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Electrospun biocomposite nanofibers of ulvan/PCL and ulvan/PEO

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ABSTRACT: Novel fibrous biocomposites comprising ulvan, a sulfated polysaccharide extracted from the green seaweed *Ulva rigida*, and either polycaprolactone or polyethylene oxide were successfully prepared using the electrospinning technique. The morphology and the chemical composition of the nanofibers were characterized using SEM and FT-IR analyses. Ulvan was found to incorporate well with the co-electrospun biocompatible polymers, conferring on the collective properties of these materials. Such nanofibrous matrices represent potentially useful materials in the biomedical sector as tissue engineering scaffolds, wound dressings, or drug delivery systems. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42153.

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INTRODUCTION

Electrospinning is a relatively simple and versatile technique for the generation of nano-/microfibers from polymeric solutions under the application of a high-voltage electric field. The applications of electrospun fibers spread in several fields, including the pharmaceutical sector, because of their high porosity, high surface area-to-volume ratio, and architectural analogy to natural extracellular matrix.[1]

The diversity of chemical structures displayed by natural and synthetic polymers, which can be electrospun provides ample opportunities for tailoring the mechanical and biological properties of fibers.[2] Natural polymers, characterized by their biocompatibility and biodegradability, are frequently preferred in the biomedical field as materials with unique properties.[3] Marine polysaccharides exhibit a wide range of bioactivities, rendering them ideal for the development of novel systems for bioapplications, such as tissue engineering and drug delivery. Up to date, only a small number of fibrous scaffolds based on marine polysaccharides have been produced by electrospinning.[4]

Ulvan is a complex anionic sulfated polysaccharide extracted from the cell walls of green seaweeds of the genus *Ulva*.[5] Pharmacological studies have demonstrated that ulvan possesses anticoagulant, antioxidant, antitumor, immunomodulating, and antihyperlipidemic activities.[6] Moreover, nanofibrous membranes based on ulvan were found to promote attachment and proliferation of osteoblasts maintaining cell morphology and viability.[7]

Efforts to produce electrospun fibers using ulvan as the sole polymer under different solvent systems and electrospinning parameters have not been successful so far due mainly to its poor rheological properties. Addition of biocompatible polymers, such as polyvinyl alcohol (PVA), polyethylene oxide (PEO), or polycaprolactone (PCL), in small amounts could improve the rheological properties and the charge carrying capacity of the ulvan electrospinning solution, which is crucial for fiber formation. Furthermore, the successful combination of ulvan with such polymers could contribute to its known beneficial pharmacological properties.

Both the water-soluble polymer PEO and the bioresorbable polymer PCL have found multiple applications in the biomaterials field.[8,9] PEO co-electrospun with ulvan should improve ulvan's nontypical solvation in water, which in turn would result in the formation of micro-aggregating gels, whereas PCL soluble in organic solvents is expected to be effective in incorporating ulvan in micro-gels.

Recently, experimenting with blends of ulvan/PVA at different ratios and solvent systems under a variety of experimental conditions, we were able to prepare nanofibers of variable sizes and morphologies.[10] Herein, the preparation of electrospun nanofibers using ulvan/PCL and ulvan/PEO blends at various compositions is reported.

