Prefabrication of *in vivo* bone constructs following *in vitro* propagation of cultured bone marrow stromal cells on hydroxyapatite/tricalcium phosphate carpets

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Statement of Purpose: Populations of adult bone marrow stromal cells (BMSCs) contain a subset of multipotent stem cells capable of differentiation towards bone, cartilage, hematopoiesis-supporting stroma, and adipocytes. We have previously shown that culture-expanded BMSCs can be combined with porous hydroxyapatite/tricalcium phosphate (HA/TCP) particles and immediately transplanted into immuno-tolerant mouse recipients to form mature cortico-cancellous bone within 8 weeks, while culture-expanded spleen stromal cells (SpSCs) form a fibro-vascular network that is devoid of bone. BMSC and SpSC constructs are too plastic at the time of transplantation to control the transplants' eventual shape, and they cannot be combined into multi-lamellar structures. Multi-lamellar structures, meanwhile, play an important role *in vivo* because they contain a stable interface of different tissue types; examples include articular cartilage and its underlying bone, as well as the cementum-dentin junction.

We therefore sought to prefabricate stable human BMSC-HA/TCP and SpSC-HA/TCP constructs in tissue culture prior to transplantation, in order to 1) control the shape of the transplants to meet precise design specifications, and 2) allow for the creation *in vivo* of laminated, multi-organ structures. These constructs were transplanted into mice to form mature bone which retained the pre-designed gross and histological characteristics.

Methods: Human BMSCs or human SpSCs were placed in tissue culture with measured aliquots of HA/TCP ceramic particles (Zimmer; Warsaw, IN) in 6-well plates. Cells were plated at densities of 0.5 million to 1.0 million per 100 mg of HA/TCP. Co-culturing continued for periods of 3 to 4 weeks using our standard media (1). During that time, each set of cells and particles formed a semi-rigid carpet that maintained its shape and form. Finally, pieces of carpets were removed from tissue culture, stacked to form laminated structures or wrapped into predetermined shapes, placed immediately into subcutaneous pockets in immuno-tolerant Bg-Nu-XID mice, and harvested after 8 weeks. H&E-stained sections of the transplants were then examined histologically. Human tissue acquisition and mouse surgery were performed under UCSF IRB and small animal protocols, respectively.

Results / Discussion: A total of 75 transplants were created and evaluated. All transplants retained their pre-designed gross characteristics. Transplants consisted of stacks of 2 or 3 layers of carpet, and each transplant contained only BMSC carpets, only SpSC carpets, or both BMSC and SpSC carpets. At the time of harvest from the mice, stacks of BMSC carpets contained extensive bone and hematopoiesis, and the individual carpet layers had fused so well with each other that the interface between layers was obliterated (Figure 1). Stacks of SpSC carpets formed a fibrovascular matrix without bone. Stacks of both BMSC and SpSC carpets showed distinct bands of bone and non-bone forming tissue which were tightly joined (Figure 2).

Conclusions: HA/TCP particles are becoming an increasingly important matrix for bone reconstruction and tissue engineering. Yet, their initial deformability prevents their immediate use as multi-lamellar structures. In this study, we have demonstrated that long-term co-culturing of BMSCs or SpSCs with HA/TCP particles does not modify the differentiation potential of the cells; it produces stable carpets that can be combined to form multi-lamellar structures with a pre-designed shape *in vivo*.

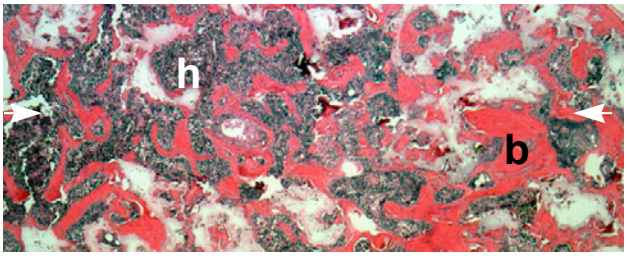

Figure 1: b=bone, h=hematopoiesis, arrows= interface between 2 adjacent BMSC layers

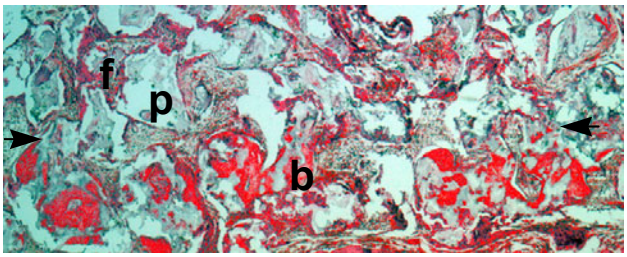

Figure 2: b=bone, f=fibrovascular tissue, p=HA/TCP particle, arrows= interface between BMSC and SpSC carpet layers

References:

[1] Mankani MH, et al. Radiology 2004; 230(2):369-376.

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