Controlling Osteogenesis by Grafting Peptide Mimetics via Orthogonal Click Chemistries to Alginate Hydrogels

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Statement of Purpose: Soluble recombinant human BMP-2 (rhBMP-2) delivery from matrices is routinely used for clinical spinal fusion procedures. However, the massive dose of growth factor required for this procedure can have dangerous side effects, such as ectopic bone formation. In contrast, much lower quantities of BMP-2 drive osteogenesis during development, suggesting that synergistic contributions from other sources, such as the extracellular matrix (ECM) can lower the dose of BMP-2 required for therapeutic interventions. Integrin-binding peptides have been shown to act together with growth factors in bone formation[1]. We have developed a new approach, utilizing orthogonal click chemistries to immobilize integrin binding peptides and growth factor mimetics to alginate polymers. Strain promoted azide-alkyne cycloaddition (SPAAC) was used to couple azide-modified cyclo-RGD (Az-cRGD) and maleimide-thiol was used to couple mimetic of BMP-2 knuckle epitope (BMP-KE) onto alginate polymers. Peptide bioactivity was demonstrated through 2D cell adhesion and 3D Mesenchymal Stem Cell (MSC) osteogenesis assays.

Materials and Methods: High molecular-weight, high G-block containing alginate (Manugel; Dupont) was dissolved in 2-(N-morpholino) ethanesulfonic acid (MES) buffer and combined with 1-ethyl-(dimethylaminopropyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) to facilitate reaction with either *N*-[(1*R*,8*S*,9*s*)-Bicyclo[6.1.0]non-4-yn-9-ylmethyloxycarbonyl]-1,8-diamino-3,6-dioxaoctane (BCN-amine) and N-β-maleimidopropionic acid (BMPH). BCN-alginate was combined with Az-cRGD and maleimide-alginate was combined with BMP-KE mimetic peptide (CGKIPKASSVPTELSAISTLYL-OH) in PBS. After dialysis and filtration, alginates were dissolved into serum free DMEM and crosslinked using $CaSO_4$. 7F2 osteoblast spreading was analyzed in ImageJ after staining for stress fibers (Alexa Fluor 488 Phalloidin) at 24 h. For 3D differentiation assays, MSCs were encapsulated into gels made from alginate, Az-cRGD-alginate, or a mixture of Az-cRGD-alginate and BMP-KE alginate. Cells were cultured for 5 days in DMEM with osteogenic supplements, 10% FBS and 0-100ng/mL recombinant bone morphogenetic peptide (rhBMP2). Cells were retrieved with alginate lyase and collagenase and lysed for measurement of Alkaline Phosphatase (ALP) activity (4-MUP) and DNA content (PicoGreen).

Results and Discussion: 300μM Az-cRGD enhanced 7F2 spreading (Fig. 1A), demonstrating higher bioactivity when compared to alginate coupled with the same cRGD density using previously established direct peptide-polymer coupling with carbodiimide chemistry[2]. Alkaline phosphatase activity of D1 mMSCs (Fig. 1B) suggests that integrin and growth factor are both required for activation of osteogenic activity. Fig. 1C demonstrates the ability of immobilized growth factor and adhesive peptide to enhance osteogenic activity as effectively, if not more so, than soluble rhBMP-2 combined with cRGD.

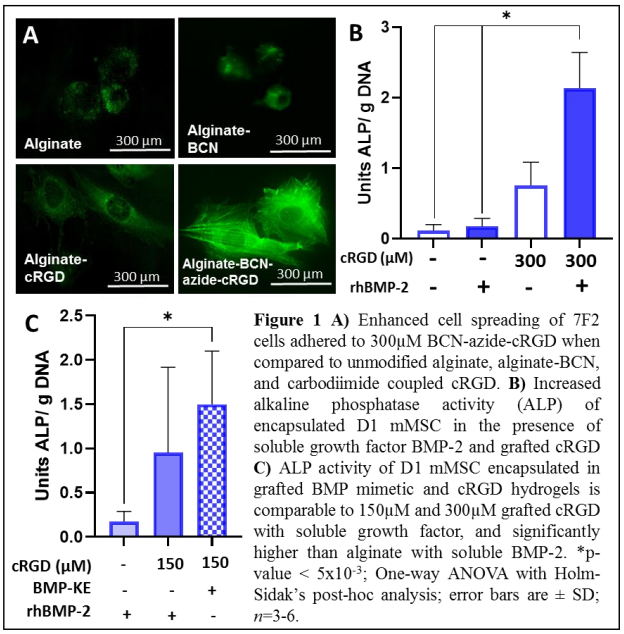

Figure 1 A) Enhanced cell spreading of 7F2 cells adhered to 300μM BCN-azide-cRGD when compared to unmodified alginate, alginate-BCN, and carbodiimide coupled cRGD. **B)** Increased alkaline phosphatase activity (ALP) of encapsulated D1 mMSC in the presence of soluble growth factor BMP-2 and grafted cRGD **C)** ALP activity of D1 mMSC encapsulated in grafted BMP mimetic and cRGD hydrogels is comparable to 150μM and 300μM grafted cRGD with soluble growth factor, and significantly higher than alginate with soluble BMP-2. *p-value < $5x10^{-3}$; One-way ANOVA with Holm-Sidak's post-hoc analysis; error bars are ± SD; n=3-6.

Conclusions: Direct conjugation of BCN to alginate facilitates bioactive peptide conjugation through SPAAC. This click chemistry more effectively couples peptide mimetics to alginate than carbodiimide coupling alone and potentially allows for new peptides to be added *in situ*, in the presence of cells. The results presented demonstrate the potential of immobilized growth factor to promote osteogenic activity as effectively as soluble growth factor without migration from the site of interest[3].

References:

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