Spatial and Temporal Interaction between Osteoprogenitors and Angioprogenitors on Composite Scaffolds

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STATEMENT OF PURPOSE:

Bone fractures are quite common and while most of these bone fractures heal naturally, severe large open bone fractures do not heal on their own, and a bone graft is needed to help regenerate bone tissue. However, due to a compromised blood supply to the affected area, many of these large traumatic bone injuries do not heal, leading to amputation[1,2]. Vessel in-growth is a crucial factor in determining the success of bone regeneration because it provides the nutrient supply, as well as waste removal pathways from the injured area[1,2]. Currently, there is a limited amount of research being done on the simultaneous growth of bone and vessel formation. Hence, a means to promote vascularized bone regeneration is needed. In this study, we evaluate the production of vascular and osteogenic markers in a co-culture model. This system consists of co-culturing endothelial cells with osteoprogenitor cells on a hydroxyapatite scaffold loaded with a collagen hydrogel. Cells were seeded in different ratios as well as different spatial distributions to determine the optimum conditions to promote vascularized bone formation.

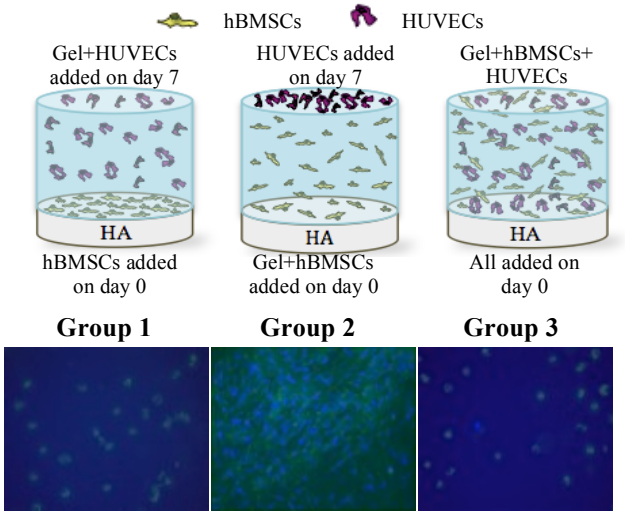


Figure 1: Schematic of groups and fluorescent microscopy images taken on Day 14 (HUVECs stained with calcein green + DAPI).

MATERIALS AND METHODS:

Composite scaffolds were prepared by casting 3 mm thick 4 mg/ml collagen hydrogels on 100% crystalline hydroxyapatite discs. Initial experiments demonstrated that Human bone marrow stem cells (hBMSCs) showed an increase in VEGF production on the composite scaffolds at day 7 when seeded alone. In the current study, optimized concentrations of hBMSCs and Human umbilical vein endothelial cells (HUVECs) were seeded in different spatial distributions: hBMSCs loaded without the hydrogel on day 0, then on day 7 HUVECs were added within the collagen hydrogel (Group 1); hBMSCs loaded within the hydrogel on day 0, then 7 days later HUVECs were seeded (Group 2) and hBMSCs and HUVECs loaded within the hydrogel on day 0 (Group 3) (Fig 1). Additionally, five ratios of hBMSCs to HUVECs were used (1:0, 5:1, 1:1, 1:5, 0:1). Production of vascular markers (vascular endothelial growth factor (VEGF), Angiogenin, and Angiopoietin-1 (Ang-1)) and an early osteogenic marker (ALP) were measured at regular intervals using ELISA and cells were observed using fluorescent microscopy. Groups were compared using 2-way ANOVA across time and Tukey's test (at $p<0.05$).

RESULTS:

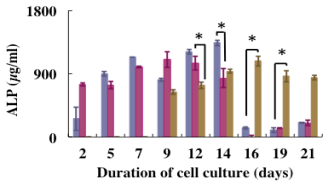
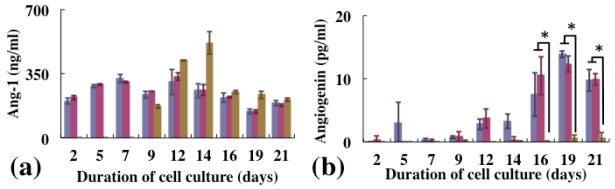


Figure 2: ALP production in each group over 21 days in vitro culture. (* indicates significant differences between groups at $p<0.05$)



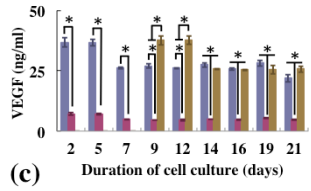


Figure 3: Production of (a) Ang-1, (b) Angiogenin, and (c) VEGF in each group. Significant differences shown only between groups at different times. * indicates $p<0.05$. (n=4/group/time)

Group 1 Group 2 Group 3

DISCUSSION AND CONCLUSIONS:

All groups had an initial peak of ALP (an early osteogenic marker), which is indicative to osteoblast differentiation (Fig 2), however, spatio-termporal variations between groups resulted in varying angiogenic profiles (Fig 3).

- Delaying the seeding of vascular cells (HUVECs) (Group 1 and 2) increased vascularization, as suggested by an increase in angiogenin, a known potent inducer of neovascularization *in vitro*[3].
- When hBMSCs are seeded in close proximity and at the same time as HUVECs (Group 3) the interaction between the two cell lines increased the production of Ang-1, which is essential for organization, integrity and maturation of neo-vasculature[4].
- Interestingly, VEGF levels were reduced if hBMSCs were seeded within the gel and the HUVECs were seeded on Day 7 (Group 2), demonstrating how crucial early hBMSC differentiation and vascular infiltration is.

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ACKNOWLEDGEMENTS:

Partially funded by NIH/NIGMS MARC U*STAR GM007717 and UTSA College of Engineering.