Experimental and Computational Investigation of Activity of Osteogenic BMP-2 Peptide in Amphiphilic PEG Hydrogels

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Statement of Purpose: Morphogenic proteins are being used extensively in cellular constructs to regulate differentiation and maturation of transplanted *cells in vivo*. However, high doses of these proteins are implicated in undesired side effects such as tumor formation and immune reaction [1]. An attractive strategy is using peptides from the active domains of morphogenic peptides [2]. Although many peptides with morphogenic activity have been identified, their use in regenerative medicine has been limited by the lack of suitable matrix for delivery and immobilization. The objective of this work was to investigate computationally and experimentally the effect of conjugation of a BMP-2 derived osteogenic peptide to an amphiphilic PEG-based hydrogel on peptide aggregation, interaction with cell surface receptors, and osteogenic differentiation of mesenchymal stem cells.

Methods: The atomic structure of the peptide and peptide-conjugated macromer were coarse-grained into different beads using the Martini model [3]. The peptide-conjugated macromer was randomly placed in the simulation box and the box was filled with water. The simulations were performed under NVT ensemble dynamics using the MARTINI force field. The temperature was held at 37°C using the Nose thermostat. The Mesocite module of Materials Studio (Accelrys) was used for the mesoscale simulations.
The BMP-2 derived peptide KIPKA SSVPT ELSAI STLYL was synthesized and conjugated to the amphiphilic lactide-PEG macromer as described [4]. MSCs were suspended in the conjugated macromer and crosslinked by UV polymerization. The cell-encapsulated gels were cultured in basal medium supplemented with ascorbic acid and β-glycerophosphate but without dexamethasone (Dex). At each time point, the encapsulated cells were analyzed for cell content, alkaline phosphatase activity (Alp), extent of mineralization, and expression of markers related to osteogenesis.

Results: Figure 1a shows course-grained structure of the BMP-2 grafted lactide-PEG macromer. The ethylene oxide, lactide, and acrylate functional unit are shown by green, red, and blue colors, respectively. Figure 2b shows the formation of aggregates when BMP-2 peptide is conjugated to 4 kDa PEG. When BMP-2 was conjugated to lactide-PEG macromer, the extent of aggregation increased compared to PEG macromer (Figure 1c). The aggregation of peptide induced by conjugation to synthetic macromers can potentially reduce bioactivity with regard to osteogenic differentiation of MSCs. Figure 2 shows the effect of conjugation and immobilization of the peptide to PEG macromer and hydrogel on osteogenic activity as measured by ALP with incubation time (7-28 days). MSCs encapsulated in PEG without peptide and incubated in osteogenic medium without Dex had very low ALP activity (OM, first column, day 14) and the addition of BMP-2 protein significantly increased ALP activity (prot, last column).

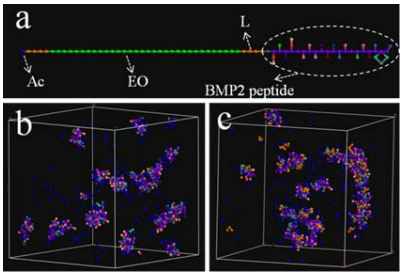

Figure 1. (a) Coarse-grained structure of BMP-2 peptide conjugated macromer; aggregate formation in BMP2-PEG (b) and BMP2-PEG-lactide conjugates (c).

ALP activity of the MSCs increased with dissolution of BMP-2 peptide in PEG matrix (P, 2nd column) but immobilization of peptide to the gel by covalent attachment reduced ALP activity (cP, 5th column) compared to P. Conjugation of the peptide to a PEG macromer followed by covalent attachment to the gel increased ALP (CE-P, 3rd column) compared to cP but conjugation of the peptide to lactide-PEG decreased ALP (cLE-P, 4th column) compared cE-P, presumably due to higher aggregation (Figure 1c).

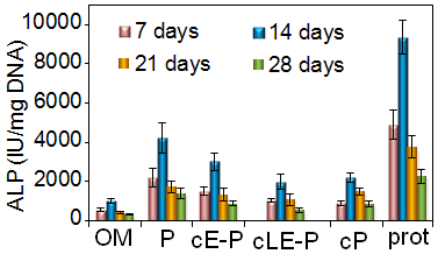

Figure 2. ALP activity of MSCs encapsulated in PEG matrix and cultured in osteogenic medium without Dex. Groups include no peptide (OM), peptide in gel (P), peptide attached to gel (cP), peptide-PEG conjugated to gel (cE-P), peptide-lac-PEG conjugated to gel (cLE-P), and BMP-2 protein in gel (prot).

Conclusions: The significantly lower activity of peptides in engineered matrices can be traced back to peptide aggregation, leading to lower bioactivity in synthetic hydrogels. Further, peptide aggregation increased non-specific cell interaction, membrane rupture, and peptide uptake by the cells (data not shown).

References: [1] Carragee EJ. Spine J. 2011;6:471-491. [2] Jabbari E. Curr Pharm Des. 2013;19:3391-3402. [3] Moeinzadeh S. J Phys Chem. 2012;116:1536-1543. [4] Moeinzadeh S. Biomacromolecules 2012;13:20173-2086.

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