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Olive Leaf Extract as a Crosslinking Agent for the Preparation of Electrospun Zein Fibers

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ABSTRACT: Incorporating active agents, reinforcing structure by crosslinking, thus changing release properties, can be listed as possible modifications in preparation methods of biopolymer fibers. This study introduces oleuropein, major component of olive leaf extract (OLE), as a natural functional crosslinker for electrospun zein fibers, owing to its antioxidant and antimicrobial properties. Incorporation of OLE causes morphological and structural changes indicated by a decrease in fiber diameter up to 27%, an increase in intensity of NH bending region due to interaction with –OH groups and observation of characteristic oleuropein bands. Extract addition also enhances thermal stability. Zein fibers without OLE is fully degraded at 600°C, whereas 10% of OLE loaded zein fibers is left undegraded. Fifty percent of initial phenolic content loaded into fibers is released which indicate the effect of OLE incorporation as accumulation of oleuropein. OLE-incorporated fibers immersed in PBS are less fused than pure zein fibers, due to the crosslinking effect. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 41338.

KEYWORDS: crosslinking; electrospinning; fibers

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INTRODUCTION

Biopolymer fibers have received much attention for biomedical uses due to their interconnected structure, high surface area, ultra-fine pore structure and biodegradability.[1,2] Their ability to mimic native extracellular matrices (ECMs) allows them to interact with the cells in the application area and enhances cell proliferation. Together with these benefits, biopolymer fibers show a great potential as wound dressings due to their excellent oxygen permeability and because they can provide evaporative water loss, they may prevent microbial burden to accelerate wound healing.[3]

Biopolymer fibers are produced by several preparation techniques such as melt-blown, phase separation, self-assembly, and template synthesis and electrospinning.[4,5] Electrospinning is prominent among other methods due to its versatility, achievement of controlled fiber diameter, convenience for scale-up, ease in application and flexibility in terms of materials used for fiber production.[6,7] The method is applicable to a wide range of biopolymers, such as collagen,[8] chitin,[9] chitosan,[10] cellulose,[11] silk fibroin,[12] hyaluronic acid,[13] and zein.[14]

Zein is the prolamine, comprising 60% of protein content in corn and consisting of one-third hydrophilic and two-thirds hydrophobic amino acid residues in its primary structure.[15] Glutamic acid, leucine, proline, and alanine comprise the amino acid composition of zein. The absence of the basic and acidic amino acids results in solubility behavior of zein in aqueous alcohol solutions,[16] which provides overcoming the disadvantage of organic solvents and makes zein an eco-friendly polymer.[14] Zein has been used in pharmaceutical industry for drug targeting[17] due to its biodegradability and hydrophobicity.[18] Zein porous scaffolds were studied in tissue engineering for bone substitution[19] and to enhance cell growth and proliferation. Zein films were also biocompatible as proliferation of liver and fibroblast cells was significant in smooth films.[20] Blend polymer solutions of zein and chitosan was also electrospun to prepare nanofibrous membrane for wound healing.[21]

One of the limitations in utilization of biopolymers as biomaterials is based on their poor mechanical properties in aqueous media. Fiber morphology collapses into film structure and porosity of the material is affected negatively which restricts their use in *in vivo* applications.[20,22,23] Because zein dissolves in ethanol, zein films have brittle characteristics, although zein fibers have poor integrity and can easily be crushed into powder form. Crosslinking is a common method to improve mechanical properties and control degradation rate of biomaterials. Agents used in this

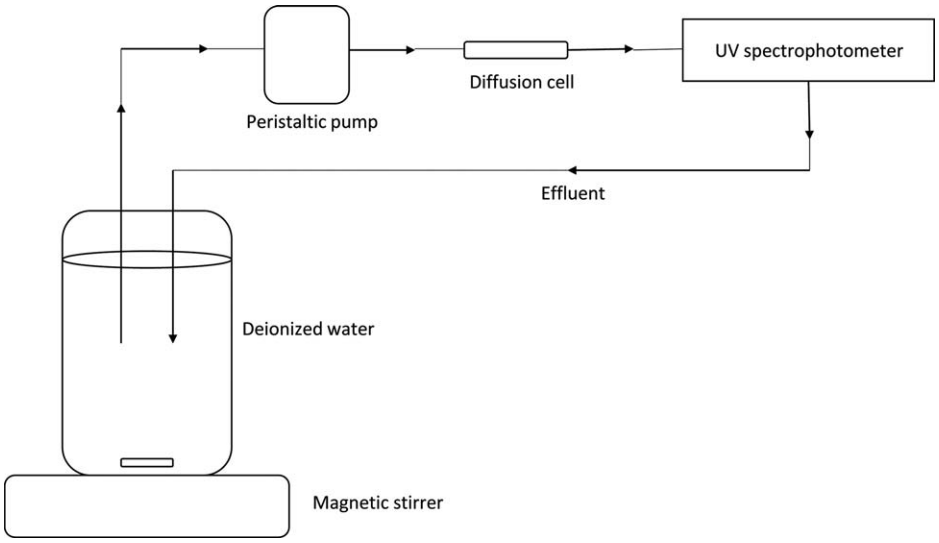

Figure 1. *In situ* measurement set-up for continuous release.

chemical modification can be listed as formaldehyde, epichlorohydrin, 1,2,3,4-butanetetracarboxylic acid,[24] glutaraldehyde,[25,26] tetrahydrofuran (THF) containing hexamethylene diisocyanate (HDI)[14,27] and glyoxal.[28] High toxicity of these agents causes a major problem for *in vivo* applications such as calcification effect of glutaraldehyde in implants.[29] Therefore, there is a great effort to discover novel natural compounds as an alternative to synthetic crosslinking agent. Citric acid[23,30] and genipin[31] are commonly used non-toxic alternatives of crosslinking agents.

