

Technical note

AC electropray biomaterials synthesis

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Received 14 February 2005; accepted 23 March 2005

Available online 12 May 2005

Abstract

A rapid, viable and safe fabrication method for biomaterials synthesis is reported using high-frequency AC electro spraying. We demonstrate its potential for polymeric nanoparticle fabrication, drug encapsulation in mono-dispersed micron-sized biodegradable polymer shells and the synthesis of 1 μm biodegradable fibers with adjustable pore sizes as bioscaffolds for tissue/orthopaedic engineering and wound care therapy. The absence of charge in the ejected drops and fibers facilitates pulmonary drug delivery, polymer encapsulation and minimizes protein/DNA denaturing or compound ionization.

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Keywords: Electro spraying; Microencapsulation; Bioscaffold; Nanoparticles; Biodegradable polymer

1. Introduction

Advances in biomaterials research have produced an arsenal of natural and synthetic materials (e.g. lactide and glycolide polymers, polyanhydrides, collagen, etc.) that can be utilized for tissue/bone engineering, targeted and controlled drug delivery and wound therapy [1]. Two key criteria are biodegradability and biocompatibility. The former requires the material to naturally decompose and absorb in vivo, either enzymatically or non-enzymatically, over a desired period of time, therefore eliminating difficult and complicated surgical procedures involved in its retrieval or removal. The latter requires that the material be non-toxic such that it does not invoke a chronic inflammatory response by the immune system. Whilst the synthesis of such materials has been successful at a laboratory scale through a host of fabrication methods, a viable, rapid, safe and economical technique that can be scaled up to mass

production lines but also scaled down to dimensions commensurate with portable devices for direct in-situ administration of the material to the patient is required.

Recently, a new electro spray mechanism using high-frequency AC electric fields above 10 kHz has been developed [2,3]. We report preliminary investigations to exploit the AC electro spray for biomaterials synthesis and propose its potential for scalability to both portable in-situ delivery devices and mass production lines. Applications are focused on two major areas, namely, micro/nano-encapsulation for drug delivery and fiber synthesis for tissue/orthopaedic engineering and wound healing.

The encapsulation of DNA, peptides, proteins and other therapeutic molecules within a biodegradable spherical shell of polymeric excipient is a vital vehicle for the controlled and targeted ophthalmic, oral, intravenous or implanted delivery of vaccines/drugs. The encapsulation shell provides a shield that isolates these substances from hostile environments thus preventing their susceptibility to decomposition, enzymatic degradation, aggregation and denaturation, and hence prolonging their half-lives in the blood stream as well as their shelf life; hydrophobic compounds therefore can be encapsulated in hydrophilic capsules to allow injection

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into the blood stream and muscle tissue. Furthermore, the diffusion of the molecules through the polymeric shell as well as the biodegradation of the shell can be controlled, therefore preventing initial bursts of dosage and thus providing a means for the slow controlled release of the drug over time. In addition, by choosing polymers with different characteristic surface properties or by modifying its surface functionality, drug specificity can be engineered to avoid uptake by the liver, spleen or other parts of the reticuloendothelial system and to locally target the diseased lesion or tumor [4]. This demonstrates the exciting possibility to design specific drug delivery systems for individuals, which could represent the future generation of drugs.

The synthesis of fibrous biomaterials, on the other hand, has also emerged as an important technology given its relevance to bioapplications such as tissue/bone engineering, wound healing therapy and vascular grafting. These biomaterials also function as a ‘skin’ for implanted non-biocompatible devices made of materials that would otherwise trigger an undesirable inflammatory response. In tissue/bone engineering, there have been collective efforts to develop highly porous bioscaffolds that progressively imitate the structure and function of the *in vivo* extracellular matrix of tissues/organs, thus providing mechanical support to the cells and maintaining the organizational definition of the tissue space whilst preserving its biocompatibility and reabsorbability.

In each of these applications, we discuss current fabrication strategies and suggest why the AC electro-spray has definite advantages over these methods and over DC electro-spraying in increasing administration efficiency, patient compliance, comfort and convenience.

