Surface versus Solution-Based C-Succinylation of Polymeric Biomaterials

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Introduction: Results of a preliminary study conducted in this laboratory indicated an enhanced growth of osteoblasts on surface c-succinylated films that have been treated with calcium hydroxide[1]. These promising results prompted the hypothesis that totally activated substrates made by solution c-succinylation will encourage even more growth, due to the greater frequency of succinic acid side groups available to attach calcium ions than those found on only surface c-succinylated films. This would result in a greater affinity of the osteoblasts to the higher frequency of calcium, therein causing more effective osseointegration. The present study tests the total c-succinylation hypothesis, and quantifies such tests through contact angle analysis and cell proliferation/ adhesion assays on 3 different materials.

Materials and Methods: Commercial molding-grade polycaprolactone (PCL) and a segmented lactide co-polymer (P-SLT) made at Poly-Med as per a patented method[2] were c-succinylated in 10% solution under heat and nitrogen purge in accordance with an established method[3]. Each were then precipitated from reaction solutions and redissolved to prepare solution-cast films. Films of the same type of P-SLT, as well as polypropylene (PP), were prepared separately using a heated, automatic hydraulic press (Carver, Wabash, IN) 5°C above melting temperature, and their surfaces were c-succinylated under heat and nitrogen purge. All c-succinylated films were then submerged in a 1% calcium hydroxide solution for 16 hours.

Human fetal osteoblasts (ATCC, Manassas, VA) were maintained under standard cell culture conditions in a 33.5°C, humidified, 5% CO_2/95% air environment in a 1:1 mixture of Dulbecco's Modified Eagle's Medium and Ham's Nutrient Mixture F12 supplemented with 10% fetal bovine serum (ATCC), 15mM HEPES, and 0.3mg/mL G418 (Invitrogen, Carlsbad, CA).

Prior to cell seeding, films were soaked in IPA, UV irradiated for 5 minutes, and affixed to a polystyrene cell culture plate using a small amount of sterile vacuum grease. Films were soaked in media overnight, then cells were seeded onto the films at a density of $3.1x10^4$ cells/cm^2. After 48 hours, cell viability was measured using a cell proliferation assay (CellTiter 96® AQueous One Solution; Promega, Madison, WI) according to manufacturer's instructions.

The contact angle of all calcified films and their untreated, control counterparts was analyzed using a PG-X Pocket Goniometer (Thwing-Albert Instrument Company, Philadelphia, PA).

Results and Discussion: As shown in Table I, the contact angle of PCL or P-SLT that has been solution c-succinylated and treated with calcium is lower than that of an untreated film of PCL or P-SLT, indicating a higher surface energy as a result of the overall treatment. Similarly, the data in Table II show that the surface c-succinylated films of polypropylene and P-SLT also exhibited comparably lower contact angles than their untreated counterparts. Compared to untreated controls, c-succinylation caused a similar change in contact angle using either the *surface* or *solution* treatment method. With respect to cell viability, osteoblasts exhibited more adhesion and proliferation on calcified, c-succinylated films for both solution and surface c-succinylation conditions as depicted in Tables I and II. As hypothesized, osteoblasts showed significantly higher proliferation and adhesion on calcified films which were based on solution c-succinylation than on those only surface c-succinylated and calcified.

Table I. Contact Angle and Osteoblast Viability Data of Solution C-Succinylated Films

Film	Contact Angle°		Osteoblast Viability (Normalized to Controls)	
	Control	Treated	Control	Treated
PCL	85	80	1.0	1.1
P-SLT	100	38	1.0	4.1

Table II. Contact Angle and Osteoblast Viability Data of Surface C-Succinylated Films

Film	Contact Angle°		Osteoblast Viability (Normalized to Controls)	
	Control	Treated	Control	Treated
PP	97	93	1.0	1.1
P-SLT	100	30	1.0	1.8

Conclusions: The data acquired in this study comparing contact angle and cell viabilities of both solution and surface c-succinylated films indicate that 1) osteoblasts adhere and proliferate more readily to calcified films than to their untreated control counterparts; 2) the contact angle is an effective method to quantify the effect of calcifying a film through direct measurement of surface energy; and 3) the solution c-succinylated and calcified films exhibit notably more osteoblast proliferation and adhesion than films which were surface c-succinylated. This directly correlates to the higher frequency of succinic acid attachment to calcium ions.

References:

1. Tate, P.L. et *al*. *Trans Soc Biomater*. 29 (2):611 (2006)
2. Shalaby SW. U.S. Patent 6,342,065 (2002)
3. Shalaby SW. U.S. Patent Appl. 10/693, 361 (2003)