BMP-2 Peptide Grafted to a Degradable Substrate Enhances Osteogenic Differentiation of Stromal Cells

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Statement of Purpose: In-vivo, bone morphogenetic proteins (BMPs) signaling is highly regulated. As a result, very high doses *recombinant human* BMP-2 (rhBMP-2) have to be loaded in the graft which is 4-5 orders of magnitude higher than that found endogenously. Such high doses cause adverse effects such as bony overgrowth and immunological reactions. An attractive alternative is to use bioactive peptides that can initiate the cascade of osteogenesis. Recently, it has been shown that a synthetic peptide, LYLTSIASLETPVSSAKPIK, corresponding to residues 73-92 of the knuckle epitope of rhBMP-2 (BMP-2 peptide), promotes calcification of bone defects [1]. The objective of this work was to determine synergistic effect of RGD cell adhesive peptide and BMP-2 osteogenic peptide, grafted to a degradable substrate, on differentiation of bone marrow stromal (BMS) cells.

Methods: Schematic diagram of a BMS cell in contact with a degradable hydrogel substrate, grafted with RGD and BMP-2 peptides, is shown in Figure 1. The substrate was a biodegradable hydrogel based on a novel poly(lactide-co-ethylene oxide fumarate) (PLEOF) macromer [2]. Acrylated GRGD (Ac-GRGD) and azide terminated BMP-2 (Az-BMP-2) peptide were synthesized manually on Rink Amide NovaGel resin with Fmoc-protected amino acids [3].

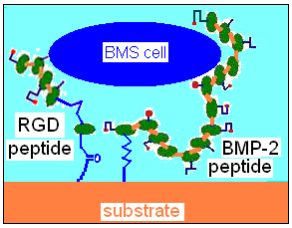

Fig 1. Schematics of BMS cell in contact with hydrogel substrate grafted with RGD and BMP-2 peptides.

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 BMP-2 peptide. BMP-2 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-BMP-2. 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 BMP-2 peptide grafted RGD-PLEOF hydrogels and cultured in osteogenic media (100 nM dexamethasone, 50 mM ascorbic acid 2-monophosphate, and 10mM β-glycerophosphate) for 21 days. At each time point, samples were removed, and alkaline phosphatase (ALPase) and calcium content were measured with p-nitrophenol and QuantiChrom Calcium Assays, respectively.

Results: Figure 2 compares alizarin red staining for the extent of mineralization on PLEOF substrate grafted with RGD and BMP-2 peptide with that of the control (no RGD and BMP-2 peptide). In general, alizarin staining was significantly less on PLEOF substrates grafted with RGD or BMP-2 compared to RGD and BMP-2 grafting.

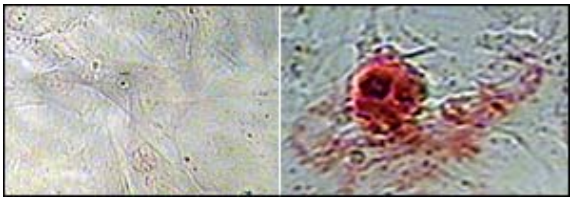

Figure 2. Alizarin red staining of BMS cells on PLEOF hydrogel without (left) and with RGD/BMP-2 peptides grafting after incubation for 3 weeks.

Figure 3 compares the ALPase concentration and calcium content of the PLEOF hydrogels grafted with RGD, BMP-2, and RGD/BMP-2 peptides with that of the control (no RGD and BMP-2 peptides) as a function of time cultured in osteogenic media. The substrates grafted with RGD or BMP-2 peptide had higher levels of ALPase and calcium compared to the control. More importantly, the substrate grafted with RGD and BMP-2 peptides had significantly higher levels of ALPase and calcium than the substrates grafted with RGD only or BMP-2 only for all time points.

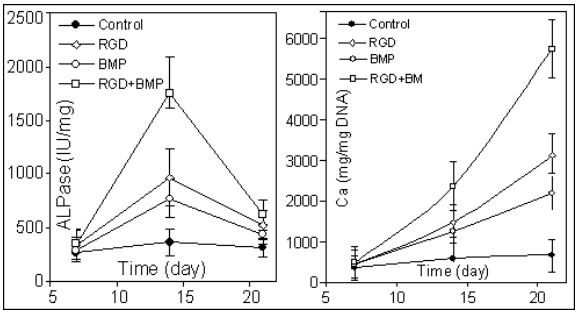

Fig 3. ALPase (left) and calcium (right) contents of BMS cells with or without RGD/BMP-2 peptides grafted to the hydrogel substrate after incubation for 3 weeks.

Conclusion: Results demonstrates that RGD and BMP-2 peptides, grafted to a biodegradable PLEOF substrate, synergistically enhance osteogenic differentiation of BMS cells.

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References:

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