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Lingyan Kong – University of Alabama

Gregory R. Ziegler – Pennsylvania State University

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or send request to lingyan.kong@ua.edu

Fabrication of Pure Starch Fibers by Electrospinning

Lingyan Kong[1] and Gregory R. Ziegler[1,*]

[1]Department of Food Science

The Pennsylvania State University

341 Food Science Building

University Park, PA 16802

For submission to: *Food Hydrocolloids*

* Corresponding author. Tel.: +1 814 863 2960; fax: +1 814 863 6132. E-mail address: grz1@psu.edu.

Abstract

Many efforts to spin starch fibers are reported in the patent and research literature. All reported spinning methods are dependent upon addition of non-starch components, *e.g.* other polymers, plasticizers or cross-linkers. In the present study, we demonstrate a method of producing pure starch fibers by an electrospinning technique. This method involves choosing an appropriate solvent for native high amylose starch and spinning on a modified electrospinning setup. Resulting starch fibers have diameters in the order of microns. Coagulation solvent composition can affect the crystallinity of the starch fibers. Post-spinning treatments were employed to increase the crystallinity and cross-link the starch fibers. The novel starch fibers have potential in various applications, *e.g.*, in the food, textile, and biomedical industries.

Keywords: starch, fiber, electrospinning, crystallinity, crosslinking

1. Introduction

Electrospinning is a technique capable of producing micro- to nano-scale fibers. Among methods of achieving fibers of this diameter, electrospinning is a peerless technique as it is cost-effective, applicable to a large variety of materials, capable of controlling fiber morphology, and easily scaled. Electrospinning is a century old process (Formhals, 1934; Morton, 1902), but largely neglected until the mid-1990s, when Reneker and co-workers (Doshi & Reneker, 1995) rekindled interest in the technique. Since then, hundreds of types of materials, including polymers, metal oxides, and ceramics, have been shown to be electrospinnable (Ramakrishna, Fujihara, Teo, Lim, & Ma, 2005).

The electrospinning process is straightforward and the apparatus required is simple. Though there are exceptions, a typical electrospinning setup includes a solution reservoir with spinnerets, a grounded collector and a high voltage power supply. When a polymer solution or melt is pumped through the spinneret, a high voltage, typically in the range of 100 to 500 kV/m, is applied between the solution and a grounded collector. With increasing voltage the electrostatic force deforms the droplet into a pointed shape. Further increase in voltage induces the formation of a Taylor cone and a jet from the needle. As the jet travels towards the collector, solvent is evaporated and sufficient entanglement of polymer chains prevents the jet from breaking into droplets (electrospraying).

The key advantages of fibrous material over other morphologies, e.g. films and foams, lie in their high surface-area-to-volume ratio, high porosity, small pore size and superior mechanical properties. Therefore, fibers have received great attention for their potential in various applications, e.g. in filtration, electronics, textiles, cosmetics and medical fields (Lu & Ding, 2008). However, electrospinning does not guarantee that nanofibers (< 100nm) are approachable for every material and method. Indeed, many electrospinning attempts obtained fibers with diameters from submicron to microns, e.g. electrospinning of polyethylene (Givens, Gardner, Rabolt, & Chase, 2006), polyurethane

(Rockwood, Woodhouse, Fromstein, Chase, & Rabolt, 2007), and dextran (Jiang, Fang, Hsiao, Chu, & Chen, 2004).

There is current interest in shifting from petroleum-based synthetic materials to utilization of bio-based materials, not only due to the environmental and economic consequences of our pervasive use and excessive dependence on petroleum-based materials, but also from the advantages of bio-based materials pertaining to their excellent biocompatibility and biodegradability. Thus a number of biopolymers, including polysaccharides, proteins and DNA, have been successfully spun into fibers, especially by electrospinning (Kong, Ziegler, & Bhosale, 2010).

Starch is among the most abundant and inexpensive biopolymers. Starch is found in plant tissues, such as leaves, stems, seeds, roots and tubers. It is also found in certain algae and bacteria. Starch exists in semi-crystalline granules of different size, shape and morphology depending on its botanical source. Nevertheless, most starches are composed of two structurally distinct molecules: amylose, a linear or lightly branched (1→4)-linked α-glucopyranose, and amylopectin, a highly branched molecule of (1→4)-linked α-glucopyranose with α-(1→6) branch linkages. The amylose/amylopectin ratio in starches varies with botanical origin.

