Osteoblastic Differentiation and Mineralization Ability of Periosteum-Derived Cells Compared With Bone Marrow and Calvaria-Derived Cells

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Purpose: Clinically, bone marrow stromal cells (BMCs) are the most common source of osteoprogenitor cells. Its harvest process, however, is invasive to patients. Previous reports have shown the potential advantages of using periosteum-derived cells (PDCs) as a source of cell-based transplant therapy. The objective of our study was to characterize the osteoblastic differentiation and mineralization ability of PDCs versus BMCs and osteoblasts (OBs).

Materials and Methods: BMCs, OBs, and PDCs were isolated from 4-week-old male Wistar rats. To characterize the differentiation ability of the cells, MTS assay, alkaline phosphatase (ALP) activity staining, picrosirius red staining, and alizarin red staining were performed. Immunohistochemistry was performed on paraffin sections of calvarial periosteum to determine the presence of mesenchymal stem cells.

Results: PDCs showed the greatest proliferation rate compared with BMCs and OBs. Matured collagenous matrix formation was observed in PDCs and BMCs. ALP-positive cells and in vitro mineralization were evident in all cell types analyzed; however, that of PDCs was not comparable to that of the OBs and BMCs. Immunohistochemistry revealed the presence of STRO-1–and CD105-positive cells in the cambium layer of the periosteum.

Conclusions: PDCs have remarkable proliferative ability, but contain only a small population of osteogenic cells compared with BMCs and OBs. Although cell activity can be affected by various factors, such as age, culture condition, additives, and so forth, PDCs are likely not the source of OBs, although they might provide matrices that indirectly aid in bone formation.
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In dental practice, many patients will have bone quantity insufficient for implant treatment. In such cases, bone augmentation techniques have enabled us to extend the application range of treatment. Tissue engineering and cell-based therapies hold great promise for treating difficult aspects of bone regeneration. The use of tissue engineering can lead to enhancement of the regeneration process and bone augmentation.[1-5] Manipulation of tissue engineering involves the combination and application of growth factors, scaffolds, and/or cell transplantation, which, together, constitute the reference standard for bone regeneration.[6] Various types of cell sources, such as bone marrow, dental pulp, periodontal ligaments, and periosteum, have been proposed for this purpose, because these cells are known to contain a certain

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population of mesenchymal stem cells (MSCs) and osteogenic cells.[1,5,7-9] Of the proposed cells, bone marrow stromal cells and periosteum-derived cells (PDCs) have already been tested in clinical trials.[10-14] Regarding the donor site, in contrast to aspirating bone marrow, harvesting periosteum is not only easier for dentists, but also less invasive for patients. At Niigata University Medical and Dental Hospital (Niigata, Japan), cultured periosteal sheet transplantation, in which small pieces of periosteum were collected from the patient, expanded in vitro, and then applied to the periodontal defect with suitable carriers or scaffolds (ie, hydroxyapatite particles, platelet-rich plasma), [10,12] has been used in clinical trials. The results from these studies have demonstrated that autologous implantation of the cultured periosteal cells combined with other elements, facilitates successive bone regeneration. To minimize the harvesting process and, subsequently, the amount of autologous bone required, autogenous periosteal cells were grafted and cultured in vitro. Using this procedure, more than 50 cases of bone augmentation clinical trials have been performed in our hospital with the aim of improving the newly formed bone quality for dental implant treatment purposes. The results have been encouraging to date, prompting successive trials.[14]

The periosteum is a specialized connective tissue that forms a fibrovascular membrane that covers bone surfaces, with the exception of areas at which articular cartilage, muscle and tendon insertion, and sesamoid bone surfaces are present.[15] This tissue can be separated into 2 distinct layers: an outer "fibrous layer" that contains fibroblasts and Sharpey's fibers and an inner "cambium layer" that contains a high number of osteogenic precursor cells.[9,16,17] The periosteum contains not only osteogenic cells, but also multipotent MSCs capable of differentiating into osteoblasts (OBs), chondrocytes, or adipocytes. Moreover, it has been reported that once the periosteum cells have been removed from the tissue, they have the potential to proliferate at much greater rates and in a more scattered pattern than bone marrow-derived progenitor cells.[15,18,19] In live isograft bone transplantation, removal of the periosteum has led to poor graft incorporation. In contrast, preservation of the periosteum has led to a marked induction of neovascularization and later integration of the transplant,[3] indicating the importance of the periosteum in the nutritional supply. Furthermore, these clinical and experimental data have demonstrated that the periosteum plays an important role in bone healing and remodeling.

The osteoblastic differentiation and mineralization ability of PDCs[11] and the good prognosis of bone augmentation with use of the periosteal sheet has been previously reported.[14] Only a few comparative studies using different cell sources and culture conditions have been published, however, and the significance and background of the findings in these trials have not yet been clarified. The objective of the present study, therefore, was to characterize and compare the osteoblastic differentiation and mineralization ability of PDCs, BMCs, and OBs.

Materials and Methods

CELL ISOLATION AND CULTURE

Twelve 4-week-old male Wistar rats (Charles River Laboratories Japan, Yokohama, Japan) were sacrificed for the present study according to the norms and regulations of the Niigata University ethical committee (approval no. 156-1). Four rats were used for each cell isolation, and the experiments were repeated at least 3 times. Cell isolation was performed immediately after death. BMCs were isolated from the femur and tibia by flushing the marrow with culture medium (α-minimal essential medium containing 10% fetal bovine serum [Nichirei Bioscience, Tokyo, Japan] and 1% penicillin/streptomycin). PDCs and OBs were taken from the calvaria by first harvesting the periosteal tissue and later dissecting the calvarial bone. The collected periosteum and calvaria were subjected to enzymatic treatment (0.1% collagenase [Sigma-Aldrich, St Louis, MO] and 0.2% Dispase II [Roche Diagnostics, Mannheim, Germany] in phosphate-buffered saline) separately, and the released cells were collected by centrifugation (1,000 rpm for 5 minutes). All types of cells were maintained in culture medium in a humidified atmosphere with 5% carbon dioxide at 37°C. All reagents for cell culture were purchased from Invitrogen (Carlsbad, CA), unless otherwise specified. The animal experiments were performed in accordance with the guidelines of the Niigata University ethical committee.

CELL DIFFERENTIATION

After the cells had reached confluence, each cell type was seeded in 35-mm dishes at a concentration of 1×10^5 cells/mL (2×10^4 cells/mL for the MTS assay). The cells were cultured under 3 different conditions: control medium, culture medium with 50 μg/mL ascorbic acid (AA); OB medium, culture medium with 50 μg/mL AA and 2 mM β-glycerophosphate (βG); and MSC medium, culture medium with 50 μg/mL AA, 2 mM βG, and 10 nM dexamethasone (Dex).

CELL PROLIFERATION ASSAY

To analyze cell proliferation, the MTS assay was performed using CellTiter 96 AQueous One Solution (Promega, Madison, WI). At days 1 and 3 of culture, the cells were treated with MTS solution in culture medium for 1 hour at 37°C, and the absorbance of the

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