Electrospinning of EVOH copolymer fiber is accomplished by dissolving the copolymers in a 2-propanol/water solution. The mixture is heated until the EVOH copolymers are fully dissolved in the solution. The copolymers fibers are then electrospun after the mixture is allowed to cool. Additionally, fully hydrolyzed PVA was able to be electrospun through use of a non-ionic surfactant. This electrospun matrix can be stabilized against disintegration and water by soaking of the matrix in methanol.
Figure 1
\[ \text{Figure 2} \]
Figure 4
Figure 11
Figure 14
Figure 18
ELECTROSPINNING OF VINYL ALCOHOL POLYMER AND COPOLYMER FIBERS


[0002] The field of the invention is electrostatic processing of synthetic polymers. Specifically, the invention relates to electrospinning of vinyl alcohol polymer and copolymer fibers.

BACKGROUND OF THE INVENTION

[0003] Polymeric biomaterials processing is an area receiving increasing attention as progress is made toward tailoring the morphology and porosity of constructs for a variety of applications, including tissue engineering, vascular grafts, tissue repair, wound healing, and drug delivery. Toward that end, electrospinning represents an attractive approach to the fabrication of fibrous biomaterials for these applications. Of particular interest is the ability to generate very small fibers (with some of the lower diameters down to about 50 nm or lower), which mimic the size scales of fibers composing the extracellular matrix of native tissues and organs. For example, biodegradable synthetic polymers such as poly(lactide) and poly(glycolide) can be electrospun to serve as scaffolds for tissue engineering. Collagen can be directly electrospun to afford 100 nm diameter fibers that exhibit banding as is found in native collagen. Non-biodegradable but biocompatible polymers such as poly(ethylene-co-vinyl acetate) (EVA) can also be electrospun and release of tetracycline hydrochloride from EVA mats has recently been reported.

[0004] In addition to the many biomedical applications, electrospun polymers can have diverse other uses. Further applications include use of the fibrous mats as fillers, solar sails and mechanical joints. The electrospun polymers may be customized by process and material variations to meet diverse requirements in broad categories of applications.

[0005] In electrospinning, polymer solutions or melts are deposited as fibrous mats with advantage taken of chain entanglements in melts or at sufficiently high polymer concentrations in solution to produce continuous fibers. A schematic of one electrospinning system is shown in FIG. 1. The fibers are derived by charging a liquid typically to 5-30 kV vs. a ground a short distance away, which leads to charge injection into the liquid from the electrode. The sign of the injected charge depends upon the polarity of the electrode; a negative electrode produces a negatively charged liquid. The charged liquid is attracted to the ground electrode of opposite polarity, forming a so-called Taylor cone at the nozzle tip and, eventually, a fiber jet as the electric field strength exceeds the surface tension of the solution. The basic elements of one laboratory electrospinning system are simply a high voltage supply, collector (ground) electrode, source electrode, and a solution or melt to be spun. The polymer solution is confined in any material formed into a nozzle with various tip bore diameters, with a very thin source electrode immersed in it or a metallic, blunt-end syringe needle attached to the high-voltage supply. The collector can be, for example, a flat plate or wire mesh, or a rotating metal drum or plate on which the electrospun fibers are continuously wound.

SUMMARY OF THE INVENTION

[0006] It is an object of the present invention to successfully and effectively electrospin vinyl alcohol polymer and copolymer fibers. Specifically, poly(ethylene-co-vinyl alcohol) and poly(vinyl alcohol) fibers are prepared in specific solutions and then electrostatically processed to form fibers that have biomedical and other diverse applications.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] FIG. 1 is a schematic drawing of a conventional electrostatic processing system for use in electrospinning polymer fibers.

[0008] FIG. 2 is a drawing of the structure of poly(ethylene-co-vinyl alcohol) EVOH (random copolymer).

[0009] FIG. 3 is a collection of micrographs of electroprocessed EVOH at the noted polymer concentration (grams polymer/mils solvent).

[0010] FIG. 4 is a graph demonstrating the average fiber diameter versus concentration of EVOH (62 mol % vinyl alcohol) electrospun from 70/30 v/v 2-propanol/water solution.

[0011] FIG. 5 is a micrograph of smooth muscle cells interacting with an electrospun EVOH mat after 7 days in culture.

[0012] FIG. 6 is a micrograph of fibroblast cells interacting with an electrospun EVOH mat after 7 days in culture.

[0013] FIG. 7 is a photograph of a matrix of EVOH fibers electrospun directly onto a human hand.

[0014] FIG. 8 is a photograph of a matrix of EVOH fibers formed between glass pipettes.

[0015] FIG. 9 is a photograph of glass pipettes attached by electroprocessed EVOH fibers.

[0016] FIG. 10 is a micrograph of electrospun 100% hydrolyzed PVA matrix.

[0017] FIG. 11 is a graph demonstrating the effect of a surfactant on the contact angle of 100% hydrolyzed PVA solution.

[0018] FIG. 12 is a micrograph of electrospun fibers of 100% hydrolyzed PVA displaying “bead-on-string” morphology.

[0019] FIG. 13 is a micrograph of 100% hydrolyzed PVA fibers electrospun from PVA/triton/acetic acid solution.

[0020] FIG. 14 is a micrograph of dried, electrospun fibers of 100% hydrolyzed PVA after (1) immersion in methanol for 24 hours and (2) immersion in de-ionized water for 24 hours.

[0021] FIG. 15 is a collection of micrograph of electroprocessed PVA.

[0022] FIG. 16 are photographs of wet, electrospun PVA mats that were and were not treated with methanol.

[0023] FIG. 17 is a micrograph of methanol treated PVA fibers.