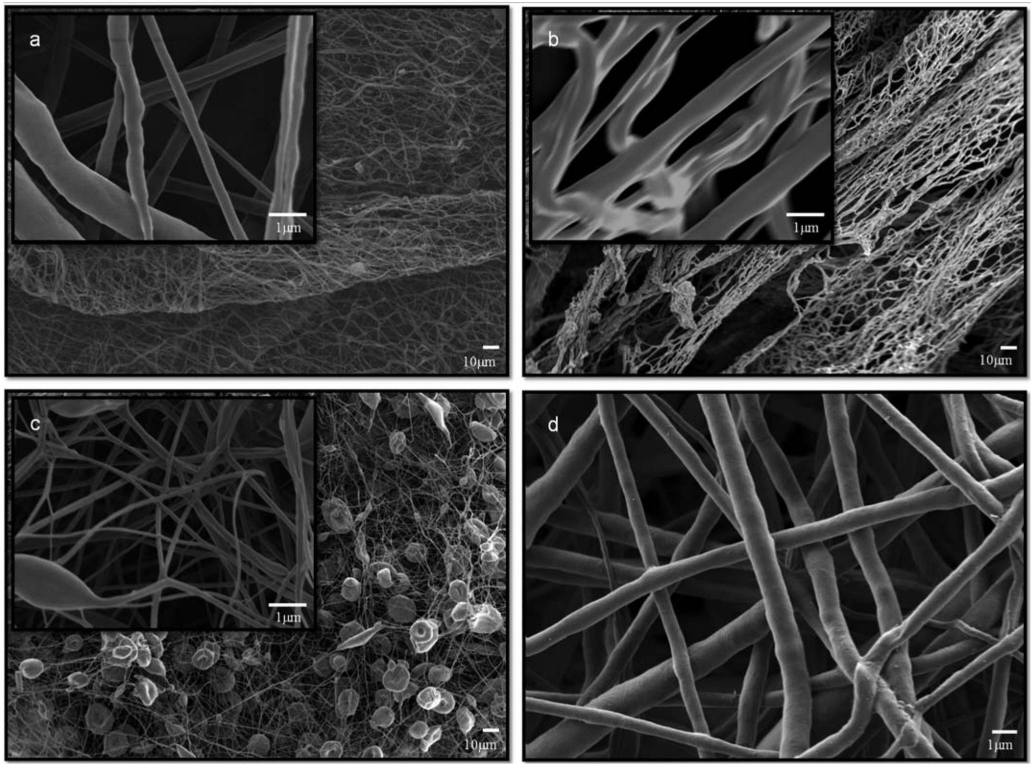

Figure 1. SEM images of fibers of ulvan/PCL at (a) 1 : 8, (b) 3 : 8, and (c) 3 : 2 ratios and (d) PCL.

