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Intrinsically water-stable electrospun three-dimensional ultrafine fibrous soy protein scaffolds for soft tissue engineering using adipose derived mesenchymal stem cells

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Soy protein, the plant protein from soybean, was electrospun into intrinsically water-stable scaffolds with large volume and ultrafine fibers oriented randomly and evenly in three dimensions (3D) to simulate native extracellular matrices of soft tissues. The 3D ultrafine fibrous scaffolds from proteins could be favored in soft tissue engineering. However, protein-based biomaterials usually suffered from poor water stability, while the highly crosslinked proteins which had water stability were usually difficult to be fabricated into fibers. Soy protein was a typical protein with intrinsic water stability, attributed to its 1.2% cysteine content. Soy protein has been developed into 3D non-fibrous structures, coarse fibers and films for tissue engineering applications, but not ultrafine fibrous structures. In this research, the disulfide crosslinks in soy protein were cleaved to facilitate its dissolution in an aqueous solvent system. The obtained solution was electrospun into bulky scaffolds composed of ultrafine fibers oriented randomly in three dimensions. Without external crosslinking, the fibrous soy protein scaffolds demonstrated long-term water stability, and maintained their fibrous structures after incubated in PBS for up to 28 days. *In vitro* study showed that the 3D soy protein scaffolds well supported uniform distribution and adipogenic differentiation of adipose derived mesenchymal stem cells. In summary, the 3D ultrafine fibrous soy protein structures could be good candidates as scaffolds in soft tissue engineering.

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Introduction

There are increasing incidences of soft tissue defects due to traumas, congenital malformation and oncological resection. Currently, autologous implantation is the primary treatment for soft tissue regeneration to regain local contour. However, the major issue of this method was that the autologous tissues could be easily absorbed and lose their volume quickly, since only 40 to 60% of the cells in the soft tissue remained viable after implantation.¹ The major reason was that the cells in the autologous soft tissues were primary cells with limited potential of expansion. To overcome this issue, tissue engineering with highly proliferative and multi-potent stem cells could be a promising alternative.

In tissue engineering, scaffolds are the key elements to provide substrates for cells to attach, proliferate, differentiate and develop into neo-tissues with satisfactory appearance and functions. The scaffolds should highly simulate the morphological and molecular features of native extracellular matrices (ECMs) to ensure that the cells cultured on them could have similar patterns of growth and differentiation. The native ECMs of soft tissues are usually three-dimensional (3D) networks composed of collagen fibrils with nano- and micro-scale diameters and random spatial orientation.² Multiple papers indicated that the morphological and molecular features of native ECMs played critical roles in guiding appropriate development and differentiation of stem cells. Regarding morphologies of scaffolds, ideally, structures with ultrafine fibers oriented randomly and evenly in three dimensions could simulate the architectures of natural ECMs of many soft tissues. Considering the materials, proteins could be optimal due to their molecular similarity to collagens, the major components in natural ECMs.

Shortcoming of proteins limited their wide applications in biomedical areas. Collagen and gelatin were the most widely studied proteins in tissue engineering applications.^{3,4} However, both of them were animal-derived, and had the concerns of potential transmission of pathogens.⁵ In addition, their poor water stability made chemical crosslinking indispensable to

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retain their morphologies and functions in aqueous environments. Nevertheless, current crosslinking methods usually had problems of either cytotoxicity or low crosslinking efficiencies. Moreover, the crosslinking was also necessary in fabrication of scaffolds from zein, the widely investigated plant protein. The improvement was limited since the fibers substantially swelled after incubated in PBS for 15 days.^{6,7} Another common protein for tissue engineering, silk fibroin lacked surface cell-binding portions and necessitated surface functionalization to facilitate cell attachment and proliferation.⁸