Oleuropein, the major component of olive leaf extract (OLE), has a great potential in this regard. It is characterized as iridoid glycoside isolated from olive tree (*Olea europaea L.*) and represents the predominant phenolic oleosides.[32] Olive leaf, common in Mediterranean coasts of Europe, has been used for medical purposes for hundreds of years. It can be suggested to treat diseases related with oxidative stress such as cancer, heart diseases, atherosclerosis, romatoid arthritis, neurodegenerative diseases and recently wound healing, due to its phenolic content.[33,34] Markin et al.[35] performed a study revealing antimicrobial effect of OLE on bacteria and fungi. This combined antimicrobial effect may be beneficial to prevent opportunistic infections rising up after long term antibiotic usage. OLE, also provides functionality when integrated to a biomaterial, which improves biocompatibility and induces specific biological responses, as decreased inflammation, increased cellular proliferation, migration, and differentiation by tailoring release properties.[36] Therefore OLE can be distinguished from other natural, non-toxic crosslinkers,[23,30] regarding these benefits.

Oleuropein also guards the plant against infections and herbivorous animals, resembling antimicrobial effect of OLE. Iridoid glycosides produce aglycone by β-glucosidase. Aglycone is responsible for the denaturation of proteins and being a crosslinking agent. Similar to glutaraldehyde, oleuropein produces poly α,β-unsaturated aldehyde by deglucosidation and oxidation. Antunes et al.[37] conducted a study, using oleuropein as crosslinking agent in collagenic films and revealed that oleuropein aglycone was capable of crosslinking more amino groups than glutaraldehyde.

The present study aims to investigate crosslinking effect of OLE in addition to its role in functionalization due to its polyphenolic content. Prepared materials can find potential applications in the field of biomaterials and tissue engineering. Using OLE may also provide a single preparation step for electrospinning as both zein and OLE can be processed with aqueous ethanol solution.

EXPERIMENTAL

Materials

Zein from maize was obtained from Sigma-Aldrich (Germany). Ethanol absolute was purchased from Merck (Germany). Olive leaves were collected from Olive Cultivation Research Station in Izmir. Folin-ciocalteu reagent and sodium chloride, used in total phenol determination, were obtained from Merck (Germany) and Carlo Erba (France), respectively. Oleuropein, used in calibration for *in situ* measurement, was purchased from Extrasynthese (France). NIH/3T3 mouse fibroblast cell line, used in cell attachment studies, was obtained from ATCC with a reference number of CRL-1658. Dulbecco Modified Eagle Medium (DMEM), fetal bovine serum (FBS), phosphate buffered saline (PBS), L-glutamine, penicillin-streptomycin solution and trypsin-EDTA were from Gibco (United Kingdom).

Preparation and HPLC Analysis of OLE

Dried olive leaves were ground by a small scale grinder. Extraction was performed in 70% aqueous ethanol with solid-liquid ratio of 1 : 20, at 180 rpm at room temperature for 2 h.[38,39] Extract was vacuum-filtered and evaporated to remove ethanol. Aqueous phase of extract was centrifuged at 4000 rpm for 5 min to remove solid residues. The liquid extracts were lyophilized for 3 days. Extraction yield was calculated as 28% (w/w).

Preparation of Zein Solutions

Zein from maize was dissolved in 70, 80, and 90% aqueous ethanol solutions (v/v) between a concentration range of 20 and 40% (w/v). Electrospinning solutions containing 30% (w/v)

