学位論文　全文要約

骨殻付き軟骨原基様組織は速やかな軟骨内骨化を生じることで骨再生を促進する

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Background

No effective treatment has been established for periodontal tissue defects caused by periodontitis, especially for irreversible large-scale bone defects such as class III furcation lesions and horizontal bone defects. Recently, we have established a three-dimensional culture clumps of mesenchymal stem cells (MSCs)/extracellular matrix (ECM) complex (C-MSC) that consist of self-produced ECM. C-MSC can be transplanted into tissue lesions without artificial scaffold to induce tissue regeneration. In addition, the cellular properties and characteristics of the ECM in C-MSCs can be regulated in vitro. Most bone formation in the developmental and healing process is due to endochondral ossification, which occurs after bone collar formation surrounding cartilage derived from MSCs. Thus, to develop a rapid and reliable bone-regenerative cell therapy, the present study aimed to generate cartilaginous tissue covered with a mineralized bone collar-like structure from human C-MSCs by combining chondrogenic and osteogenic induction.

Methods

Human bone marrow-derived MSCs were cultured in a xeno-free/serum-free (XF) growth medium. Confluent cells that formed cellular sheets were detached from the culture plate using a micropipette tip. The floating cellular sheet contracted to round clumps of cells (C-MSCs). C-MSCs were maintained in XF-chondro-inductive medium (CIM) and XF-osteo-inductive medium (OIM). The biological and bone-regenerative properties of the generated cellular constructs were assessed in vitro and in vivo.

Results

In vitro, C-MSCs cultured in CIM/OIM formed a cartilaginous tissue covered with a mineralized matrix layer, whereas CIM treatment alone induced cartilage with no mineralization. Furthermore, VEGF message expression was increased in the C-MSCs cultured in CIM/OIM. *In vivo*, transplantation of the cartilaginous tissue covered with a mineralized matrix induced a more rapid bone reconstruction via endochondral ossification in the severe combined immunodeficiency mouse calvaria defect model than that of cartilage generated using only CIM. After 4 weeks of implantation, earlier bone formation was observed. Over time, as blood vessels flowed into the graft, the safranin-positive cartilage matrix disappeared, TRAP-positive cells were observed near the vessels, bone formation was observed, and bone marrow structure was observed inside. The process of bone tissue regeneration shows aspects of endochondral ossification. Furthermore, bone tissue formation was observed regardless of the implantation site.

Conclusions

These results highlight the potential of C-MSC culture in combination with CIM/OIM to generate cartilage covered with a bone collar-like structure, which can be applied to novel bone-regenerative cell therapy.

Material from: Morimoto S., Kajiya M., Mizuno N. et al. A Cartilaginous Construct with Bone Collar Exerts Bone-Regenerative Property Via Rapid Endochondral Ossification.
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