Soy protein, the major protein in soybeans, has received much attention as an alternative to animal-originated proteins in tissue engineering. Biomedical applications using both pure soy protein or blends of soy protein and other polymers or macromolecules have been reported. It was found that soy protein films were non-toxic and promoted cell proliferation.⁹ Soy protein membranes were prepared for wound dressing with controlled antibiotic release.¹⁰ Soy protein reinforced with tricalcium phosphate has also been investigated for orthopedic biomedical applications.¹¹ Soy protein granules were used as bone filler for wound dressing.¹² Three-dimensional soy protein scaffolds developed from 3D printing and freeze drying did not invoke an allergic reaction in *in vivo* study.¹³ The similar freeze dried 3D soy protein scaffolds could retain their shapes in phosphate buffered saline (PBS) for up to 14 days after crosslinking with transglutaminase, and *in vitro* study indicated that the soy protein scaffolds well supported growth of mesenchymal stem cells.¹⁴ Moreover, hydrolyzed soy protein covalently bonded to poly(ethylene glycol) (PEG) was fabricated into hydrogel for drug release.¹⁵ Soy protein was also crosslinked with chitosan and made into films to support cell growth.¹⁶ However, in most cases, the soy protein structures have been prepared with 20–100% of glycerol, which could remarkably jeopardize water stability of the protein products, and thus reduced their potential for biomedical applications that required long-term water stability. Blend of soy protein and PEG was electrospun into two-dimensional (2D) scaffolds.¹⁷ Though there has been no emphasis on its natural water stability in the above mentioned applications, soy protein could be potentially water-stable due to the 1.2% of cysteine in its amino acid compositions.¹⁸

In this research, soy protein isolate has been directly used for electrospinning after dissolved in an aqueous solvent system containing reductant. The obtained soy protein scaffolds demonstrated 3D structures with ultrafine fibers distributed spatially and randomly to mimic the native ECMs. The 3D ultrafine fibrous soy protein scaffolds were morphologically stable in phosphate buffered saline (PBS) for up to 28 days. The *in vitro* study showed that the 3D ultrafine fibrous soy protein scaffolds well supported proliferation and adipogenic differentiation of adipose-derived mesenchymal stem cells.

Experimental

Materials

Soy protein isolate (PRO-FAM 646) with about 90% of protein was kindly supplied by ADM International, Decatur, IL. As

indicated in the data sheet, the protein contained about 1.2% of cysteine. Sodium dodecyl sulfate (SDS, 99.0%) was supplied by Hoefer Inc., San Francisco, CA, and cysteine was supplied by Amresco LLC., Solon, OH. Other chemicals, including sodium carbonate, sodium bicarbonate, acetone, sodium hydroxide, isopropyl ethanol and potassium chloride were purchased from BDH chemicals Inc., West Chester, PA.

Molecular weight measurement

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to evaluate the molecular weights of soy protein as is and soy protein treated with alkaline. About 100 μ L NuPAGE® LDS Sample Buffer (1 $\times$) was used to dissolve 1 mg of each sample. The mixture was heated at 70 $^{\circ}$ C for 30 min and vortexed prior to loading. About 10 μ L of soy protein solution was loaded into each slot of the polyacrylamide gel. After electrophoresis, the gel was fixed in 10% acetic acid and 65% 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.

Electrospinning of 3D fibrous soy protein scaffolds

To fabricate 3D ultrafine fibrous soy protein scaffolds, soy protein, cysteine (10 wt% of soy protein) and SDS with the same weight were dispersed in 0.3 M sodium carbonate–bicarbonate buffer at a soy protein-to-buffer weight ratio of 1 : 3. Cysteine was used here to cleave the disulfide bonds in soy protein. As comparison, soy protein hydrolyzed in 0.16 M sodium hydroxide was also dissolved with SDS of the same weight ratio and dispersed in the same sodium carbonate buffer at a soy protein-to-buffer weight ratio of 1 : 3 without cysteine. The hydrolyzed soy protein was used because it was commonly blended with other polymers and macromolecules in electrospinning.¹⁵ The mixture was heated at 90 $^{\circ}$ C under stir for 1 h to obtain transparent soy protein 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 of syringe was negatively charged and the receptor was positively charged. The obtained soy protein scaffolds were annealed at 130 $^{\circ}$ C for 2 h and immersed in 70% methanol for 1 h for coagulation. Subsequently, the electrospun 3D soy protein 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. The two-dimensional (2D) soy protein scaffolds were electrospun onto an insulated board using the same solution for 3D electrospinning, and washed under the same washing conditions.

Morphologies of 3D and 2D fibrous soy protein scaffolds

Morphologies of electrospun soy protein scaffolds using untreated soy protein and hydrolyzed soy protein were observed under Hitachi S-3000N scanning electron microscope (SEM) at magnifications of 70 $\times$ and 350 $\times$. Gross appearance of 3D and 2D fibrous soy protein scaffolds was photographed using a

digital camera. Morphologies of the 3D and 2D fibrous soy protein scaffolds were observed under the same SEM, and the interior structure of the 3D soy protein scaffolds after removal of SDS was observed in wet state using confocal laser scanning microscope (CLSM) in both longitudinal and transverse directions.