Many attempts have been made to fabricate starch fibers as reported in the research and patent literature (Kong & Ziegler, 2012). Initially some tried producing amylose fibers (Hiemstra & Muetgeert, 1959), because the amylose component of starch is largely linear and thus was assumed to readily associate side-by-side under favorable conditions. However, the high cost of purifying amylose is an obstacle for scaled production, since amylose is the minor component of most common starches. Attempts to utilize native starches or modified starches generally required a large amount of non-starch components, including plasticizers, cross-linker resins or other polymers (Gordon, Cabell, Mackey, Michael, & Trokhan, 2006).

In the present study, we present a method of producing pure starch fibers by electrospinning that involves using an appropriate solvent for native high amylose starch and spinning starch with a modified electrospinning setup. Additionally, a post-spinning heat treatment and cross-linking, were employed to further improve the starch fiber properties, including water stability.

2. Experimental

2.1. Materials.

Starch (Gelose 80) was kindly provided by Penford Food Ingredients Company (Centennial, CO) and used as received. Gelose 80 is a corn starch with amylose content of about 80%. Ethanol (200 proof) and dimethyl sulfoxide (DMSO) was obtained from VWR International (Radnor, PA). Glutaraldehyde was purchased from Sigma-Aldrich, Inc (St. Louis, MO).

2.2. Electrospinning

Spinning dope was prepared by dissolving starch (15% w/w) in a 95% aqueous DMSO solution. The starch dispersion was heated in a boiling water bath with continuous stirring for about one hour and allowed to cool to room temperature. A 10 mL syringe (Becton, Dickinson and Company, Franklin Lakes, NJ) with a 20-gauge blunt needle was used for electrospinning.

The electrospinning setup used in this study, referred to as “electro-wet-spinning,” contained a high voltage power supply (ES40P, Gamma High Voltage Research, Inc., Ormond Beach, FL), a syringe pump (81620, Hamilton Company, Reno, NV), and a grounded metal mesh immersed in ethanol. The fibrous mat deposited on the surface of the coagulation bath was washed with ethanol and dried in a desiccator containing Drierite under vacuum. Electrospinning was conducted at room temperature in this study. Feed rate was set at 4 ml/h, spinning distance at 7.5 cm and voltage at 7.5 kV.

2.3. Post-spinning treatments

The as-spun dried fibers were subject to a further heat treatment intended to increase the starch crystallinity and a cross-linking treatment to render improved water stability. For the heat treatment, a

sample of starch fiber mat was placed in a 50% (v/v) aqueous ethanol solution and heated at 70 ºC for one hour, after which the sample was washed with ethanol and dried as above. For the cross-linking treatment, a sample starch fiber mat on a metal mesh was place over a petri dish in a desiccator with Drierite. Ten (10) mL of 25% (v/v) aqueous glutaraldehyde solution was dispersed evenly in the petri dish. The desiccator was kept in an incubator at 40 ºC for 24 hours for the glutaraldehyde to vaporize and cross-link the starch fibers.

2.4. Morphological characterization

Fiber morphology was examined using an Olympus BX41 optical microscope (Hitech Instruments, Edgemont, PA) equipped with cross polarizers and a SPOT Insight QE camera (SPOT Diagnostic Instruments, Sterling Heights, MI). Image analysis was completed using SPOT analytical and controlling software. Observation of fibers was also performed using a FEI Quanta 200 ESEM (FEI, Hillsboro, OR) in low vacuum mode at an accelerating voltage of 20 KeV. Fiber diameter was measured from the ESEM images. Three images were used for each fiber sample and at least 50 different segments were randomly measured to obtain an average diameter.

