

## OSTEOBLASTIC CELLS TO STUDY CELL/BIOMATERIAL INTERACTIONS: A COMPARITIVE STUDY

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**INTRODUCTION:** To study interactions of osteoblastic cells with biomaterials, osteoblast-like cell lines from different species are often used. Although cell lines can be used for biocompatibility testing, it is more convenient to use freshly isolated osteoblastic cells to study cell adhesion and the osteoconductive and -inductive properties of the biomaterials. Consequently a sufficient amount of easily obtained and well-characterized osteoblastic cells capable of differentiating is a useful tool to study biomaterial/cell interactions essential for bone tissue engineering.

Osteoblastic cells were derived from adult and fetal rat via different isolation techniques. The isolation and in vitro proliferation of primary cultures and the osteogenic potential of subcultures were compared.

**METHODS:** Osteoblastic cells were isolated (enzymatically or explant cultures) from fetal (21 day) Wistar rat calvaria and long bones (trabecular bone) and from adult (3 month old) Wistar rat long bones (trabecular bone and periosteum) [1, 2].

*Explant culture.* Bone pieces (from both fetal calvaria and long bones and adult long bones) and periosteal explants (from adult long bones) were cultured in L-ascorbic acid 2-phosphate containing medium.

*Enzymatic isolation.* Bone pieces (from fetal calvaria and long bones) were digested sequentially in a trypsin II-S-collagenase IA solution at 37°C for 10, 20, 30, 50 and 70 minutes. Rat calvaria cells and long bone-derived cells obtained from the last three respectively two digestion steps were pooled and plated at a concentration of 20000 cells/cm<sup>2</sup>. They were cultured in L-ascorbic acid 2-phosphate containing medium.

*Subculturing.* After confluence, the cells derived from fetal calvaria, long bones and adult long bones were plated (20000 cell/cm<sup>2</sup>) in osteogenic medium (containing L-ascorbic acid 2-phosphate and  $\beta$ -glycerophosphate) and cultured for 21 days. After 4, 14 and 21 days, they were analyzed for their osteoblastic markers.

Periosteum-derived cells were passaged for up to 4 months and at each passage, they were analyzed for their osteogenic differentiation.

*Analysis.* Osteogenic differentiation was evaluated based on alkaline phosphatase activity (early marker), morphology, nodule formation and mineralization of a collagen containing extracellular matrix (late marker).

**RESULTS:** Calvaria cells were easier to obtain and the amount of cells released by enzymatic isolation was higher than for the long bone cells. The expansion of the cells in primary culture was highest for fetal calvaria cells and adult periosteum-derived cells compared to fetal and adult long bone cells.

All cultures expressed high alkaline phosphatase activity in secondary culture except for calvaria cells obtained by spontaneous outgrowth and periosteum-derived cells. Periosteum-derived cells had initially a negligible alkaline phosphatase activity. Little cuboidal cells could be observed in 25 % of the cultures already at early passages. These cultures showed an increasing alkaline phosphatase activity starting at P1 until P7.

Enzymatic isolation of fetal calvaria and long bone cells favoured the osteogenic differentiation. Enzymatically isolated calvaria cells formed well defined three-dimensional nodules which mineralized restricted to this area. In contrary, cultures derived from fetal as well as adult long bones mineralized in ill defined deposits throughout the culture and only formed occasionally nodular-like structures. Periosteum-derived cells at passages 3-5 formed numerous nodules. From that passage on, nodule formation capacity decreased until completely absent. The mineralization pattern of these subcultured cells became diffuse and comparable to the cultures derived from fetal and adult long bones.

### DISCUSSION & CONCLUSIONS:

The present study demonstrates that considering the isolation method, proliferative capacity and the osteogenic potential, the enzymatically released

However, disadvantages of this type of cells are related to their advanced differentiation stage. Consequently, it is difficult to expand them into large amounts or to keep them in culture for long periods (> 21 days). The latter renders these cells less suitable for long-term in vitro test systems in which the biomaterial only degrades over a period for up to 3 months. The use of progenitor cells may circumvent these problems. Periosteum-derived cells are in a less differentiated stage, which can be favourable to expand them more easily and to keep them for longer periods in vitro.

**REFERENCES:** <sup>1</sup> H. Declercq, N. Van den Vreken, E. De Maeyer, R. Verbeeck, E. Schacht, L. De Ridder and M. Cornelissen (2003) Isolation, proliferation and differentiation of osteoblastic cells to study cell/biomaterial interactions: comparison of different isolation techniques and source. Submitted for publication. <sup>2</sup> H. Declercq, L. De Ridder and M. Cornelissen (2003) Isolation, proliferation and osteogenic differentiation of periosteum-derived cells. Submitted for publication.