Encapsulation of Drug Reservoirs in Fibers by Emulsion Electrospinning: Morphology Characterization and Preliminary Release Assessment

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In this paper, we prepared composite fibers via electrospinning from either W/O or O/W emulsion. SEM images demonstrate the beads-in-string structures in these fibers and proved this technique to be an effective method for microencapsulation. As a practical application, Ca-alginate microspheres, which serve as reservoirs for hydrophilic drugs, were prepared in a reverse emulsion and then incorporated into poly (L-lactic acid) (PLLA) fibers by electrospinning. With the bovine serum albumin (BSA) loaded into the microspheres, the beads-in-string structure thus entrapped hydrophilic proteins in hydrophobic polymeric matrix. In the in vitro release test, BSA, which was released from composite fibers, achieved prolonged release profiles and lower burst release rates than those from naked Ca-alginate microspheres. In comparison with other well-established techniques to prepare microcapsules, such as solvent evaporation and spray-drying techniques, emulsion electrospinning features partly competing, partly complementary characteristics. Extension to other emulsion systems will be able to fabricate new types of functional structures.

Introduction

Biopolymer microcapsules have been extensively investigated for the entrapment and controlled release of drugs (compounds, proteins, or peptides) due to the benefits of maximizing the safety, effectiveness, and reliability of these agents.[1] According to oral, injection, or transdermal administration, these microcapsules may be molded to different dosage forms with predictable release behaviors. For tissue engineering, growth factors, such as nerve growth factor (NGF), glial cell line derived neurotrophic factor (GDNF), basic fibroblast growth factor (BFGF), and so on, could be applied by releasing from microcapsules,[2] and these microcapsules thus serve as scaffolds with additional advantages to adjust the microenvironment on the implanted site and transmit signals to modulate the behavior of incorporated cells or host tissues.[3]

Poly(lactic acid) (PLA) and its copolymer poly(lactic-*co*-glycolic acid) (PLGA) are the most popular materials for biomedical application, owing to their biocompatibility and biodegradability.[4] However, the successful encapsulation of water-soluble agents in these hydrophobic polymers still presents challenges in high drug loading and their denaturation during formulation process.[5,6] It is reasonable that a core−shell structure should be introduced to reserve drugs inside and control the transport of internal agents across the wall.[7] Therefore, technologies of fabricating this structure in a mild, effective, and productive manner are desired.

Among those methods for encapsulation, the solvent evaporation and spray-drying techniques have been well-established due to their simple procedures and the potential for industrial scale-up.[8] Spray-drying is a straightforward method but confined to compounds which may be unaffected by high temperature. Comparatively, the solvent evaporation technique is featured by the application of a second aqueous phase which provides a mild process for microencapsulation and the convenience of controlling particle size in a wide range. However, this makes the leakage of hydrophilic drug unavoidable and thus becomes an obstacle to achieving high encapsulation efficiency.[9] Furthermore, since they are hardened from droplets, products from both techniques cannot serve as tissue engineering scaffolds directly unless incorporated into another polymer matrix or sintered together.[10,11]

Alternatively, fibers can serve as both drug vehicles and tissue engineering scaffolds.[12] A promising technique to prepare polymer fibers has been represented by electrospinning from a variety of polymers for applications in biomedical area.[13−16] However, it is difficult to encapsulate functional materials, because most electrospinning processes are confined to homogeneous polymer solution in which all components should be dissolved. Recent advances in coaxial electrospinning have achieved the core−shell structure and proved its function as drug delivery devices.[17−20] However, a special apparatus and careful selection of operation parameters are needed to ensure desired results. Inspired by the work on two-phase electrospinning by Sanders et al.,[21,22] we employed emulsions in the electrospinning technique and developed it as an effective approach to encapsulate functional particles.

Figure 1 outlines the major steps of our methods: (1) emulsification of core materials in a solvent; (2) dissolution of fiber-forming polymers in the continuous phase; (3) electrospinning process of the resulted system; (4) harvesting the composite fibers on the receptor.