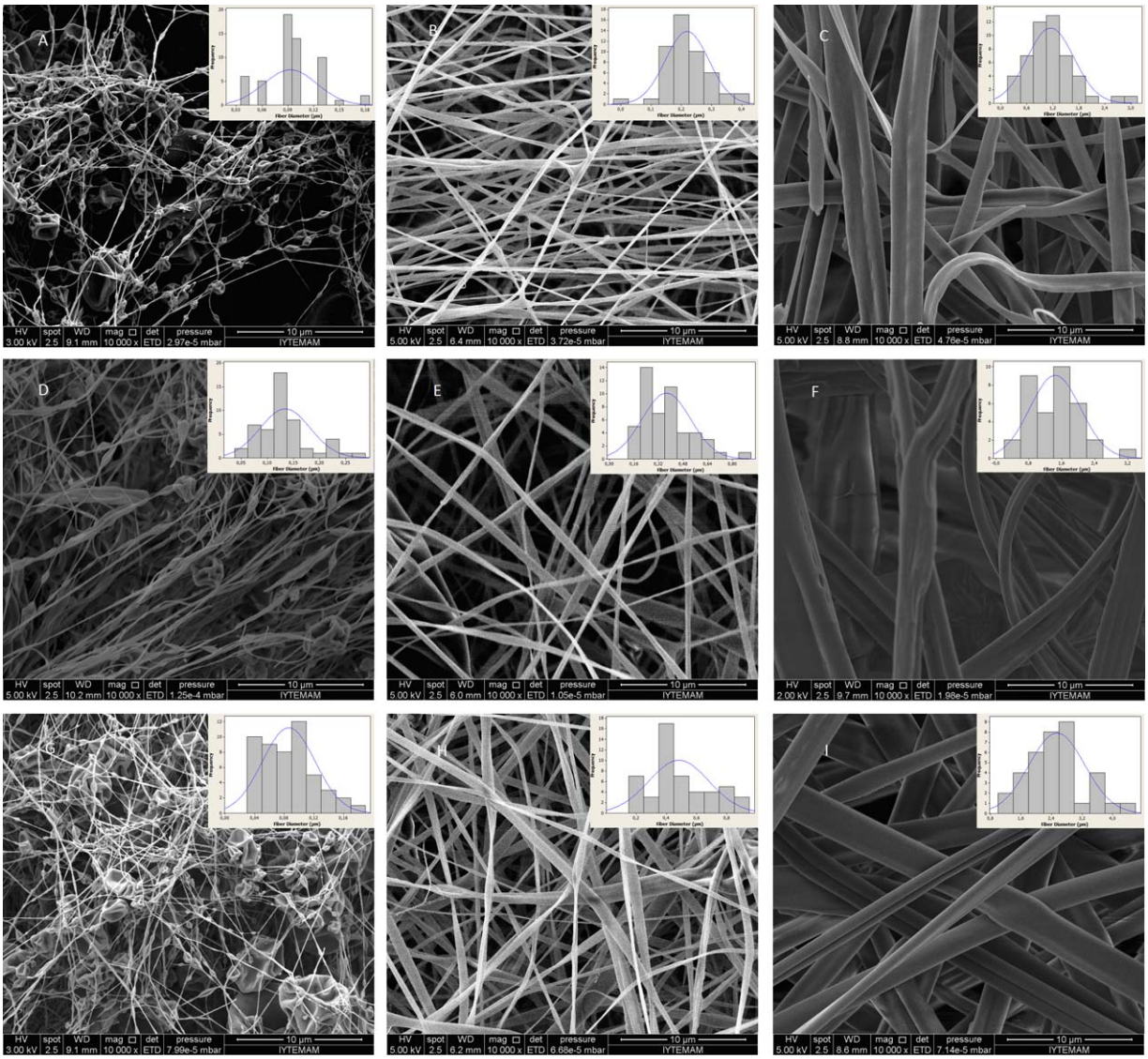

Figure 2. The morphology of fibers prepared at varying zein concentrations and applied voltage in 70% aqueous ethanol solution. Applied voltage is A–C: 15 kV, D–F: 20 kV, and G–I: 25 kV. Zein concentrations are 20% for A, D and G, 30% for B, E, and H, 40% for C, F, and I. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

zein in 70% aqueous ethanol were mixed with lyophilized OLE at concentrations of 5, 7.5, and 10% (w/v). Aqueous ethanol phase (70%) (v/v) of OLE containing 5, 7.5, and 10% dry matter was also used to dissolve 30% zein (w/v) to observe the effect of the single preparation step of the polymer solution.

Electrospinning of Zein

The prepared zein solutions were fed into a 5-mL plastic syringe with a needle having a tip diameter of 0.6 mm. The flow rate of the pump was adjusted to 6 mL/h. The fibers were deposited on a constant target of aluminum foil under 15, 20, and 25 kV. The distance between the needle tip and the target was 10 cm.

Scanning Electron Microscopy

Morphology of zein fibers were observed by SEM (FEI Quanta 250 FEG) after gold sputtering. Images were taken by applying an electron voltage of 5 kV. The size distribution was generated by measuring and processing the diameter of fibers with Image J software.

Fourier Transform Infrared Spectroscopy (FTIR)

Alterations in bond structures of fibers were observed by FTIR spectroscopy (Shimadzu-8400S). Powdered zein fibers were mixed with potassium bromide and IR spectra were collected by operating in the region from 4000 to 400 cm^{-1}, averaging 40 scans at 2 cm^{-1} resolution.

Thermogravimetric Analysis (TGA)

The degradation behavior of zein fibers were analyzed by TGA (Shimadzu TA 50) by scanning from 25 to 600°C at a heating rate of 10°C/min under nitrogen atmosphere.

In Vitro Release Studies

OLE loaded zein fibers of 25 mg were immersed in 4 mL of deionized water in 12-well plate for batch release and incubated

Table I. The Average Diameter of Fibers Prepared at Different Process Parameters

Parameter	Values	Average diameter (μm) (±standard deviation)
Zein[a] (%) (w/v)	20	0.13 ± 0.05
	30	0.37 ± 0.16
	40	1.44 ± 0.62
Ethanol[b] (%) (v/v)	70	0.48 ± 0.20
	80	0.65 ± 0.24
	90	0.81 ± 0.36
Voltage[c] (kV)	15	0.22 ± 0.07
	20	0.37 ± 0.16
	25	0.48 ± 0.20

Constant values for the parameters were
[a] 70% aqueous ethanol solution and 20 kV voltage,
[b] 30% zein content and 25 kV voltage,
[c] 70% aqueous ethanol solution and 30% zein content.

at 37°C for 1, 2, 3, and 6 days. The entire release medium was collected for each time interval and subjected to total phenol content determination by Folin–Ciocalteu method. *In situ* release experimental set-up consisted of a peristaltic pump, a diffusion cell and a UV-spectrophotometer (Figure 1). Ten milligrams of OLE loaded zein fiber was placed in the diffusion cell and 50 mL of deionized water was pumped through the system. Zein fibers without OLE were used as control. Effluent was returned to the source after spectrophotometric measurement. Absorbance data were collected at 280 nm for 210 min., and the amount of the soluble phenolic compounds was determined by OLE calibration curve.