2.5. Thermal analysis

Approximately 5 to 6 mg of starch fiber sample was weighed in a 60 μL stainless steel differential scanning calorimeter (DSC) pan (Perkin-Elmer Instruments, Bridgeville, PA) and ethanol/water mixtures of different volume ratios were added to obtain a 10% (w/w) dispersion. Pans were hermetically sealed and stored overnight for moisture equilibration. Samples were equilibrated at 20 ºC, and then heated to 170 ºC at a scanning rate of 2 ºC/min in a Thermal Advantage Q100 DSC (TA Instruments, New Castle, DE). The DSC was calibrated with indium and an empty sample pan was used as a reference. Data was analyzed using the TA Universal Analysis software (Universal Analysis 2000 v.4.2E, TA Instruments-Waters LLC, New Castle, DE).

2.6. Wide angle X-ray diffraction

WAXD patterns were obtained with a Rigaku MiniFlex II desktop X-ray diffractometer operated at 15 mA and 30 kV (Rigaku Americas Corporation, TX). Samples were exposed to Cu K-alpha radiation (0.15405 nm) and continuously scanned between 4 and 30 º 2θ at a scanning rate of 1º/min with a step size of 0.02º. Data were analyzed with Jade v.8 software (Material Data Inc., Livermore, CA). To calculate the degree of crystallinity, an amorphous halo was subtracted from the overall X-ray diffraction pattern. The overall area was calculated as the area between the linear baseline and data points. The amorphous halo was generated by Jade software using the cubic spline fit option. The degree of crystallinity was calculated as the proportion of the crystalline area of the overall area multiplied by 100.

3. Results and Discussion

A crucial issue with fiber spinning from most biopolymers is to find an appropriate solvent or solvent system capable of dissolving the polymer and promoting sufficient chain entanglements. In previous attempts to electrospin starch (Stijnman, Bodnar, & Hans Tromp, 2011), water was used as the solvent, but fiber formation was unsuccessful. Such failure might be explained by the conformation of two components of starch in aqueous solution. Moderate heating below 100 ºC, while able to gelatinize the starch and form a homogeneous dispersion, may not completely disrupt starch helices, and these helices may cause rapid recrystallization upon cooling (Ziegler, Creek, & Runt, 2005). Sufficient long-range chain entanglements, required for continuous fiber formation, cannot be established without untwisting helices into random coils. Starch may undergo a helix-to-coil transition upon intensive heating at or above 160 ºC (Creek, Ziegler, & Runt, 2006; Ziegler et al., 2005). But electrospinning with traditional equipment seems impractical at such a high temperature. Furthermore, the highly-branched structure of amylopectin gives it a globular bulky hydrodynamic shape, which is not easily elongated and aligned in the extensional flow field of the spinneret.

Electrospinning of starch with other polymers, such as polycaprolactone (PCL) (Jukola, Nikkola, Gomes, Reis, & Ashammakhi, 2008), poly(vinyl alcohol) (PVA) (Sukyte, Adomaviciute, & Milasius, 2010), and polylactic acid (PLA) (Sunthornvarabhas, Chatakanonda, Piyachomkwan, & Sriroth, 2011) has been demonstrated. However, addition of starch was shown to be detrimental to the electrospinnability of the polymer mixture. For instance, the maximum possible amount of potato starch tolerable when electrospinning poly(vinyl alcohol) was 3% (w/w), less than a half of the PVA used (7% w/w) (Sukyte et al., 2010). It is reasonable to hypothesize that the beads formed in some electrospun bicomponent fibers (Jukola et al., 2008; Sukyte et al., 2010) are actually starch separated from the polymer phase. Therefore, in these mixtures starch may be acting simply as a filler to replace a limited portion of the actual fiber-forming polymer. Similar situations have also been confronted in electrospinning mixtures with other polysaccharides *e.g.* electrospinning PVA with alginate, cellulose, and chitosan (Kong et al., 2010). In a recent study (Toskas et al., 2011), PVA was electrospun in a mixture with the seaweed polysaccharide ulvan. Smooth fibers were not obtainable beyond an ulvan-to-PVA weight percentage ratio of 1.6 to 3.6.

