Modulation of Osteogenic and vasculogenic Differentiation of Stromal Cells in a Collagen Scaffold

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Statement of Purpose: Bone marrow stromal (BMS) cells are a heterogeneous population of cells with multi-lineage differentiation potential. BMS cells have the ability to differentiate into mature phenotypes that are different from their tissue of origin. Since endothelial precursor cells have been identified in the adult bone marrow, the objective of this work was to determine the capacity of BMS cells to undergo vasculogenic as well as osteogenic differentiation in-vitro when cultured in collagen type I scaffold in complete osteogenic media.

Methods: 3-D collagen type I scaffolds served as a scaffold on which marrow stromal cells were grown for differentiation purposes. Rat BMS cells were harvested from the bone marrow of young adult male Wister rats. The isolated BMS cells were maintained and expanded in basal medium. Then, the cells were seeded into collagen type I scaffolds [1] and cultured in osteogenic media consisting of DMEM supplemented with 10% FBS, 10 mM sodium β-glycerol phosphate, 50 µg/ml L-ascorbic acid, 10^{-8} M dexamethasone and 8 µg/ml gentamicin for 3, 6, and 9 days. The cell cultures were terminated and subsequently processed for RT-PCR, immunohistochemical and cytochemical analyses [2]. Lineage specific proteins were localized by immunofluorescence using confocal laser scanning microscopy and mRNA transcript analysis was performed by Real-Time quantitative PCR (RT-qPCR).

Results: The expression pattern of key osteogenic markers is shown in Figure 1.

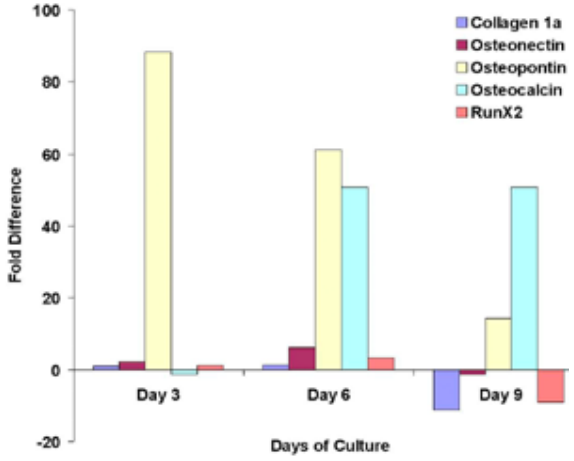
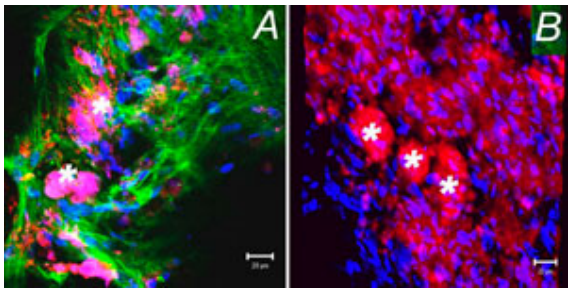
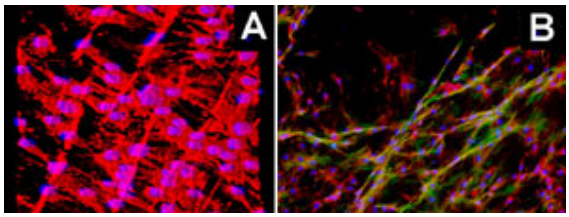


Figure 1. The expression pattern of osteogenic markers for BMS cells seeded on collagen type 1 scaffold and cultured ion osteogenic media.

BMS cells demonstrated an initial upregulation of osteopontin which returned to baseline level following 6 days. In contrast, osteocalcin showed a sustained upregulation beyond day 3. Transcripts for type I collagen and osteonectin were actively expressed until day 6 and gradually down regulated. Early mineralization was evident beginning at day 6 with extensive deposits at day 9 in osteogenic cultures. By day 9 BMS cells began to form multiple foci of multilayered nodular structures as a result of coalescing cellular aggregates.



After 9 days, vessel-like structures were produced. These structures were positive for various vascular antigens Pecam-1, Flk-1, α-SMA and tomato lectin. Moreover, nascent capillary-like vessels were also seen amidst the osteoblasts in the osteogenic culture.



Discussion: It appears that the specific structural organization of the collagen scaffold and the supplements of osteogenic media not only enhanced the osteoblastic differentiation but also supported the process of generating microvascular structures (plexus of nascent capillary-structures), in addition to the elongated vessel-like structures which were obviously coated by the α-SMA [3]. The development of both nascent capillary-like vessels and smooth muscle containing vessel-like structures in our study probably contributed from a common vascular progenitor of BMS cells. In addition, these cells produced mineralized matricellular deposits.

Conclusion: These results demonstrate that BMS cells can be differentiated in-vitro into vasculogenic as well as osteogenic pathways when cultured in ostegenic media, leading to neo-vascularization.

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References:

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[2] Jabbari E, He X, Valarmathi MT, Sarvestani AS, Xu W, J. Biomed. Mater. Res. in Press (2008).