[0024] FIG. 18 displays graphs of the elastic moduli of PVA electrospun mats.
DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0025] One alternative of the invention is directed to the electrospinning of poly(ethylene-co-vinyl alcohol) (hereinafter EVOH). EVOH (FIG. 2) is derived from hydrolysis of polyethylene-co-vinyl acetate, and this structure is idealized as there is likely a small residual amount of acetate groups from incomplete hydrolysis. The material is commercially available in a range of compositions, most commonly encompassing vinyl alcohol contents of about 55-70 mol %. EVOH is a semi-crystalline polymer with a typical melting point ($T_m$) range of about 170-190°C, depending upon the vinyl alcohol content, and a typical glass transition temperature ($T_g$) of 45-50°C, essentially independent of vinyl alcohol content. The polymer absorbs water because of favorable hydrogen bonding interactions with —OH groups, and water acts as a plasticizer which lowers the $T_g$ of EVOH well below room temperature at 100% relative humidity. EVOH is a suitable vinyl alcohol derivative for use in electrostatic processing of matrices for at least several reasons: (1) fibers of the material will be highly hydrophilic due to the vinyl alcohol repeat units, yet water-insoluble due to the ethylene repeat units; (2) the biocompatibility of EVOH is reported to be good; and (3) the secondary hydroxyl functionalities afford opportunities for derivatization either before or after electrospinning. Further, electrospinning of EVOH represents a straightforward and simple means to fabricate porous structures.

[0026] EVOH is known to be soluble principally in DMSO and lower alcohols. It has been discovered in some embodiments that EVOH is soluble above certain temperatures in about 50/50% to 90/10% v/v 2-propanol/water (preferably 70/30%—i.e., typical rubbing alcohol). In one embodiment, the temperature above which EVOH is soluble is between about 30°C and about 120°C. In another embodiment, that temperature is between about 50°C and about 100°C. In another embodiment, that temperature is between about 60°C and about 70°C. In another embodiment, that temperature is about 65°C. In another embodiment, that temperature is between about 75°C and about 85°C. In another embodiment, that temperature is about 80°C. In some embodiments, it has been found that EVOH dissolved at higher temperatures remains in solution for several hours after returning to room temperature before the EVOH begins to precipitate. Further, even after precipitation, it has been observed in some embodiments that the EVOH dissolves again readily and in a short period of time upon reheating, often at temperatures lower than that at which it was originally dissolved. In one embodiment, EVOH was dissolved by heating to about 80°C for 2-3 hours, then it was allowed to precipitate, upon cooling, and finally was dissolved again by reheating to about 50°C for about 10 minutes. After cooling, these room-temperature solutions show remarkable proclivity toward electrospinning.

[0027] The use of rubbing alcohol as a solvent affords the opportunity to electrospin disinfect EVOH directly onto living tissue and this is indeed possible, suggesting an interesting approach for wound coverage and healing. Electrospun mats also appear to be favorable substrates for cell culturing as demonstrated by preliminary seeding experiments with smooth muscle cells and fibroblasts.

[0028] In a further alternative of the invention, poly(vinyl alcohol), PVA, is an important commodity polymer used in textiles, adhesives, and in crosslinked form, as hydrogels for medical applications. PVA is typically derived from poly(vinyl acetate) by hydrolysis or alcoholysis, and the extent of the conversion to vinyl alcohol units is important. The present invention is directed toward 99-100% hydrolyzed PVA because of its good biocompatibility. A primary difficulty overcome in the present invention is the suitability of fully hydrolyzed PVA for use in forming electrospun fiber matrices. The present invention also addresses treatment of resulting PVA fiber matrices with a lower alcohol such as methanol to stabilize the fibers against disintegration in contact with water.

[0029] 1. Experimental

[0030] EVOH

[0031] Sample preparation. Several samples of EVOH were obtained from Soarus LLC (Arlington Heights, Ill.) having compositions of 56-71 mol % vinyl alcohol repeat units, 2-propanol (isopropanol) was obtained from Aldrich and 70/30% v/v alcohol/water solutions were made using distilled, deionized water. Solutions of EVOH and 2-propanol/water with compositions ranging from 2.5-20% w/v % were prepared by warming the appropriate amounts of polymer and solvent to about 80°C for about 2-3 hours until complete dissolution of polymer occurred. Dissolution was also accomplished at 65°C and 70°C, although this generally required more time. Solutions for electrospinning were cooled to room temperature (ca. 22-24°C). Precipitation of polymer occurred, but not for several hours after reaching room temperature. For example, a 50 ml solution of EVOH (62 mol % vinyl alcohol) in 2-propanol/water (10 w/v %) in a 100 ml round-bottom flask did not show signs of precipitation until after about 7 h at room temperature, and thus it was possible to use room-temperature solutions for electrospinning within several hours of preparation. The precipitated mixture could be solubilized again by warming back to about 50°C for about 10 minutes, and subsequent precipitation and re-dissolution could be repeated many times.

[0032] Electrospinning. The electrospinning set-up (FIG. 1) utilized in this study consisted of a syringe and blunt-end needle, a ground electrode (stainless steel sheet on a drum whose rotation speed can be varied) ca. 30 cm from the needle, and a Spellman CZE1000R high voltage supply (0-30 kV CZE1000R; Spellman High Voltage Electronics Corp.) with a low current output (limited to a few μA). A positive voltage (15 kV) was applied to the polymer solution with the distance between the syringe tip and the target surface being ca. 20 cm. EVOH-2-propanol-water solutions were prepared as described above and electrospun within 2 to 3 h after cooling to room temperature. The solution was delivered via a syringe pump to control the mass flow rate, which ranged from 10-18 ml/h. The resulting fibers were collected on a rotating (1000 rpm) metal drum to produce a sheet of non-woven fabric. Interestingly, dielectrics (e.g., a plastic petri dish) interposed between the syringe and grounded target are easily coated, as is a human hand.