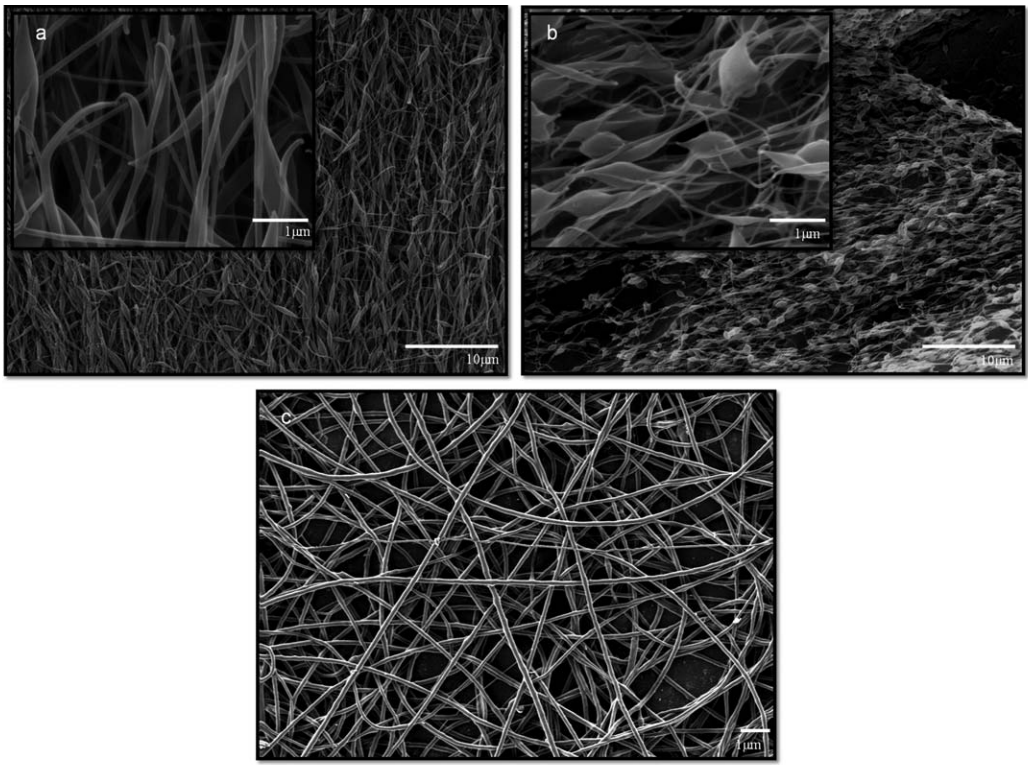

Figure 2. SEM images of fibers of ulvan/PEO at (a) 1 : 1, (b) 2 : 1 ratios, and (c) PEO.

EXPERIMENTAL

Materials

PCL (average M_n 80,000), PEO (average M_v 900,000), *N*,*N*-dimethylformamide (DMF), and dichloromethane (DCM) were purchased from Sigma–Aldrich. The extraction and chemical analysis of ulvan were performed as previously described.[10]

Size Exclusion Chromatographic Analysis

The molecular weight distribution of ulvan was determined by high pressure size exclusion chromatography coupled with refractive index detection (HPSEC–RI). The analyses were conducted using a LKB 2248 pump equipped with a Shodex RI-102 detector and a BIO BASIC SEC-1000 (Thermo Scientific, 300 mm × 7.8 mm) column using ultrapure water as the mobile phase. The lyophilized ulvan samples were dissolved in ultrapure water (1 mg/mL), heated for 20 min at 70°C and filtered through 0.45 μm membrane filter before analysis. Column calibration was performed with pullulan standards.

Preparation of Electrospinning Solutions

A 8% w/v PCL solution was prepared in DCM/DMF (80/20, v/v). Appropriate amounts of ulvan were added in the PCL solution to yield blends of ulvan/PCL at 1 : 8, 3 : 8, and 3 : 2 ratios. A 2% w/v ulvan solution and a 4% w/v PEO solution were prepared separately in deionized water and were stirred until uniform gels were formed. Subsequently, the ulvan and PEO solutions were mixed in appropriate volumes to afford blends of ulvan/PEO at 1 : 1 and 2 : 1 ratios. All electrospinning solutions were stirred for 24 h to ensure their homogeneity.

Electrospinning Conditions

A γ-High Voltage Research DC power supply generator provided voltages of 11 and 9 kV for ulvan/PCL and ulvan/PEO solutions, respectively. The polymer solutions were loaded into 10-mL disposable syringes fitted with tip-ground-to-flat needles (21 and 23 gauge for ulvan/PCL and ulvan/PEO solutions, respectively), which were mounted on a horizontally positioned Harvard PHD 2000 programmable syringe pump. The feed rate was 0.5 mL/h for ulvan/PCL solutions and 0.1 mL/h for ulvan/PEO solutions. The nanofibers were deposited on aluminum foil wrapped on a RC-6000 (NaBond Technologies) rotating drum collector at a rotation speed of 400 rpm and a tip-to-collector distance of 10 cm. Temperature and relative humidity were 20 ± 2°C and 60 ± 5%, respectively. The fibrous mats were stored in a desiccator under vacuum for 48 h to ensure removal of residual solvents.

Characterization

A PHILIPS Quanta Inspect (FEI Company) scanning electron microscope with tungsten filament (25 kV) was used for SEM analyses of the nanofibers which were sputter-coated with gold. The diameters of 25 fibers from each SEM image were measured using the embedded image analysis software and the average fiber diameter was determined. The chemical composition of the fibers was analyzed by Fourier Transform infrared spectroscopy using the attenuated total reflection (ATR) method on a FTIR Bruker Tensor 27.