Utilizing an appropriate solvent system to dissolve the starch is an alternative approach to relying on another fiber forming polymer. Amylose helices adopt a random coil conformation in a certain range of aqueous dimethyl sulfoxide (DMSO) concentration (De Vasconcelos, Pereira, & Fonseca, 2001). Therefore, DMSO or a DMSO/water mixture would be a candidate for the electrospinning process. However, DMSO is relatively nonvolatile compared with other solvents commonly used for electrospinning. Thus in our initial trials, even though the starch dispersion in aqueous DMSO could form a jet at an elevated voltage, we were unable to deposit solid fibers on a grounded metal mesh collector since the DMSO solution could not be evaporated sufficiently under standard ambient conditions. Therefore, we turned to a modified setup for electrospinning called “electro-wet-spinning.” Theoretically any solvent that is miscible with DMSO but incompatible with starch can be used as a

coagulation bath. As the solution jet reaches the coagulation bath, DMSO is extracted and the starch collapses in a fibrous form.

3.1. Fiber morphology

When a voltage was applied between the needle tip and the coagulation bath, starch dispersion dopes were accelerated towards the coagulation bath, and a continuous jet was obtained at a critical voltage. A fiber mat was deposited on the bath surface, the size of which was dependent upon the electric field strength. After drying, the appearance and texture of the starch fiber mat resembled a piece of bath tissue, though not as flexible. The starch fibers within the mat were randomly oriented, with an average diameter of 2.60 ± 0.85 μm (Fig. 1). The fiber surface appeared smooth and the fibers were largely continuous. Some breaks existed, possibly indicating that the starch fibers were relatively brittle, which was confirmed by their behavior on handling. Extensive drying in a desiccator eliminated ethanol as well as moisture, which play a role as plasticizer of the starch fibers. A thin section of the fiber mat was observed under optical microscopy with normal light and between crossed polarizers, respectively (Fig. 2). Although the birefringence from thicker sections, *e.g.* the right side of the micrograph, may result from multiple refractions by overlaying fibers, the single fibers do show birefringence. The birefringence obtained using crossed polarizers results from orientation of starch chains in the fiber axis direction.

3.2. Thermal analysis

Thermal transitions appeared when starch fibers were heated in solvents of intermediate water:ethanol concentrations (Fig. 3 & 4 and Table 1). At aqueous ethanol concentrations between 40 and 60 % (v/v), both exothermic and endothermic peaks were observed. It is likely that this corresponded to the crystallization of amorphous starch followed by melting, and suggested that an annealing treatment could be applied to increase the crystallinity of the starch fibers, perhaps altering their mechanical properties. Starch fibers were held at 65 ºC for 30 minutes in 50 % (v/v) aqueous

ethanol. After this heat treatment, the exotherm was seen to disappear and the endotherm increased slightly to 114.4 ºC and 14.2 J/g. At higher ethanol concentrations there appeared to be insufficient water for annealing to occur.

Table 1. Thermal analyses of starch fibers in various ethanol/water mixtures.

Ethanol/water	Exothermic			Endothermic		
(v/v)	Tp[a] (ºC)	Range[b] (ºC)	ΔH (J/g)	Tp (ºC)	Range (ºC)	ΔH (J/g)
0/100	-	-	-	-	-	-
20/80	-	-	-	108.6	100.7 – 130.9	4.2
40/60	40.9	36.0 – 47.5	3.5	71.2	61.8 – 77.5	5.2
50/50	54.5	48.5 – 62.1	1.1	111.2	101.2 – 120.3	7.7
60/40	61.4	56.1 – 67.0	0.9	125.1	113.7 – 136.1	11.8
80/20	-	-	-	158.6	150.6 – 164.3	2.9
100/0	-	-	-	-	-	-

[a] Tp represents peak temperature.

[b] The onset and end temperatures were reported.

3.3. Post-spinning heat treatment

Since the fibers were deposited in a random manner, wide-angle X-ray diffraction patterns were obtained from the fiber mat with an X-ray powder diffractometer. As spun, the dried starch fibers were largely amorphous (Fig. 5). This agrees with DSC thermograms. After annealing, peaks at 8º, 13.8º, 15.9º, 17.7º, 19.4º and 21º, characteristic of a V-type diffraction pattern, were observed. Based on a hexagonal crystal structure proposed for V-type starch, the unit cell dimensions were calculated to be a=b=25.9 Å and c=5.6 Å. The parameters are close to reported values but smaller (Takeo, Tokumura, & Kuge, 1973), indicating that the helices are closely packed in a hexagonal arrangement after extensive drying. The degree of crystallinity of the heat-treated starch fibers was estimated to be 43 %. It is thus reasonable to suggest that amylose helices rearrange and crystallize during the moderate annealing of the fibers in the ethanol/water mixture. This observed % crystallinity was higher than most values previously reported for V-type starch (Lay Ma, Floros, & Ziegler, 2011).