Cell Attachment on Zein Fibers

NIH/3T3 mouse fibroblast cell line was maintained in DMEM supplemented with L-glutamine, 10% fetal bovine serum, 100 μg/mL streptomycin and 100 U/mL penicillin in an atmosphere of 5% CO_2 at 37°C. Cells were subcultured every 48 h.

For *in vitro* attachment, fiber-coated coverslips were prepared.[40,41] Zein and OLE loaded zein solutions were electrospun onto autoclaved glass coverslips (18 mm × 18 mm) for 20 min. until a thin layer was formed. Fiber-coated coverslips were then subjected to UV sterilization for 1 h and after washing with copious amounts of PBS, they were placed in six-well tissue culture plates. Cells were seeded onto coverslips at a density of 10^5 cells/well and allowed to attach for 1 week. Polarized light microscope with Differential Interference Contrast (DIC) module (Olympus) was used to visualize cell attachment on coverslips of electrospun zein fibers.

RESULTS AND DISCUSSION

Fiber Morphology

Zein fibers had diverse appearance ranging from bead formation to ribbon-shaped morphology due to the increased zein content as observed in SEM micrographs (Figure 2). Bead formations

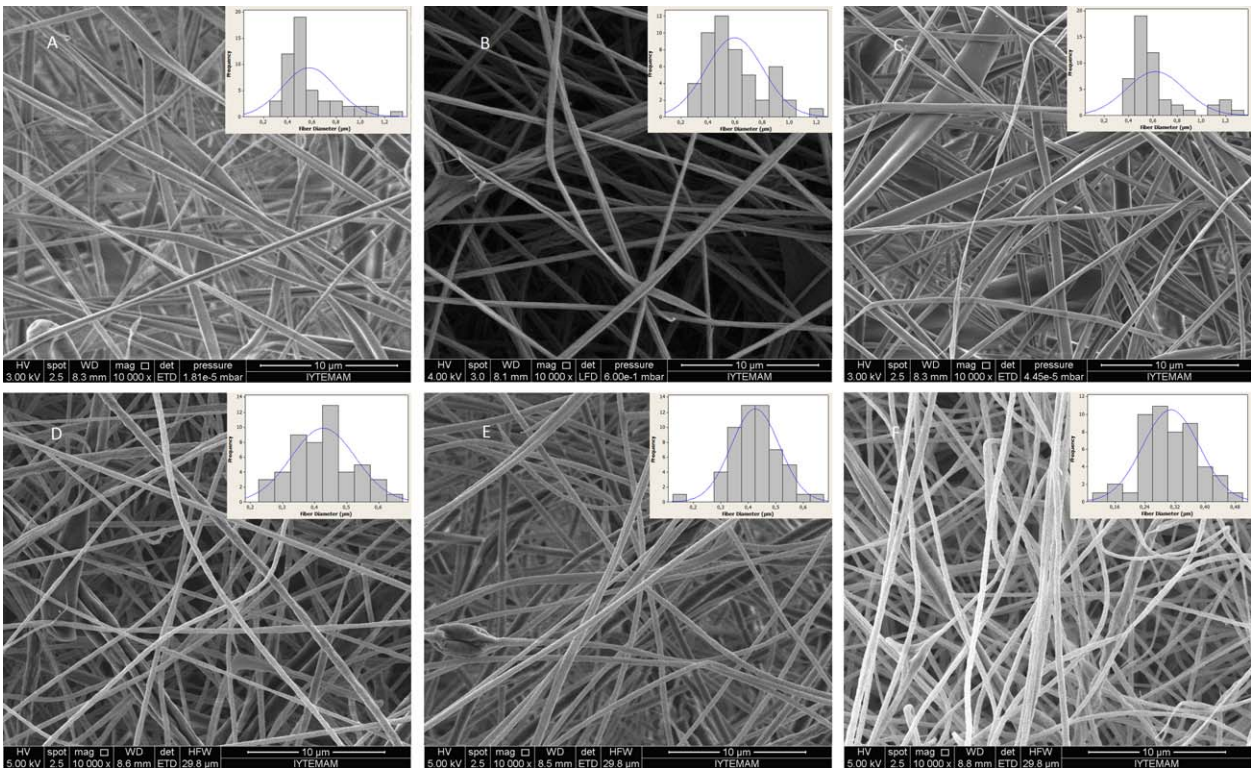

Figure 3. The morphology of OLE loaded fibers prepared at varying conditions. A-C: fibers prepared with lyophilized extract; D-F: fibers prepared with extract in aqueous ethanol solution. Solutions consist of 30% zein in 70% aqueous ethanol. OLE concentrations are 5% for A and D, 7.5% for B and E, 10% for C and F. Fibers were prepared under 20 kV. Scale bar is 10 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