2. Materials and methods

A schematic depiction of the experimental apparatus is shown in Fig. 1. A high-frequency (>10 kHz) AC electric field, generated using a high-voltage output transformer (Industrial Test Equipment 113459-1), RF amplifier (Powertron 250 A, 10 Hz–1 MHz) and function/arbitrary waveform generator (Hewlett-Packard 33120A), is applied across a metal hub micro-syringe (Hamilton N733) filled with the working fluid and a ground electrode consisting of a copper strip placed 5 mm away. The syringe is mounted at an inclination angle of 50° to the horizontal to provide an adequate hydrostatic head to deliver the fluid to the syringe tip.

The polymeric excipient consisted of poly-DL-lactic-acid (PLA) with molecular weight 6000–16,000 (Polysciences Inc. 22505), received in the form of small white crystalline pellets in sealed containers to prevent hydrolysis by moisture in the air. The pellets were subsequently stored refrigerated in tightly closed con-

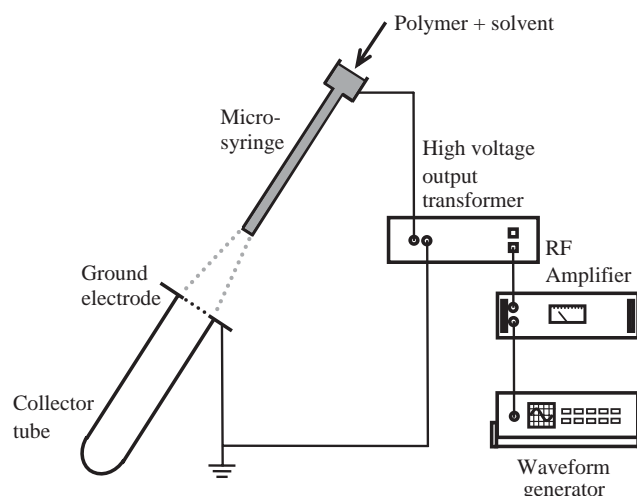


Fig. 1. Schematic of the AC electro-spray apparatus used for microencapsulation and fiber synthesis.

tainers; dessicant was used to absorb the surrounding moisture. The solvent used to dissolve PLA is a 20% 1-butanol (Fisher Scientific ACS grade A399-500) and 80% methylene chloride (Spectrum Chemicals HPLC grade HP732) mixture. The density, viscosity, interfacial tension and relative permittivity of butanol are 810 kg/m³, 3 mPa s, 26.28 mN/m and 17.8, respectively, whereas those for methylene chloride are 911 kg/m³, 0.244 mPa s, 28.12 mN/m and 12.6, respectively.

The concentration of the polymeric excipient in the solvent depends on the nature of the application. For electro-spray microencapsulation, 10 mg of PLA was dissolved in 2 ml of the 20%/80% butanol/methylene chloride mixture. A water-in-oil microemulsion is created by adding 0.5 ml of deionized (DI) water into the polymer/solvent solution followed by vigorous agitation; the microemulsion was stabilized by adding trace amounts of surfactant (99% hydrolyzed poly-vinyl-alcohol, Aldrich Chemical Co.). The mixture was then electro-sprayed directly using high-frequency AC electric fields at 20 kHz and 4.5 kV (peak-to-peak) to produce compound drops below 10 μm in dimension. Polymer solidification occurred in-flight leaving behind a hardened polymer shell containing an aqueous core. To allow for an extended flight length such that complete solidification is ensured before impact, a 5 mm hole was drilled into the ground electrode behind which a tubular collector greater than 3 cm in length was placed, as shown in Fig. 1. The collector was then rinsed with DI water and its contents passed through a porous membrane filter (Millipore Type GS 0.22 μm) to recover the microspheres. To test for encapsulation, an aqueous phase fluorescent dye uranine/sodium fluorescein (Sigma F6377 fluorescent sodium salt) was added to DI water in a subset of experiments. The recovered microspheres were then thoroughly washed with DI water to

remove any traces of the dye external to the microspheres. Any evidence of fluorescence in the microspheres after the rinse then suggests that the fluorescence labeled DI water is encapsulated within the microspheres.

