Effect of Chemical and Enzymatic Crosslinking of Novel Porous Soy Protein Scaffolds on Human Mesenchymal Stem Cell Morphology and Growth

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Statement of Purpose: Natural biopolymers have been widely explored for tissue engineering applications. Our group has been investigating the use of soy protein in particular due to its abundance as a natural renewable resource, as well as its thermoplastic qualities that allow for ease and versatility in processing. Modifications of soy protein structures through fabrication methods such as extrusion and blending with composites have been attempted previously [1,2]. Crosslinking with glyoxal/heat treatment [1], formaldehyde [3], and transglutaminase [4] have resulted in unique products with varying mechanical properties, textures, and degradation profiles. However, beyond preliminary cytotoxicity studies of soy films [2], the behavior of cells within 3D soy protein constructs has not yet been explored. This study examines the interaction of human mesenchymal stem cells (hMSC's) seeded on novel chemically and enzymatically crosslinked soy protein porous scaffolds to reveal the range of cell responses that can be achieved with this material.

Methods: ***Scaffold Fabrication:*** An aqueous slurry of 3-5% (w/w) soy protein isolate (SPI, NOW Foods, Bloomingdale, IL) with glycerol was heat treated at 90°C and subsequently freeze-dried to form porous scaffolds. To produce enzymatically crosslinked scaffolds, transglutaminase (Ajinomoto, Japan) was dissolved in the slurry and incubated for 1 hour prior to lyophilization. For chemically crosslinked scaffolds, freeze-dried scaffolds were subsequently crosslinked with 10-15 mmol 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, Sigma-Aldrich, MO) coupled with 4-6 mmol N-hydroxysuccinimide (NHS, Sigma-Aldrich, MO) in 95% ethanol. ***Scaffold Characterization:*** The moisture content of scaffolds was determined by measuring the difference of weight before and after drying at 40°C (n=5). Porosity was characterized using mercury-infused porosimetry (n=3). Uniaxial unconfined compression was performed using a strain rate of 2 mm/min on a mechanical tester (JLW Instruments, IL). Compressive modulus was determined from stress/strain data (n>4). ***Cell Culture:*** hMSC's were expanded to passage 5 and seeded ($2x10^5$ cells/scaffold) onto the soy scaffolds. Cultures were maintained in DMEM with 10% FBS for up to 14 days. Proliferation was measured with the Picogreen DNA quantification kit (n>4), and cell viability/morphology was assessed using scanning electron microscopy (SEM) and laser scanning confocal microscopy with Live/Dead staining. Data were analyzed using unpaired Student's t-test and reported as mean ± standard error.

Results: Moisture content within the soy scaffolds ranged from 6-9%, with the EDC-treated scaffolds having the least amount of retained moisture. Scaffolds possessed a 90-93% porosity regardless of crosslinking treatment. The compressive modulus varied 3 orders of magnitude between enzyme crosslinked/control groups (50 Pa) and EDC crosslinked scaffolds (1000 Pa). Results from cell culture studies revealed that hMSC's seeded onto control and enzyme-treated scaffolds had a more elongated and spread morphology compared to cells seeded on EDC-treated scaffolds (**Fig. 1A**). Cells attached to enzyme-treated scaffolds showed extensive cell spreading and proliferation, forming a dense cell layer on the surface and within the pores of the scaffolds. DNA amount in enzyme-treated scaffolds showed consistent increases during the entire culture period resulting in a 3-fold higher amount than control and 6-fold higher amount than EDC-treated scaffolds after 14 days (**Fig. 1B**).

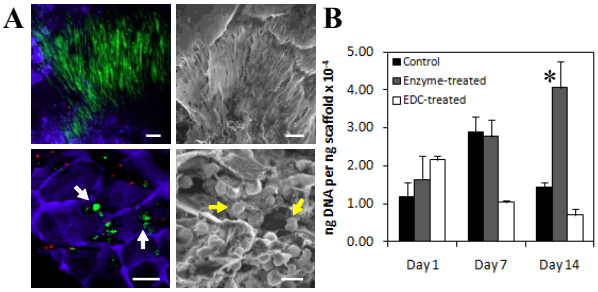

Figure 1. (**A**) Confocal microscopy (left column) and SEM (right column) of cells seeded on scaffolds at day 7. Blue = scaffold, green = live cells, red = dead cells. Top row: enzyme-treated scaffolds (scale =100 μm); Bottom row: EDC-treated scaffolds (scale=50 μm). Arrows highlight representative cells. (**B**) DNA quantification for all groups at days 1, 7, and 14. *:Significant difference at day 14 ($p < 0.005$).

Conclusions: The results from this study showed that a range of physical and bioactive properties can be achieved with porous soy protein scaffolds. EDC crosslinking of soy scaffolds significantly increased scaffold mechanical properties yet resulted in lower proliferative capacity of seeded cells and more rounded cell morphologies. Enzyme crosslinking, on the other hand, did not significantly change the mechanical properties but greatly enhanced cell proliferation. Future work will elucidate mechanisms responsible for these differences in cell behavior, as well as optimize the mechanical and bioactive properties of our soy scaffolds towards specific tissue engineering applications.

References: [1] Vaz CM *et al*. Polym Degrad Stabil. 2003; 81:65-74. [2] Silva SS *et al*. J Mater Sci-Mater M. 2005;16:575-579. [3] Chen L *et al*. Biomaterials. 2008; 29:3750-3756. [4] Tang CH *et al*. Food Res Int. 2006; 39:87-97.

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