Protein-based Biomaterials Accelerate Osteogenic Differentiation

Yeji Kim and Julie C. Liu
School of Chemical Engineering, Purdue University, West Lafayette, IN, 47907

Introduction: Bone is a dynamic and highly vascularized tissue. It has an excellent ability to heal and remodel without leaving scar tissue. However, large bone defects caused by trauma or fracture require surgical procedures for treatment. Tissue engineering of bone is emerging as a promising approach to regenerate large bone defects that do not self-heal. In this study, we are investigating the use of protein-based biomaterials as potential scaffolds for bone regeneration. These biomaterials are modular and include multiple protein domains in a single material. In particular, we have included a short bioactive peptide in the biomaterial as a material-based cue to promote stem cells to differentiate and produce a bone matrix.

Methods: An artificial protein containing resilin repeats from *Anopheles gambiae*[1] and a peptide derived from bone morphogenetic protein-2 (BMP-2)[2] was cloned and transformed into *E. coli*. Resilin-based proteins were expressed in *E. coli* at 37 °C with isopropyl β-D-1-thiogalactopyranoside (IPTG, Denville Scientific, NJ) either in a shake-flask or a fermentor (BioFlo 110, New Brunswick, CT). Proteins were purified by a salting-out and heating method[3]. Protein purity in this study was ≥95%, which was confirmed by SDS-PAGE analysis.

Purified proteins at 1 mg/mL were adsorbed onto tissue culture polystyrene (TCP) overnight at 4 °C. Human mesenchymal stem cells (hMSCs) were seeded on TCP, protein containing a BMP-derived peptide (RZ-BMP), or protein containing a scrambled BMP peptide sequence (RZ-sBMP). Osteogenic differentiation of hMSC was characterized by Alizarin red S staining. Statistical difference between groups was determined by Tukey's multiple comparison tests.

Results: DNA encoding resilin-based proteins was successfully cloned. Proteins were also successfully expressed and purified. Proteins were produced at a yield of 18 mg/L (shake-flask) and 40 mg/L (fermentor).

The bioactivity of resilin-based proteins was investigated by culturing hMSCs on adsorbed proteins. After 11 days of culture, cells seeded on RZ-BMP showed the most calcium deposition compared to cells cultured on TCP or RZ-sBMP (Figure 1). This result suggests that RZ-BMP accelerated osteogenic differentiation of hMSCs. For all groups, the mineralization increased over time from day 11 to day 13 (Figure 1B).

Some cells on RZ-sBMP appeared to have significant calcium deposits, which suggest that the resilin sequence in the biomaterial may be interacting with cells. However, more homogeneous mineralization was observed on cells seeded on RZ-BMP.

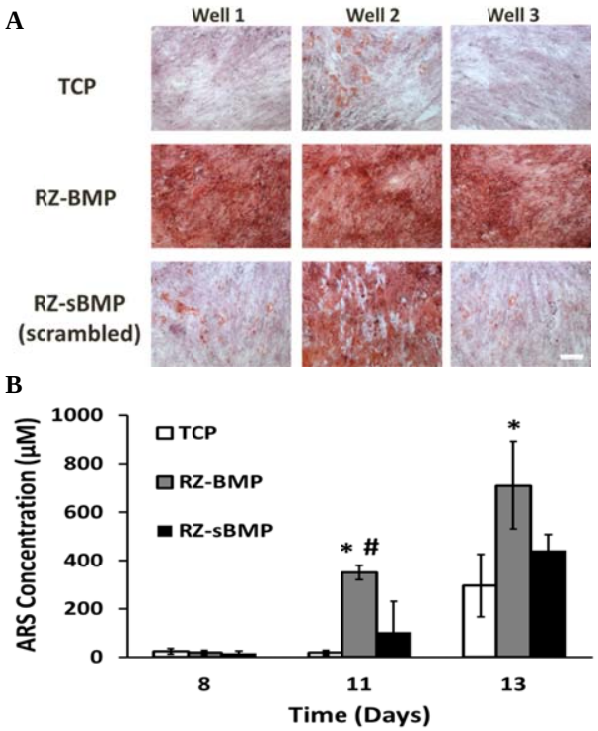

Figure 1. Calcium deposition of hMSCs. A) At 11 days of culture, hMSCs on RZ-BMP showed the most mineralization compared to cells on TCP or RZ-sBMP, as assessed by Alizarin red S staining. The scale bar represents 250 μm. B) At 11 and 13 days of culture, the calcium content of cells on RZ-BMP is statistically higher than on TCP. This result indicates that RZ-BMP accelerated osteogenic differentiation of hMSCs. (* $p < 0.05$ compared to TCP, # $p < 0.05$ compared to RZ-sBMP)

Conclusions: The protein containing a BMP-derived peptide (RZ-BMP) accelerated osteogenic differentiation of hMSCs as assessed by Alizarin red S staining. Thus, the BMP-derived peptide retained bioactivity within our biomaterial, and the cells interacted with the peptide in a sequence-specific manner. Incorporation of bioactive peptides as material-based cues may be useful for accelerating bone healing. Furthermore, our protein-based biomaterials can be engineered to provide additional functional sites such as proteolytic degradation sites.

References:

1. Lyons, R. E. *et al*, Protein Engineering Design and Selection, 2007. 20(1): p. 25-32.
2. Saito, A., *et al*., Biochimica et Biophysica Acta-Proteins & Proteomics, 2003. 1651(1-2): p. 60-67.
3. Renner, J. N. *et al*, Protein Expression and Purification, 2012, 82: p. 90-96.