[0033] The following Table 1 demonstrates electroprocessing of various EVOH copolymers with various ethylene contents.
<table>
<thead>
<tr>
<th>Table 1. Electrospinning of ethylene-vinyl alcohol copolymers with various ethylene contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>EVOH 68% VA</td>
</tr>
<tr>
<td>EVOH 68% VA</td>
</tr>
<tr>
<td>Soarnol DC5203 (VA 68%)</td>
</tr>
<tr>
<td>Soarnol FT803 (VA 62%)</td>
</tr>
<tr>
<td>Soarnol AF4403 (VA 56%)</td>
</tr>
</tbody>
</table>

Note: Addition of glycerol to the EVOH solution improves the flexibility and mechanical properties of the spun fibers. Other low molecular weight polyethylene glycols could also be used.

[0034] Cell seeding. For the cell seeding and biocompatibility testing, an EVOH solution (62 mol % vinyl alcohol, 10% w/v solution of 70/30 v/v 2-propanol/water) was electrospun over the top of a polystyrene 6-well culture plate by placing the plate in the polymer fiber stream for a few minutes to produce a thin mat (a few fibers thick) on the upper surface of the plate. The mat was then cut into four circles of ca. 12 mm radii, which were then placed individually into sterile culture wells (24 well culture plates). After the mats were set, approximately one ml. (10^6) of smooth muscle cells or fibroblasts were added to the tops of the mats for seeding. After a seeding time of one hour, each culture well was topped off with media and warmed to 37°C. The media was composed of Dulbecco’s Modified Eagle Medium (DMEM) and F12 Nutrient Mixture (F12) (2:1 DMEM:F12 with high glucose plus L-glutamine, sodium pyruvate and pyridoxine hydrochloride) supplemented with 15% fetal bovine serum and 1% Penicillin-Streptomycin (10,000 Units/ml). Once the media was added, the culture wells were covered with a sterile cover and placed in an incubator for 7 days. The media was changed only once, at day 3, during this cell culture period. After 7 days in culture, the individual mats were removed from their respective wells and fixed in 3% glutaraldehyde.

[0035] Microscopy. Scanning electron microscopy (SEM) of the electrospun samples and the cell-seeded mats was performed with a JEOL JSM-820 JE (JEOL Ltd.) electron microscope. Fiber samples required only mounting on an aluminum stub and sputter coating with gold for analysis. The cell-seeded samples were dehydrated with a series of ethanol/water solutions, critical point dried, mounted on stub, and sputter-coated with gold for analysis by SEM.

[0036] Whole Cell immobilization Alternative. For whole cell applications, and EVOH fibrous matrix can be used to entrap cells for various applications including whole cell biocatalysis and bioconversion, diagnostic devices, biosensors and biofiltrations, among others. The electrospinning technology provides a simple yet generic approach to immobilize cells. The novel immobilization approach facilitates much broader applications of whole cell based technology previously impeded by the cost and stabilities of such biocatalysts.
In one experiment, overnight grown E. coli cells were harvested by centrifugation and mixed with EVOH to form a 70% polymer in isopropyl alcohol, 30% aqueous suspension. The resulting suspension was electrospun into a mat using the methodologies. In another experiment designed to avoid exposure of E. coli cells to high voltage, cells were inoculated on one side of a pre-spun mat, which was then sealed with another layer of electrospun EVOH mat. The immobilized cells in each experiment, after washed with large amounts of water, were placed in a buffered solution supplemented with glucose 5 g/L. The cellular metabolism activity was monitored by measuring the pH in the solution. In both cases, a rapid acidification of medium was observed, indicating that cellular metabolism activity was preserved despite the high voltage exposure of the cells in the first case.

Midair electrospinning. Similar to conventional electrospinning, midair electrospinning uses the same experimental set-up (FIG. 1) as the earlier example. However, in order to precipitate fibers before they reach the rotating drum, the distance from the needle tip to the drum is increased, for example, from 20-30 cm to about 30-40 cm. In this embodiment the field strength of 0.5 kV/cm was maintained and was controlled by increasing the applied potential at the needle tip. Increasing the distance from the needle tip to the rotating target allows the polymer jet to experience a longer “flight time.” While not wanted to be bound by the following statement, it is believed that with added time of flight, the 2-propanol-water solvent more completely evaporates from the jet allowing the fibers to fully develop. When this phenomenon is observed, it seems as though sections of fibrous mat appear in “mid-air.”

generally speaking, a plurality of objects may be inserted into the electric field and positioned adjacent each other. As EVOH fibers form on the objects, they are slowly moved apart. The fiber matrix begins, therefore, to build on itself. The speed and angle at which the objects are moved with respect to one another can create a broad range of “mid-air” geometries.

A mat as shown in FIG. 8 is an example of this midair spinning. This bench example was created by inserting two glass pipettes into the electric field. The pipettes are inserted parallel to and adjacent each other. As the fibrous media begins to form on and between the pipettes, the pipettes are moved slowly apart. The fibrous mat builds on itself as the pipettes are moved apart to form the mat shown. The spread of fibers and the width of the mat, among other variables, can be fine-tuned and adjusted as needed.

Mechanical joint created from mid-air electrospinning. The fusing of two 12 in. Pasteur pipettes end to end can be accomplished by electrospinning EVOH fibers in the space that separates the two pipettes. The electrospinning set-up used a syringe and needle, a grounded stainless steel plate (20 cm x 40 cm) 45 cm from the needle, and a Spellman model CZE1000R high voltage supply. A positive voltage (22.5 kV) was applied to the polymer solution. EVOH/2-propanol-water solutions were first heated to about 80 °C and electrospun within 2 to 3 h of cooling to room temperature. The Pasteur pipettes (not grounded) were held approximately 10-20 cm from the grounded electrode plate inside the electric field. After approximately 30-90 seconds a fibrous media formed between the two pipettes joining them together. In one embodiment, twisting and turning the pipettes inside the field affords a more uniform and stronger bond. If the two pipettes are pulled apart while in the electric field, fibers will collect to fill in gaps created from the pulling. FIG. 9 shows an example of this electrospinning.