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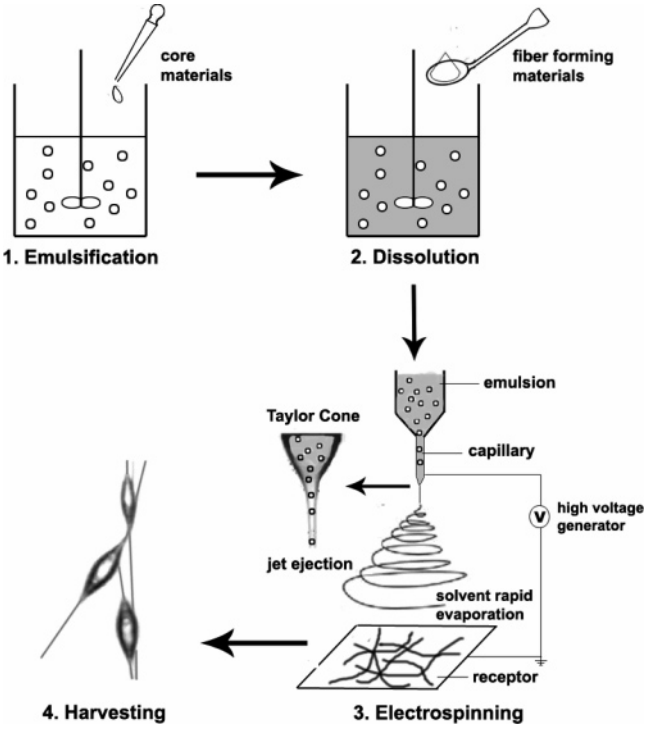

Figure 1. Schematic overview over the four major steps of microencapsulation in fibers by emulsion electrospinning.

Materials and Methods

Materials. Alginate (viscosity of 2% water solution is about 3500 cps at 25 °C) and the surfactant AOT (sodium bis(2-ethylhexyl) sulfosuccinate) were brought from Beijing Chemical Reagent Company. Bovine serum albumin (BSA) (fraction V) was purchased from Sigma. The polymer used for electrospinning, poly(L-lactic acid) (PLLA, M_w = 500 000), was provided by Shandong Institute of Medical Appliances, China. Dichloromethane (AR) was purchased from Atoz Fine Chemicals Co., Ltd. All the reagents were used as received.

Preparation of Ca-Alginate Particles in Reverse Emulsion. AOT (500 mg) was fully dissolved in 25 mL dichloromethane. Then, 3 mL alginate−water solution (1.0 wt %) was added dropwise into the dichloromethane phase. The mixture was vortexed under mechanical stirring by a high-speed mixer at 12 000 rpm for 10 min. After this process, 1 mL calcium chloride solution (5 wt %) was added to this mixture to cross-link alginate by Ca^{2+}. The cross-linking step took another 10 min. To entrap bovine serum albumin (BSA) in Ca-alginate particles, the procedure was the same as the one just described above, except that BSA was mixed with alginate solution previously.

Fiber Encapsulation by Electrospinning. After the above step, PLLA was added to the emulsion and dissolved in the external phase to obtain 3−12 wt % concentration. The electrospinning process was carried out at room temperature in a vertical spinning configuration using a 0.9-mm-i.d. flat-end needle. The applied voltages were in the range 10−20 kV, driven by a high-voltage power supply (Beijing Machinery & Electricity Institute, China). Fibers were collected on an electrically grounded aluminum foil placed at 8−15 cm below the needle tip. The process was carried out until the required thickness of the fibrous mats was reached. Samples were further processed for observations under scanning electron microscopy (SEM, Hitachi S450, Japan).

In Vitro BSA Release from Scaffolds. The release study was carried out in 0.9% NaCl−water solution. Accurately weighted amounts of electrospun mats were placed in Eppendorf tubes containing 10 mL of the release medium. Three parallel samples for each release curve were cut from different parts of the same mats. The tubes were then incubated at 37 °C under the rotation speed of 100 rpm. At selected time intervals, the BSA concentrations were determined by Ultraviolet Spectrophotometer (U-4100, Hitachi, Japan).