Water stability

Stem cells usually takes long time to attach, proliferate and differentiate during cell culture, and thus requires long-term water stability and dimensional integrity of scaffolds. Water stability of 3D ultrafine fibrous soy protein scaffolds was measured in phosphate buffered saline (PBS) with a liquor ratio of 20 : 1 at 37 °C. At various time points of 0, 3, 7, 14, 21 and 28 days, scaffolds were taken out from the solution, rinsed three times in distilled water and freeze dried. Morphological change of the obtained soy protein scaffolds were observed under SEM.

Cultivation of adipose derived mesenchymal stem cells (ADMSCs)

Adipose derived mesenchymal stem cells (ADMSCs, ATCC® PCS-500-011™, Manassas, VA) were cultured on electrospun 3D and 2D soy protein ultrafine fibrous scaffolds and commercial 3D scaffolds (Biomerix 3D Scaffold™, Fremont, CA) to evaluate their potential for soft tissue engineering. Commercial 3D scaffolds had open-cell macroporous structures of polycarbonate polyurethane-urea (PCPU) with void content higher than 90%.

Cell seeding

Each sample was separated into specimens with individual weight of 10 mg of each scaffold were prepared. Before cell culture, the soy protein samples were treated under 120 °C for 1 h in an autoclave, while the commercial 3D scaffolds were immersed in 70% aqueous ethanol overnight. All the scaffolds were rinsed in PBS and then placed in 48-well culture plates (TPP® Techno Plastic Products, Switzerland). ADMSCs with a density of 3×10^5 cells per mL were seeded onto the scaffolds, followed with sequential addition of 500 μ L Dulbecco's modified Eagle's medium (DMEM, with 10% FBS, 1% penicillin and streptomycin solution). The scaffolds were at 37 °C in a humidified 5% CO₂ atmosphere.

Cell attachment and proliferation

Cell viability at different attachment and proliferation stages was quantitatively investigated using MTS assay. During cell culture, the samples were removed from the wells and washed with PBS at different time points (0, 5, 10 and 15 days). Here, 0 day referred to 4 hours after cell seeding. The scaffolds were placed in new 48-well plates containing 450 μ L per well 20% MTS reagent (CellTiter 96® Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI) in DMEM and incubated at 37 °C in a humidified 5% CO₂ atmosphere for 3 h. After incubation, 150 μ L solution from each well was pipetted into a 96-well plate and the optical density at 490 nm was measured on

a UV/Vis multiplate spectrophotometer (Multiskan® Spectrum, Thermo Scientific, Waltham, MA). MTS solution in DMEM without cells served as blank. For each point of data, at least four samples were tested.

Adipogenic differentiation of ADMSCs

Five days after the ADMSCs were seeded and cultured on the scaffolds, DMEM was replaced by adipogenic differentiation medium (Adipocyte Differentiation Tool Kit, ATCC® PCS-500-050™, Manassas, VA) and cultured under the same condition. Adipose differentiation medium was renewed every 3 or 4 days.

Biochemical assays

Adipogenic differentiation was evaluated by quantification of Oil red O (Sigma, St. Louis, MO, USA) stained intracellular lipid in the cells on the scaffolds. Scaffolds cultured with ADMSCs were removed from adipogenic differentiation medium at different time points (5 days, 10 days and 15 days) after cell seeding, and then fixed in PBS containing 10% formaldehyde (Sigma, St. Louis, MO) for at least 1 h. The 5 day samples were collected 12 hours after induction of adipogenic differentiation. The fixed samples were rinsed in 60% isopropanol (Sigma, St. Louis, MO, USA), and then stained with 60% Oil red O solution for 10 min followed by repeated washing with PBS, and destained in 100% isopropanol for 15 min. The optical density of the solution was measured at 500 nm using a UV/Vis multiplate spectrophotometer (Multiskan® Spectrum, Thermo Scientific). For each point of data, at least 6 specimens under each condition were used for measurement in order to calculate the means and standard deviations.