Aqueous PVA solutions (10 wt %) were prepared by dissolving PVA powder (99.4% hydrolyzed, number average MW=115,000 g/mol) (Aldrich Chemical, Milwauk ee, Mich.) in de-ionized water at 80 °C with constant stirring for at least 12 h. When the solution was cooled to room temperature, Triton X-100 (Sigma-Aldrich Corp. St. Louis, Mo.) in the concentration range between 0.03 to 1.5 v/w % was added. The mixture was stirred further for 10-15 minutes before electrospinning. The configuration of the electrospinning setup used in this study is shown in FIG. 1. It consists of a syringe with a flat-end metal needle, a syringe pump for controlled feeding rates, a grounded cylindrical stainless steel mandrel, and a high voltage DC power supply (Spellman, CZE1000R, Spellman High Voltage Electronics Corp. Hauppauge, N.Y.). In a typical electrospinning experiment, PVA solution was transferred into a syringe and delivered to the tip of the syringe needle by the syringe pump at a constant feed rate (0.7-2.0 mL/h). A 25 kV positive voltage was applied to the PVA solution via the stainless steel syringe needle. The subsequently ejected polymer fiber was collected on the rotating, cylindrical stainless steel mandrel, which was rotated and moved longitudinally simultaneously during the electrospinning process. The distance between the tip of the needle to the surface of the mandrel was about 10 cm. Electrospun PVA mats were stabilized against disintegration in water by treatment with methanol for several hours (8-24 h; 24 h is typical). Then, the methanol-treated PVA mat was dried in a ventilated hood at room temperature for 24 hours.

Without any addition of the surfactant, electrospinning of 99-100% hydrolyzed PVA aqueous solutions has not occurred to any significant extent, with electrospaying of droplets being the typical result. While not wanting to be bound to the following statement it is believed that the surface tension of aqueous PVA solutions might be too high to facilitate electrospinning. It is believed that electrospinning usually occurs when the electric force on the surface of the charged polymer overcomes the surface tension of the polymer solution. A non-ionic surfactant, Triton x-100, which is widely used in recovery of biological membrane components under denaturing conditions, was used to lower the surface tension of the pure PVA solution. Triton x-100 is an ethoxylated alcohol. Other ethoxylated alcohols can be used. Other nonionic surfactants that can be used including, but not limited to, ethoxylated alkyl phenols, ethoxylated fatty acids, and pluronic block copolymers.

Contact angles of PVA solutions were measured on hydrophobically-modified microscope glass slides using a goniometer (NRL.C.A. Goniometer, Ramé-Hart Inc, Mountain Lakes, N.J.) to track changes in surface tension of 100% hydrolyzed PVA/Triton solutions as a function of surfactant concentration. The surfaces of microscope glass slides were modified using octadecytrichlorosilane (OTS) as follows. Glass slides were sequentially cleaned by ultrasonication in acetone (1 min.), isopropyl alcohol (1 min.) and 4:1:1 H2O:NH4OH:H2O2 (10 s) then immersed in a solution
of 0.1 v/v % OTS in anhydrous toluene at 40°C for 30 minutes. The slides were then, rinsed with dried toluene three times and dried at 110°C for 20 minutes. In a contact angle measurement, a droplet of PVA solution was vertically placed on an OTS-modified glass slide from a fixed height, and the contact angle was directly measured from the focusing lens of the goniometer. Since OTS provided hydrophobic surfaces on the substrate, de-ionized water on the hydrophobic OTS surface has a relatively high contact angle (102°), and this angle was expected to decrease upon addition of surfactant.

[0046] The experiments showed that the contact angle of 10 wt % pure PVA aqueous solution was as high as pure de-ionized water, about 100°. By addition of small amount of surfactant, the contact angle of the PVA solution was significantly lowered (FIG. 11). The contact angle of the PVA solution decreased dramatically with an increase of Triton x-100 concentration initially, then leveled off when the surfactant concentration reached about 0.3% yielding a contact angle of about 60°. Further addition of surfactant did not affect the contact angle. Interestingly, the polymer solution with about 0.5% of Triton x-100 was able to be electrospun, as were all solutions with greater amounts of surfactant. The PVA solution electrospun well when its contact angle was about 55-60°, resulting in PVA fibers of about 100-200 nm in diameter as shown by the scanning electron microscopy (SEM) in FIG. 10.

[0047] The morphology of PVA mats was characterized using scanning electron microscopy (SEM) (JEOL JSM-820, JEOL (U.S.A.), Inc., Peabody, Mass.) and polarized light microscope (Leica DM IRBE, Leica Microsystems AG, Wetzlar, Germany). For optical microscopy, PVA fibers were collected on a microscope glass slide during electrosprining process. The slide was soaked in methanol for 1 hour and then dried in air. A few drops of water were placed on top of the fibers (both untreated and treated) and examined simultaneously under light microscope before the water was evaporated.

[0048] Occasionally, the fibers electrospun from PVA/Triton solution have polymer beads as “product” along the polymer fibers, known as “beads-on-string” morphology (FIG. 12). Experiments show that in some embodiments these beads can be reduced or removed by adding acetic acid to the PVA/Triton solution prior to electrosprining to afford smoother fibers. The amount of acetic acid added is about 5-14% by weight relative to the weight of the PVA in solution. While not wanting to be bound to the following statement, it is believed that this results from the increase of the net charge density of the polymer solution by addition of acetic acid. Likewise, other acids could increase the net charge density of the polymer solution. In some embodiments, higher net charge density not only favors formation of polymer fibers without beads but also leads toward formation of thinner fibers. Addition of surfactant also facilitates formation of smooth fibers because the surface tension drives toward the formation of beads. It is believed that reducing surface tension with surfactant favors formation of fibers without beads. Moreover, additional isopropanol also helps in electrospinning since it increases the volatility of the solvent and can further lower the surface tension. FIG. 13 is the SEM picture of electrospun 100% hydrolyzed PVA mat obtained from PVA/Triton/acetic acid solution.