For the synthesis of biodegradable fibers, a water-in-oil microemulsion is not required. The fibers were generated by directly spraying higher polymer concentrations (0.05–0.1 g of PLA) dissolved in the 2 ml 20%/80% butanol/methylene chloride solution described above under the same conditions. Due to the higher concentration of polymeric excipient in the solvent, partial solidification of the meniscus occurred. However, as a result of hydrodynamic stresses, thin long jets of fibers were extruded from the meniscus. In some instances, the polymer drops continued to be ejected from the meniscus tip concurrently with the fiber formation, which occurred a small offset away from the tip. In place of an electrode orifice and collector, however, the fibers are sprayed directly onto the ground electrode to form a fibrous mat consisting of a mesh/network of single-strand fibers.

Electrospray images were obtained using a high-speed video camera (Kodak Ektapro 1000 Imager and High Spec Processor) at record rates between 1000 and 6000 fps. The camera was connected to a telescopic lens and background illumination was provided by a fiberoptic lamp (Dolan-Jenner PL-800). The drops and fibers were inspected using an inverted microscope (Olympus IX71) with 10× to 60× magnification objectives. A scanning electron microscope (Oxford Instruments INCAEnergy) was also used to obtain images of the drops and fibers.

3. Results and discussion

In AC electro spraying, the Maxwell stress arising due to the applied electric field overcomes the capillary stresses on the liquid and acts to stretch and deform the protruding meniscus. The drop subsequently pinches off

due to viscous or inertial effects, the latter occurring at higher frequencies above a viscous-capillary pinch-off frequency that is typically between 20 and 50 kHz. Fig. 2 shows the resulting drop ejection mechanisms: viscous pinch-off leads to tip streaming (Fig. 2a) whereas inertial pinch-off results in a long slender microjet, at the tip of which the drop detaches (Figs. 2b and c). The AC electro spray is thus capable of producing mono- and poly-dispersed electroneutral drops below 10 μm. Scanning electron microscopy (SEM) images as will be shown also reveal that the AC electro spray can produce submicron particles that cannot be observed using conventional microscopy.

A distinct advantage of using high-frequency AC over its traditional DC counterpart is that the micron-sized AC drops produced are typically larger and are electroneutral [2]. For direct local administration to target organs in respiratory drug delivery, an optimum drop size of approximately 2.8 μm is required for optimum dose efficiency for the maximum amount of drug to reach the lower respiratory airways [5]. The DC charged drops undergo Rayleigh fission and are typically too small (~10 nm) to penetrate into the respiratory airways. Moreover, due to the charged nature of the nanometer DC electro spray drops, they are prone to surface adsorption and the encapsulated drug content potentially susceptible to destabilization as a result of electroporation or ionization.

Rayleigh fission, on the other hand, does not occur in the electroneutral AC drops. Furthermore, the current and hence the power requirement is low [2], demonstrating the potential for the miniaturization of the AC electro spray to portable devices. The absence of charge also precludes the need for cumbersome ancillary equipment such as corona discharge tips to neutralize charged drops [6,7] before delivery to the patient is possible. In addition, drops are produced at lower voltages using AC fields. Typically, the critical onset voltage for drop ejection in AC electro sprays is around 1 kV (peak-to-peak) in comparison to several kV in DC electro sprays; extremely large DC voltages up to 30 kV

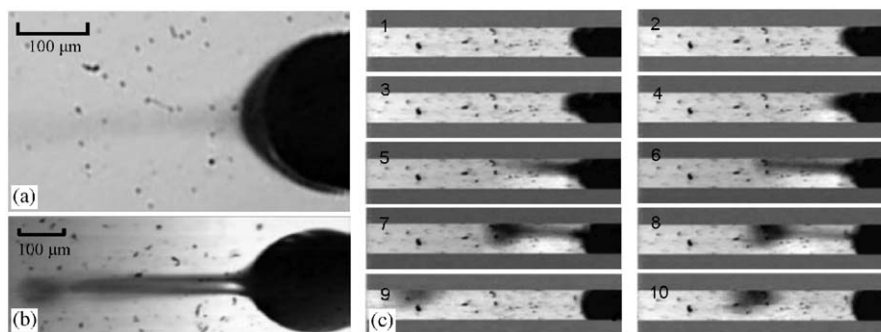


Fig. 2. AC electro spray drop ejection mechanisms. (a) Drop ejection due to viscous pinch-off (tip streaming); (b) drop ejection due to inertial pinch-off resulting in microjet formation; (c) sequences of images at 6000 frames/s showing the development of the microjet and subsequent drop detachment.