RESULTS AND DISCUSSION

Hot water extraction of the dried green alga *U. rigida* allowed for the isolation of ulvan with a 28.3% w/w yield. Analysis by HPSEC-RI revealed that ulvan had a molecular weight distribution centered around 1,800 kDa, whereas chemical analyses showed that the ulvan used in the current study was composed

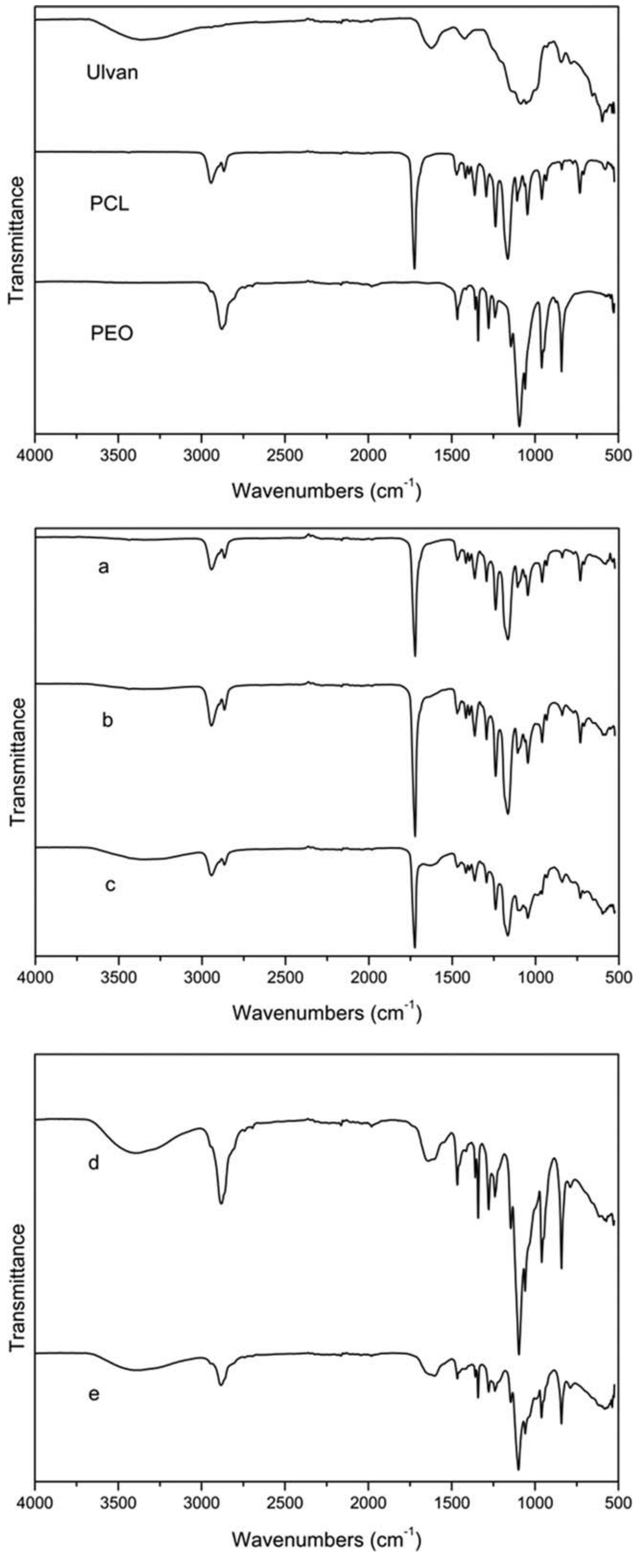

Figure 3. FT-IR spectra of (A) ulvan, PCL and PEO; (B) ulvan/PCL at (a) 1 : 8, (b) 3 : 8, and (c) 3 : 2 ratios; (C) ulvan/PEO at (d) 1 : 1 and (e) 2 : 1 ratios.