were dominant in fibers having 20% zein content dissolved in 70% aqueous ethanol solution. Beads were converted into fibers as zein content of the solution increased. Ribbon shaped fibers were observed with an increase in zein content, most abundantly in fibers having 40% zein. Koombhongse et al.[42] defined the beads as "toroids" and the ribbon shapes as collapsed tubular skins. They explained the fact as evaporation of the solvent while atmospheric pressure collapsed the tube, the tube became flat and formed a ribbon shape afterwards. Arinstein and Zussman[43] also explained this phenomenon as "fiber buckling" which resulted from rapid evaporation of the solvent. They stated that when solvent evaporated rapidly, polymer formed a "shell" around the fiber that resisted solvent evaporation. This resistance resulted inhomogeneous pressure distribution. Residual solvent in the fiber prevented deformation in the fiber at initial stage. As evaporation proceeded, pressure drop across fiber shell was developed due to increase in external pressure. With the removal of solvent, fiber showed tendency to deform by means of buckling.

The average diameters of zein fibers also showed that fiber diameter was mostly affected by zein content of the electrospinning solution. The average diameters of zein fibers prepared at varying conditions are tabulated in Table I. When the average diameters of 50 zein fibers were evaluated, it can be seen that average fiber diameter increased 10 times as zein content was increased from 20 to 40% (w/v). Increase of standard deviations in direct proportion to zein content of the solution, as seen in Table I, also indicated that the uniformity of the fiber diameter distribution was decreased. This observation indicates that homogeneous fiber morphology can be obtained under process conditions of 30% zein content (w/v). Increase in fiber diameter under increasing voltage can be explained by decreased distribution of bead formation.[1] It can be seen that bead formation is mostly dominant under working voltage of 15 kV when zein concentration was 20% (Figure 2), and fiber formation began to emerge as voltage was increased to 25 kV. Solvent content also affected fiber diameter due to influence on net volume charge density of electrospun jet.[44] Torres-Giner et al.[44] stated that increasing ethanol content caused increase in fiber diameter because of increase in evaporation rate and facilitation in solidification and formation of fibers. They also noted that this increasing effect was significant as ethanol content was superior to 80%, which align with our findings depicted in Table I.

Table II. Diameter of Fibers Prepared with Different Concentrations and Phases of OLE

Extract phase used in preparation	Extract concentration (%) (w/w)	Average diameter (μm) (±standard deviation)
Dry powder extract	5	0.58 ± 0.22
	7.5	0.60 ± 0.21
	10	0.62 ± 0.24
Aqueous ethanolic extract	5	0.43 ± 0.10
	7.5	0.43 ± 0.09
	10	0.31 ± 0.07

Zein concentration was kept constant at 30% (w/v) and fibers were formed under voltage of 20 kV.

In this study, zein content was found to be the most influencing factor for the diameter of electrospun zein fibers in accordance with the findings in the related literature.[14,44] Fiber diameter decreased with a decrease in zein content, with the lowest for the fibers prepared from 70% aqueous ethanol solutions having 20% zein content. Bead formation did not allow regular fiber formation.

Effect of OLE was also reflected on fiber morphology. Ribbon-shaped zein fibers without extract were replaced with curved form in OLE-loaded zein fibers due to possible crosslinking effect of oleuropein (Figure 3). Yao et al.[14] also revealed that zein fibers were curved when crosslinked with tetrahydrofuran containing 1% hexamethylene diisocyanate (HDI). When the

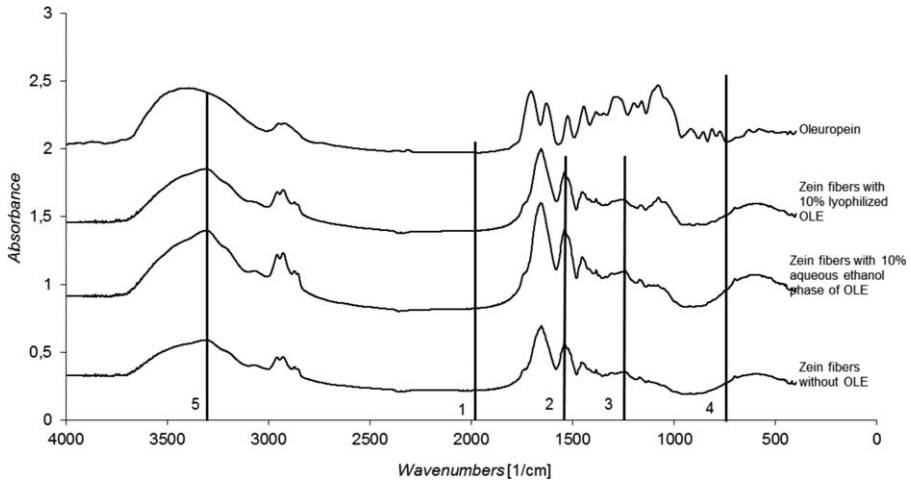

Figure 4. FTIR spectra of zein fibers with and without OLE and oleuropein standard. Electrospinning solutions were prepared in 70% aqueous ethanol containing 30% zein. The applied voltage was 20 kV. Vertical lines 1, 2, and 3 represents characteristic zein bands; amide I, II and III respectively. Vertical line 4 refers to oleuropein and vertical line 5 represents the interactions between polyphenols in olive leaf extract and zein protein.