[0049] It is known that PVA without any crosslinking is typically soluble in water. Generally, when an electrospun PVA mat, which is white and opaque, is immersed in water, the mat becomes clear instantaneously as the micro- and nanofibers of the PVA dissolves in water. Thus, the unique nanofibrous structure of the material is lost in aqueous environment, and the mat gradually dissolves in water.

[0050] A novel treatment was developed to preserve the nanofibers using low alcohols such as methanol. To do this, first the electrospun PVA mat was immersed in methanol for several hours (12-48 hours) typically 24 hours was used. Then, it was dried at room temperature in a vented area, such as in a hood, to remove the residue methanol. Methanol is a poor solvent/or non-solvent of PVA. After the methanol treatment, the electrospun mat was still white and opaque as the original mat. When a piece of methanol-treated electrospun PVA mat was immersed in water, the white opaque feature still remained and the PVA mat was found to be insoluble in water at room temperature for at least several weeks and stable at temperatures up to 60-65°C for at least 12 hours. The SEM picture of the wetted methanol treated electrospun 100% hydrolyzed PVA mat showed that the nanofiber of the mat was well preserved by this method (FIG. 14).

[0051] Differential scanning calorimetry (DSC) (Perkin-Elmer Pyris 1C, PerkinElmer Instruments, Shelton, Conn.) was used to characterize the thermal properties of the electrospun PVA mats. A piece of PVA mat (5-10 mg) was placed in an aluminum sample pan and heated from 30 to 250°C at 5°C/min under N2. A melting peak was observed at approximately 230°C for all samples. See Table 2. The degree of crystallinity was calculated by dividing ΔH by the heat required for melting a 100% crystalline PVA sample (ΔHf=138.6 J/g).

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Tm (°C)</td>
</tr>
<tr>
<td>ΔH (J/g)</td>
</tr>
<tr>
<td>Degree of crystallinity (%)</td>
</tr>
</tbody>
</table>

[0052] The mechanical properties of the electrospun PVA mats were characterized using dynamic mechanical analysis (DMA) instrument (Rheometric RSA II, Rheometric Scientific Inc, Piscataway, N.J.). For DMA tests, the specimens were cut into a rectangular bar with a typical geometry of 6 mm wide, 21 mm long (between the grips) and 0.08 mm thick. The elastic modulus (E) and loss modulus (E') were obtained during a frequency sweep (1-100 rad/s) in tension at room temperature. See Table 5. The DMA tests were performed on the dry mats before and after methanol treatment under ambient conditions. A methanol-treated (24 h), water-swollen (over 10 days) mat was also studied, and water was constantly sprayed on the mat during the measurements to prevent dehydration of the sample.
TABLE 3 Elastic Moduli of Electrospun PVA Mats at 1 Hz

<table>
<thead>
<tr>
<th>Sample</th>
<th>Electrospun mat without methanol soaking</th>
<th>Electrospun mat soaked in methanol for 20 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry Mat</td>
<td>93 ± 12 MPa</td>
<td>133 ± 162 MPa</td>
</tr>
<tr>
<td>Wet Mat</td>
<td>—</td>
<td>6.7 ± 0.7 MPa</td>
</tr>
</tbody>
</table>

[0053] II. Results and Discussion

[0054] Electrospraying occurs when the applied electrical voltage exceeds a critical electrical potential at which the electrostatic force overcomes the surface tension of the polymer solution. A droplet of polymer solution at the needle tip is deformed into a conical shape, the Taylor cone, from which a jet of polymer solution is initiated. Taylor established the dependency of the critical potential of forming a cone at the end of a capillary tube according to

\[
\frac{V_c^2}{H} = \frac{4}{3} \left( \ln \frac{2L}{R} - \frac{3}{2} \right) (0.1197 \eta R)
\]

[0055] where \( V_c \) is the critical electrical voltage, \( H \) is the separation between the tip of the capillary and the ground, \( L \) is the length of the capillary, \( R \) is the radius of the capillary, and \( \eta \) is the surface tension of the solution. From the above equation, one can see that the critical electric voltage of formation of Taylor cone is proportional to the surface tension of the solution provided that \( H, L \), and \( R \) are fixed. Thus, solutions with high surface tension require a higher voltage to initiate the Taylor cone and thus the fiber jets.

[0056] EVOH

[0057] The solubility characteristics of EVOH in 2-propanol/water is first discussed. It is not uncommon for polymer-solvent mixtures to be soluble above a certain temperature, referred to as an upper critical solution temperature (UCST), with partial or complete insolubility below it. In electrospraying, fluid emerges from the tip of a small-bore nozzle at a high potential with respect to an opposing counter electrode. The liquid forms a so-called Taylor cone from whose tip emerges a thin filament or jet of liquid. Effectively, a thin "skin" of liquid is pulled off the surface of the cone to form the jet of liquid that is accelerated toward the electrode. Polymer chain entanglements serve to stabilize the jet and a fiber forms as solvent evaporates. (In the absence of a fairly high concentration of polymer, the liquid jet will often undergo breakup into droplets via a Rayleigh instability, with the result being electrospaying rather than spinning.) While not wanting to be bound to the following statement, it is believed that the thermodynamic instability of EVOH/rubbing alcohol solutions provides an additional, early stabilization of the jet to form polymer fiber, perhaps accounting for the great ease of electrospraying.

