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Electrospun ultrafine fibrous wheat glutenin scaffolds with three-dimensionally random organization and water stability for soft tissue engineering

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ABSTRACT

Wheat glutenin, the highly crosslinked protein from wheat, was electrospun into scaffolds with ultrafine fibers oriented randomly and evenly in three dimensions to simulate native extracellular matrices of soft tissues. The scaffolds were intrinsically water-stable without using any external crosslinkers and could support proliferation and differentiation of adipose-derived mesenchymal stem cells for soft tissue engineering. Regeneration of soft tissue favored water-stable fibrous protein scaffolds with three-dimensional arrangement and large volumes, which could be difficult to obtain via electrospinning. Wheat glutenin is an intrinsically water-stable protein due to the 2% cysteine in its amino acid composition. In this research, the disulfide crosslinks in wheat glutenin were cleaved while the backbones were preserved. The treated wheat glutenin was dissolved in aqueous solvent with an anionic surfactant and then electrospun into bulky scaffolds composed of ultrafine fibers oriented randomly in three dimensions. The scaffolds could maintain their fibrous structures after incubated in PBS for up to 35 days. *In vitro* study indicated that the three-dimensional wheat glutenin scaffolds well supported uniform distribution and adipogenic differentiation of adipose derived mesenchymal stem cells.

1. Introduction

Large soft tissue defects resulted from traumatic injury, oncologic resection and congenital malformation usually lead to non-self-healable contour deformities, and thus adversely influence emotional and physical well-being of patients. To date, regeneration of large-volume soft tissue defects, especially loss of adipose tissue, has been frequently conducted by implanting autologous fat flaps or synthetic materials. However, the two approaches have limitations, such as post-surgery volume loss of up to 40–60% (Tachi and Yamada, 2005), donor site morbidity and foreign-body reactions (Patrick, 2004). Tissue engineering is a relatively new but attractive approach for large-volume adipose regeneration. Currently, adipose derived mesenchymal stem cells (ADMSCs) are increasingly attracting attention as cell sources for adipose tissue engineering (Sterodimas et al., 2010). ADMSCs are available in large quantity, can be easily harvested using non-invasive collecting methods, such as lipoaspiration, and can differentiate rapidly and readily into adipocytes. To guide proper growth of ADMSCs and development of functional artificial tissues, suitable scaffolds are still in need (Rahaman and Mao, 2005).

Three-dimensional (3D) ultrafine fibrous scaffolds may be advantageous over two-dimensional (2D) scaffolds and 3D non-fibrous scaffolds in adipose tissue engineering. The 3D ultrafine fibrous scaffolds are more structurally similar to the native adipose extracellular matrices (ECMs), which are composed of collagen fibrils oriented randomly in three dimensions (Bellas et al., 2013). In soft tissue engineering, it is particularly important for the 3D scaffolds to have certain volumes, since large tissues are usually required to reconstruct contour defects, and the cell activities could be better supported in 3D than in 2D scaffolds (Daquinag et al., 2012). So far, most of the scaffolds for adipose tissue engineering were 3D non-fibrous scaffolds composed of proteins (Phull et al., 2013), polysaccharides (Tan et al., 2009) and synthetic polymers (Hoo et al., 2013). Among them, proteins were preferred as they had compositions similar to the native ECMs, and therefore, could

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stimulate proper cellular response and activities to promote neotissue formation (Itoi et al., 2010).

Water stability was of vital importance for the protein scaffolds in stem cell tissue engineering, because scaffolds should be incubated in medium for long time in stem cell culture. However, most of the protein scaffolds degraded fast due to poor water stability, and thus required crosslinking, which suffered from either cytotoxicity or low crosslinking efficiency (Jiang and Yang, 2011; Jiang et al., 2012; Jiang et al., 2010). Besides, proteins were usually blended with synthetic polymers to improve the biocompatibility of synthetic polymers (Prabhakaran et al., 2013). In terms of adipose tissue engineering, limited applications of 3D ultrafine fibrous scaffolds could be found, not to mention 3D fibrous protein scaffolds with water stability.

Wheat glutenin (WG), a protein in wheat, has intrinsic water stability because it has up to 2% cysteine in the disulfide crosslinked molecules with molecular weights as large as 500 to 10,000 kDa (Wieser, 2007; Huebner et al., 1974). WG could be preferred over other proteins for adipose tissue engineering due to its biocompatibility, biodegradability and low risk to transmit pathogens (Reddy and Yang, 2011). Wheat glutenin films showed good water stability and better supported attachment and proliferation of osteoblasts than PLA films (Reddy et al., 2011).

Till now, limited research has been done on developing tissue engineering scaffolds from pure wheat glutenin due to its poor processability attributed to the highly crosslinked molecular structure. Due to the high crosslinking degree, WG does not dissolve in common solvents, and thus has difficulty to be electrospun or freeze dried solely. So far, successful fabrication of fibrous structures containing WG involved either existence of cytotoxic proteins or blending with synthetic polymers after hydrolysis of WG. Electrospinning of wheat gluten, the mixture of WG and gliadin, has been reported (Woerdeman et al., 2005). In addition, soluble wheat gluten has been mixed with PVA and electrospun (Dong et al., 2010). However, gliadin was proved cytotoxic (Elli et al., 2003), while PVA could remarkably reduce the tendency of scaffold-cell interaction. The disulfide crosslinks in WG should be broken while the backbones should be preserved before WG could be dissolved and electrospun into 3D structures (Cai et al., 2013).

