Two-Phase Electrospinning from a Single Electrified Jet: Microencapsulation of Aqueous Reservoirs in Poly(ethylene-*co*-vinyl acetate) Fibers

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Introduction. Electrospinning is recognized as a unique and useful technique to fabricate nonwoven mats of polymeric fibers with diameters ranging from several microns down to less than 100 nm.[1] Fibers can be electrospun from solutions and melts, and processing conditions (surface tension, solution concentration, conductivity) can be adjusted to control fiber diameter.[2−5] Of particular interest in our laboratories[6] and elsewhere[7−9] is the application of electrospinning to biomaterials processing, including the development of tissue engineering scaffolds, drug delivery platforms, and wound care materials.

We report here a new and potentially useful expansion of the scope of electrospinning, namely, the encapsulation of aqueous domains within thin polymer fibers. This work derived from an interest in incorporating water-soluble small molecules and/or macromolecules (e.g., drugs, enzymes, growth factors, DNA), and perhaps living cells, that could be immobilized for long times and/or released in a controlled manner. It was recently reported that small droplets of water in oil can be prepared by electrospraying[10] using coaxial jets, and while such an approach could in principle be extended to electrospinning, we have initially sought to employ a single needle and an aqueous dispersion in a polymer/organic solvent mixture to achieve microencapsulation of water reservoirs in polymer fibers. The bulk of our initial work has focused on fibers of poly(ethylene-*co*-vinyl acetate) (EVA, Elvax-40 from Dupont, 40 mol % vinyl acetate), as this material is known to be biocompatible and useful as a drug delivery matrix.[11,12] EVA has also recently been electrosprayed to form microspheres[13] and electrospun to yield microfibers.[14] We demonstrate that EVA fibers containing aqueous reservoirs are easily electrospun and that osmotic swelling can lead to expansion and eventually the bursting of these reservoirs.

Experimental Section. In a typical experiment, a 13% w/w solution of poly(ethylene-*co*-vinyl acetate) in dichloromethane was mixed in a 40:1 ratio with a 0.55 g/mL solution of bovine serum albumin (BSA, MW ∼ 66 kDa) dissolved in phosphate-buffered saline (PBS). The cloudy suspension containing dispersed water droplets was drawn into a 5 mL syringe, which was then placed in a syringe pump (kdScientific, New Hope, PA) and electrospun from a blunt tip, 18 gauge needle at 10 kV and a pump rate of 7 mL/h. Polymer fibers were collected on glass slides[15] secured to both sides of a grounded metal target, which was then placed 15 cm from the needle tip and rotated at ca. 100 min^{-1}. These conditions give relatively thick fibers[16] (ca. 5−20 μm).

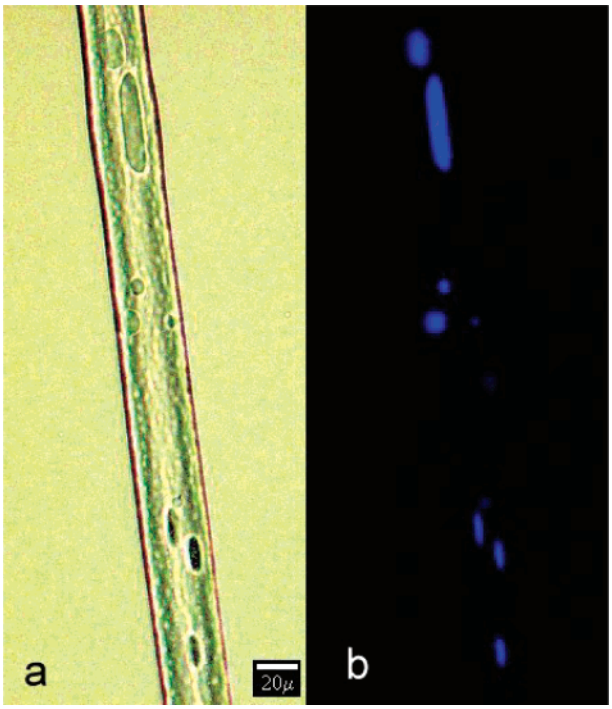

Figure 1. Visualization of fluorescently labeled protein encapsulated in reservoirs using visible (a) and ultraviolet (b) light.