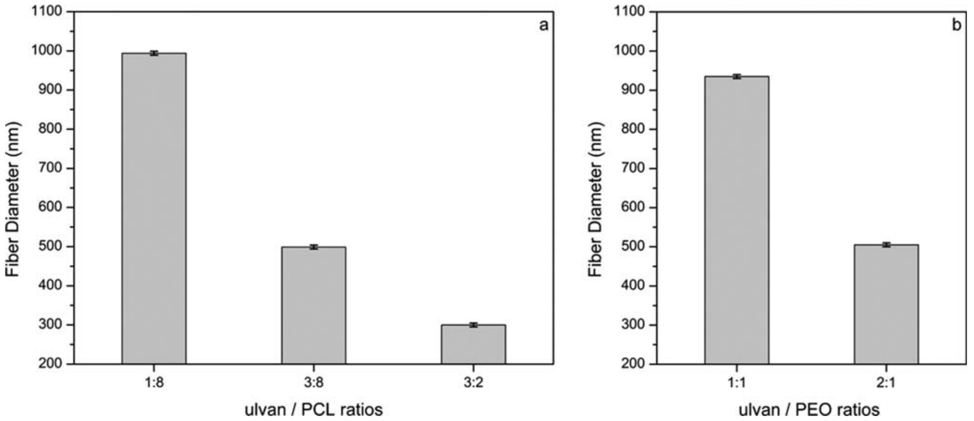

Figure 4. Average diameters with standard error bars of (a) ulvan/PCL and (b) ulvan/PEO nanofibers in relation to the polymer ratios.

of 39.7% carbohydrates, 49.8% sulfate, and 0.3% protein on a dry weight basis.

Ulvan was blended with either PCL or PEO and their mixtures were electrospun into nanofibers of different sizes and morphologies. DCM/DMF (80 : 20, v/v), which was used in the past for the electrospinning of PCL/fucoidan,[11] was selected as the most suitable solvent for the fabrication of ulvan/PCL fibers. Three mixtures of different compositions of ulvan/PCL (1 : 8, 3 : 8, and 3 : 2) were thus prepared. The low solubility of ulvan did not allow complete solubilization of the polysaccharide particles, which however were evenly dispersed in the solution for ulvan concentrations up to 12% w/v. Higher concentrations of ulvan led to aggregation and precipitation phenomena. Fine nanofibers were successfully generated from ulvan/PCL blends with 1 : 8 and 3 : 8 ratios [Figure 1(a,b)]. The hybrid fibers displayed a smooth and uniform surface indicating that PCL and ulvan were homogeneously mixed. However, in the blend with 3 : 2 ratio, both fibers and beads coexisted [Figure 1(c)]. When the concentration of PCL is low, the critical chain entanglement state is not achieved. In this case, interpenetration and sufficient interaction of the macromolecular chains with ulvan is not attained, resulting in beads formation.[12,13] The size of the beads is found to be approximately 1 μm, as also previously observed with other ulvan systems.[10] The average diameters of the nanofibers resulting from ulvan/PCL blends with 1 : 8, 3 : 8, and 3 : 2 ratios were found to be 994 ± 5, 499 ± 5, and 300 ± 5 nm, respectively, whereas the average diameter of PCL fibers is approximately 1 μm [Figure 1(d)]. The results show that the diameter of ulvan/PCL nanofibers decreases as the ulvan content increases. It is noteworthy that the ulvan/PCL fiber mats produced with the 1 : 8 and 3 : 8 blends exhibited highly interconnected spider-web-like structures which are rarely observed in electrospun matrices.[14]

Aqueous solutions of ulvan and PEO were separately prepared and mixed in 1 : 1 and 1 : 2 ratios to give clear solutions that were subsequently electrospun. Spindle-like nanofibers were obtained when the ratio of ulvan/PEO was 1 : 1 [Figure 2(a)], whereas beaded fibers with smaller diameters were produced when the ratio was 2 : 1 [Figure 2(b)]. The electrospinnability of the solutions was reduced when the ulvan content increased. The average diameters of the ulvan/PEO fibers resulting from blends with 1 : 1 and 2 : 1 ratios were 935 ± 5 and 505 ± 5 nm, respectively. Surprisingly, the diameter of the ulvan/PEO composite fibers was increased when compared with the average diameter of PEO nanofibers measuring 151 ± 23 nm [Figure 2(c)].

