A Novel Three-Dimensional Culture System to Study vasculogenesis and Osteogenic Differentiation of BMS Cells

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Statement of Purpose: Bone marrow stromal cells (BMS) are fundamentally a heterogeneous population of cells with multilineage differentiation potential. When BMS cells were seeded onto a three-dimensional tubular scaffold fabricated from aligned type I collagen strands, and cultured in osteogenic media, they underwent simultaneous maturation and differentiation into osteoblastic and vascular cell lineages. In addition these cells produced mineralized matricellular deposits. This *in vitro* model provides an excellent model to study the concurrent temporal and spatial regulation of both osteogenesis and vasculogenesis during bone development.

Methods: Rat BMS cells were isolated from Wister male rats maintained and expanded for two passages in basal medium as described [1]. Then, these cells were seeded into collagen-gel tubes at density of $1x10^6$ cells and cultured either in basal or osteogenic media for 3, 6, and 9 days [2]. The cells were subsequently processed for RT-PCR, immunohistochemical and cytochemical analysis. Lineage specific proteins were localized by immunofluorescence using laser scanning confocal microscopy (Zeiss 510 Meta) and mRNA transcript analysis was performed by real-time PCR (RT-qPCR).

Results / Discussion: Figure 1 and 2 show the expression pattern of various osteogenic and vasculogenic markers in this culture system on day 9. Figure 3 shows the mRNA expression profiles of various osteogenic markers. The expression pattern of key osteogenic markers significantly differed in response to basal and osteogenic media. Tubes containing BMS cells when cultured in osteogenic media demonstrated an initial upregulation of osteopontin which returned to baseline level following 6 days. In contrast, tubes with BMS cells cultured in ostegenic media showed a sustained upregulation of osteocalcin beyond day 3. The Alkaline phosphatase activity and calcium content (alizarin red staining) significantly increased over the observed period of time in osteogenic medium. Surprisingly, sheets of abundant α-smooth muscle positive cells were observed in these tubes seeded and cultured with BMS cells. Moreover, nascent capillary-like vessels were also seen amidst the osteoblasts in osteogenic cultures.

Conclusions: This novel 3-D culture system augmented the maturation and differentiation of BMS cells into osteoblasts. Substantial upregulation of osteoblastic phenotypic markers is reminiscent of *in vivo* expression pattern. This model system has considerable potential for investigating the development of vascularized osteogenesis *in vitro* as well as for bone tissue engineering. Finally, these cytodifferentiated cells may be useful for therapeutic purposes in regenerative medicine.

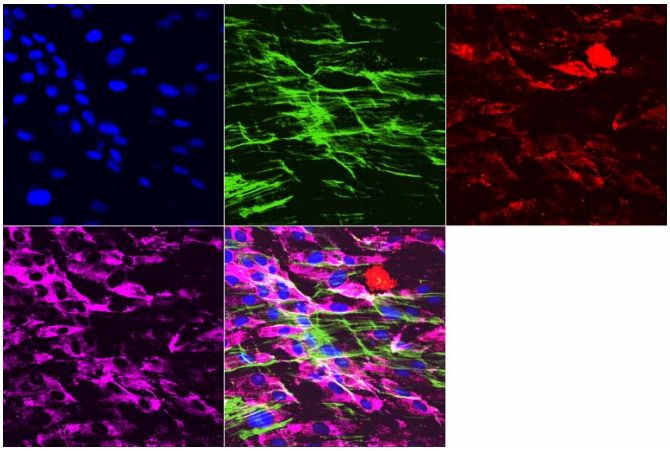

Fig.1. Expression pattern of osteogenic markers. (a) DAPI – nuclei, (b) Phylloidin – actin, (c) Osteonectin, (d) Osteopontin, (e) Merged image.

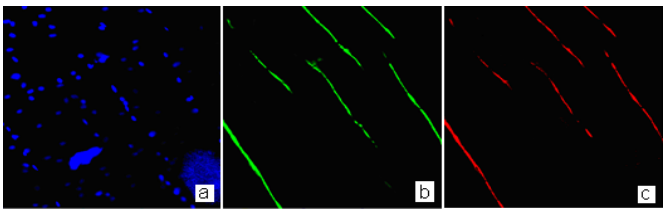

Fig.2. Expression of vasculogenic marker. (a) DAPI – nuclei, (b) Phylloidin – actin, (c) α-Smooth Muscle Actin.

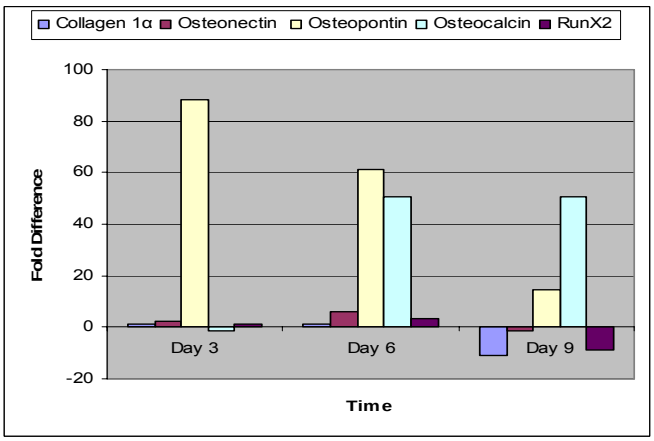

Fig. 3. mRNA expression profile of various osteogenic markers.

Reference:

[1] Jabbari E, He, X. Polym. Prepr. 47-2 (2006) 353-354.
[2] Yost MJ, Baicu CF, Stonerock CE, Goodwin RL, et al. Tissue Eng. 10 (2004) 273-84.