Differential Phenotypic Characteristics of Heterogeneous Cell Populations in the Periosteum

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Introduction: Periosteum or periosteum-derived progenitor cells have demonstrated a potential for stimulative applications in repairs of various musculoskeletal tissues [1,2,3]. It has been found that the periosteum contains mesenchymal progenitor cells capable of differentiating into either osteoblasts or chondrocytes depending on the culture conditions [4,5]. Considering that the periosteum is a heterogeneous multi-layered membrane, consisting of an outer fibrous layer and an inner cambium layer, however, we hypothesized that the phenotypic characteristics of periosteal progenitor cells are distinctively specific to the fibrous and cambium layers, respectively. We also hypothesized that the phenotypic distinction between the layers diminishes in a standard monolayer cell culture. To test these hypotheses, we developed a new cell harvest method to sequentially harvest the fibrous and cambium cells, respectively, from a periosteum-bone complex.

Methods: Fresh explants of intact bone and periosteum complex were harvested from the medial proximal tibia of NZW rabbits. After a brief treatment with 0.25% trypsin for 30 mins, these bone-periosteum explants were sequentially digested with 0.2% Type II collagenase two times for 120 mins each at 37°C. As the collagenase digestion took place first on the outer fibrous layer of the periosteum, most of the fibrous layer cells were liberated during the first 120 min period of the collagenase digestion, and the released periosteal fibrous cells were harvested. The remaining explants were further digested for the second 120 min, and the periosteal cambium layer cells were harvested. The two groups of cells, harvested from the periosteal fibrous and cambium layers respectively, were cultured in an α-MEM culture medium, supplemented with 10% FBS, 100μM dexamethasone, 20mM β-glycerol phosphate, and 50μg/ml ascorbic acid, and the cell morphologies were monitored. The alkaline phosphatase (AP) and osteocalcin (OC) productions were quantitatively measured using a p-nitrophenyl phosphate reaction method (ALP-10, Sigma) and a Gla-OC-EIA kit (Takara Inc., Japan), respectively, and normalized with respect to the cell number.

Results: The cell morphology was distinct between the fibrous and cambium cells in a monolayer cell culture system. The periosteal fibrous cells showed a fibroblastic spindle shape and the periosteal cambium cells an oval shape (Fig. 1). The fibrous cells showed a rapid increase in the cell population, whereas the population of the cambium cells was only moderately increased.

Immediately after the cell harvest (Day 0), the cambium layer cells showed significantly higher AP activity than the fibrous layer cells. On Day 3, however, the AP activity of the cambium cells dramatically decreased to the level of the fibrous cells. The AP activity of the cambium cells slightly increased later (Day 9), but that of the fibrous cells remained low throughout the experiment (Fig. 2).

The OC production also showed a significantly higher level for the cambium cells than the fibrous cells in Day 3 culture, but the OC level for both cell types gradually decreased with time (Fig. 3).

Discussion: A separate histological evaluation of periosteum (the results not shown in this abstract) revealed that the cambium layer has a higher cell population than the fibrous layer, and that the cambium cells have a rounder cell shape than the fibrous cells. The cell morphology shown in Fig. 1 was consistent with the histological findings. However, the rapid mitotic pattern of the fibrous cells in the monolayer culture system appeared to be different from the histological observation of the periosteum in situ. In this study, a small number of fibrous cells were found to be also present among the harvested cambium cells and quickly grew to form their colony with time.

The biological phenotypes of the fibrous and cambium cells were distinct. The cambium cells demonstrated higher osteogenic characteristics (higher AP and OC level), as compared to the fibrous cells. However, these differences significantly diminished with time in the monolayer culture system. These findings suggest that the monolayer culture system may not be suitable to maintain the specific phenotypes of the periosteal cambium cells.