3.4. Cross-linking treatment

Current technologies in spinning starch fibers may include the addition of cross-linking agents in order to improve the wet stability of the starch fibers (Bailey, Mackey, & Trokhan, 2011). Cross-linkers used in previous reports include polyamide-epichlorohydrin resin, glyoxylated polyacrylamide resin, urea formaldehyde, melamine formaldehyde, polyethylenimine type resin and glyoxal. In this study, cross-linking of the starch fibers was conducted by exposing the fibers to glutaraldehyde in the vapor phase. This method has been applied to cross-link electrospun chitosan fibers (Schiffman & Schauer, 2007), collagen fibers (Rho et al., 2006), and gelatin fibers (Sisson, Zhang, Farach-Carson, Chase, & Rabolt, 2009). The appearance and size of the cross-linked starch fibers remained unchanged when compared with the starch fibers without cross-linking.

The wet stability of the as-spun starch fibers, heat-treated fibers and cross-linked fibers were compared by a simple experiment. Fiber mats of the same size were dropped into water and observed using optical microscopy. When placed in water, both the as-spun and heat-treated starch fiber mats became soft, and lost integrity when picked up with tweezers. On the contrary, the cross-linked starch fiber mat did not disintegrate when placed into water, and can be recovered from the water without losing its fibrous structure. Optical micrographs of these fiber mats after immersion in water for 10 minutes are shown in Fig. 6. The as-spun and heat-treated starch fibers lost their fibrous structure after wetting and formed a gel like structure. Even though the heat-treated starch fibers were highly crystalline, a sufficiently large amount of amorphous structure susceptible to plasticization by water apparently remained. The cross-linked starch fibers retained the original fibrous structure. The cross-linking mechanism of glutaraldehyde has been described (El-Tahlawy, Venditti, & Pawlak, 2007) as the reaction between terminal aldehydes and hydroxyl groups of starch to the formation of acetals. In this way, glutaraldehyde bridges the starch helices into a network, which is not disintegrated by water.

3.5. Effect of coagulation bath composition

The starch fibers without guests were spun into a series of coagulation baths with varying ethanol concentrations (Fig. 7A). The starch fibers recovered from pure ethanol were largely amorphous with a very weak V-pattern. Decreasing ethanol content in the coagulation bath induced V-type X-ray diffraction patterns. In the as-spun starch fibers, amylose might have formed inclusion complex with some native lipids and ethanol. After drying, the ethanol was extracted and evaporated, leaving empty helices (Le Bail, Bizot, Pontoire, & Buléon, 1995). The crystallinity of the V-type starch fibers increases from 25% to 33% as ethanol content decreased from 90% to 60% (v/v). The starch fibers deposited into pure ethanol collapsed so rapidly that the starch polymers had insufficient time to transform into helical structures and arrange in a crystalline pattern. Adding water to the coagulation bath slowed down the precipitation of starch fibers and thus allowed the starch molecules to pack into V-type arrangement. After heat treatment of the starch fibers at 70 ºC in 50% (v/v) ethanol, peaks at 8, 13.8, 15.9, 17.7, 19.4 and 21 º 2θ, characteristic of a V-type diffraction pattern, were observed (Fig. 7B). Starch fibers from different coagulation ethanol contents resulted in similar V-type patterns with crystallinity, from 40% to 43%.