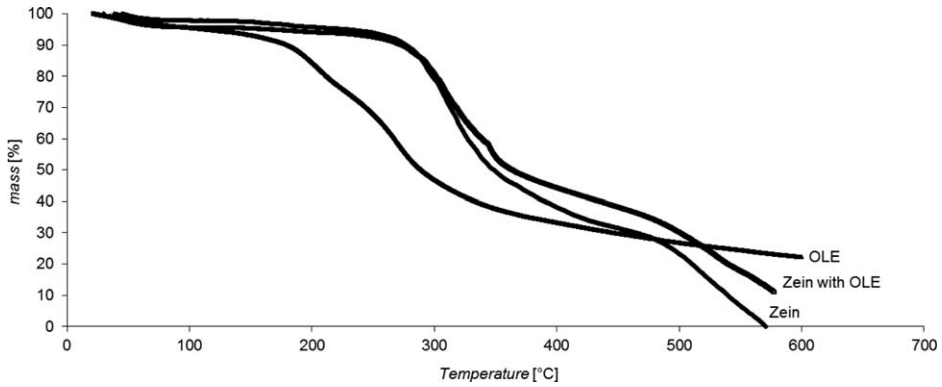

Figure 5. TGA thermogram of zein fibers, aqueous ethanol phase of OLE incorporated zein fibers and OLE. OLE in aqueous ethanolic phase contained 10% dry matter.

effect of lyophilized and aqueous ethanol phase of OLE were compared, a decrease in fiber diameter could be observed in fibers prepared with OLE in aqueous ethanol solution (Table II). The increasing amount of OLE caused significant decrease in fiber diameter as well. When zein fibers with and without OLE that were produced under the same process parameters were compared, it was observed that fiber diameter was decreased between the range of 17 and 27%. The difference of standard deviations between the diameters of the fibers prepared from solutions containing dry powder and aqueous ethanolic extract also indicated that fiber morphology was more homogeneous when aqueous ethanolic extract was used in fiber preparation. Difference in fiber morphology and diameter distribution in OLE-loaded zein solutions was based on extract form used in polymer mixture. Aqueous ethanolic extract caused decrease in fiber diameter in proportion with extract concentration, whereas dry powder extract did not cause decrease in fiber diameter. Fibers in ribbon shape were dominant in case of dry powder extract utilization, but zein fibers were transformed to more curved like morphology in aqueous ethanolic extract usage. These differences emerged from integration of extract with the polymer solution. Aqueous ethanolic extract showed a better integration with zein than dry powder extract, which led to more homogeneous mixture of polymer and crosslinker.

Fourier Transform Infrared Spectroscopy (FTIR)

OLE-incorporated zein fibers were subjected to FTIR spectroscopy to observe the differences in bond structure as an indicator of crosslinking effect. Zein proteins show a typical absorption referring amide I band between 1600–1690 cm^{-1}, amide II between 1480–1575 cm^{-1} and amide III between 1229–1301 cm^{-1}.[45–47] These characteristic bands of peptide backbone were observed in zein fibers at 1665, 1545, and 1269 cm^{-1} for amide I, II, and III, respectively, and can be seen in Figure 4. Each band was marked with a vertical line. Intensity difference of amide bands between zein fibers containing OLE and pure zein fibers used as control is an indicator of change in bond structure. Intensity increase of the band in the region of 3200–3500 cm^{-1} and peak at 1643 cm^{-1} also indicated OLE incorporation into fibers when FTIR spectra of OLE loaded and pure zein fibers were compared. Specifically, fibers prepared with aqueous phase of OLE exhibited higher intensities in comparison with fibers prepared with lyophilized OLE, which might indicate better incorporation and bond formation. The region between 3200–3500 cm^{-1} and peak at 1643 cm^{-1} represent NH bending of amide groups[48] of protein which interacts with hydroxyl (-OH) groups of polyphenols as a possible crosslinking interaction. Band intensity differences between fibers prepared with lyophilized and aqueous form of OLE might result from homogeneity of extract in preparation

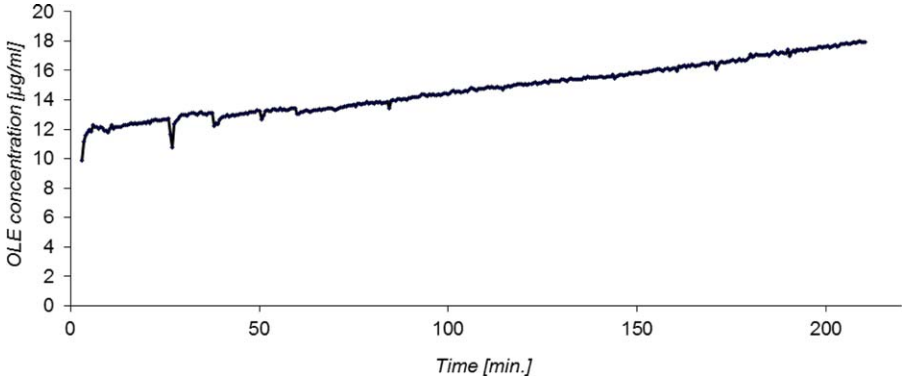

Figure 6. *In situ* cumulative release of OLE from zein fibers.

