Controlling Cell Differentiation with Protein-engineered Microenvironments

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Introduction: Protein-based biomaterials have emerged as powerful tools for tissue engineering applications. Recombinant DNA techniques can be used to precisely tune material properties at the molecular level, and multiple peptide modules can be incorporated into a single material. In this study, we describe a new approach to incorporate bioactive cues, which elicit specific cell repsonses, into the backbone of bio-inspired biomaterials. Our biomaterials are modular and include multiple protein domains in a single material. In particular, we included short bioactive peptides in the biomaterials to serve as material-based cues in cellular microenvironments, which promote specific cell differentiation. The goal of this study was to demonstrate the versatility of modular proteins containing bioactive cues, which promote cell differentiation towards distinct lineages. The bioactive cues included a peptide derived from bone morphogenetic protein-2 (BMP-2) for osteogenic differentiation or vascular endothelial growth factor (VEGF) for endothelial differentiation.

Methods: Modular proteins containing the bioactive cues and resilin repeats (RZ) from *Anopheles gambiae*[1] were expressed in *E. coli* at 37 °C with isopropyl β-D-1-thiogalactopyranoside in a fermentor. Proteins were purified by a salting-out and heating method.[2]

Purified proteins were adsorbed onto tissue culture polystyrene (TCP) overnight at 4 °C. Human mesenchymal stem cells (hMSCs) were seeded on various surfaces including TCP or one of proteins listed on Table 1. For osteogenic differentiation, cells were cultured in osteogenic medium and characterized by evaluating alkaline phosphatase (AP) activity, calcium deposition, and expression of bone-related genes. For endothelial differentiation, cells were grown in medium containing 5% fetal bovine serum, and von Willebrand Factor (vWF) expression and the uptake of acetylated low density lipoprotein (ac-LDL) were determined. Statistical differences between control and experimental groups were determined by Dunnett's test.

Table 1. Modular proteins used in this study

Protein name	Sequence serving as bioactive cue
RZ-BMP	BMP-derived peptide[3]
RZ-scBMP	Scrambled peptide
RZ-QK	VEGF-mimicking peptide[4]

Results: First, cells cultured on RZ-BMP showed an increase in AP activity (Figure 1A), calcium deposition (Figure 1B), and gene expression of Runx2 and type I collagen (data not shown) compared to cells on TCP. These results suggest that RZ-BMP accelerated osteogenic differentiation of hMSCs. Cells appeared to respond to RZ-BMP in a sequence-specific manner as cells on RZ-BMP showed enhancement of many osteogenic markers compared to cells on RZ-scBMP (Figure 1 and data not shown).

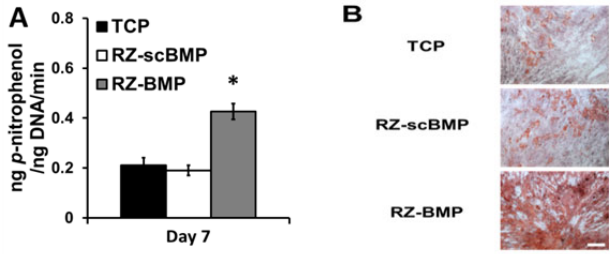

Figure 1. RZ-BMP proteins accelerated osteogenic differentiation of hMSCs. Compared to cells on TCP, hMSCs on RZ-BMP showed increased (A) AP activity at day 7 and (B) calcium deposition at day 11. * $p < 0.05$ compared to TCP. Calcium deposition was measured by Alizarin red S staining. Scale bar represents 250 μm.

Second, we replaced the BMP-2 peptide with the VEGF-mimicking peptide in the protein backbone. Cells grown on RZ-QK behaved in a similar manner to positive control cells (cells cultured on TCP in endothelial differentiation medium). At day 12, cells on RZ-QK displayed an increase in ac-LDL uptake (Figure 2A) and vWF expression (Figure 2B), compared to proliferating cells at day 0.

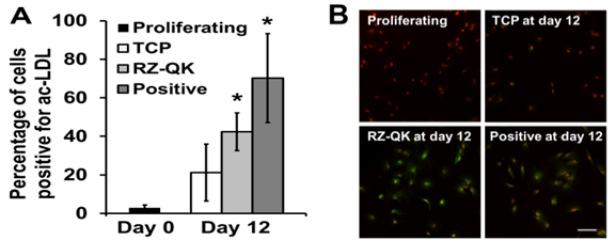

Figure 2. RZ-QK proteins promoted endothelial differentiation of hMSCs At day 12, cells on RZ-QK proteins exhibited higher ac-LDL uptake (A) and increased vWF expression (B, Green: vWF, Red: nuclei), compared to proliferating cells at day 0. * $p < 0.05$ compared to day 0. Scale bar represents 100 μm.

Conclusions: The protein-based biomaterials containing the BMP-2 peptide or VEGF-mimicking peptide promoted osteogenic or endothelial differentiation, respectively. Thus, our results demonstrated that cell differentiation can be modulated by switching bioactive cues in protein-engineered microenvironments.

References: 1. Lyons, R. E. *et al*, Protein Engineering Design and Selection, 2007. 2. Renner, J. N. *et al*, Biomacromolecules, 2012. 3. Saito, A. *et al*., Biochimica et Biophysica Acta - Proteins & Proteomics, 2003. 4. D'Andrea. L. D. *et al*., Proceedings of the National Academy of Sciences, 2005.