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論 文 審 査 の 結 果 の 要 旨

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論　文　題　目
Combination of Carbonate Hydroxyapatite and Stem Cells from Human Deciduous Teeth Promotes Bone Regeneration by Enhancing BMP-2, VEGF and CD31 Expression in Immunodeficient Mice
（乳歯歯髄由来間葉系幹細胞および炭酸アパタイト担体を併用した骨再生治療への応用）

論文審査担当者

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〔論文審査の結果の要旨〕

Regenerative medicine has been developed as a new therapeutic alternative to transplantation and allows the regeneration of dysfunctional tissues using tissue-specific stem cells. Tissue engineering is a widely used approach for the regenerative treatment of parenchymal tissue defects, including that of bone tissue. The key to cell-based regenerative medicine is stem cells, and currently, one of the most widely utilized stem cells in regenerative medicine are somatic mesenchymal stem cells (MSCs). Many studies have suggested that various types of MSCs have the potential for bone regeneration in the craniofacial area. Stem cells from human deciduous teeth (SHEDs) are expected to be more effective than that of dental pulp (DPSCs) because SHEDs are easily obtained by less invasive techniques, as deciduous teeth are usually discarded. Based on this background, the research group of this applicant has intensively focused on SHEDs. Artificial bone materials, such as hydroxyapatite (HAP) and β-tricalcium phosphate composites, have been applied in the orthopaedic and prosthodontic fields. Un-sintered carbonated hydroxyapatite (CAP), in which a part of the HAP components is replaced by carbonate, is the main inorganic component of bone and teeth in a living body and has excellent bio-affinity and shape retention. In addition, because it contains carbonate, it is highly soluble in acid and susceptible to resorption by osteoclasts. Therefore, this study was conducted to evaluate bone regeneration ability after SHED transplantation, CAP transplantation, and the transplantation with a combination of SHEDs and CAP in immunodeficient mice with skull bone defects.

In this study, upper right primary canine teeth were extracted from 11-year-old male patients undergoing orthodontic treatment at Hiroshima University Hospital; the SHEDs were isolated and cultured. To prevent immunogenic and graft rejections, immunodeficient mice (n = 5 for each group/4 groups) were used and artificial calvarial bone defects (5 mm in diameter) were created. To the defects, SHEDs and CAP granules were transplanted with an atelocollagen sponge as a scaffold. The CAP used in this study was subjected to a grinding with a nano-grinder to produce granules with a mean particle size of approximately 110 nm. The mean particle size was validated by scanning electron microscopy and particle size analysis. Bone formation was 3-dimensionally analyzed by microcomputed tomography at 4 and 12 weeks after transplantation. Hematoxylin and eosin (H&E) and Masson trichrome (MT) staining were performed to assess bone formation, whereas immunohistochemical (IHC) staining was done using specific antibodies for bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor-A (VEGF-A), and cluster of differentiation (CD) 31.

The results showed that at $t = 0$, in all groups, no bone was observed in the calvarial area. In the control group at 4 and 8 weeks, wound closure at the bone defect area was seen,

but only a few newly regenerated bones were observed. At the centre of the calvarial defects in the other groups, significant wound closure with newly generated bone was observed relative to that in the control group. The SHED+CAP group showed significantly larger bone volume at 4 and 8 weeks after transplantation compared to that of the other groups. With H&E staining, newly formed bone was clearly observed in the SHED, CAP, and SHED+CAP groups. In contrast, only a few newly formed bone was observed in the control group.

MT staining revealed that the percentage of mature bone areas was significantly higher in the SHED+CAP transplantation group than in all other groups. The results of IHC staining showed that the SHED+CAP group had a prominent BMP2-stained area at the centre of the scaffold. It is shown by IHC staining that the SHED+CAP transplantation group showed significantly enhanced percentage of VEGF-A expressing areas in the transplantation site relative to those of the other groups. In conjunction with CD31 expression, a significant increase in new blood vessels was observed in the SHED+CAP group, but only a few new blood vessels were observed in the control group. These histological and immunohistochemical analyses suggested that combining SHEDs with CAP served to enhance the expression of BMP-2, VEGF, and CD31, and to promote bone regeneration.

To reduce inflammatory responses and achieve optimal resorption behaviour, using materials with a composition similar to natural bone minerals is important. CAP resorption properties are due to the tendency of carbonates to reduce crystallinity in the apatite structure, thereby the CAP is expected to promote bone remodelling or turnover. CAP undergoes osteoclastic resorption; therefore, its absorption rate closely matches that of natural bone. Making apatite carriers into smaller-sized granules may facilitate transplantation and lead to earlier bone formation and carrier resorption. To this end, in the present study, CAP granules were crushed to an average size of 110 nm, and CAP transplantation was performed in calvarial defects in immunocompromised mice. Dental-derived MSCs are easily obtained non-invasively. The high cell proliferation capacity of MSCs may favour tissue regeneration during transplantation and easily ensure the number of MSCs before transplantation. In addition, a previous in vitro study demonstrated that SHED have a higher expression of BMP-2 compared to that of BMSCs or DPSCs. In this study, the expression levels of VEGF-A and CD31 were significantly higher in the SHED and SHED+CAP groups than in the other two groups. The present study suggests that, compared to SHED transplantation or CAP transplantation alone, transplantation of SHED–CAP in combination results in the enhanced expression of BMP-2 and VEGF-A and more effective induction of angiogenesis and osteogenesis.

As described above, this dissertation clearly demonstrates that the transplantation of SHEDs in combination with CAP granules serve to induce more effectively new bone formation than the transplantation of either SHED or CAP alone does and that the transplantation of SHEDs and CAP granules can enhance the expression of BMP-2 and promote angiogenesis by enhancing the expression of VEGF-A and CD31. Accordingly, this study suggests that transplantation of SHEDs combined with CAP may be a promising tool for bone regeneration in alveolar defects.

Therefore, all the committee members admitted that this dissertation has sufficient value to confer the Doctor of Philosophy (Ph.D) to Nurul Aisyah Rizky Putranti.