Hybrid-type bone substitute composed by porous hydroxyapatite and hBM-MSCs

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Statement of Purpose: We are involved in the research and development of bone substitutes using tissue engineering with the aim of decreasing the level of surgical invasion for patients requiring multiple bone transplants. We created a hybrid-type bone substitute for use in grafts and compared bone formation in bone defects. It has been reported that 5 mm diameter, 2 mm thick discs of hydroxyapatite (HA) ($Ca_{10}[PO_4]_6[OH]_2$; Pentax, HOYA, Japan) with porosity of 85% are useful as a scaffold. This type of HA was therefore used to incorporate the cells in this study. We created a hybrid-type bone substitute using human bone marrow-derived mesenchymal stromal cells (hBM-MSCs) and evaluated its osteogenic capability in bone tissue *in vivo*. The synergistic effect of hybrid-type bone substitute which has a osteo-conductivity by HA and osteo-inductility by hBM-MSCs was examined.

Methods: We used MSCs that had undergone long-term cryopreservation at −80°C. The cells had originally been obtained after primary culture of the surplus iliac bone tissue collected during secondary bone grafting performed in the Department of Plastic and Cosmetic Surgery at the Kitasato University School of Medicine. Eleven samples were obtained from 6 male and 5 female patients aged 5–18 years (mean age: 8.9 years). No subjects had any infections or diseases of note. We recultured hBM-MSCs *in vitro* following cryopreservation for ≥10 years, divided the cells into osteogenic differentiation and nondifferentiation MSC groups, and compared the cells in the two groups. *In vitro* expression of osteoblast markers was then measured. Furthermore, we created a hybrid-type bone substitute and transplanted it into 5-mm diameter holes in the left and right sides of the skulls of 8-week-old male nude rats. Eight weeks later, the skulls were examined by micro-computed tomography (MicroCT). We then performed microscopic analysis of grafts after hematoxylin and eosin (HE) staining and calculated the surface ratio. Immunohistochemical staining with an anti-human osteocalcin antibody was al. HEso performed and NEO STEM™ expression was confirmed by fluorescence microscopy.

Results: With regard to osteoblast marker expression, alkaline phosphatase (ALP) and osterix were significantly elevated in the osteogenic differentiation MSC group (Figure 1). MicroCT revealed osteogenesis in the hybrid-type bone substitute grafts (Figure 2). HE staining clearly indicated new bone formation within porous hydroxyapatite (HA) in the hybrid-type bone substitute grafts. Immunohistochemical staining indicated anti-human osteocalcin antibody expression and NEO STEM™ expression in the sites exhibiting new bone formation (Figure 3). The volume of bone formation was also significantly higher in the osteogenic differentiation MSC group than in the nondifferentiation MSC group (Figure 4). ($P < 0.05$).

Conclusions: We observed excellent osteogenic capability of porous HA and hybrid-type bone substitute,and we believe that our hybrid-type bone substitute can be adapted for future clinical application.

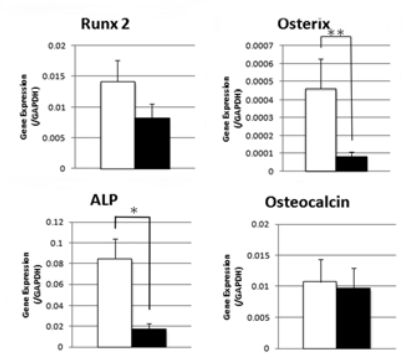

Figure 1. Osteoblast marker expression according real time RT-PCR
Significant differences were noted for ALP and osterix expression but not for Runx2 or osteocalcin (*p<0.05, **p<0.01)

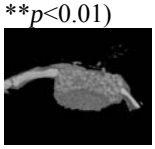

Figure 2. Micro CT images
Hybrid-type bone substitute grafting

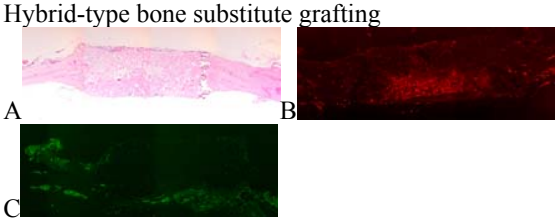

Figure 3. Histological and immunohistochemical staining
A. HE staining: Clear new bone formation in HA pores of the hybrid-type bone substitute.
B. Immunohistochemical staining: Anti-human osteocalcin antibody
C. Immunohistochemical staining: NEO STEM™

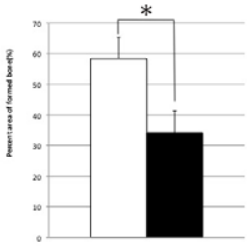

Figure 4. Bone formation volume

References: Shimakura Yasuhito. J Craniofac Surg 2003;14:108-116. Matsuo Aoi. J Craniofac Surg 2008;19:693-700. Takase Chikara. Kitasato Med J 2010; 40: 122-128.