Assessing the Osteogenic Differentiation of Human Mesenchymal Stem Cells Co-Cultured with Human Vein Endothelial Cells on a Peptide Amphiphile Nanomatrix

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Statement of Purpose

To date, there has not been a bone regeneration strategy that convlusively provides both an osteogenic and angiogenic environment conducive to bone tissue growth. Thus, the purpose of this study is to evaluate the effects of crosstalk between human mesenchymal stem cells (hMSCs) and human umbilical vein endothelial cells (HUVECs) in a self-assembled peptide amphiphile (PA) nanomatrix. PAs are developed to mimic the native extracellular matrix (ECM) of various tissues by forming a self-assembling nanomatrix. We have previously shown that PA-RGDS enhances the osteogenic differentiation of hMSCs[1]. It was hypothesized that co-cultured hMSCs and HUVECs on the PA-RGDS nanomatrix will result in synergistic osteogenic and angiogenic response.

Materials and Methods

A total of 9 different conditions were tested in the following experiments. There were 3 different growth substrates: PA-RGDS, PA-Short (PA-S), and plasma treated tissue culture plate (TCP). PA-S served as a negative substrate control. In addition to the growth substrate, there were three different cellular conditions used: hMSCs, HUVECs, and a co-culture consisting of both hMSCs and HUVECs. All experiments were performed with a cell density of 20,000 cells/cm^2. Alkaline phosphatase (ALP) activity was measured using a flourometric Sensolyte FDP Alkaline Phosphatase Assay Kit at 1, 7, 14, and 28 days. Von Kossa staining was performed after 28 days. The stained images were quantified to find the fraction of each image occupied by mineralization.

Results and Discussion

With respect to the osteogenic differentiation of hMSCS, the results of this study indicate that co-culturing hMSCs with HUVECs on a PA-RGDS enhances the osteogenic response. Alkaline phosphatase activity was elevated for the co-cultured condition on PA-RGDS. The peak activity was measured at day 14, whereas peak ALP activity for the monocultured hMSCs on PA-RGDS was measured at day 28. The day 14 peak was significantly greater than all other conditions suggesting that differentiation was induced at a much earlier stage in the co-cultured condition. As expected, HUVECs cultured by themselves expressed little to no ALP, but the addition of the endothelial cells promoted the hMSCs to up regulate the production of ALP. For the first three time points, the measured co-culture conditions consistently had greater amounts of quantified ALP. This suggests that there is a synergistic effect between the hMSCs and HUVECs, regardless of the coating conditions.

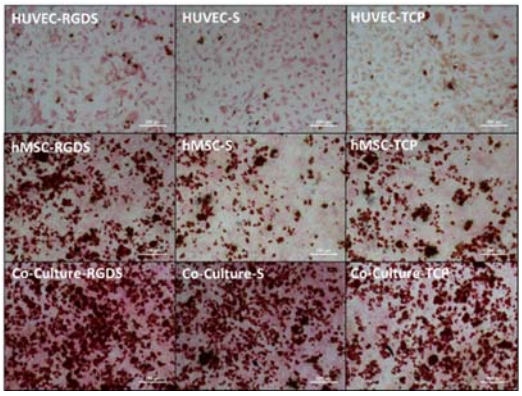

Figure 1: Von Kossa stain after 28 days indicating the mineralization in each of the different conditions. Row 1:HUVECs, Row 2: hMSCs, Row 3: Co-culture. Column 1: PA-RGDS, Column 2: PA-S, Column 3: Tissue culture plate.

Von Kossa staining (Figure 1) revealed that the co-culture condition grown on PA-RGDS seemed to have the largest amount of mineralized colony formation. Quantified results indicated that all co-culture conditions had a larger fraction of the image occupied by mineralization compared to the mono-cultured hMSC equivalents.

Conclusion

This study has shown that PA nanomatrices have the potential for being used in bone tissue engineering applications. In addition, co-culturing hMSCs and HUVECs on the PA-RGDS nanomatrix was shown to promote the differentiation of the hMSCs and induce the formation of endothelial cell networks. The PA-RGDS nanomatrix further supported the growth and function of hMSCs and HUVECS. The co-cultured cells on the PA-RGDS nanomatrix had the greatest ALP activity, the largest fraction of each surface occupied by mineralization, and the greatest evidence of endothelial network formation.

Reference: 1. Anderson JM et al. Acta Biomaterialia. 2011;7:675-682.

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