Effect of Osteopontin-Derived Pepide on Vasculogenic Differentiation of Bone Marrow Stromal Cells

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Statement of Purpose: The high clinical failure rates with allografts and slow bone regeneration observed with implanted natural and synthetic biomaterials are generally attributed to the lack of vascularization in the interior parts of the implant. An attractive alternative is to use peptides, based on the active domains of ECM proteins to initiate the cascade of vascularized osteogenesis. Compared to proteins, peptides, grafted to TE scaffolds exhibit much higher biological stability, making them very attractive as a synthetic graft for rapid stabilization and dressing of skeletal injuries. It has been demonstrated that the osteopontin-derived peptide SVVYGLR (ODP peptide) has as potent activity for tube formation by endothelial cells as vascular endothelial growth factor (VEGF), which is known to be the strongest angiogenic factor [1]. The objective of this work was to determine the effect of OPD peptide grafted to bioresorbable poly(lactide-co-ethylene oxide fumarate) (PLEOF) scaffold on osteogenic and vasculogenic differentiation of bone marrow stromal (BMS) cells.

Methods: Acrylated GRGD (Ac-GRGD) and azide terminated ODP peptide were synthesized manually on Rink Amide NovaGel resin with Fmoc-protected amino acids. PLEOF macromer, Ac-GRGD peptide, propagyl acrylate, methylene bisacrylamide (BISAM) crosslinker, ammonium persulfate (APS) initiator, and tetramethylethylene diamine (TMEDA) were mixed in PBS and the mixture was allowed to crosslink to produce PLEOF hydrogel containing RGD peptide and unsaturated acetylene functional group for grafting ODP peptide. ODP peptide was grafted to the PLEOF hydrogel with "click chemistry" by the reaction of acetylene groups of the hydrogel and the azide groups of the Az-ODP. BMS cells were isolated from the bone marrow of young adult male Wistar rats and bioactivity of BMP-2 peptide was assessed with BMS cells. BMS cells were seeded on grafted RGD-PLEOF hydrogels and cultured in osteogenic media (100 nM dexamethasone, 50 mM ascorbic acid 2-monophosphate, and 10mM β-glycerophosphate). At each time point, samples were removed, and alkaline phosphatase (ALPase) and calcium content were measured with p-nitrophenol and QuantiChrom Calcium Assays, respectively, and the expression of PECAM was quantified by qRT-PCR. The expression of PECAM was imaged anti-body staining and confocal fluorescent microscopy.

Results:
In-vitro experimental groups included substrates without ODP peptide (control group; with RGD), grafted with RGD and with a mutant version of ODP peptide (PLEOF+mODP), and grafted with RGD and ODP peptides (PLEOF+ODP). When RGD and ODP peptides were grafted to PLEOF, a small increase in mineralization was observed (data not shown). But grafting RGD and ODP peptides significantly increased PECAM expression of BMS cells, as shown in Figure 1, after 28 days of culture.

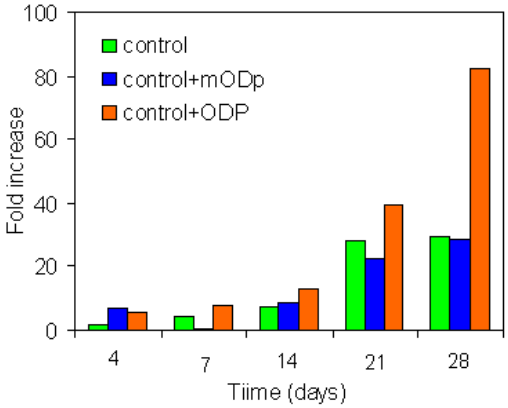

Figure 1. PECAM expression of BMS cells.

Figure 2 shows the confocal microscopy image of the BMS cell seeded PLOEF-ODP samples, stained with antibody against PECAM. parallel sheets of cells positive for PECAM is observed in the image which was not observed in PLEOF or PLEOF-mODP. These results demonstrate that the osteopontin-derived peptide can potentially initiate the cascade of vascularization.

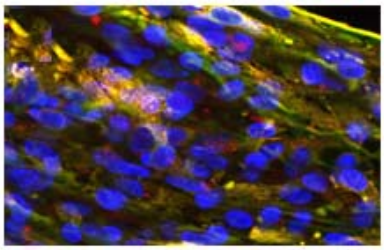

Figure 2. Confocal image of PLEOF-ODP stained with antibody against PECAM.

Conclusion: Results demonstrates that RGD and ODP peptides, grafted to a biodegradable PLEOF substrate, synergistically enhance vasculogenic differentiation of BMS cells.

References

1. Hamada Y, Egusa H, et al. Dent. Mater. J. 26(4): 487-492 (2007).

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