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ISOLATION, CULTURE AND IDENTIFICATION OF BONE CELLS FROM NEWBORN RATS

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SUMMARY

Osteoblasts are the skeletal cells responsible for the synthesis; deposition and mineralization of the extracellular matrix of bone.\

In our study osteoblastic cells were isolated from the calvarial bones of newborn Wistar rats. Neonatal rat calvariae were dissected free from adherent tissue; washed in Ca2+- and Mg2+-free PBS solution and sequentially digested with 1 mg/ml trypsin (25 min; 37°C) and 2 mg/ml collagenase (120 min; 37°C)). After confluence; the cells derived from neonatal fetal calvaria were passaged and plated in osteogenic medium DMEM/F12 containing L-ascorbic acid; dexamethasone and glycerophosphate. Osteoblasts were characterized for alkaline phosphatase and for identification of mineralization was used Von Kossa; Alizarin Red. The mucosubstances were stained with Alcian Blue.

The characteristics of osteoblast cells were studied for morphology observation; alkaline phosphatase staining after citocentrifugation and Alizarin Red; Von Kossa; Alcian Blue staining after osteogenic induction. The culture expressed high alkaline phosphatase activity. A small number of cuboidal cells could be observed in 30% of the cultures already at early passages. Calvaria cells were easier to obtain and the amount of cells released by enzymatic isolation is very good. Enzymatic isolation of fetal calvaria cells favoured the osteogenic differentiation and the three-dimensional nodules production with mineralization but this method is more complicated. The enzymatic digestion period can be controlled by the alternative utilization of the two substances leading to the obtainment of a sufficient cell number for the posterior studies.

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