4. Conclusion

In conclusion, pure starch fibers were, for the first time, fabricated by the modified electrospinning technique *i.e.* "electro-wet-spinning". Continuous jets from high amylose corn starch in DMSO/water solution could be observed when a high voltage was applied. Smooth and uniform starch fibers were fabricated. The diameter of starch fibers was in the order of microns under the described experimental parameters. Further studies are required to systematically examine the effect of numerous experimental variables on fiber diameter. Thermal analysis of the starch fibers in 40% and 50% (v/v) ethanol solutions showed the presence of an exotherm at about 40 and 55 ºC, respectively, which is attributed to crystallization of starch molecules. Hence, a post-spinning heat annealing treatment at 65 ºC was employed. The heat treatment was able to significantly increase the crystallinity of the starch fibers,

which may contribute to an improvement in mechanical strength of the starch fiber mat. Cross-linking of starch fibers was successfully conducted using vapor-phase glutaraldehyde. Cross-linked starch fibers show improved wet stability. Decreasing ethanol concentration in the coagulation bath resulted in increased crystallinity of the starch fibers. Further heat annealing treatment could enhance the crystallinity to similar level, i.e. around 43%.

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References

Bailey, V. A., Mackey, L. N., & Trokhan, P. D. (2011). Fiber comprising unmodified and/or modified starch and a crosslinking agent. US Patent 7938908.

Creek, J. A., Ziegler, G. R., & Runt, J. (2006). Amylose crystallization from concentrated aqueous solution. *Biomacromolecules*, *7*(3), 761–770.

De Vasconcelos, C. L., Pereira, M. R., & Fonseca, J. L. C. (2001). Solvent composition and rheology of starch–DMSO–water solutions. *Journal of Applied Polymer Science*, *80*(8), 1285–1290.

Doshi, J., & Reneker, D. H. (1995). Electrospinning process and applications of electrospun fibers. *Journal of Electrostatics*, *35*(2-3), 151–160.

El-Tahlawy, K., Venditti, R. A., & Pawlak, J. J. (2007). Aspects of the preparation of starch microcellular foam particles crosslinked with glutaraldehyde using a solvent exchange technique. *Carbohydrate Polymers*, *67*(3), 319–331.

Formhals, A. (1934). Process and apparatus for preparing artificial threads. US Patent 1975504.

Givens, S. R., Gardner, K. H., Rabolt, J. F., & Chase, D. B. (2006). High-temperature electrospinning of polyethylene microfibers from solution. *Macromolecules*, *40*(3), 608–610.

Gordon, G. C., Cabell, D. W., Mackey, L. N., Michael, J. G., & Trokhan, P. D. (2006). Electro-spinning process for making starch filaments for flexible structure. US Patent 7029620.

Hiemstra, P., & Muetgeert, J. (1959). Process for the production of amylose articles by extrusion of aqueous sodium hydroxide solution thereof into concentrated aqueous ammonium sulphate solution. US Patent 2902336.

Jiang, H., Fang, D., Hsiao, B. S., Chu, B., & Chen, W. (2004). Optimization and characterization of dextran membranes prepared by electrospinning. *Biomacromolecules*, *5*(2), 326–333.

Jukola, H., Nikkola, L., Gomes, M. E., Reis, R. L., & Ashammakhi, N. (2008). Electrospun starch-polycaprolactone nanofiber-based constructs for tissue engineering. *AIP Conference Proceedings*, *973*(1), 971–974.

Kong, L., & Ziegler, G. R. (2012). Patents on fiber spinning from starches. *Recent Patents on Food, Nutrition & Agriculture*, *4*(3), 210–219.

Kong, L., Ziegler, G. R., & Bhosale, R. (2010). Fibers spun from polysaccharides. In R. Ito & Y. Matsuo (Eds.), *Handbook of carbohydrate polymers: development, properties and applications* (pp. 1–43). New York: Nova Science Pub Inc.

Lay Ma, U. V, Floros, J. D., & Ziegler, G. R. (2011). Formation of inclusion complexes of starch with fatty acid esters of bioactive compounds. *Carbohydrate Polymers*, *83*(4), 1869–1878.

Le Bail, P., Bizot, H., Pontoire, B., & Buléon, A. (1995). Polymorphic transitions of amylose-ethanol crystalline complexes induced by moisture exchanges. *Starch - Stärke*, *47*(6), 229–232.