Statistical analysis

All the data obtained were analyzed by the one-way analysis of variance with Scheffé test with a confidence interval of 95%. A *p* value smaller than 0.05 indicated statistically significant difference. The error bars in figures indicated standard deviations, and the data labeled with the same symbols or characters indicated there was no significant different among the data points.

Results and discussion

Fig. 1 compares the distribution of molecular weights of soy protein in lane 2 and alkaline treated soy protein in lane 3. In lane 2, the band around 22 kDa belonged to the 11S globulin, and the bands between 50 and 70 kDa belonged to 7S globulin.¹⁹ The molecular weights of raw soy protein met the relevant requirement for fiber spinning.²⁰ On the contrary, only smear with gradient shade from 3 to 100 kDa, instead of obvious bands could be found in the NaOH treated sample.

Fig. 2 demonstrated the solubility of untreated and hydrolyzed soy protein in SDS aqueous solution with or without cysteine as reductant. It can be found that in Fig. 2a, untreated soy protein could not be dissolved without reduction, because the intermolecular and intramolecular disulfide bonds could not be interrupted by SDS in aqueous environments. The

Fig. 1 SDS-PAGE of soy protein. (1) Standard protein markers; (2) soy protein; (3) soy protein hydrolyzed in 0.16 M NaOH.

Fig. 2 Solubility of (a) soy protein dispersed in carbonate buffer without cysteine; (b) soy protein dissolved in carbonate buffer with 10% cysteine; and (c) soy protein treated with 0.16 M NaOH dissolved in carbonate buffer without cysteine. All solution contained 32.5 wt% of soy protein and 17.5 wt% of SDS.

covalent bonds preserved the entanglements of polypeptides, limited their interaction with SDS and exposure of hydrophilic domains in the buffer, and thus did not dissolve. In Fig. 2b, viscous and transparent solution indicating successful dissolution of soy protein in aqueous solution could be observed. Cysteine functioned as an effective reductant in mild alkaline condition, since the sulfhydryl groups ($-SH$) could deprotonate into $-S^-$, which could initiate thiol-disulfide exchange to break the disulfide crosslinks in the soy protein. Resultantly, the liberated molecules in soy protein could effectively expose the hydrophilic portions in water, as well as entangle with SDS *via*

hydrophobic portions. The negative charges brought by the sulfate groups of SDS on the protein-SDS complexes resulted in strong intermolecular electrical repulsion that pushed polypeptides away from each other, and thus disentangled them from aggregations. The effects of exposure of hydrophilic portions, existence of strong negative surface charges and disentanglement of polypeptides synergistically increased the solubility of soy protein in aqueous environments. In Fig. 2c, the solution was transparent but much less viscous as that in Fig. 2b. As shown in Fig. 1, the molecules of soy protein were destroyed after hydrolysis. The principle of dissolution of hydrolyzed soy protein was similar to that for the raw soy protein. However, due to the remarkably increase in the number of polar terminal groups after hydrolysis and the reduced molecular weights, the dissolution was easier and faster, resulting in thin solution.

As shown in Fig. 3a and b, hydrolyzed soy protein could only be electrospun into beads with irregular shapes, while the raw soy protein dissolved in the carbonate buffer could be electrospun into ultrafine fibers with uniform diameters, as shown in Fig. 3c and d. Fiber spinning was highly affected by molecular entanglement, which was determined by the molecular weight and linearity of macromolecules. Regarding the hydrolyzed soy protein, the linearity of macromolecules might be contributed to the breakage of disulfide bonds by alkaline. However, a lack of interactions among the short molecules prevented effective drawing of molecules and resulted in formation of beads with small length-to-diameter ratios. On the contrary, during dissolution of raw soy protein, linearity of molecules was achieved by cleavage of disulfide bonds after adding cysteine, while the mild alkaline pH might not affect molecular weight severely. The resultant solution of raw soy protein with long and linear molecular chains could maintain certain degree of molecular entanglement among each other. During electrospinning, the drawing force could be efficiently transferred among molecules

Fig. 3 Electrospun structures from hydrolyzed soy protein (a) and (b) and from raw soy protein solution (c) and (d). All electrospinning solution contains 32.5 wt% of soy protein and 17.5 wt% of SDS. (a) and (c) Top view of SEM images at magnification of 70 $\times$, (b) and (d) top view of SEM images at magnification of 350 $\times$.

and led to alignment of polypeptides and formation of ultrafine fibers with large length-to-diameter ratio.