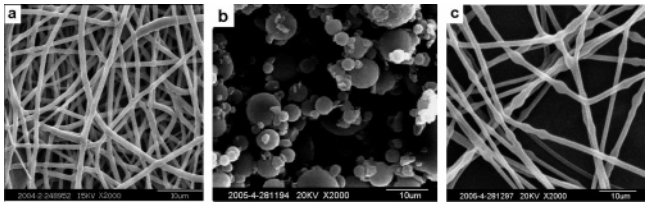

Figure 2. (a) The morphology of electrospun fibers from PLLA solution, (b) Ca-alginate microspheres prepared in W/O emulsion, and (c) electrospun fibers from PLLA emulsion (applied voltage in electrospinning: 15 kV).

Results and Discussion

In advance, smooth fibers, with average size at 2.21 ± 1.15 μm, can be achieved by electrospinning from PLLA solution with the addition of AOT (Figure 2a). And then, in the first step of emulsion electrospinning, Ca-alginate gel beads were fabricated in an water-in-oil emulsion, which was based on some existing formulas, including pH value, alginate and Ca^{2+} concentration, and the rate of the addition of the cross-linking agent.[23,24] Following this method, the microbeads were dispersed in dichloromethane and achieved an average diameter of 7.16 ± 2.36 μm (Figure 2b). Second, PLLA was dissolved in the external phase of the above emulsion and molded by electrospinning. Although this system contained a multiphase composition, the PLLA emulsion can also be electrospun into fibers readily. Figure 2c shows that fibers from the emulsion appear to be a little different from the bead-on-string structure,[25,26] and alginate microbeads are not exposed on the surface of fibers, although they have larger diameters than fibers. Once the applied voltage had risen to 20 kV, the whole morphology became different. The fibers lost their diameter dramatically, and beads transformed to spindles (Figure 3a,b).

Moreover, the dispersion of inner beads and their location in fibers were investigated after removal of the polymer component by chemical vapor etching. A 6-h exposure to saturated vapor of ethanol and dichloromethane (1:1 volume ratio) was proper to dissolve and spread the polymer component from the inner surface of the beads. Thus, Figure 4 shows that most microspheres with different diameters are incorporated into the fibers at random. A few of them were congregated, which was supposed to exist in the emulsion (Figure 4c).

The existing theory of the electrostatically driven bending instability leading to elongation of polymer jets and formation of fibers allows the estimation of the effect of system parameters on fiber morphology.[29] We suppose that, when the emulsion flows through a long capillary and forms rapidly expanding and bending fluid jets after that, the dispersed phase has the tendency to accumulate in the center of the liquid for the elongation effect along the direction of fluid during its flight in the air. This helps microbeads settle into fibers rather than on surfaces.

For the application of protein delivery, the stability of the composite fibers in solution was also examined. First, the Ca-alginate particles cannot be redissolved in 0.9% NaCl−water solution for at least a week. Besides that, we conducted two comparative experiments. In one experiment, Ca-alginate/PLLA fibers were immersed in the release medium for 30 days. Then, SEM observation found that fibers kept their original shapes despite some conglutination with each other (Figure 3c). In another experiment, composite fibers were submerged in phosphate-buffered saline (PBS) for 6 days. Because Ca^{2+} ions

