Osteoinductive Polymer Scaffolds for Bone Tissue Engineering: A Surface-Modification Approach

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Introduction: Tissue engineering has emerged as a viable alternative to traditional bone grafting procedures by providing defect sites with engineered synthetic constructs for guided bone regeneration. Many currently available materials unfortunately suffer from a lack of biological activity, which can significantly limit the extent of cellular interaction and tissue integration at the interface between the host and implanted biomaterial. The addition of cell-binding signals, in the form of short-chain oligopeptides, can endow synthetic materials with the biological cues needed to mimic native cell-matrix protein interactions and improve host integration [1]. In this study, oligopeptides derived from human growth factor, bone morphogenetic protein-2 (BMP-2), were covalently immobilized to the surface of poly(lactic-*co*-glycolic) acid (PLAGA) polymer thin films and evaluated *in vitro* for osteoinductive potential using rat bone marrow-derived mesenchymal stem cells (rMSCs).

Methods: Oligopeptides derived from amino acid residues 73-92 of the mature human BMP-2 protein [2] were custom synthesized (Anaspec®, San Jose, CA) and used for surface modification of PLAGA thin films. PLAGA thin films were fabricated by solvent casting and surfaces functionalized via a water-soluble carbodiimide. Activated PLAGA surfaces were incubated with BMP-2 oligopeptides for 12 and 24 hours (B & C) and rinsed thoroughly with ultra-pure dIH_2O. Unmodified PLAGA thin films containing no BMP-2 oligopeptides (D & F) were used as negative controls for comparison. To confirm surface modification, thin films were characterized by static contact angle measurements and x-ray photoelectron spectroscopy (XPS). Rat MSCs were cultured on PLAGA thin films and evaluated for biological activity by assessing for: 1) cellular proliferation by total DNA content and 2) mineral deposition by calcium ion assay.

Results & Discussion: Surface-modified PLAGA thin films (B & C) demonstrated a decrease in surface contact angle with a corresponding increase in surface free energy, compared to unmodified thin films (D & F). XPS demonstrated an increase in surface nitrogen (N1s) and sulfur (S2p) content on modified thin films (B & C), with enrichment in amine groups ($N\text{-}H_x$) and multiple sulfur species (S-H, S-S), confirming the surface immobilization of hydrophilic BMP-2 oligopeptides.

	B	C	D	F
C1s	65.3%	65.4%	65.8%	65.3%
N1s	0.70%	1.20%	0.09%	0.11%
S2p	0.02%	0.13%	0%	0%

Table 1: XPS analysis of PLAGA thin films. Modified films (B & C) demonstrated increased surface nitrogen (N1s) and sulfur (S2p), compared to unmodified negative controls (D & F).

Rat MSCs cultured on surface-modified PLAGA thin films (B & C) demonstrated an increase in cellular proliferation by Day 14, reflected by an increase in dsDNA content, compared to negative controls. Additionally, increased calcium deposition was measured for surface-modified groups (B & C) by Day 14, compared to negative controls (D & F).

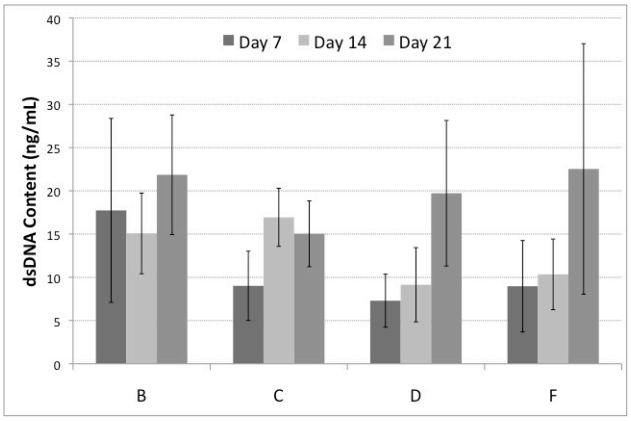

Figure 1: Total DNA content obtained from rMSCs cultured on surface-modified thin films. Increased DNA content from cells cultured on surface-modified films (B & C) by Day 14, compared to unmodified films (D & F), indicating increased cell numbers.

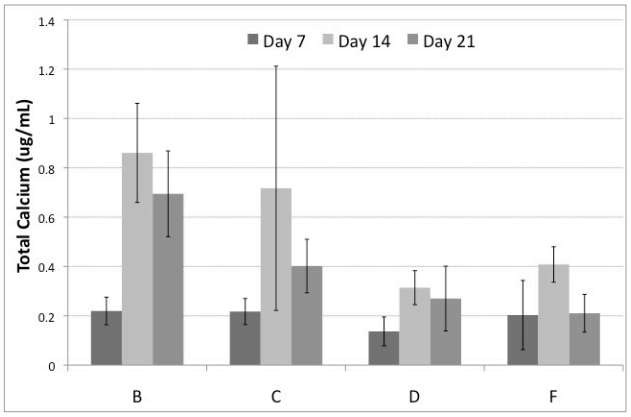

Figure 2: Total calcium deposition by rMSCs. Surface-modified films (B & C) demonstrated more mineralization by Ca^{+2} assay by Day 14, compared to negative controls (D & F).

Conclusions: BMP-2 derived oligopeptides were successfully immobilized to the surface of PLAGA thin films using carbodiimide-mediated chemistry. Surface-modified films were biologically active, while supporting the *in vitro* proliferation and osteoinduction of rMSCs. This study demonstrates that oligopeptide fragments derived from human BMP-2 can be utilized in the fabrication of biomimetic, bioactive materials for directed bone tissue regeneration.

References:

[1] Yang XB, et al. Bone. 2001;29: 523-531.
[2] Saito A, et al. Biochem Biophys Acta 2003;1651:60-67.