An Olympus optical microscope (BX-51) equipped with digital cameras was used to visualize fibers under normal and ultraviolet illumination. To confirm the presence of BSA in what were believed to be pockets of aqueous solution when the electrospun fibers were viewed by optical microscopy, fluorophore-tagged BSA was prepared with AlexaFluor 350 (Molecular Probes, Eugene, OR), and 1 mL of the tagged protein solution was combined with 0.55 mL of untagged BSA. This solution was then combined with the EVA solution as described earlier, and fibers were collected as noted previously. These fibers were viewed using ultraviolet illumination with a blue DAPI[17] filter. To prepare fibers with hyperosmotic aqueous reservoirs, a 1.28 M aqueous solution of dextrose was mixed with a 13% w/w EVA solution in CH_2Cl_2; the solution was electrospun, and again fibers were collected onto glass slides as described above.

Results. Observation of polymer fibers spun from two-phase suspensions of EVA−CH_2Cl_2/BSA−water under a light microscope typically reveals the presence of what appear to be small[18] and larger domains within the perimeter of the fibers (Figure 1a), and we believe these to be water-filled, containing both BSA and PBS, especially in the case of the larger domains. The fiber was spun using fluorescently labeled BSA in the suspension and when viewed under ultraviolet light (Figure 1b) demonstrates that BSA indeed resides in these larger domains.

We noticed that, upon immersion of these fibers in water, the aqueous reservoirs would swell slightly,

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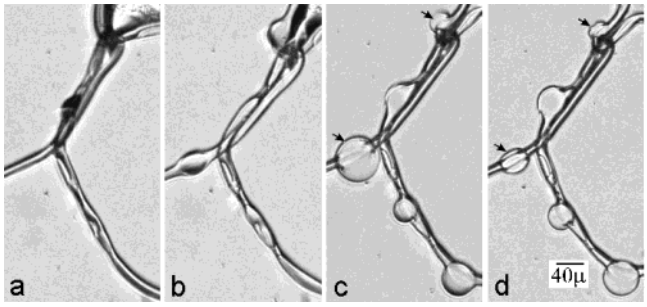

Figure 2. Swelling of aqueous reservoirs due to osmotic pressure: (a) time zero, (b) 6 min, (c) 32 min, (d) 115 min. Arrows indicate reservoirs that deflated during observation.

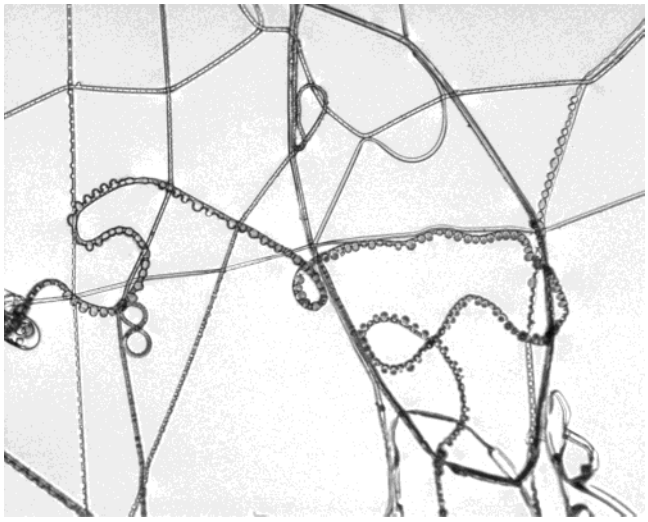

Figure 3. Electrospun EVA fibers showing extensive strings of swollen microencapsulated aqueous domains.

presumably due to water diffusion through the thin fiber walls driven by the presence of BSA and salt in the reservoirs. To amplify this effect, EVA fibers were electrospun from dispersions in which the aqueous phase contained 1.28 M dextrose. When the resulting fibers were exposed to a lower molarity solution, in this case 1× PBS,[19] the aqueous pockets swelled to several times their initial diameters (Figure 2a−d). Swelling of the individual pockets occurred at different rates, suggesting the presence of nonuniform wall thicknesses. Several of the swollen pockets, or blisters, were also observed to decrease in size (Figure 2c,d) back to near their original volumes after they had reached a maximum inflation, suggesting that the blister walls had ruptured[20] with concomitant leakage of the contents of the pockets.[21] Some fibers show extensive strings of blisters while others have few or none (Figure 3). Electron micrographs of EVA fibers containing aqueous glucose pockets which had burst indicate that the walls of the blisters ruptured with significant plastic deformation (Figure 4a). In one case, a hole almost a micron in diameter was left at the base of the bubble after rupture (Figure 4b).