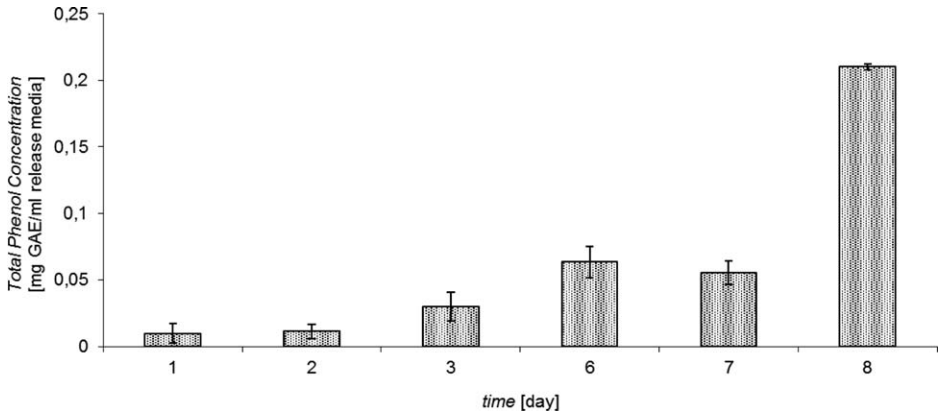

Figure 7. Batch release of OLE released from zein fibers in terms of GAE. Fibers contained aqueous ethanol phase of OLE with 10% dry matter.

of electrospinning solutions. Aqueous form of OLE was more integrated into zein structure. More homogenous electrospinning solutions were formed because aqueous ethanol phase of extract was used as solvent which provided better integration of the extract and polymer. OLE incorporation was also reflected in comparison of oleuropein and zein fibers' FTIR spectra. Typical oleuropein band at 1090 cm^{-1},[38] indicated by Line 4 was present in zein fibers prepared with both lyophilized and aqueous ethanol phase of OLE. As expected, oleuropein band was not observed in the FTIR spectrum of pure zein fibers. The results overall indicated that zein fibers prepared with aqueous form of OLE was incorporated into zein structure successfully at a higher degree with a potential of crosslinking reaction.

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) was used to evaluate and compare thermal stabilities and decomposition behavior of zein fibers, aqueous ethanol phase of OLE incorporated zein fibers and OLE as seen in Figure 5.

Initial weight loss of 10% was observed between 50 and 150°C, which was attributed to vaporization of water from the samples. The second weight loss occurred at 300°C as 50% for zein and OLE loaded zein fibers, related to thermal degradation of polymer structure, in agreement with other studies.[44] OLE showed different degradation profile within the same temperature range, initial mass loss due to water evaporation, an additional mass loss of 55% occurred at 180°C and degradation rate decreased after 300°C. OLE had 22% of mass undegraded at 600°C at the end of analysis and it was estimated that degradation would be complete at temperatures higher than 800°C. The degradation profile of zein and OLE-incorporated zein fibers also differed after 300°C, as degradation rate of zein was higher than OLE-incorporated zein fibers. Zein was fully degraded at 600°C, whereas OLE-incorporated zein fibers still had 10% of its mass undegraded. Mixing aqueous ethanol phase of OLE with zein may have caused irreversible changes in the structure that increase the mechanical properties and establishing resistance to thermal degradation as well.

In Vitro Release Studies and Fluid Uptake Properties of the Fiber

The release study was performed by two methods as *in situ* continuous measurement and batch release. *In situ* continuous measurement was performed to observe saturation in the system. Zein fibers prepared with OLE in aqueous ethanol phase, containing 10% dry matter, were used for both continuous and batch

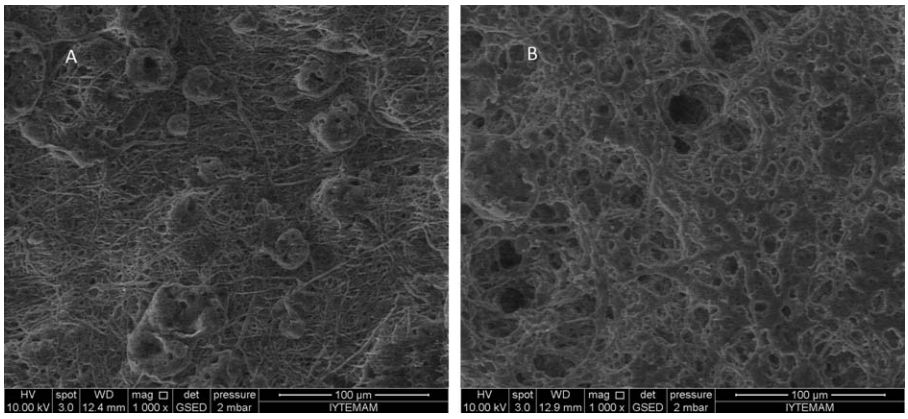

Figure 8. SEM micrographs of A. Zein fibers without OLE and B. OLE-loaded (10%) zein fibers after subjected to 6 days of batch release at 37°C. Scale bar is 100 µm.