are not uncommon [8]. One reason why DC electro-spraying has failed to emerge as a viable option in commercial devices is because of safety issues regarding such high voltages. High-frequency AC with negligible current, despite the high field involved, is inherently safe as the field does not have sufficient time to penetrate the body.

3.1. Electrospray encapsulation

Current encapsulation technologies involve polymer solvent extraction from a double emulsion by evaporation, phase separation (coacervation) or spray drying [9], the latter involving the addition of a third compound to the polymer solution dissolved in the organic solvent in order to reduce the solubility of the polymer. While these methods are relatively effective, the process is slow. Furthermore, the encapsulation can only be carried out within the context of laboratory or mass production settings. Electrospray microencapsulation, on the other hand, is a powerful technique that could be parallelized for rapid mass production but can also be miniaturized to portable devices that encapsulate and deliver the drug concurrently on demand by medical practitioners and patients alike. An encapsulation method based on DC electro-spraying using co-axial liquid jets has been proposed [10]. In this method, DC electric fields are employed to extrude a liquid jet of aqueous phase containing the material to be encapsulated. The stresses associated with this liquid jet concurrently pull along with it an outer annulus sheath of immiscible organic liquid containing a photopolymeric excipient thus producing a co-axial bi-layered jet which subsequently breaks up to form a spray of compound drops. In order to solidify the photopolymer, a beam of UV light is passed through the drops.

A major drawback of the DC electro-spray-based co-axial jet method is evident in the need for a complicated two needle sheath device design in which the inner needle used to electro-spray the aqueous liquid containing the encapsulation material is housed within an annular sheath of organic liquid solvent and polymer contained by an outer needle. This is because the direct electro-spraying of the polymer dissolved in an organic solvent, i.e. dielectric liquids which have low ionic concentrations and hence surface charge density, is not possible since only electrolytes which possess free charge can be sprayed using DC electric fields. Moreover, this poses a further disadvantage in that the encapsulation materials are restricted to substances that are only soluble in aqueous phases, which precludes a large array of organic soluble drug compounds. In addition, the extremely high DC electric fields used pose considerable concern in the denaturing of biological particles when protein/DNA is to be encapsulated.

We have demonstrated the potential of the AC electro-spray device as a viable and attractive alternative to both encapsulation technologies described above. The recovered compound microspheres are shown in Fig. 3a. Evidence of the encapsulation can be observed in Fig. 3b in which the DI water used to create the water-in-oil microemulsion was labeled with the uranine fluorescent dye; any traces of the aqueous phase dye external to the microspheres would have been washed away during the rinse. SEM images of the microspheres are shown in Fig. 3c. A relative monodispersed distribution of drop sizes were obtained as shown in Fig. 3d, with population mean $\sim 3.7\mu\text{m}$ and standard deviation $1.9\mu\text{m}$; the characterization method however was not able to account for microsphere sizes below $1\mu\text{m}$ (the SEM images in Fig. 3c, and those to be shown subsequently, indicate however a rich presence of submicron particles). The monodispersity can be controlled by varying system parameters such as the applied voltage and frequency. Aggregation of the encapsulated drops was observed resulting from capillary action as the contact line of water receded during evaporation, providing a convenient method of collecting the drops.

Organic phase soluble therapeutic molecules can also be encapsulated with the AC electro-spray by dissolving the drug with the polymeric excipient in the organic solvent and electro-spraying directly. Given that the ejected drops do not possess net charge, post-ejection neutralization procedures are also not required. The AC electro-spray therefore has the advantage over DC electro-spraying of not only being simpler in design but also provides greater flexibility for the encapsulation of organic soluble drug compounds and liposomes [1].

