CALCIFICATION AND OSSIFICATION IN OSTEOBLASTIC CELL CULTURES.

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INTRODUCTION: When cultured in appropriate osteogenic media, osteoblastic cells form a calcified extracellular compartment[1-3]. These cultures are often used as reference models to study biomaterials on their osteoinductive capacity, including the formation of a mineralized matrix. To be equivalent to bone extracellular matrix, this compartment has to contain collagen I fibers on which bone hydroxyapatite crystals are deposited. Although many papers describe mineralization in cultures of osteoblastic cells, no clearcut correlations between the origin of the cultured cells and the observed mineralization patterns are described. We evaluated and compared mineralization in cultures of UMR-106 osteosarcoma cells and freshly obtained osteoblastic cells derived from different tissue sources.

METHODS: *Cell cultures* -UMR-106 osteosarcoma cells were purchased from ECACC. Osteoblastic cells were obtained from fetal rat calvaria and long bones (enzymatically and by explant) and adult rat long bones, periost (explant) and bone marrow. To increase the number of cells standard culture medium was used (DMEM, 10%FBS, 100µM L-ascorbic 2-phosphate). To induce differentiation, cells were cultured in osteogenic medium (α-MEM, 10% FBS, 4mM L-glutamine, 100 µM L-ascorbic acid 2-phosphate, 10 mM β-glycerophosphate). As a reference culture UMR-106 osteosarcoma cells were used.

Assays for osteogenic differentiation

Alkaline phosphatase activity (a parameter for early osteoblast differentiation) was measured spectrophotometrically (using p-nitrophenylphos- phate as a substrate and absorbance measurements at 405 nm) and histochemically (using BCIP/NBT as a substrate and aceton fixed cells).

Mineralization of the extracellular matrix was evaluated by histochemical methods (Von Kossa and Alizarin Red S staining), TEM and infrared spectrometry.

RESULTS: Data concerning ALP-activity and mineralization in the different cell cultures are summarized in Table 1.

Table 1. ALP activity (mM pNP/mg protein) and mineralization patterns in different cell cultures

	ALP	mineralization
UMR	> 45	dispersed
fetal long bones/exp	> 10	dispersed
fetal long bones/enz	> 15	dispersed
adult long bones/exp	> 10	dispersed
fetal calvaria/exp	5	absent
fetal calvaria/enz	> 15	nodules
periost exp	> 45	nodules
bone marrow	> 45	nodules

(exp = explant / enz = enzymatically)

Despite the differences in mineralization patterns, infrared analysis showed that the mineral formed in all cell cultures was hydroxyapatite . Electron microscopy showed the presence of needle like structures in the extracellular compartment, representative for hydroxyapatite crystals. In calvaria, periost and bone marrow derived cell cultures, the extracellular compartment contained collagen I fibers and matrix vesicles in which crystal formation was observed. Collagen fiber formation in UMR and long bone derived cultures was not obvious.

DISCUSSION & CONCLUSIONS: To investigate biomaterials on their osteogenic potential, meaning the stimulation of osteoblastic cells to form an organic matrix and the consequent mineralization of the matrix and to be sure that the biomaterials not just are associated with dystrophic calcification, it is important to use the right cell cultures. Osteoblastic cells derived from long bones and commercially available osteosarcoma cell lines should not be used, because in these cell culture models ossification is hard to distinguish from non-osteoblastic calcification. Freshly obtained cell cultures from either periost, calvaria or bone marrow are preferred.

REFERENCES: [1] JN Beresford, SE Graves, CA Smoothy (1993) *Formation of mineralized nodules by bone derived cells in vitro: a model of bone formation?* Am J med Gen **45:**163-178, [2] L Malaval, F Liu, P Roche, J Aubin (1999) *Kinetics of osteoprogenitor proliferation and osteoblast differentiation in vitro.* J Cell Biochem **74**: 616-627, [3] E Stringa, C Filanti, D Giunciuglio, A Albini, P Manduca (1995) *Osteoblastic cell from rat long bone. I. Characterization of their differentiation in culture.* Bone **16:** 663-670.E. Schneider, et al (2000) *J Biomech* **33**:1471-77.