The FT-IR spectra of ulvan/PCL and ulvan/PEO fibers, as well as of the respective spectra of the three polymers for comparison purposes, are depicted in Figure 3. Pure ulvan exhibited the characteristic absorption of the saccharide structures at 3361 cm^{-1}, assigned to the —OH stretching [Figure 3(A)]. The FT-IR spectra corresponding to the different blends of ulvan/PCL were similar to each other [Figure 3(B)]. Nevertheless, various small differentiations were observed as the relative proportion of ulvan/PCL changed. Specifically, the characteristic absorption band of the —OH groups was 3389, 3350, and 3340 cm^{-1} for ulvan/PCL ratios of 1 : 8, 3 : 8, and 3 : 2, respectively, revealing a shift to higher wavelengths as the ulvan content increased. In the case of ulvan/PEO hybrid fibers [Figure 3(C)], the absorption band of the —OH groups was shifted to lower wavelengths of 3390 and 3398 cm^{-1} for blends with 1 : 1 and 2 : 1 ratios, respectively. The gradual shift of the above-mentioned absorption band indicates a strong interaction and good compatibility of ulvan with PCL and PEO in the fiber mats. The —OH groups in ulvan are suggested to be involved in hydrogen bonding between chains and are capable of forming hydrogen bonds with –COOH groups in PCL. Moreover, the –COOH groups in ulvan may also form hydrogen bonds with the oxygen atoms of PEO.

The chemical integrity and the incorporation ratios of ulvan in the two copolymers can be deduced from the comparison of specific absorption bands in the FT-IR spectra. Specifically, the intensity of the stretching band of —C=O groups of uronic acids in ulvan (approx. 1640 cm^{-1}) is visible and proportionally increasing in the 1 : 8, 3 : 8, and 3 : 2 ratios of ulvan/PCL in contrast to the intensity of the stretching band of —C=O groups of PCL (approx. 1730 cm^{-1}) which is decreasing [Figure 3(B)]. Similarly, comparison of the relative intensities of the C–H stretching bands of PEO (2,820–2,980 cm^{-1}) and of the stretching band of —C=O groups of ulvan supports the efficient blending and chemical integrity of the ulvan/PEO fibers [Figure 3(C)]. In particular, it is evident that as the ulvan content gets higher, the intensity of the stretching band of —C=O groups of ulvan increases proportionally, whereas the intensity of the C–H stretching bands of PEO decreases.

As it can be observed in Figure 4, the increase of the ulvan content in the polymer blends with either PCL or PEO exerts a significant impact on the average diameter of the electrospun fibers, which is proportionally decreasing. The smallest nanofibers with an average diameter of 300 ± 5 nm were observed for the ulvan/PCL blend with a 3 : 2 ratio.

CONCLUSIONS

Ulvan, a marine sulfated polysaccharide, blended with the biodegradable polymers PCL and PEO, was successfully electrospun into fibrous matrices. The morphology of these biocomposite nanofibers was examined by SEM analyses which showed that the fiber average diameters decrease when the ulvan content is increasing (Figure 4). Moreover, FT-IR analyses revealed the strong interaction and good compatibility between ulvan and the two copolymers. The preparation of a hybrid spider-web-like mat based on a marine polysaccharide is reported for the first time. The collective properties of ulvan/PEO and ulvan/PCL nanofibers can lead to novel applications. Ulvan, as a marine heparin-analogue of algal origin, in combination with the water soluble PEO can impart antithrombogenic properties. In addition, this biocomposite can be used as a drug release and wound healing medium. On the other hand, PCL which demonstrates a long biodegradation period, up to 18 months, can yield through ulvan incorporation a long-term drug release and tissue engineering scaffolding material. Therefore, these fibrous scaffolds incorporating ulvan could represent new promising biomaterials.

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