Proepicardial Cells Modulate the Osteogenic Potential of BMS Cells in Aligned Collagen I Scaffold

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Statement of Purpose: It is well-established that the process of neo-vascularization or neoangiogenesis is coupled to the development and maturation of bone. Bone marrow stromal (BMS) cells comprise heterogeneous population of cells that can be differentiated *in vitro* into both mesenchymal and non-mesenchymal cell lineages. The objective of this work was to determine the effect of neo-vascularization on the osteogenic potential of BMS cells. When both rat BMS cells and quail proepicardial (PE) cells were seeded onto a three-dimensional tubular scaffold engineered from aligned type I collagen and co-cultured in osteogenic media, simultaneous maturation and differentiation of osteoblastic and vascular cell lineages were observed. In addition, these cells produced abundant mineralized extracellular matrix. This culture system provides a useful *in vitro* model to investigate the functional role of neo-vascularization in the proliferation and differentiation of BMS derived osteoblasts.

Methods: The collagen type I tubular scaffolds were fabricated as described [1]. The PE cells were isolated from the precardial cavity of quail as described [2]. Bone marrow stromal cells were isolated from the bone marrow of Wistar rats as described [3]. Rat BMS cells were seeded in the collagen tubes at a density of $2x10^6$ cells per tube and cultured in osteogenic medium for 3 days. The osteogenic medium was supplemented with dexamethazone, β-glycerophosphate, and ascorbic acid. Next, on day 3, the PE cells were seeded onto the same tubes and culture was continued in osteogenic media for another 7 days. These differentiated cells were subjected to immunohistochemical and cytochemical analysis. Various osteogenic and vasculogenic lineage specific markers were examined by immunofluorescence using laser scanning confocal microscopy (Zeiss 510 Meta).

Results/Discussion: Figure 1 shows the increased alkaline phosphatase activity. Figure 2 shows the expression pattern of various other osteogenic markers. Figure 3 shows the expression pattern of vasculogenic markers in this co-culture system on day 10. The expression of both early and late osteogenic markers showed significant increase. The Alkaline phosphatase activity and mineralized deposits showed significant increase over the observed period of time. Moreover, extensive arborization of nascent capillary-like structures was seen.

Conclusions: Co-culturing BMS along with PE cells in this 3-D model system modulated the maturation and differentiation of BMS cells derived osteoblasts. The upregulation of osteoblastic phenotypic markers with concurrent deposition of abundant extracellular/ matricellular materials indicated that the PE cells augmented osteogenesis. Hence, this model not only reinforces the intimate association of the vascular endothelium with bone development and function but it is also a very useful *in vitro* model to study the neo-vascularized osteogenesis.

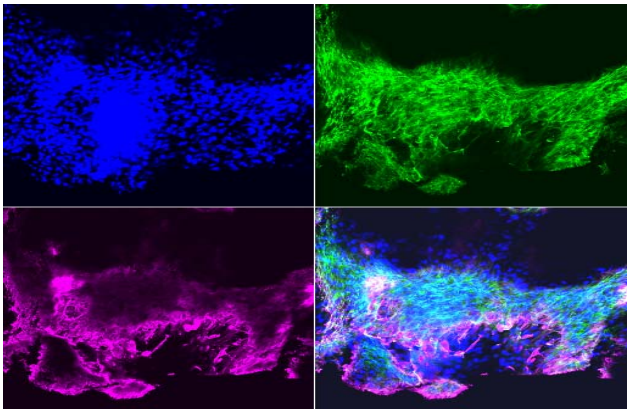

Fig. 1. Expression pattern of alkaline phosphatase (AP). (a) DAPI – nuclei, (b) Phylloidin – actin, (c) AP, (d) Merged.

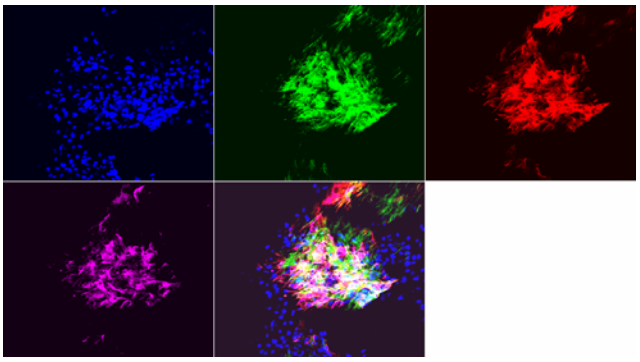

Fig. 2. Expression pattern of osteogenic markers. (a) DAPI – nuclei, (b) Phylloidin – actin, (c) Osteocalcin, (d) Osteopontin, (e) Merged.

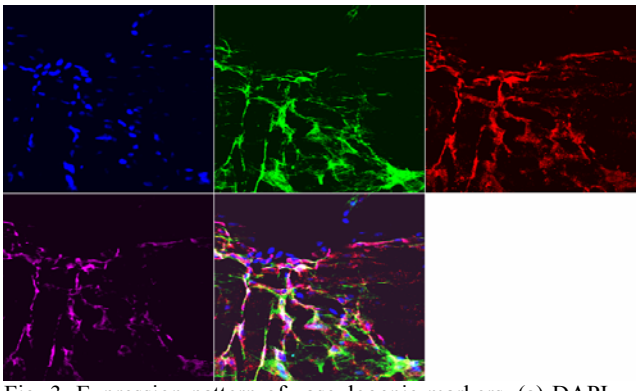

Fig. 3. Expression pattern of vasculogenic markers. (a) DAPI – nuclei, (b) Phylloidin – actin, (c) α-Smooth muscle actin (d) Osteopontin, (e) Merged.

Reference:

[1] Yost MJ, Baicu CF, Stonerock CE, Goodwin RL, et al. Tissue Eng. 10 (2204) 273-84.
[2] Nesbitt T, Lemley A, Davis J, Yost MJ, Goodwin RL, Potts JD. Microsc Microanal. 12 (2006) 390-8.
[3] Jabbari E, He, X. Polym. Prepr. 47-2 (2006) 353-354.