BLOOD VESSELS & MESENCHYMAL CELLS: CAN THEY MAKE BETTER BONE?

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INTRODUCTION: Mesenchymal cells are commonly used as a source of cells for new bone production. *In vivo* models clearly demonstrate that bone marrow-derived mesenchymal cells have an osteogenic potential, with new bone usually associated with a vascular supply. The blood vessels are generally thought to simply provide a source of nutrients for the donor cells rather than a direct role. *In vitro* models of osteogenesis provide valuable data on the regulating factors and genes involved in bone formation but most models rarely produce a piece of true bone and rely on markers of osteogenesis such as type I collagen, alkaline phosphatase or bone sialoprotein. Here, we present an *in vitro* organ culture model of osteogenesis where a piece of true bone forms from a human bone marrow plug. In the presence of endothelial cell-derived factors, a larger piece of bone develops more rapidly. Endothelial cell-derived factors stimulate a five-fold increase in adherent mesenchymal cell numbers within one week and these cells also differentiate into bone if pelleted and grown in organ culture. In addition, the endothelial cell-derived factors allow a plug of marrow to adhere to a slice of human bone and develop into a nodule of new human bone. These data suggest that blood vessels and endothelial cells play a direct role in osteogenesis and may aid in the production of larger, quicker and better bone.

METHODS: Human bone marrow was obtained with full consent from the opened sternum of patients undergoing cardiac surgery. A total of 133 organ cultures were established from 12 patients, age range 47 – 67 years. (10 males, 2 females). Bone marrow was dissected free from existing bone under a dissecting microscope and either grown as a plug in organ culture or mechanically dissociated into a single cell suspension. Marrow plugs or cells were established in αMEM, BGJb or conditioned media removed from confluent endothelial cells – ECCM (also αMEM). Cell cultures were harvested and counted at 7 days and if necessary pelleted for organ culture. Organ cultures were fixed at weekly intervals for up to six weeks and processed for histology and immunocytochemistry.

RESULTS: During the six-week culture period, αMEM allowed fibroblastic cell survival but no bone formation. When supplemented with β-glycerophosphate (+P), bone formation occurred with a frequency of 72% in 3-4 weeks, BGJb media produced bone with a frequency of 93 % in the same time period but ECCM (which is αMEM harvested from confluent endothelial cell cultures produced bone with a frequency of 95% within 7-10 days (Figure 1).

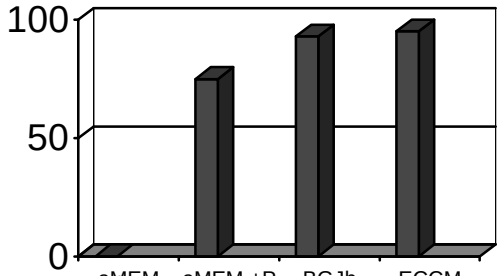

Fig 1. Frequency of bone formation of human bone marrow plugs in organ culture.

The bone resembled true bone with osteocytes embedded in a mineralized matrix and osteoblasts on the periphery.

DISCUSSION & CONCLUSIONS: In this report we show that endothelial cell-derived factors, released into tissue culture medium stimulate rapid bone differentiation from human bone marrow-derived mesenchymal cells. This bone develops in approximately one third of the time required in control cultures. Indeed, instead of being "spent" medium, this ECCM can stimulate a five-fold increase in mesenchymal cell number and these cells also can progress to true bone formation.

Normal and pathological bone formation requires a vascular supply, often associated with the removal of cartilage. We have recently demonstrated that ECCM will also specifically destroy hypertrophic chondrocytes. We suggest that during development, endothelial cells produce factors which can aid in the destruction and removal of hypertrophic chondrocytes, blood vessels transport osteogenic cells to the appropriate site and the endothelial cell products can stimulate cell proliferation and then differentiation of the osteogenic cells into new bone. The data presented here indicate that rather than just providing nutrients and a means for the appropriate cells to reach their destination, the endothelial cells play a direct and important role in bone formation. Thus, endothelial cells do help make better bone.

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