[0058] A critical concentration of polymer in solution must typically be exceeded in order to observe electrospraying. Below this concentration, chain entanglements are insufficient to stabilize the jet, leading to spraying of droplets. In one embodiment of the invention, 62 mol % vinyl alcohol copolymer in 70/30 v/v 2-propanol/water was studied, and it was found that spinning commenced about 2.5 and 5% w/v of polymer as indicated by the scanning electron micrographs in FIG. 3. Below this concentration, droplets were formed which coalesced into ill-defined shapes on the grounded target. At 5% w/v, a hybrid structure of fibers and beaded fibers is seen. The micrographs also indicate that the average fiber diameters of the electrosprayed samples increased with increasing polymer concentration, which is common. This trend is more clearly evident from a plot of average fiber diameter vs. concentration shown in FIG. 4. Rather thick fibers were seen at 20% w/v, and at 15% w/v fibers appeared to be fused at overlapping junctions, perhaps the result of incomplete solvent evaporation. The most uniform fibers derived with our experimental conditions were afforded at 10% w/v, and hence this concentration was routinely employed for our experiments. Micrographs of other EVOH copolymers (not shown) revealed no significant change in fiber diameter with copolymer composition in the range studied (56-71 mol % vinyl alcohol). The electrosprayed mats became noticeably more flexible upon immersion in water, consistent with the lowering of \( T_g \) due to plasticization by water.

[0059] FIGS. 5 and 6 are scanning electron micrographs of smooth muscle cells and fibroblasts, respectively, allowed to interact with thin EVOH mats for 7 days. The mats were spun over the top of the cavity of a 3 cm diameter plastic culture dish, then removed as free-standing webs. The dish was held with little movement in the electrospraying jet and hence the mats were composed of a random collection of fibers. Due to using a low efficiency seeding procedure in this preliminary study, minimal cells had settled onto the EVOH matrix. Those cells that did seed were interacting in a natural fashion (e.g., web-like, flattened structures versus a dead, rounded cell) with the matrix, suggesting that electrosprayed EVOH is an effective substrate for cell culturing.

[0060] The use of rubbing alcohol as a solvent affords the opportunity to electrospray disinfect EVOH directly onto living tissue, suggesting an interesting approach for wound care. Electrospun fibers are electrically charged and thus mild static dissipation will generally occur upon grounding, but very low currents typically employed in common systems render it safe for deposition directly on tissue. This is demonstrated in FIG. 7, where a hand was coated over the course of 30 minutes with a thick mat of EVOH. It is also noted that tubes can be readily prepared by electrospraying EVOH onto a syringe needle or other cylindrical objects. Such structures may be useful as nerve guidance channels, among other applications.

[0061] An attractive feature of EVOH is the presence of \( \equiv O \) hydroxyls that can be derivatized either before or after electrospraying. Derivatization may be desirable, for example, to (1) change the moisture absorbing properties of the mat, (2) tailor surface wettability of electrosprun fibers, (3) covalently attach compounds that can be slowly released via hydrolysis of, for example, an ester linkage, (4) covalently attach compounds that confer surface specificity for various biological reactions or cell line growth, and/or (5) attach compounds that are capable of sequestering molecules from blood, urine, intestinal, or wound fluids.
Derivatized materials that can be electrospun have several potential applications. Two specific examples are noted herein. First, it might be highly desirable to use the reactive sites to couple peptides (or other agents) to these sites prior to electrospinning a matrix of unique properties. For example, a matrix enriched in specific subdomains of type I collagen (AP 1 site) may be manufactured. By isolating this 15 amino acid site from collagen, or synthesizing the peptide in vitro, and coupling this sequence to the EVOH backbone, a matrix of unique properties can be fabricated. The matrix can be highly porous (allow cells to penetrate) and composed of filaments enriched in the P15 site designed to be a side chain. In use, such a matrix may be very effective as a seeding site for the formation of bone (P15 site promotes the deposition of bone specific markers). In this type of application the matrix has been customized and enriched in specific peptide moieties that have biological activity. In addition to functioning as a backbone for a specific subset of peptide sequences, this type of matrix can also be supplemented with complex mixtures of peptides, allowing a natural matrix with a synthetic backbone to be fabricated.

Reactive side groups within the EVOH can also be used to fabricate diagnostic filtration systems. For example, antibodies can be coupled to the EVOH backbone and then the derivatized EVOH can be spun into a fibrous mat (it can also be prepared as an aerosol droplet preparation). The electrospun matrix provides a solid phase site for the antibodies. This type of matrix can be placed into a device like a syringe filter. Ablood sample with an antigen of interest or other sample with materials to be evaluated can be injected into the matrix and allowed to incubate for an interval of time. A wash solution can subsequently be passed through the matrix to rinse un-reacted antigens or other materials from the matrix. A second antibody coupled with a chromophore, enzyme or other detection agent can then be passed through the matrix to detect the presence of the antigens that are now bound to the antibodies coupled to the EVOH backbone. One advantage of this approach is that a relatively large amount of material may be passed through the matrix to pull materials out and concentrate the antigens or other materials. An advantage of this system is that the matrix with antibodies or other binding agents bound to it would provide a large surface area for reactions to occur. With modifications this type of device can be used to detect airborne materials. For example bacterial samples can be detected with the appropriate detection agents.

This type of EVOH backbone can be used as a backbone to detect binding protein pairs or even gene sequences. For gene sequences, cDNA nucleotides (or mRNA), for example can be coupled to the matrix. The immobilized sequences can be reacted with a mixture of unknowns. Hybridization would occur and complimentary sequences could be pulled out of the unknown. The attractive feature of this type of system is that a large amount of genetic material could be retained and reacted in a very small volume of material. In a similar fashion protein binding partners can be identified and detected from a solution, again the attractive feature is the large amount of material that can be placed within the small volume of an electrospun matrix.

Modifications to this basic design can include coupling protein G or protein R to the matrix. Now antibodies can be mixed with a sample containing an unknown in solution. The antibodies can bind antigen (if present) within the solution. Now the antibody antigen solution can be passed through the matrix. The protein G and R can bind the antibodies and serve as a solid phase chromatography support to pull the bound antigens from solution. The antigens can be released from the matrix by acid shock or detected as described in the preceding discussion. While this discussion concentrates on diagnostic applications for the clinical setting, this basic design could be used to prepare a chromatography column, for example. The efficiency of the electrospinning process can be exploited to capture antigens for purification. The process can be used to prepare custom columns that would be far more stable than the columns in present use.