Discussion and Conclusions. In electrospinning, 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

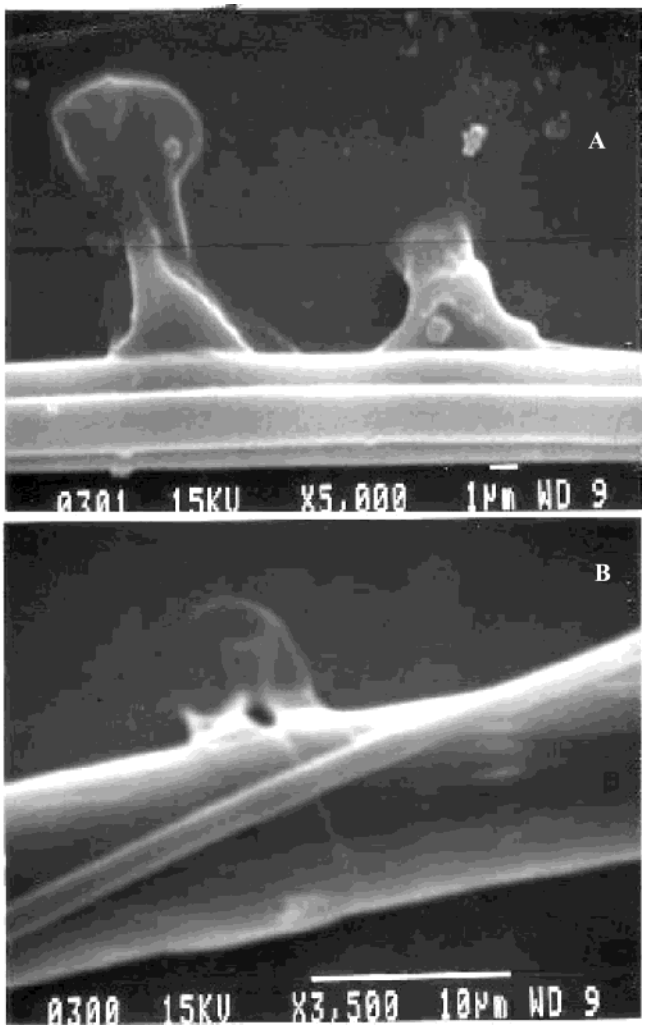

Figure 4. (a) Electron micrograph showing plastic deformation of capsule membrane (left). The reservoir on the right underwent a more violent rupture. (b) Electron micrograph showing ruptured membrane of an encapsulated reservoir, with a resultant hole of nearly 1 μm in diameter.

as solvent evaporates. We suggest that water droplets suspended in EVA−CH_2Cl_2 undergo deformation at the Taylor cone along with the surrounding polymer solution and break up into droplets during polymer fiber formation via a Rayleigh instability. This phenomenon is frequently manifested in unconfined liquid[22] as well as liquid-within-liquid columns[23] and is seen in elongated droplets of polymers in electric fields.[24] In fact, we occasionally observe (optical micrographs not shown) EVA fibers with extended "hourglass" structures characteristic of a liquid column in the process of breakup. The observation that some fiber segments are richly populated with blisters while others are devoid of such features also suggests that as a droplet of aqueous solution in the suspension approaches the instability region of the Taylor cone, the droplet breaks up into many smaller domains that become encapsulated within the polymer fiber.[25] We are currently exploring the efficacy of various additives (e.g., surfactants) to control water droplet size in the fibers and extending this work to other polymer systems (e.g., biodegradable polyesters).

