Availability of Hybrid-type Bone Substitute by Cryopreserved Human Bone tissue Derived Mesenchymal Cells

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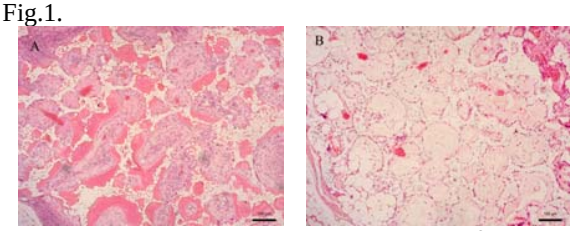
Statement of Purpose: Bone grafting in the alveolar defect is one of the most important therapeutic modalities for patients with dental implant treatment . However, with an ambulatory surgery, harvesting a sufficient amount of bone is difficult, and repeated operations are often required because deformation of the alveolar defect may occur because of the grafted bone absorption and periodontal disease, which imposes a heavy burden on the patients. The burden may be reduced if the banking of human bone tissue derived mesenchymal cells (BMCs) could be made possible, that is, if cryopreserved autologous BMCs, those that have been harvested from the patient's own bone tissue, could be cultured and expanded with serum-free medium and can be thawed and cultivated for grafting at a later date. In the current study, #1 the availability of a hybrid-type bone substitute by thawing and cultivating BMCs that have been cryopreserved for more than 10 years, and hydroxyapatite disk [$Ca_{10}(PO_4)6(OH_2)$; Pentax, Tokyo, Japan; porosity: 85%, pore diameter: 50-300 μm, diameter: 5 mm, and thickness: 2 mm], and #2 the utilization of serum-free medium [STK; DS Pharma Biomedical, Osaka, Japan] for culture, were investigated.

Methods: :#1 Osteogenic Potentialand Cytogenetic safety of BMCs after long-term Cryopreservation: In the 7 specimens of the cryopreserved (for more than 10 years) BMCs, we investigate the alkaline phosphatase (ALP) activity, calcium-producing capability and gene expressions (*Runx2, osterix, osteocalcin*) and compare non-differentiated group (Dif(-)-group) with osteogenic differentiated group (Dif(+)-group) statistically. we transplant both hybrid-type bone substitutes into subcutaneous of back of nude mice and investigate osteogenesis. we examined chromosomal morphology, presence or absence of abnormalities of the p53 tumor-suppressor gene, and expression of the myc oncogene.**#2 The Utilization of Serum-Free Medium for Culture by fresh (non-cryopreserved) BMCs :** In the *in vitro* study, alkaline phosphatase, calcium, and osteocalcin were used to assess the osteogenic differentiation of formed osteoblasts. This hybrid-type bone substitute was used to histologically examine the osteogenic potential *in vivo* of formed osteoblasts.

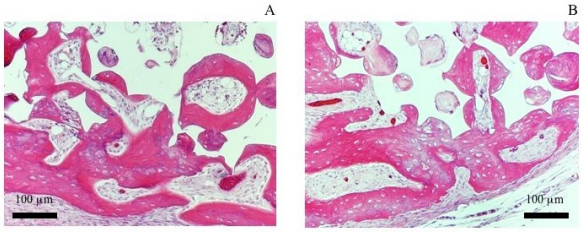
Results: #1 There are significantly more high ALP activity and calcium-producing capability in Dif(+)-group than in Dif(-)-group *in vitro*. The generated bone was positive for fluorescent immunostaining of human osteocalcin, which confirmed that the new bone was made from human-derived cells.only *in vivo* (Fig.1). The morphologic and gene abnormality were not accepted by cryopreserved BMCs. **#2** When cultured with serum-free medium, BMCs exhibited osteogenic potential *in vitro* and *in vivo*. No statistically significant difference was found in osteogenic potential between BMCs cultured with serum-free medium and with FBS-added medium. Specimens for both serum-free medium and FBS-added medium indicated the presence of bone matrix, bone lacunae, and osteocytes, showing the good formation of bone tissue(Fig2).

Fig.1.



HE staining in the *in vivo* study: New bone formation was observed in osteogenic differentiated group, while not observed in non-differentiated group.
A: osteogenic differentiated group at 10weeks.
B: non-defferentiated group at 10weeks

Fig.2



HE staining in the *in vivo* study: Specimens for both serum-free medium and FBS-added medium indicated the presence of bone matrix, bone lacunae, and osteocytes, showing the good formation of bone tissue at 8weeks.

Conclusions:

1.With BMCs cryopreserved more than ten years, osteogenic potential was maintained. The BMCs cryopreserved more than ten years may be clinically useful.

2. Osteogenic potential of BMCs was confirmed.when cultured with serum-free medium.

3. Hybrid-type Bone Substitute by cryopreserved human bone tissue derived mesenchymal cells will be useful.

References:

1. Kumazawa K, Sugimoto T, Yamazaki Y, Takeda A, Uchinuma E: Osteogenic Potential, Multipotency, and Cytogenetic safety of human bone tissue-derived mesenchymal stromal cells (hBT-MSCs) after long-term cryopreservation. The Kitasato Medical Journal (In press) 2013.

2.Ishiguro M ,Yamazaki Y, Baba K、Kumazawa K, Sugimoto T, Takeda A, Uchinuma E:Assessment of the Osteogenic Potential of Maxilla-Derived Mesenchymal Stromal Cells and the Utilization of Serum- Free Medium for Culture Thereof. The Kitasato Medical Journal (In press) 2013.