Acta Biomaterialia 8 (2012) 694–703

Contents lists available at SciVerse ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Novel soy protein scaffolds for tissue regeneration: Material characterization and interaction with human mesenchymal stem cells

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ARTICLE INFO

Article history:
Received 29 June 2011
Received in revised form 19 September 2011
Accepted 29 September 2011
Available online 11 October 2011

Keywords:
Soy protein
Scaffold
Transglutaminase
Stem cell
Degradation

ABSTRACT

Soy protein modified with heat treatment and enzyme crosslinking using transglutaminase in maltodextrin was used to fabricate novel, porous three-dimensional scaffolds through lyophilization. Physical properties of scaffolds were characterized using scanning electron microscopy, mercury intrusion porosimetry, moisture content analysis and mechanical testing. Human mesenchymal stem cells (hMSC) were seeded and cultured in vitro on the scaffolds for up to 2 weeks, and changes in stem cell growth and morphology were examined. The resulting scaffolds had rough surfaces, irregular pores with size distributions between 10 and 125 μm, <5% moisture content and compressive moduli ranging between 50 and 100 Pa. Enzyme treatment significantly lowered the moisture content. Increasing amounts of applied enzyme units lowered the median pore size. Although enzyme treatment did not affect the mechanical properties of the scaffolds, it did increase the degradation time by at least 1 week. These changes in scaffold degradation altered the growth and morphology of seeded hMSC. Cell proliferation was observed in scaffolds containing 3% soy protein isolate treated with 1 U of transglutaminase. These results demonstrate that controlling scaffold degradation rates is crucial for optimizing hMSC growth on soy protein scaffolds and that soy protein scaffolds have the potential to be used in tissue engineering applications.
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1. Introduction

Tissue engineering involves the fabrication of constructs which aid in the repair and regeneration of damaged tissue, providing proper structure, function and integration with the host tissue. One frontier of tissue engineering lies in using a biomaterial scaffold to deliver cell-based therapy. Porous scaffolds provide three-dimensional microenvironments, which can mimic the extracellular matrix and can allow for cell infiltration and space for matrix deposition by cells to form new tissue. An ideal scaffold material should stimulate the formation of tissue which is structurally and functionally robust, while being safe and cost-efficient to obtain, process and manufacture [1,2]. The use of natural proteins to form biomaterials is an attractive therapy because of the ability of the natural material to control stem cell adhesion and growth through inherent binding sites. Human mesenchymal stem cells (hMSC) seeded on collagen and silk protein scaffolds have been shown to proliferate and differentiate into osteoblasts and chondrocytes that were fully functional, biocompatible and able to form tissues resembling native tissue structure and function [3,4].

Soy protein, an isolated component of the soybean, has recently emerged as an attractive alternative to animal-derived protein sources for biomedical applications. The US has led the world production of soybeans for over 50 years, generating 81 million metric tons in 2008 [5]. Soybeans are a natural and abundant resource, which contains 40% pure protein [6]. Two major subunits in the globular structure of soy protein include conglycinin (7S) and glycinin (11S), which contain all amino acids but are rich in glutamate, aspartate and leucine [6]. Soy protein exhibits versatility in processing and is shown to have good biodegradable and biocompatible qualities [7–13].

Different processing strategies of soy protein have been developed to alter its material properties. Thermal and chemical modifications have the capability of tailoring bulk and surface properties during the fabrication of soy structures [10,12]. Heat treatment of soy protein has been shown to induce thermoplasticity, which allows a wide variety of shapes and structures to be formed, including films, granules/pellets and gels [9,13–15]. Glyoxal and tannic acid have been used to extend degradation times of extruded soy protein pellets [12], and soy protein films crosslinked with varying amounts of formaldehyde were capable of controlling model drug release [13]. Transglutaminase, a physiological

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doi:10.1016/j.actbio.2011.09.036

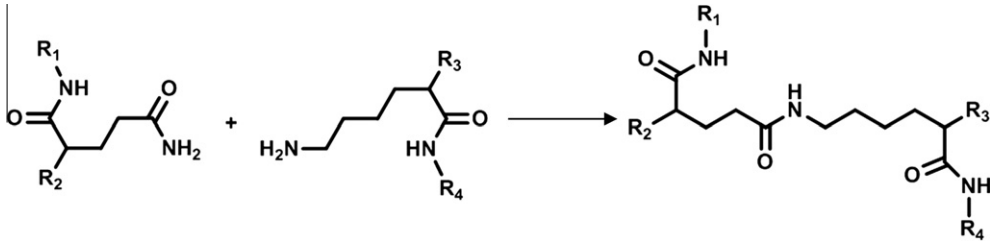


Fig. 1. Reaction of transglutaminase between glutamine and lysine.

enzyme, has been applied to soy protein in the food industry to modify the textures and mechanical properties of gels [14–16]. The enzyme facilitates the reaction of the γ-carboxamide group of a glutamine side chain with the ε-amino group of lysine side chain to form a ε(γ-glutamyl) lysine linkage (Fig. 1). Previous research suggests that microbial transglutaminase can increase the mechanical stability of soy protein scaffolds by forming intermolecular or intramolecular covalent bonds [14–16].