Fig. 4 compares the morphology of 2D and 3D ultrafine fibrous soy protein scaffolds. As shown in Fig. 4a and b, the 2D scaffold was flat structure with limited thickness and the 3D scaffold was a fluffy fibrous sphere with diameter as large as 2 cm. The 2D scaffold had fibers oriented randomly in the planar directions but no fibers oriented in the thickness direction as shown in Fig. 4c and e. On the contrary, the interior structures of 3D the fibrous sphere could be found oriented randomly in both horizontal and vertical directions, as illustrated in Fig. 4d and f, the mechanism of formation of 3D ultrafine fibrous structures could be referred to our previous research.²¹ In wet

state as shown in Fig. 4g and h, the 3D soy protein scaffolds could preserve their microscopic appearances. The CLSM image from the 45 degree angle illustrated that the wet soy protein scaffolds still had fibers oriented and distributed randomly in all the directions, while the 2D soy protein scaffolds also maintained their fibrous morphologies. Furthermore, fiber ends in the thickness directions and random fiber arrangements in the horizontal directions could be observed. The distribution and water stability with significantly affected cell behaviors on the soy protein scaffolds.

In Fig. 5, the scaffold still maintained their fibrous structures after immersed in PBS at 37 °C for up to 4 weeks, though the diameters of the fibers increased. It could be inferred that the fibers may retain their fibrous morphologies longer than 28 days. This duration was much higher than other soy protein scaffolds even with crosslinking, as compared in Table 1. The good water stability of the electrospun ultrafine soy protein fibers could be attributed to re-formation of disulfide bonds in soy protein fibers after heat treatment. It could be inferred that, by using the strategy of de-crosslinking before fiber spinning and re-crosslinking after fiber formation, the intrinsic water-stability due to the disulfide crosslinks in soy protein could be efficiently employed in biomedical applications.

Fig. 6 demonstrates that ADMSCs in electrospun 3D ultrafine fibrous soy protein scaffolds could distribute more uniformly and penetrate more deeply compared with those in 2D soy protein fibrous scaffolds or in 3D commercial PCPU scaffolds. In Fig. 6c, the cells could be found 165 μm under the surface, and distributed uniformly on certain horizontal planes of the

Fig. 4 Morphology comparisons between 2D and 3D electrospun soy protein scaffolds. Digital photo of (a) 2D soy protein electrospun scaffolds and (b) 3D electrospun soy protein scaffolds; top view of SEM image of (c) 2D electrospun soy protein scaffold and (d) 3D electrospun soy protein scaffold at magnification of 70 $\times$; side view of SEM image of (e) 2D electrospun soy protein scaffold and (f) 3D electrospun soy protein scaffold at magnification of 70 $\times$; 45 degree view of CLSM images of (g) 2D electrospun soy protein scaffold and (h) 3D electrospun soy protein scaffold in wet state at magnification of 100 $\times$.

Fig. 5 (a) As-spun soy protein fibers, (b) to (f) soy protein fibers immersed in PBS at 37 °C for (b) 3, (c) 7, (d) 14, (e) 21 and (f) 28 days. Scale bar = 300 μm .

Table 1 Comparison among soy protein 3D fibrous scaffolds, 3D non fibrous scaffolds without/with crosslinking

Sample	In PBS (days)
3D fibrous scaffolds	>28
3D soy protein/glycerol sponges ¹³	4
3D soy protein/glycerol sponges crosslinked with TG 1 U ¹⁴	7
3D soy protein/glycerol sponges crosslinked with TG 20 U ¹⁴	11

Fig. 6 CLSM montage images in sequential sections at 15 μ m intervals under magnification of 60 $\times$ demonstrating the penetration of ADMSCs in (a) 2D and (c) 3D soy protein electrospun scaffolds, and (b) commercial 3D porous scaffold. The light blue dots represent the cell nucleus stained with Hoechst 33342 dyes. The first pictures that labels with "1" in each figure are the combination of all the other pictures in the figure.

3D ultrafine fibrous soy protein scaffold. However, in Fig. 6a, cells could not be observed 60 μ m below the surface of the soy protein 2D scaffold, though the distribution of cells in each plane was uniform. The tight packing of fibers in the horizontal directions and lack of space in the thickness direction contributed to the small penetration of cells in the 2D structures, and thus the resultant neo-tissue of this scaffolds might not serve the goals in large volume reconstruction in soft tissue regeneration. As shown in Fig. 6b, cells could be observed 60 μ m under the surface of the commercial 3D porous scaffold.