3.2. Fiber synthesis

Conventional indirect fabrication techniques such as crystal deposition/polymer precipitation and the use of molds have been somewhat successful in producing three-dimensional bioscaffold architectures [11]. There has also been a parallel effort for synthesizing bioscaffold prototypes by direct deposition methods such as polymer melt spinning [12], pressure assisted microsyringe extrusion [13], fused deposition modeling [14], selective laser sintering [15], stereolithography [16] and three-dimensional printing [17]. Nevertheless, design issues remain in overcoming the limitations of reduced or compromised porosity, large scaffold sizes, reproducibility, extensive effort consumption, cost and scalability [18].

An attractive direct deposition method is electrospinning [19–23]. Whilst electrospinning utilizes the same principle of electrostatic atomization in DC electro-spraying, the spraying of polymeric liquids in electrospinning results in the persistence of an elongated jet that would have otherwise broken up into drops due to hydrodynamic instabilities had a monomeric liquid been

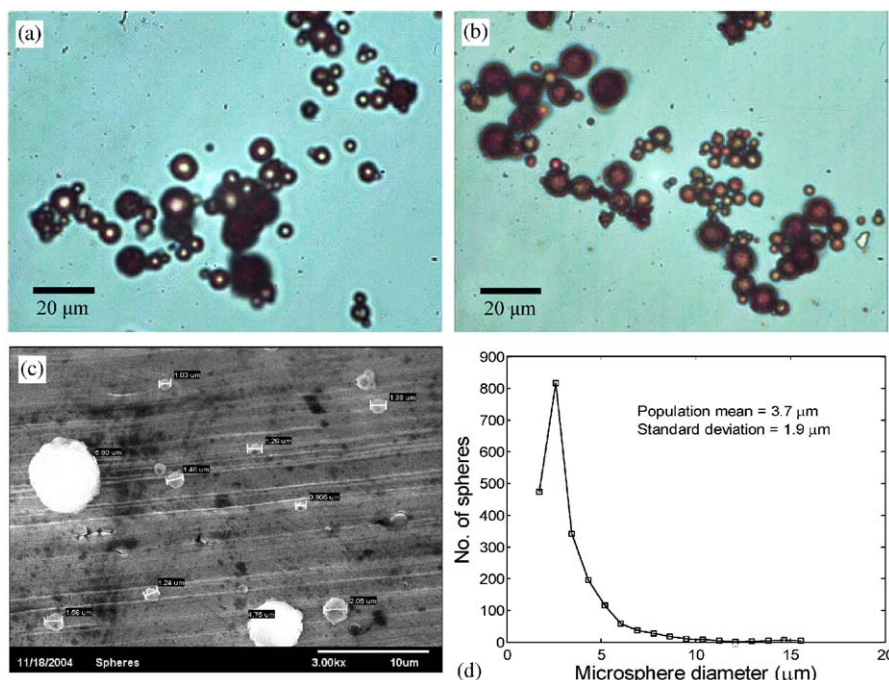


Fig. 3. Microspheres produced by the AC electrospay. (a) DI water encapsulated within microspheres; (b) DI water and uranine dye encapsulated within microspheres; (c) SEM micrograph of 1–10 μm biodegradable microspheres; (d) size distribution of the microspheres. Although the visual method of size characterization in (d) could only account for microspheres above 1 μm , the SEM image in (c) and those shown subsequently indicate that sub-micron particles are present in abundance.

used [24]. The exposure of the jet to the atmosphere causes the polymer to solidify thus creating a polymeric fiber that can be wound by a rotating ground electrode or deposited as a fibrous mat onto a flat ground electrode plate. DC electrospinning has a significant advantage in its ability to produce 100 nm diameter fibers far smaller than the typical 100 μm structures that can be achieved by the conventional fabrication methods discussed above. These smaller fibers therefore provide a greater surface contact area for the adhesion of cells and for the diffusion of encapsulated dermatological/osteogenic growth factors.

