論　文　内　容　要　旨

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）

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主指導教員：谷本　幸太郎 教授

（医系科学研究科　歯科矯正学）

副指導教員：加来　真人 教授

（医系科学研究科　生体構造・機能修復学）

副指導教員：内藤　真理子 教授

（医系科学研究科　口腔保健疫学）

Nurul Aisyah Rizky Putranti

（医系科学研究科　医歯薬学　専攻）

(Introduction) 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 regeneration 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 in regenerative medicine is the somatic mesenchymal stem cell (MSCs). Many studies have suggested that various types of MSCs have the potential for bone regeneration in the craniofacial area. The effectiveness of SHED compared to that of DPSCs is expected because SHED is easily obtainable by less invasive techniques, as deciduous teeth are usually discarded. Based on this background, our group has repeatedly focused on SHED in our investigations. Artificial bone materials, such as hydroxyapatite (HAP) and ß-tricalcium phosphate composites, have been applied in the orthopaedic and prosthodontic fields. Un-sintered carbonated hydroxyapatite (CAP), in which a part of the HAP crystal structure is replaced by carbonic acid, 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 carbonic acid, it is highly soluble in acid and susceptible to resorption by osteoclasts. Therefore, this study aimed to compare bone regeneration ability and evaluate bone regeneration after SHED transplantation, CAP transplantation, and the combination of SHED and CAP transplantation in immunodeficient mice with skull bone defects.

(Material and methods) 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) with artificial calvarial bone defects (5 mm in diameter) were developed, and stem cells from human deciduous teeth (SHEDs) and carbonate hydroxyapatite (CAP) granules were transplanted with an atelocollagen sponge as a scaffold. The CAP used in the experiments was subjected to a grinding procedure using a nano-grinder to produce a mean particle size of approximately 110 nm. The mean particle size was validated by scanning electron microscope (SEM) and particle size analysis. A 3D analysis using microcomputed tomography were performed, at 4 weeks and 12 weeks after transplantation, histological (H&E staining and MT staining) and immunohistochemical evaluations of markers of bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF), and cluster of differentiation (CD) 31 were performed.

(Result) At t0, in all groups, no new bone formation was observed in the calvarial area. In the control group at t1 and t2, 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 had significantly greater 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 areas were observed in the control group.
In the MT staining, the percentage area of mature bone was significantly higher in the SHED+CAP transplantation group than in all other groups. IHC staining for BMP-2 and for VEGF and CD31 was performed to observe bone formation and angiogenesis. The SHED+CAP group had a prominent BMP2-stained area at the centre of the scaffold. IHC staining for VEGF-A were performed, the SHED+CAP transplantation group showed significantly enhanced percentage of VEGF-A area in the transplantation site relative to those of the other groups. After IHC staining for 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. There were significantly more blood vessels in the SHED+CAP group. Histological and immunohistochemical evaluations showed that combining SHEDs and CAP enhanced the expression of BMP-2, VEGF, and CD31, and promoted bone regeneration.

(Discussion) To reduce inflammatory responses and achieve optimal resorption behaviour, using a material with a composition similar with natural bone minerals is important. CAP resorption properties are due to the tendency of carbonates to reduce crystallinity in the apatite structure, thereby promoting bone remodelling or turnover. CAP undergoes only 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 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, our 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 and CD31 were significantly higher in the SHED and SHED+CAP groups than in the other two groups. Our study suggests that, compared to SHED transplantation or CAP transplantation alone, SHED–CAP transplantation can enhance BMP-2 and VEGF expression and induce superior angiogenic and osteogenic potential.

(Conclusion) Transplantation of SHED in combination with carbonate apatite granules can induce more new bone formation than transplantation of either SHED or carbonate apatite alone does, and transplantation of SHED and carbonate apatite granules can enhance the expression of BMP-2 and promote angiogenesis by enhancing the expression of VEGF and CD31. This study demonstrates that the combination of SHEDs and CAP transplantation may be a promising tool for bone regeneration in alveolar defects.