However, the distribution of cells were highly uneven, because the cells only adhered on the walls of the scaffold.

As demonstrated in Fig. 7, 3D soy protein scaffolds were remarkably better than 2D soy protein scaffolds and commercial 3D porous scaffold to support cell growth. More attachment and higher proliferation rates of ADMSC were found on 3D soy protein fibrous scaffolds. The amount of cells attached on 3D scaffolds was 163% and 210% times of that on 2D scaffolds and commercial 3D scaffolds. After cultured up for 2 weeks, the proliferation of cells on soy protein 3D fibrous scaffolds was 227% and 114% higher than that on 2D scaffolds and commercial 3D scaffold at the same time point, respectively. The different cell culture results should be attributed to the differences of scaffold materials and cell accessibility induced by the differences in scaffold structure. After 1 week of cell culture, cells were found at least 165 μ m beneath the surface of 3D scaffold, while cells could not be found 45 μ m under the surface of 2D scaffold. The tight packing of fibers in the 2D scaffolds restricted penetration of cells vertically, while the multiple pores with much larger sizes and significantly higher porosity of 3D scaffolds facilitated migration and penetration of cells into the interior of the structures. For the commercial 3D porous scaffold, though cell could still be observed 75 μ m under the surface of the scaffold, it should be noticed that the distribution of cells was highly uneven, and cells can only be found on the wall structure of the scaffold. The unevenly distribution of cells in commercial 3D porous scaffold may cause the formation of uneven soft tissue in long term *in vivo* soft tissue repairing process.

In Fig. 8, the content of newly secreted fat by each ADMSC on soy protein 2D fibrous scaffolds was 28% higher than that on commercial 3D porous scaffold after cultured in differentiation medium for 15 days. This result demonstrated that although more cells could proliferate on commercial 3D porous scaffold, the differentiation degree of each ADMSC on 2D soy protein fibrous scaffolds was still higher than that commercial 3D porous scaffold. It proved that soy protein could better support the adipogenic differentiation of ADMSCs, and was consistent with the report that basal cell culture medium added soy

Fig. 7 MTS assay results of attachment (0 day) and proliferation (5 days, 10 days, 15 days) of adipose-derived mesenchymal cells on 2D, 3D soy protein electrospun scaffolds and on commercial 3D porous PCPU (polycarbonate polyurethane-urea) scaffold. Data labeled with the same symbols were NOT significantly different from each other.

Fig. 8 Adipogenic differentiation at different time intervals (5, 10 and 15 days) of ADMSCs was analyzed using Oil red O staining. Data labeled with the same symbols were NOT significantly different from each other.

peptides could significantly increase the proliferation of human ADMSCs.²²

Moreover, the content of newly secreted fat by each ADMSC on soy protein 3D fibrous scaffold was 34% and 73% higher than that on soy protein 2D scaffolds and commercial 3D porous scaffold, respectively. For 2D and 3D soy protein scaffolds fabricated by the same raw materials, the difference of the Oil red O OD value should be only attributed the difference of scaffold structure, porosity and fiber orientations. It was believed that a 3D randomly oriented fibrous environment is needed to guide cells to grow and differentiate into stereoscopic topographies, and cells cultured on flat 2D substrates may differ considerably in morphology and differentiation pattern from those cultured in more physiological 3D environments. Therefore, it could be concluded that the soy protein 3D fibrous scaffold could better support ADMSC for adipogenic differentiation.

Conclusion

Soy protein has been successfully electrospun into intrinsically water-stable scaffolds with large volume and ultrafine fibers oriented randomly and evenly in three dimensions for regeneration of soft tissues. The disulfide crosslinks in soy protein were cleaved during the dissolution of soy protein in the aqueous solvent system with reductant. Afterwards, the soy protein solution was electrospun into bulky scaffolds composed of ultrafine fibers oriented randomly in three dimensions to mimic native ECMs. Without any external crosslinking, the soy protein scaffolds showed substantial water stability by retaining their fibrous morphologies after incubated in PBS for 28 days. *In vitro* study showed that the 3D soy protein scaffolds better supported uniform distribution and adipogenic differentiation of adipose derived mesenchymal stem cells comparing to the 2D soy protein scaffolds and the 3D commercial scaffolds.

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