Using AC electrospinning, fibrous mats consisting of a mesh/network of single strand 1 μm diameter fibers as shown in Fig. 4a have been successfully synthesized using polymer concentrations of 0.05 g. At higher polymer concentrations of 0.1 g, a single composite 10 μm fiber, as depicted in Figs. 4b–d, is produced when single micron fiber strands are entangled. There is a high level of controllability of both pore and fiber sizes by varying polymer concentration, field intensity, frequency and spray duration. The AC electrospinning process, however, differs slightly from DC electrospinning in that the solidified fibers are extruded from a partially solidified meniscus due to extensional stresses and dynamic pressure as opposed to in-flight polymer solidification in DC electrospinning.

The ability to produce smaller fibers and controllable structure sizes using AC and DC electrospinning there-

fore constitutes an attractive bioscaffold fabrication method over conventional techniques. Moreover, it is easier to encapsulate skin/osteogenic growth factors in the fibers using electrospinning. In addition, the conventional fabrication techniques are not suitable for direct implementation for wound care therapy unlike electrospinning. Indeed, fibers have been directly electrospun in situ onto living tissue and human hands to demonstrate its potential in wound healing [25]. Nevertheless, static discharge occurs during grounding since the DC fibers are charged [22]. Furthermore, the typically large DC voltages used raises safety concerns for general public usage. AC electrospinning thus has the added advantage of lower voltages, safety and potential for portability. Additionally, only a small subset of biodegradable polymers, specifically those that are aqueous or acid soluble such as poly ethylene oxide and collagen, can be spun using DC electrospinning [23,24], thus limiting its utility. However, the trade-off in using AC fields in place of DC fields is that the fiber sizes are limited to 1 μm ; nevertheless, this is still considerably smaller than the structures obtained using conventional techniques.

3.3. Polymeric nanoparticles

The SEM images in Figs. 4c and d also reveal a monodispersion of 100 nm crystal-like polymer particles, obtained through the tip streaming mechanism

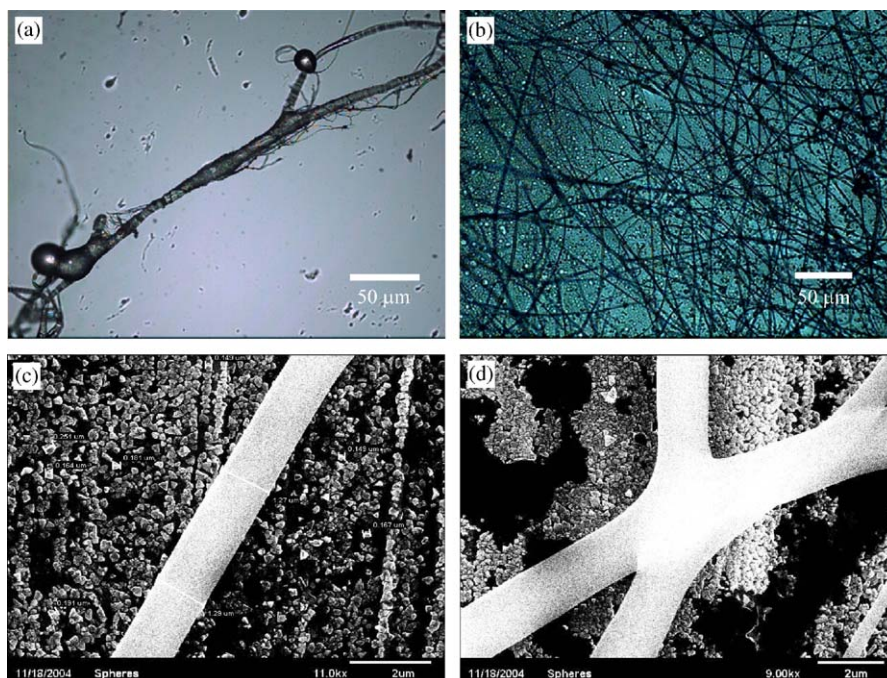


Fig. 4. AC electrospay fiber synthesis. (a) A 10 μm compound fiber. (b) A network of 1 μm single strand fibers produced by the AC electrospay. SEM images of (c) a single-strand fiber, and (d) a network junction of single strand fibers. Both (c) and (d) are magnified from the fiber mesh network in (b). The compound fiber in (a) consists of an entanglement of sub-micron fiber strands. In (c) and (d), the background shows submicron polymer particles similar to those obtained in Fig. 3c, obtained concurrently with the fiber synthesis.