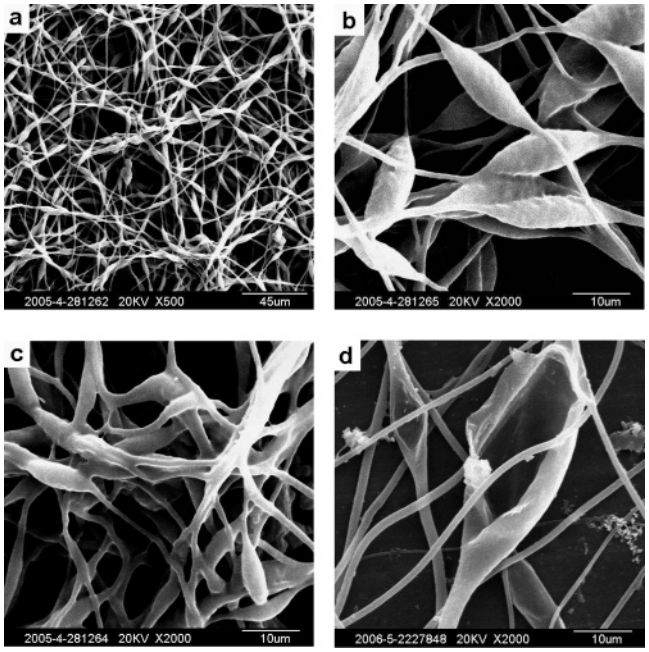

Figure 3. The morphology of fibers obtained via electrospinning of the emulsion of Ca-alginate and PLLA (applied voltage in electrospinning: 20 kV). (a) Fibers before release test, 500× magnification; (b) fibers before release test, 2000× magnification; (c) fibers which were immersed in 0.9% NaCl water solution for 30 days; (d) fibers which were immersed in PBS for 6 days.

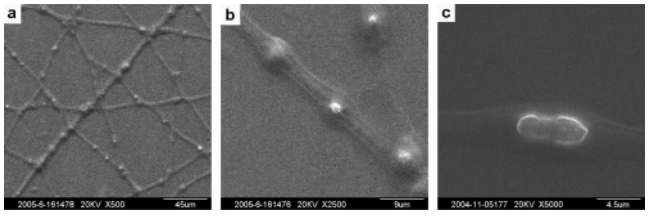

Figure 4. The SEM images of composite fibers after solvent etching.

in Ca-alginate were combined with phosphate and finally replaced by Na^+, the microspheres suffered from redissolution.[27] The picture of this sample (Figure 3d) showed that some holes were left in fibers, which hinted at the sites where the Ca-alginate microspheres were entrapped and implied that the composite fibers in 0.9% NaCl solution can be invulnerable for a longer period of time.

In this study, bovine serum albumin (BSA) was used as a model drug and incorporated into alginate gel beads before its gelation.[28] The relatively mild gelation process enabled BSA to retain its full biological activity.[23] After the electrospinning process, the protein of BSA was immobilized in the hydrophobic PLLA with negligible effects on the final structures and thus demonstrated a sustained release behavior compared to the naked alginate microbeads (Figure 5). On the other hand, the beads-in-string structure was further proven by the release profiles of entrapped drug.

Figure 5 shows the release behaviors of BSA from the composite fibers. Initially, a burst release was observed in two samples, due to the proteins that were either adsorbed on the surface or loosely associated with the surface. This was followed by a period of slow release, which may be attributed to the diffusion of the drug out of the fibers. However, the sample with the spindle-like structure always had a higher cumulative release percentage than the other one at the initial 50 h. This may be because inner beads were closer to the surface of spindles which were elongated by a stronger electrostatic force.

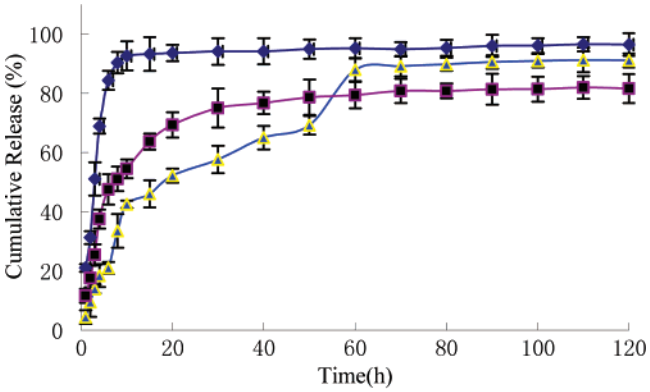

Figure 5. The release profiles of BSA from (◆) naked Ca-alginate microspheres; (▲) fibers via emulsion electrospinning whose morphology is corresponding to Figure 2c; (■) fibers via emulsion electrospinning whose morphology is corresponding to Figure 3a.