Exploration of the in vitro and in vivo biocompatibility of soy, albeit limited, shows promise for the use of soy protein as a biomaterial for drug delivery and tissue engineering purposes [8,10,13,17,18]. Soy curd containing protein and all other soybean components was shown to decrease the level of proinflammatory cytokine production of mononuclear cells from human peripheral blood and to promote osteoblast proliferation [9]. The in vitro biocompatibility of soy protein alone has been explored with L929 mouse fibroblasts in membranes and fiber forms [7,8,10]. For all studies, soy protein substrates were able to sustain cell viability, with no cytotoxic effects. Soy protein blends with cellulose and chitosan were also shown to be biocompatible [7,17,19].

The behavior of cells within three-dimensional soy protein constructs has not yet been explored. This is the first study to investigate the use of three-dimensional porous soy scaffolds for tissue engineering applications. The overall aims were: (1) to successfully fabricate soy protein into a porous, three-dimensional scaffold structure; (2) to investigate the effect of transglutaminase and maltodextrin modification on the material properties of soy protein scaffolds; and (3) to determine how scaffold properties affect hMSC growth and proliferation. hMSC are precursors to tissue-forming cells such as osteoblasts, chondrocytes and myocytes and can be obtained from an autologous source [20,21]. Understanding the basic interaction between hMSC and the soy protein scaffold provides insight into developing a potentially safer, lower-cost and more effective biomaterial system which delivers cell therapy for tissue regeneration.

2. Methods and materials

2.1. Scaffold fabrication

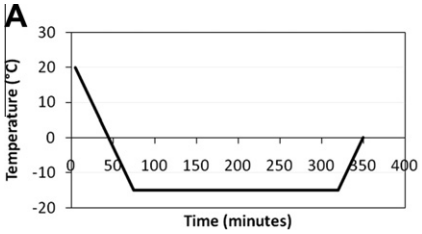
Soy protein isolate (SPI) containing ~83% pure soy protein (as verified by bicinchoninic acid analysis from Thermo Fisher Scientific (Rockford, IL, USA)) was obtained from Now Sports (Bloomingdale, IL, USA). Mixtures of 3 and 5 wt.% SPI were dissolved in Millipore water and were homogenized at 5000 rpm for 5 min. The slurries were heated at 90 °C for 1 h. Upon cooling of the solutions to room temperature, glycerol from Sigma–Aldrich (St. Louis, MO, USA) was added in the same weight percentage as SPI. The slurry was homogenized again at 5000 rpm for 5 min. Slurries were cast in 7 cm diameter aluminum weigh boats at volumes of 18 ml and 13 ml for 3 and 5 wt.% SPI solutions, respectively. ACTIVA TI microbial transglutaminase containing maltodextrin from Ajinomoto (Fort Lee, NJ, USA) was added to individual slurries in 1 and 20 U of enzyme activity to 1 g of SPI. The added ACTIVA TI formulation included 1 wt.% transglutaminase and 99 wt.% maltodextrin. Maltodextrin (DE = 4.0–7.0) from Sigma–Aldrich (St. Louis, MO, USA) was added in the same weight to gram protein percentage as 1 U transglutaminase as separate control samples. The slurries were incubated at 37 °C for 1 h.

All slurries were freeze-dried using a VirTis AdVantage BenchTop lyophilizer (Gardiner, NY) via a three-step process: ramping down temperature, solidification and sublimation. The lyophilizer temperature was lowered from 20 to −15 °C at a rate of 0.5 °C min^{-1} to cool down the slurries (Fig. 2A). The slurries were solidified by holding the lyophilizer temperature constant at −15 °C for 5 h (Fig. 2A). Ice from the water within the slurries was sublimed at 0 °C using a pressure of 100 mtorr for at least 40 h to create a porous scaffold structure. In summary, 3% and 5% SPI heat treatment (HT) and maltodextrin (MD) control groups as well as 1 and 20 U transglutaminase-crosslinked groups (TG 1 U and TG 20 U) were fabricated (Fig. 2B).

2.2. Material characterization

2.2.1. Scaffold microstructure and porosity

Both dry and water-hydrated scaffolds were imaged using scanning electron microscopy (SEM). Dry scaffolds were dessicated for at least 2 h prior to imaging. Hydrated scaffolds were first dehydrated in 95% ethanol for 3 h then rinsed in water for 30 min, and the scaffolds immersed in water were lyophilized prior to imaging. Cross sections of all scaffolds were obtained through liquid nitrogen fracture and coated with 9 nm of osmium. SEM was performed ($n = 2$ per group) using a LEO Gemini 1525 FEG SEM with an acceleration voltage of 15 kV (Oberkochen, Germany)



B

Controls	Enzyme-crosslinked
Heat treatment only (HT control)	One unit (TG 1U)
One unit maltodextrin only (MD control)	Twenty units (TG 20U)

Fig. 2. (A) Protocol used to freeze slurries. Slurries were cooled down by ramping the lyophilizer temperature down from 20 to −15 °C at a rate of 0.5 °C min^{-1}. Slurries were frozen for at least 5 h at −15 °C to ensure complete solidification before sublimation. (B) Summary of scaffold groups fabricated.

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