNA of BMP-2 complex (data not shown).

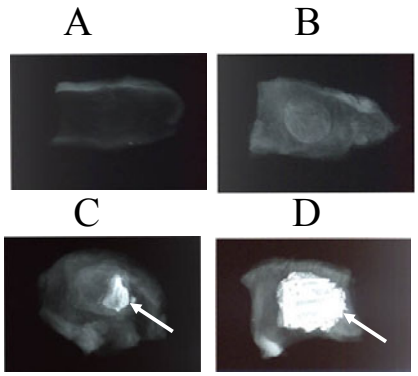

Figure 1. Soft x-ray photographs of ectopically formed bone 1, 2, 3, and 4 weeks after implantation of collagen sponges with PGA fiber incorporation at collagen/PGA fiber weight ratio of 0.2 impregnated with PBS (A), MSC (B), naked plasmid DNA (C), and transfected MSC by ationized dextran-plasmid DNA-BMP-2 complex (D). The dose of plasmid DNA was 10 μg/ml. Arrows indicate the newly formed bone.

Conclusions: We conclude that combination of plasmid DNA-impregnated PGA-reinforced collagen sponge and the perfusion method was promising to promote the *in vitro* gene expression for MSC and *in vivo* ectopic bone formation.

References:

1. Lou, et al., J Orthop Res 1999; 17**:** 43-50.