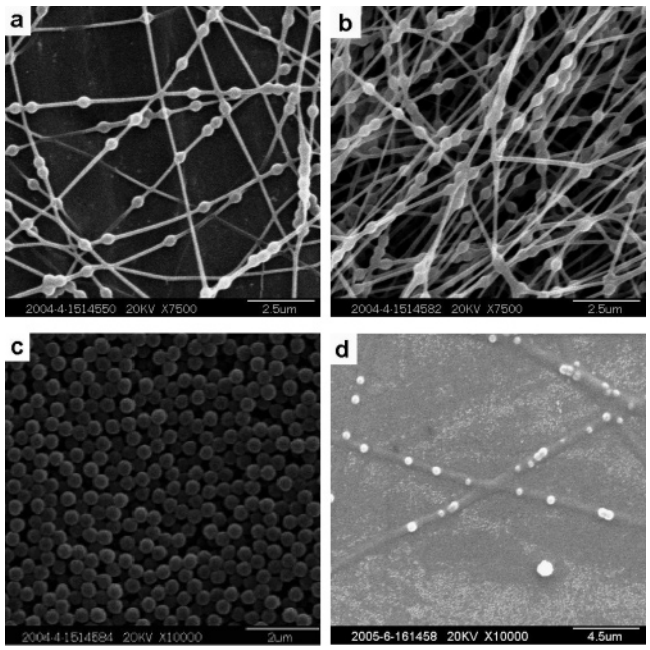

Figure 6. SEM micrographs: (a) electrospun fibers by emulsion (1 g PVA dissolved in 9 mL water and 1 mL original emulsion); (b) electrospun fibers by emulsion (1 g PVA dissolved in 8 mL water and 2 mL original emulsion); (c) PS microbeads in original emulsion; (d) composite fibers after solvent etching.

In fact, alginate microbeads served as the reservoirs for drug loading. It is possible that encapsulating one or several collaborative agents in hydrophobic polymer fibers may be realized simply by dissolving these agents in alginate solution previously.

Further consideration leads us to investigate whether other emulsions could be electrospun for microencapsulation. Here, we chose the products of styrene emulsion polymerization, which is a model of emulsion for practical and theoretical analysis. From Figure 6c, polystyrene (PS) beads were the contents of oil-in-water emulsion. We dissolved poly(vinyl alcohol) (PVA) in the emulsion and applied a 20 kV voltage to this system. After the electrospinning process, the composite fibers were collected on a receptor which was 20 cm below the needle (Figure 6a). By improving the proportion of PS balls to PVA in emulsions, the resulting fibers demonstrated different distances between two adjacent balls. In other words, the average distance of two adjacent balls in the same fiber was reduced by a more dense distribution of PS balls in the emulsion (Figure 6b). Solvent etching, a 4-h exposure of composite fibers to

saturated vapor of ethanol and water (1:3 volume ratio), also proved the existence of beads in fibers (Figure 6d).

Actually, powered by a strong electrostatic field, the whole process of emulsion electrospinning is a combination of jet ejection, rapid solvent evaporation, and subsequent product drying (Figure 1). Different from the spray-drying and solvent evaporation techniques mentioned above, electrospinning works at room temperature and normal air conditions, which may protect susceptible agents from harsh treatments and enhance the encapsulation efficiency by avoiding separating final products from a second medium. Furthermore, the separated steps for emulsion preparation and electrospinning enable us to bring the versatility of both well-established techniques. Extension to other particles and carrier materials will enable fabrication of new types of functional structures.

Conclusion

In this paper, we prepared composite fibers with beads-in-string structures via electrospinning from either W/O or O/W emulsion and thus proved this technique as an effective method for microencapsulation. In both experimental cases, SEM images demonstrated the formation of the beads-in-string structure.

To immobilize hydrophilic protein in hydrophobic polymer, Ca-alginate microspheres were prepared in W/O emulsion and served as drug reservoirs for bovine serum albumin (BSA). After dissolving with poly(L-lactic acid) (PLLA) to its continuous phase, the emulsion was spun into fibers by the electrospinning technique. Our study on morphology confirmed that Ca-alginate microspheres were encapsulated in electrospun fibers. In the in vitro release test, BSA was retained in composite fibers for 120 h, in contrast to that in naked microspheres, which was almost completely released in the first 10 h. Our next work will aim to correlate the drug release behavior with the microstructure of fibrous mats. In addition, the interaction of scaffolds with living cells will also be investigated.

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