Some possible derivatization chemistries at the hydroxyl groups of EVOH and PVA include, but are not limited to, esterification (e.g., with acyl halides, acid anhydrides, carboxylic acids, or esters via interchange reactions), ether formation (for example, via the Williamson ether synthesis), urethane formation via reactions with isocyanates, sulfonation with, for example, chlorosulfonic acid, and reaction of β-sulfato-ethylsulfonyl anilide to afford an amine derivative that can be converted to a diazo for reaction with a wide variety of compounds. Such chemistries can be used to attach a wide variety of substances to EVOH and PVA, including but not limited to crown ethers (Kimura et al., J. Polym. Sci., Part A Polym. Chem., 21, 2777, 1983), enzymes (Chase and Yang, Biotechnol. Appl. Biochem., 27, 205, 1998), and nucleotides (Overberger and Chang, J. Polym. Sci., Part A Polym. Chem., 27, 3589, 1989).

The foregoing examples of derivatization mechanisms are non-limiting examples. Other derivatizations, whether by different mechanisms or involving different reactants, are considered within the scope of this invention.

One possible application of EVOH mats is used after modification as a replacement for polystyrene styrene-supported quaternary ammonium salts which are often used as phase transfer catalysts in organic synthesis. The catalytic activity of the modified EVOH fiber can be much higher than polystyrene-based systems due to high surface area of the fibers. In addition, it is considered more environmentally friendly than polystyrene.

PVA

The surface tension of aqueous PVA solutions exhibits a marked dependence on the degree of hydrolysis of the PVA. The rise of surface tension becomes more pronounced as the degree of hydrolysis approaches 100%. For example, the surface tension increases from 51 to 54 dyn/cm when the degree of hydrolysis is increased from 87 to 95%, and then sharply increases to about 69 dyn/cm when the degree of hydrolysis is about 99.5%. The surface tension of pure water is about 73 dyn/cm. The inability to electrospin 100% hydrolyzed PVA water solution was apparently the result of its high surface tension, and that the critical electric field needed to overcome the surface tension to form a Taylor cone may be beyond the limit of a pilot set-up. In order to facilitate the electrospinning of 100% hydrolyzed PVA, a small amount of diluted Triton X-100, a non-ionic surfactant, was used to lower the surface tension of the PVA solution. When 25 kV was applied to the 100% hydrolyzed PVA/Triton solution with an about 10 cm needle tip to target
distance, a fine jet of polymer was immediately ejected from the polymer droplet at the tip of the needle. Continuous PVA fibers were deposited on the rotating cylindrical stainless steel mandrel and collected in the form of a non-woven, fibrous mat. Contact angle measurements on hydrophobically-modified glass surfaces were used to monitor the efficacy of Triton X-100 to lower and so adjust the surface tension of 100% hydrolyzed PVA/water solutions. The contact angle of 10 wt % of 100% hydrolyzed PVA water solution without Triton X-100 was as high as pure deionized water, 101.2±0.4°. By addition of a small amount of surfactant, the contact angle of the 100% hydrolyzed PVA solution was significantly lowered (Fig. 11). The contact angle of the aqueous 10 wt %, 100% hydrolyzed PVA solution decreased dramatically with an increase of Triton X-100 concentration and then leveled off when the surfactant concentration was about 0.3 v/w %, yielding a contact angle of about 60°. Further addition of surfactant has little effect on the contact angle of the polymer solution. The electrospinning feasibility of 100% hydrolyzed PVA/Triton solutions was examined at 2.5 kV/cm. When the surfactant to PVA concentration was below 0.06 v/w %, corresponding to contact angle of about 86°, electrospaying of small droplets was dominant. Electrospaying resulted in the formation of combinations of isolated droplets and small pieces of PVA film, the latter presumably due to coalescence of wet droplets followed by evaporation of water. When the surfactant to PVA was between 0.1 to 0.2 v/w %, corresponding to 77-65°, electrospaying was accompanied with some electrospaying of fibers. Electrospaying of fibers began to dominate when the surfactant concentration was about 0.3 v/w %. The 100% hydrolyzed PVA solution electrospun particularly well when its contact angle was about 54°-60°. The diameters of the resulting PVA fibers ranged from 200 to 700 nm with the majority in the 500-500 nm range as observed by SEM in Fig. 15a.

[0071] Stabilization of PVA Fibers Against Dissolution in Water. When an electrospun PVA mat, which is white due to light scattering from the fibrous structure, is immersed in water, the mat instantaneously shrinks and becomes a clear, gelatinous material. Thus, the unique nanofibrous structure of the electrospun material is lost in an aqueous environment. PVA can be chemically crosslinked with a variety of substances including glutaraldehyde, acrylamide or formaldehyde. Recently, others have prepared PVA nanofiber aggregates using the tetrafunctional crosslinking agent glyoxal prior to electrospinning. The present invention sought to avoid chemical crosslinking in order to mitigate the introduction of reactive species that could compromise biocompatibility. Approaches are known that produce physical hydrogels of PVA through partial crystallization, in particular the alternate freeze-thaw technique applied to PVA solutions. However, an apparently new and simple treatment to physically crosslink electrospun, 100% hydrolyzed PVA fibers using lower alcohols such as methanol was discovered. Soaking of an electrospun PVA mat in methanol for at least 12 h preserves the integrity of the mat when it is immersed in water. Scanning electron micrographs revealed that the fibrous morphology of an electrospun mat without methanol treatment was completely destroyed when the mat was immersed in water (Fig. 15c). A methanol-treated mat (Fig. 15d) appears to retain a significant degree of fibrous character even after 3 weeks of immersion in water (Fig. 15e).

