

## LOW CALCIUM IS EXPANDING THE OSTEOPROGENITOR CELL FRACTION IN VITRO: ANOVEL PROCEDURE FOR BONE RERENERATION IN VIVO

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**INTRODUCTION:** Tissue engineering of bone requires three essential elements, enrichment of the cellular components, growth and differentiation factors and a scaffolding matrix. The novel aspect of our studies include the use of periosteal cells as opposed to bone marrow, the reproducible and facile expansion of these cells, and the ability to characterize the mineral and matrix that is formed both in vitro and in vivo.

**METHODS:** We have previously described a two dimensional culture method [1] supporting the growth of osteogenic cells by using low Ca medium (0.25mM). The cells that were grown exhibited proliferative capacity, responsiveness to mechanical stimulation and to PTH, positive stain for alkaline phosphatase and osteocalcin and matrix mineralization. A 10 fold increase in progenitor, small cell fraction [2] was isolated by flow cytometry (FACS), from subcultures grown in low calcium in comparison to subcultures grown in 1nM calcium. This cell fraction of progenitors were placed in DBM cylinders (demineralized rat femur

cortical bone), and implanted in subcutaneous thoracic site of DA young rats [3].

**RESULTS:** New bone was formed after four weeks in vivo resembling membranous bone. X-ray microradiography, histology and infrared spectroscopy (FITR) showed bone apposition characteristic to normal bone.

**DISCUSSION & CONCLUSION:** The results of these experiments propose a novel procedure for expansion of osteoprogenitors that form bone in vivo.

**REFERENCES:** <sup>1</sup>I. Binderman, E. Berger, N. Fine, Z. Shimshoni, A. Harell, D. Somjen (1989) *Connect Tissue Res* **20**:41-7. <sup>2</sup>R. Zohar, J. Sodek, C. McCulloch (1990) *Blood* **90**:3471-81. <sup>3</sup>EM. Nimni, S. Bernick, D. Ertl, SK. Nishimoto, W. Paule, BS. Strates, J. Villanueva (1988) *Clin Orthop Relat Res* **234**:255-66.