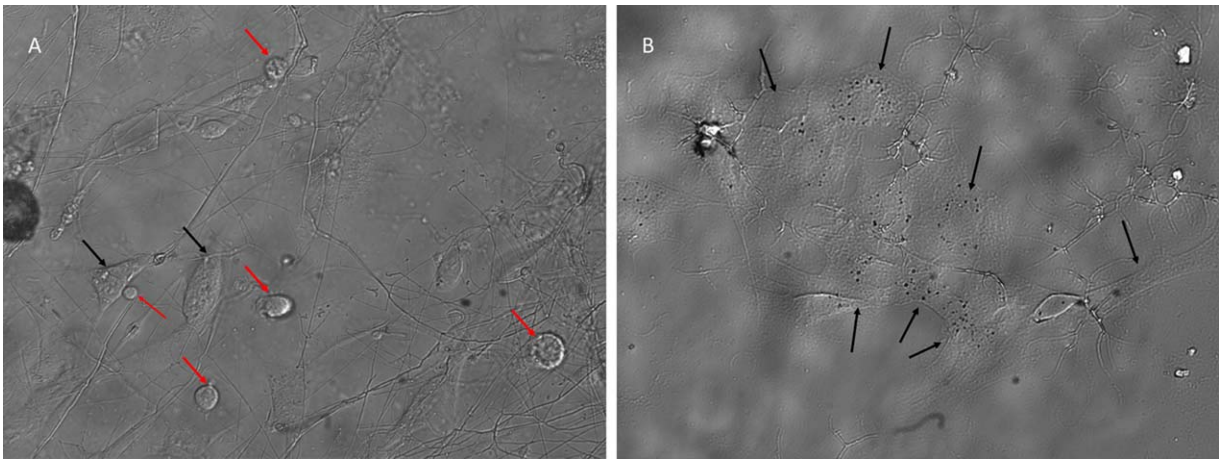

Figure 9. Phase contrast microscope images of cells seeded on A. Pure zein fibers and B. OLE-loaded zein fibers after 1 week of incubation. Black arrows show cells that spread and proliferated better than cells indicated by red arrows. Images were taken at a magnification of 20×. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

release measurements. Zein fibers prepared without OLE were used as blank and the blank subtracted values were used in calculations. Figure 6 shows cumulative OLE release in terms of concentration, which was calculated using OLE calibration curve. The profile indicated that there was a continuous release from OLE-incorporated zein fibers, offering sustained release of incorporated compounds. The released amount of extract in elapsed time was not sufficient for the system to reach saturation.

Fiber samples were also immersed in deionized water to perform batch release during 6 days. Release media were subjected to total phenol analysis to determine the phenolic properties of the extract after release. Figure 7 shows the amount of the soluble phenolic compounds in Gallic Acid Equivalent (GAE). It was observed that amount of phenolic compounds in release media of OLE-incorporated zein fibers increased in a time-dependent manner. Soluble phenolic content reached to highest level in 8 days. Zein fibers released approximately 50% of initial phenolic content at the end of 8-day period. The results of *in situ* measurement of OLE and soluble phenolic content determination might indicate that the excess amount of OLE deposited on the surface of the fibers was released in sustained manner. The high amount of soluble phenolics on the 8th day of the batch release period might result from degradation of zein fibers in release media due to the burst release of OLE incorporated into fiber structure.

Zein fibers after subjected to 6 days batch release were also investigated by SEM. Collapse of fiber formation and fused morphology were clearly observed in pure zein fibers [Figure 8(a)], whereas pore structures were present in OLE-incorporated fibers [Figure 8(b)]. OLE incorporation caused decrease in fiber diameter because it integrated within zein structure, therefore swelling properties of the fibers also changed. Fiber structure was maintained in zein fibers containing OLE more than pure zein fibers, which may indicate crosslinking effect.

Cell Attachment on Zein Fibers

Biocompatibility of OLE-loaded zein fibers was investigated by seeding NIH3T3 mouse fibroblast cells on fibers. Zein fibers without OLE was used as control. Figure 9(a,b) show cells on pure and OLE-loaded zein fibers, respectively. Cells were allowed to attach for 1 week. Attachment of the cells on pure zein fibers can be observed in Figure 3(a) by red arrows. Cells on pure zein fibers did not spread over fiber surface when compared with cells on OLE-loaded zein fibers. Morphology of cells on OLE-loaded zein fibers were also changed due to spreading which may indicate better proliferation of these cells [Figure 3(b)]. It can be concluded that OLE addition enhanced fibroblast spreading and proliferation with regard to better fiber formation and providing higher surface area than pure zein.

CONCLUSIONS

In this study, OLE has been introduced as a natural, non-toxic crosslinker due to its oleuropein content. The incorporation of OLE affected morphology, water stability, bond structure and thermal properties of the electrospun zein fibers. The decrease in fiber diameters and homogeneous fiber morphology could be attributed to the effects of OLE, which are based on alterations in bond structure as depicted in FTIR analysis and resistance to thermal degradation indicated by TGA analysis. As well as reinforcing water stability of zein fibers in aqueous media, which referred to crosslinker effect, OLE addition also functionalized zein fibers, due to sustained release of phenolic contents. OLE-loaded zein fibers were also found biocompatible with fibroblast cells which proliferated and spread on fiber surface better than pure zein. Our results align with the findings of the related studies in the field. To the best of our knowledge, this is the first study to present OLE as a crosslinking agent in zein fibers which are commonly used in tissue engineering applications. In the light of these findings it can be proposed that OLE has a potential to be used as a crosslinker in zein fibers as well as providing functionality attributed to its high antioxidant capacity and antimicrobial property.

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