[0072] Fig. 16 shows a visual comparison of a wet, electrospun PVA mat prior to and after methanol treatment. Without methanol treatment, even though the PVA electrospun mat does not dissolve in water, it completely loses its mechanical integrity, forming a soft, gelatinous mass (Fig. 16). In contrast, the water-swollen, methanol-treated PVA mat remains as such (see also Fig. 15f) and is elastic. These observations can be verified with individual fibers using a light microscope. In seen in Fig. 17, methanol-treated (one hour) PVA fibers remain intact in contact with water after several days.

[0073] The methanol treatment served to increase the degree of crystallinity, and hence the number of physical crosslinks in the electrospun PVA fibers. This may occur by removal of residual water within the fibers by the alcohol, allowing PVA-water hydrogen bonding to be replaced by intermolecular polymer hydrogen bonding resulting in additional crystallization. Extraction of residual surfactant may also promote some local crystallization. Toward that end, we determined the degrees of crystallinity for treated and untreated mats from DSC experiments, the results of which are summarized in Table 2. The original, 100% hydrolyzed PVA mat was partially crystalline with degree of crystallinity about 52%. Soaking in methanol affords an additional about 7% crystallinity, or an increase of about 13% relative to the initial amount. There is little dependence of the degree of crystallinity on methanol soaking time beyond about 8 h of immersion.

[0074] The results of dynamic mechanical testing of PVA electrospun mats are shown in Fig. 18. The frequency sweep from 1 to 100 rad/s indicates that, in a cases, the elastic (or storage) modulus, E', increases with frequency over the frequency range. This is a typical characteristic of the viscoelastic response of polymers. Note that data on wet PVA mats without methanol treatment are not available since these materials have essentially no mechanical integrity. Methanol treatment not only preserved fibrous structure of the PVA mat but also significantly increase the mechanical strength of the mat. The elastic modulus of the dry mats increased by a factor of 10 after methanol treatment. When immersed in water, methanol-treated PVA mats swelled significantly, thus softening the material yet allowing the characteristics of a mechanically stable hydrogel. It is also worth noting that the loss modulus, E", of the wet, methanol-treated mat decreased with increasing frequency in contrast to the dry mats. Apparently, less energy per cycle is able to be dissipated by the methanol-treated, water-plasticized mats.

[0075] Conclusions

[0076] Electrospinning of EVOH copolymers from 2-propanol-water solutions is a straightforward and general route for the production of highly fibrous and porous EVOH materials for various biomedical applications.

[0077] The addition of small amounts of Triton surfactant affords the reproducible electrospinning of fully hydrolyzed PVA. Importantly, the electrospun PVA fibers can be stabilized against disintegration in water by a soak in methanol. It is concluded that methanol treatment increases the degree of crystallinity of the PVA fibers, thereby increasing the number of physical crosslinks responsible for fiber stabilization in water.

[0078] While the invention has been described with reference to specific embodiments thereof, it will understood
that numerous variations, modifications and additional embodiments are possible, and accordingly, all such variations, modifications, and embodiments are to be regarded as being within the spirit and scope of the invention.

What is claimed is:

1. A polymer matrix comprising electrospun poly(ethylene-co-vinyl alcohol) fibers.

2. A solution for use in electrostatic processing of a polymer, the solution comprising poly(ethylene-co-vinyl alcohol) and 2-propanol.

3. The solution described in claim 2, wherein the solution comprises about 50/50% to 90/10% v/v 2-propanol/water.

4. The solution described in claim 3, wherein the solution comprises about 70/30% v/v 2-propanol/water.

5. A method of preparing a solution for use in electrostatic processing of a polymer, the method comprising:
   providing an effective amount of poly(ethylene-co-vinyl alcohol);
   providing a solution of 2-propanol and water;
   dissolving the poly(ethylene-co-vinyl alcohol) in the 2-propanol and water by heating the mixture thereof to a temperature above an upper critical solution temperature;
   allowing the mixture to cool to a temperature below the upper critical solution temperature.

6. The method described in claim 5, wherein the poly(ethylene-co-vinyl alcohol) content is in the range of about 55-70 mole %.

7. The method described in claim 5, wherein the effective amount of poly(ethylene-co-vinyl alcohol) to 2-propanol and water is at least about 2.5 w/v %.

8. The method described in claim 7, wherein the effective amount of poly(ethylene-co-vinyl alcohol) to 2-propanol and water is at least about 5 w/v %.

9. The method described in claim 5, wherein the mixture is heated to at least about 50° C.

10. The method described in claim 5, wherein the mixture is heated to at least about 60° C.

11. The method described in claim 5, wherein the mixture is allowed to cool below about 40° C.

12. The method described in claim 5, further comprising the step of reheating the mixture to a temperature lower than the upper critical solution temperature.

13. A method of electrospinning poly(ethylene-co-vinyl alcohol) fibers comprising:
   providing an effective amount of poly(ethylene-co-vinyl alcohol);
   providing a solution of 2-propanol and water;
   dissolving the poly(ethylene-co-vinyl alcohol) in the 2-propanol and water by heating the mixture thereof to a temperature above an upper critical solution temperature;
   allowing the mixture to cool to a temperature below the upper critical solution temperature; and
   electrospinning the cooled mixture.

14. The method described in claim 13, wherein the poly(ethylene-co-vinyl alcohol) content is in the range of about 55-70 mole %.

15. The method described in claim 13, wherein the effective amount of poly(ethylene-co-vinyl alcohol) to 2-propanol and water is at least about 2.5 w/v %.

16. The method described in claim 13, wherein the effective amount of poly(ethylene-co-vinyl alcohol) to 2-propanol and water is at least about 5 w/v %.

17. The method described in claim 13, wherein the mixture is heated to at least about 50° C.

18. The method described in claim 13, wherein the mixture is heated to at least about 60° C.

19. The method described in claim 13, wherein the mixture is allowed to cool below about 40° C.

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