Endothelial cell/osteoblast coculture ratios for angiogenesis and bone formation

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Statement of Purpose: In the human body, cells interact with other cell types in order to perform their desired functions. In *in vitro* cultures, coculturing cells may have greater potential for achieving preferred functions of cells, such as bone regeneration and angiogenesis. The ratio of endothelial cells to osteoblasts in culture appears to have an effect on the function of cells. There have been mixed results in studies done with endothelial cell/osteoblast cocultures in a 1:1 ratio, but studies with high endothelial cell to osteoblast ratios more consistently show increased microcapillary-like structures.[1,2,3] There has not been a study which formally compares various coculture cell ratios for bone and vessel formation. The objective of this study is to observe the behavior of endothelial cells and osteoblasts in cocultures of varying ratios on different surfaces and determine the best coculture ratios for bone regeneration and vasculogenesis for use in tissue engineering applications.

Methods:

Human umbilical vein endothelial cells (Invitrogen; Carlsbad, CA) and normal human primary osteoblasts (Lonza; Hopkinton, MA) were cultured per vendor protocol. Endothelial cells (EC) were labeled with PKH 67 (green) and osteoblasts (OB) were labeled with PKH 26 (red). After labeling, EC and OB were mixed in even endothelial:osteoblast (EC:OB) ratios (1:1), low EC:OB ratios (1:2, 1:3, 1:5, 1:10), and high EC:OB ratios (10:1, 5:1, 3:1, and 2:1) (n=3). Endothelial cell and osteoblast monocultures were used as controls. The cell mixtures were seeded on 24 well polystyrene tissue culture plates and collagen cell matrix (Cayman Chemical; Ann Arbor, MI) in endothelial cell media without VEGF. Cells on plastic were visualized at days 1, 3, 7, and 12 using phase contrast and fluorescence microscopy. Cells on collagen were imaged on day 1 and day 6 using fluorescence microscopy.

Results:

Angiogenic networks were seen at day 6 in the collagen angiogenesis assay in cocultures with a high EC:OB. (Fig. 1a) Cultures with low EC:OB ratios showed an even dispersion of both cells throughout the collagen with no organization. On plastic, angiogenic-like networks were seen at day 3 only at EC:OB ratios of 2:1, 3:1, and 5:1 but this result was not consistent in all replicates. (Fig. 1b)

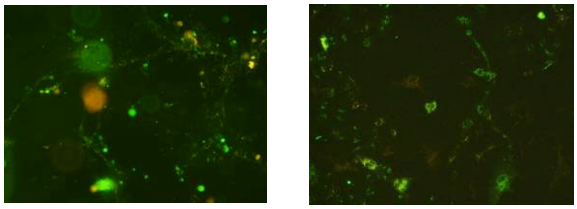

Figure 1. (a) Network in collagen at day 6, 5:1 ratio (b) Network on plastic at day 3, 3:1 ratio

Angiogenic networks were seen in the endothelial cell monoculture at day 12 only. (Fig. 2)

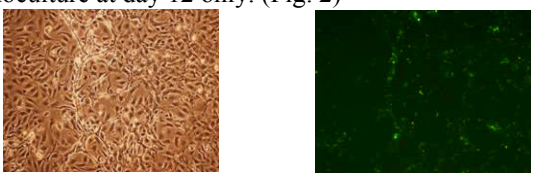

Figure 2. (a) EC monoculture at day 12 with angiogenic network under phase contrast and (b) fluorescence

At low EC:OB ratios on plastic, the endothelial cells aggregated with osteoblasts into strut-like formations and small islands in areas that eventually became bone nodules. Bone nodules were seen at day 12 on cocultures with low EC:OB ratios on plastic. (fig. 3) There were no bone nodules seen in osteoblast monocultures, cocultures with high EC:OB ratios, or in collagen. In these cultures the osteoblasts were evenly interspersed throughout the endothelial cells. In 1:1 cocultures, no network formation or bone nodules were seen.

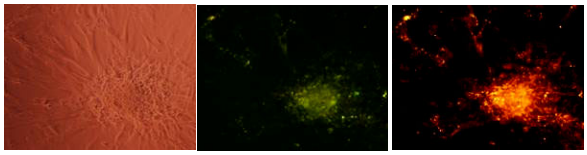

Figure 3. Bone nodule on plastic at day 12, 1:5 ratio (a) phase contrast, (b) HUVECs, (c) Osteoblasts

Conclusions:

Increased angiogenesis networks were seen in cocultures with high EC:OB ratios in the collagen angiogenesis assay. There were vessel like formations on plastic in cultures with ratios less than 10:1 but analysis was limited at days 7 and 12 due to dilution of the cell membrane label as the cells replicated. Further studies with immunocytochemistry are necessary to confirm network formation. Bone formation was seen in low EC:OB ratios only on plastic in cultures where endothelial cell aggregation was noted. This may indicate that endothelial cells have a positive effect on bone formation and the coculture response is altered by the material surface. The results from this study suggest that by manipulating coculture ratios, vascularization and bone formation can be increased, but cell ratios will need to be tailored to fit the desired response and material.

References:

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