Lu, P., & Ding, B. (2008). Applications of Electrospun Fibers. *Recent Patents on Nanotechnology*, *2*, 169–182.

Morton, W. J. (1902). Method of dispersing fluids. US Patent 705691.

Ramakrishna, S., Fujihara, K., Teo, W.-E., Lim, T.-C., & Ma, Z. (2005). *An Introduction to Electrospinning and Nanofibers*. Singapore: World Scientific.

Rho, K. S., Jeong, L., Lee, G., Seo, B.-M., Park, Y. J., Hong, S.-D., Roh, S., et al. (2006). Electrospinning of collagen nanofibers: Effects on the behavior of normal human keratinocytes and early-stage wound healing. *Biomaterials*, *27*(8), 1452–1461.

Rockwood, D. N., Woodhouse, K. A., Fromstein, J. D., Chase, D. B., & Rabolt, J. F. (2007). Characterization of biodegradable polyurethane microfibers for tissue engineering. *Journal of Biomaterials Science -- Polymer Edition*, *18*(6), 743–758.

Schiffman, J. D., & Schauer, C. L. (2007). Cross-linking chitosan nanofibers. *Biomacromolecules*, *8*(2), 594–601.

Sisson, K., Zhang, C., Farach-Carson, M. C., Chase, D. B., & Rabolt, J. F. (2009). Evaluation of cross-linking methods for electrospun gelatin on cell growth and viability. *Biomacromolecules*, *10*(7), 1675–1680.

Sukyte, J., Adomaviciute, E., & Milasius, R. (2010). Investigation of the possibility of forming nanofibres with potato starch. *Fibres & Textiles in Eastern Europe*, *18*(5), 24–27.

Sunthornvarabhas, J., Chatakanonda, P., Piyachomkwan, K., & Sriroth, K. (2011). Electrospun polylactic acid and cassava starch fiber by conjugated solvent technique. *Materials Letters*, *65*(6), 985–987.

Takeo, K., Tokumura, A., & Kuge, T. (1973). Complexes of Starch and its Related Materials with Organic Compounds. Part. X. X-Ray Diffraction of Amylose-Fatty Acid Complexes. *Starch - Stärke*, *25*(11), 357–362.

Toskas, G., Hund, R.-D., Laourine, E., Cherif, C., Smyrniotopoulos, V., & Roussis, V. (2011). Nanofibers based on polysaccharides from the green seaweed Ulva Rigida. *Carbohydrate Polymers*, *84*(3), 1093–1102.

Ziegler, G. R., Creek, J. A., & Runt, J. (2005). Spherulitic Crystallization in Starch as a Model for Starch Granule Initiation. *Biomacromolecules*, *6*(3), 1547–1554.

Figure Captions

Fig. 1. (a) Scanning electron micrograph of electrospun pure starch fibers and (b) diameter histogram of electrospun pure starch fibers

Fig. 2. Optical micrographs of electrospun pure starch fibers under normal light (left) and between crossed polarizers (right). Scale bar represents 50 μm in both pictures.

Fig. 3. Thermograms of electrospun starch fibers heated in various ethanol/water mixtures (v/v): (a) 0/100, (b) 20/80, (c) 40/60, (d) 50/50, (e) 60/40, (f) 80/20, and (g) 100/0.

Fig. 4. Thermograms of electrospun starch fibers (a) heated in 50% (v/v) ethanol and (b) scanned in 50% (v/v) ethanol after being held at 65 ^{o}C for 30 minutes.

Fig. 5. X-ray diffraction patterns of (a) as-spun electrospun starch fibers and (b) electrospun starch fibers after post-spinning heat treatment.

Fig. 6. Optical micrographs of electrospun starch fiber mats immersed in water after 10 minutes: (a) as-spun starch fibers, (b) heat-treated highly crystalline starch fibers, and (c) starch fibers cross-linked by vapor phase glutaraldehyde. Scale bar is 50 um that is applied to all figures.

Fig. 7. X-ray diffraction patterns of as-spun starch fibers (A) and heat-treated starch fibers (B) from coagulation baths with different ethanol concentrations: (i) 100%, (ii) 90%, (iii) 80%, (iv) 70%, and (v) 60% (v/v).