In this research, WG was extracted from wheat gluten and treated under reductive conditions. The obtained WG was dissolved with surfactant in aqueous solution and then electrospun into 3D ultrafine fibrous scaffolds with random orientation and water stability. As indicated in the *in vitro* study using ADMSCs, the 3D ultrafine fibrous WG scaffolds could better support proliferation and adipogenic differentiation of ADMSCs than 2D ultrafine fibrous WG and commercial 3D non-fibrous scaffolds.

2. Materials and methods

2.1. Materials

Wheat gluten (Whetpro 80) with about 80% protein content, was a gift from Archer Daniels Midland Co., Decatur, IL. Sodium dodecyl sulfate (SDS, 99.0%) was supplied by Hoefer Inc., San Francisco, CA, and urea (99.0%) was purchased from Oakwood Chemical Inc., West Columbia, SC. Sodium carbonate, sodium bicarbonate, sodium sulfate, acetone, potassium chloride and sodium hydroxide were purchased from BDH chemicals Inc., West Chester, PA. Cysteine was supplied by Amresco LLC., Solon, OH. Amylase was a gift from Novazyme, Franklinton, NC.

To obtain WG for electrospinning, gliadin and starch were removed. To extract gliadin, wheat gluten was dispersed in 70% (v/v) ethanol at a solid to liquid weight ratio of 1:4 and stirred overnight at room temperature. The mixture was centrifuged at 8000 rcf for 10 min. The precipitate was considered as gliadin-free-gluten. To further remove starch, the precipitated gliadin-free-gluten was dried at 50 °C, pulverized and then dispersed in 1% amylase at 4:1 ratio of enzyme solution to the gliadin-free-gluten for treatment at 50 °C for 48 h. The pH was adjusted to 5.0 using sodium acetate buffer. The obtained WG was rinsed in distilled water for three times, dried at 50 °C and pulverized.

2.2. Controlled cleavage of disulfide crosslinks in WG

Glutenin was treated under mildly alkaline and reductive condition to break the disulfide crosslinks and preserve the backbones (Xu and Yang, 2014). Wheat glutenin was treated at 70 °C with an 8 M urea solution-to-WG weight ratio of 10:1. About 10% of cysteine based on the weight of WG was added into the solution. The pH was adjusted to around 10.5 using 50% NaOH solution and the treatment time was 12 h. After treatment, the solution was centrifuged at 8000 rcf for 20 min to precipitate undissolved WG. The supernatant was adjusted to pH 4 using hydrochloric acid and sodium sulfate to precipitate dissolved WG. The precipitate was washed three times with distilled water under centrifugation at 8000 rcf for 20 min. The collected WG was dried under 50 °C and pulverized.

2.3. Molecular weight measurement

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to evaluate the molecular weights of WG before and after treatment. About 100 μL NuPAGE® LDS Sample Buffer (1×) was used to dissolve 1 mg of each sample. The mixture was heated at 70 °C for 30 min and vortexed prior to loading. About 10 μL of WG solution was loaded into each slot of the polyacrylamide gel. After electrophoresis, the gel was fixed in 10% acetic acid and isopropyl ethanol for 1 h, stained with Coomassie Brilliant Blue G-250 for 2 h at room temperature. The stained gel was then destained in 10% acetic acid overnight until a clear background could be observed. The molecular weights of the protein standard markers ranged from 4 to 250 kDa.

2.4. Electrospinning of 3D and 2D fibrous WG scaffolds

To fabricate 3D ultrafine fibrous WG scaffolds, extracted WG and SDS with the same weight were dispersed in 0.3 M sodium carbonate-bicarbonate buffer at a WG-to-buffer weight ratio of 1:3, according to our previous study (Xu et al., 2014). The mixture was heated at 90 °C under stir for 1 h to obtain transparent spinnable WG solution. The solution was loaded into a syringe and electrospun under a voltage of 45 kV with a distance of 25 cm between the receptor and syringe needle. The needle was negatively charged and the receptor was positively charged. The post-treatment of WG scaffolds included annealing at 130 °C for 2 h and treatment in 70% methanol for 1 h. Subsequently, the electrospun 3D WG scaffolds were washed in 60% acetone solution with 10% potassium chloride for 5 days to remove SDS and in distilled water for 2 days, and then freeze dried. To study the influences of structures of scaffolds on the cell proliferation and differentiation, 2D WG scaffolds were electrospun under the same conditions except an insulation board was inserted before the collection board. The SDS was also removed using the same treatment.

2.5. Morphologies of 3D fibrous WG scaffolds

Gross appearance of 3D fibrous WG scaffolds in dry and wet states were recorded using a digital camera. Morphologies of the 3D fibrous WG scaffolds were observed under Hitachi S-3000N scanning electron microscope (SEM), and the interior structure of the 3D WG scaffolds after removal of SDS was observed in wet state using confocal laser scanning microscope (CLSM) in both longitudinal and