Patterning Bone Formation Using Proteins Derived from Seashell and Bone

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Statement of Purpose: The goal of this research is to evaluate whether osteogenic proteins such as bone morphogenetic protein 2 (BMP-2) and those extracted from mother of pearl (nacre) can exert spatial control over bone formation when micropatterned to poly(ethylene glycol) (PEG) hydrogels. The autologous bone graft is the current gold standard in clinical practice for the repair of skeletal trauma[1]. However, drawbacks include donor site morbidity, limited supply, and increased risk of infection[1,2]. Allogeneic and synthetic graft materials are widely available, but they generally have reduced osteogenic activity due to storage and processing that destroys osteoprogenitors[3], requiring codelivery with the powerful osteogenic agent BMP-2. BMP-2, however, is contraindicated in cancer patients due to its role in cancer proliferation, and reports of ectopic bone growth, swelling and airway obstruction are increasing[4,5]. Thus, there is a need to develop delivery systems capable of spatially controlling the presentation of BMP-2 and similar therapeutics to promote bone regeneration while minimizing undesirable side effects. Proteins extracted from the nacre of mollusks are also investigated for their regenerative potential. Nacre is osteogenic and non-immunogenic and has been shown in vitro and in vivo to induce both osteoblast differentiation and mineralization in several species during both animal and human clinical trials.[6] Nacre proteins have also demonstrated high spatial control over calcium crystal formation. Therefore, it holds potential as an alternative to BMP-2, capable of spatially controlling bone formation.

Methods: BMP-2 and extracted nacre proteins from the shells of giant oysters (Pinctada maxima) were reacted with acrylate-poly(ethylene glycol)-succinimidyl valerate (PEG-SVA) under basic conditions. Poly(ethylene glycol) diacrylate (PEGDA) hydrogel disks (1-cm diameter, 0.4 mm thick) were formed by UV photopolymerization in glass molds separated by 0.4 mm spacers. The proteins were then covalently photo-conjugated to the substrate by exposing the hydrogels coated with protein solution to white light (nacre was bound either through a photomask or not) followed by repeated rinse cycles in PBS. W-20-17 mouse bone marrow stromal cells were cultured in growth media supplemented with PEG-conjugated BMP-2 for three days and assayed via alkaline phosphatase (ALP) staining and enzyme activity. Mouse bone marrow stromal cells (W-20-17) were then seeded onto the protein-conjugated hydrogels and assayed via ALP staining and enzyme activity after 3 days.

Results: Increased ALP staining and enzyme activity of W-20-17 cells cultured in growth media supplemented with PEG-conjugated BMP-2 shows increased ALP activity over non-supplemented controls in a dose-dependent manner (Fig. 1). ALP staining shows increased activity on hydrogels containing surface-bound BMP-2 and nacre proteins when compared to negative controls containing only surface-bound RGDS peptides (Fig. 2), and increased mineralization in patterns containing PEG-nacre proteins (Fig. 3).

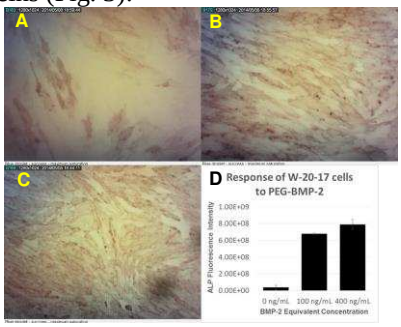

Fig. 1: W-20-17 cells stained for ALP after 3 days exposure to PEG-BMP-2 equivalent to **A.** 0 ng/mL, **B.** 200 ng/mL, and **C.** 400 ng/mL unconjugated BMP-2. **D.** Relative ALP activity was also assayed via fluorescence.

Fig. 2. ALP stained W-20-17 cells on PEGDA hydrogel substrates containing **A.** no proteins, **B.** surface-bound BMP-2, and **C.** surface-bound nacre proteins.

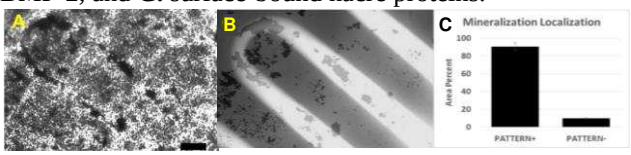

Fig. 3. **A.** Nacre-patterned mineralization after 14 days. **B.** Fluorescently labeled nacre proteins overlaid with mineralization. **C.** 90 % of the mineralization has contiguous contact with the pattern. Scale bar is 200 µm.

Conclusions: This work shows that PEG-SVA can serve as a covalent linker in order to immobilize BMP-2 and nacre proteins onto a PEG hydrogel substrate without decreasing osteogenic activity. This work further demonstrates the capability to spatially control osteoblast mineralization. Future considerations will include alternate bioactive compounds and linking methodologies in order to further optimize this platform for tissue engineering and drug delivery applications.

References:

1. Giannoudis PV, et al. Injury. 2005;36 Suppl 3:S20-7.
2. Marino JT, Ziran BH. Orthop Clin North Am. 2010;41(1):15-26
3. Valimaki VV, Aro HT. Scand J Surg. 2006;95(2):95-102.
4. Kang MH, et al. Exp Cell Res. 2010;316(1):24-37.
5. Perri B, et al. Spine J. 2007;7(2):235-239.
6. Rousseau M, et al. Comp Biochem Physiol , Part B: Biochem Mol Biol. 2003;135B(1):1-7.