We believe that "two-phase" electrospinning may have a number of potential applications including drug delivery, active filters, tissue engineering, and sensing.

The concentrations of species in the aqueous reservoirs may be tailored to provide various functions, including buffering and osmotic gradients. It may also be possible to transport molecules through the fiber "walls" by Fickian diffusion. Moreover, tailoring of the polymer "shell" around the droplets to be responsive to various stimuli (e.g., electric or magnetic fields, pH) can in principle afford a novel means to release macromolecules trapped in the reservoirs on demand, and we are presently exploring these avenues. Finally, we suggest that under the proper conditions it should be possible to trap living cells[26] within the reservoirs, with the prospect of developing microencapsulated cell bioreactors and artificial organs that are resistant to immune system attack.

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(15) Short electrospinning times (ca. 10−30 s) were employed to isolate only a few fibers on the slide for easy visualization by optical microscopy. However, relatively thick (ca. 300 μm) mats which are free-standing and mechanically durable can be electrospun at longer times.
(16) Relatively thick fibers of EVA spun from chloroform or methylene chloride are common at concentrations of 13−15 wt % (ref 14). However, we find that the aqueous dispersion allows electrospinning from lower concentration polymer solutions which would otherwise electrospray. For example, fibers were successfully produced from EVA in methylene chloride solutions as low as 3.25 wt %, and fiber diameters were smaller (ca. 1 μm) compared with the 13 wt % solutions (ca. 7 μm). A detailed study of the dependence of fiber diameter on polymer concentration and on the concentration of additives (e.g., salts) is in progress.
(17) 4′,6-Diamidino-2-phenylindole solution is used with fluorescence microscopy: excitation, approximately 360 nm; emission, approximately 460 nm.
(18) Some or most of the small domains may be air bubbles as we see these in cast films from the suspensions where aggregated water phases are very large (many tens of microns) in diameter. Air bubbles are likely the result of the vigorous mixing used to prepare the suspensions.
(19) Typical formulation: 137 mM NaCl, 10 mM sodium phosphate buffer pH 7.4, 2.7 mM KCl.
(20) Stress (σ) in a thin-walled spherical vessel is defined as $\sigma = Pr/2t$ where P is pressure, r the inner radius of the vessel, and t the thickness of the wall. Solving for the osmotic pressure inside the blister (P) gives $P = 2\sigma t/r$. The tensile stress of EVA is ca. 900 psi for Elvax-40 (obtained from the manufacturer). Given blister diameters of ca. 20−40 μm (Figure 3) and assuming a blister wall thickness at rupture of about 1 μm, we calculate values for the pressure at rupture to be between about 45 and 90 psi. The osmotic pressure generated (calculated from the van't Hoff equation, $\pi = cRT$) by a 1.3 M solute in water is ca. 460 psi, significantly larger than the pressure needed for rupture of an EVA membrane given our assumptions.
(21) To test this, 0.1 M sodium ferrocyanide was added to the aqueous phase of the dispersion, and the resulting electrospun fibers were immersed in an aqueous solution containing 0.035 M ferric ammonium sulfate. Upon immersion of the fibers, swelling of several pockets was observed and blue regions characteristic of Prussian Blue ($Fe_4[Fe(CN)_6]_3$, resulting from reaction of the ferrocyanide and the ferric salt) were seen outside of the ruptured blisters, demonstrating osmotically triggered release of their contents.
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(25) Microdomain volumes were estimated using digital image analysis (ImageTool, UTHSCSA). Multiple images were taken from a droplet of EVA−CH_2Cl_2/sucrose−water placed between a glass slide and cover slip and of several electrospun fibers from the same solution. Bromphenol Blue was added to provide contrast between the two phases. For the droplet, 96% of the observed volumes were greater than 0.125 pL, while in the fibers 97% were less than 0.125 pL Although a small fraction of the observed domains within fibers could have resulted from entrainment during the electrospinning process, these results appear to indicate that droplet breakup occurs during electrospinning.
(26) Preliminary work (collaboration with Prof. Rachel Chen, Department of Chemical Engineering, VCU) indicates that it is possible to trap small bundles of yeast cells in the aqueous pockets within an EVA fiber.

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