(Fig. 2a) concurrently during AC electrospay fiber synthesis. These nanoparticles, however, are not generated from charged drops as in DC electrospays. They are also not obtained in DC electrospinning. Their generation therefore represents a significant opportunity for the rapid fabrication of biodegradable polymeric nanoparticles using AC electrospaying, which are currently synthesized by relatively slow and complex methods such as emulsion solvent evaporation/extraction [26], nanoprecipitation or emulsion photo-cross-linking [27]. To stabilize the nanoparticles, large amounts of surfactant/co-surfactant are added, limiting both polymer solid content and polymer application [28].

4. Conclusion

We have demonstrated the capability of the AC electrospay as a viable, safe and attractive alternative for micro/nano-encapsulation, bioscaffold production as well as polymeric nanoparticle fabrication over conventional fabrication techniques as well as DC electrospaying/electrospinning. Whilst conventional techniques of biomaterials synthesis involve slow and complex processes (e.g. evaporation, phase separation and extraction) and are subject to several limitations as discussed above, AC electrospaying is relatively quick and has the potential to be scaled up for rapid mass

production. Efforts are currently underway to fabricate parallel arrays of micro-syringe tips such that the synthesis can be carried out cost effectively on a large production scale. Moreover, the AC electrospay technique also presents an opportunity for direct in-situ administration of the material to the patient, which cannot be achieved with conventional fabrication techniques.

In addition, AC electrospaying also has several advantages over DC electrospaying/electrospinning. Unlike its DC counterpart, the AC electrospay is capable of producing larger micron sized polymeric encapsulation shells, which is within the optimal size range for maximum delivery to the lower respiratory airways [5]. It has also a greater flexibility to encapsulate organic phase soluble therapeutic molecules and drug compounds as well as liposomes. Moreover, the polymer microspheres and fibers that are ejected do not possess net charge and hence eliminates the possibility of surface adsorption or destabilization of the encapsulated material due to electroporation or compound ionization. Furthermore, post-neutralization procedures involving ancillary equipment are not required, thus simplifying the spray design. The absence of charge also stipulates negligible current through the spray and hence the power requirement is low. As such, the AC electrospay benefits from the potential to be miniaturized to portable devices for direct patient delivery. Perhaps the most important drawback of DC

electrospraying, however, is the danger involved in using high voltages. The lower threshold voltages involved and the use of AC electric fields nevertheless renders AC electrospraying inherently safe as a portable device for general public use.

Acknowledgements

The authors thank K. Hidlay and S.-C. Wang for their valuable assistance in the experiments. They also gratefully acknowledge the help of T. Hall in acquiring the SEM images in Figs. 3 and 4.

References

- [1] Chasin M, Langer R, editors. Biodegradable polymers as drug delivery systems. New York: Marcel Dekker; 1990.
- [2] Yeo LY, Lastochkin D, Wang S-C, Chang H-C. A new AC electrospray mechanism by Maxwell–Wagner polarization and capillary resonance. *Phys Rev Lett* 2004;92:133902.
- [3] Chang H-C, Yeo L, Lastochkin D, Wang S-C, Gagnon Z, Maheshwari S. Method and apparatus for AC electrospray, United States Patent Application, 2004 (Patent Pending).
- [4] Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. *Adv Drug Del Rev* 2004;56:1649–59.
- [5] Ijsebaert JC, Geerse KB, Marijnissen JCM, Lammers J-WJ, Zanen P. Electro-hydrodynamic atomization of drug solutions for inhalation purposes. *J Appl Physiol* 2001;91:2735–41.
- [6] Grace JM, Marijnissen JCM. A review of liquid atomization by electrical means. *J Aerosol Sci* 1994;25:1005–19.
- [7] Clopeau M, Prunet-Foch B. Electrohydrodynamical spraying functioning modes: a critical review. *J Aerosol Sci* 1994;25:1021–36.
- [8] Kenawy E-R, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders EH, Wnek GE. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinylacetate), poly(lactic-acid), and a blend. *J Controlled Release* 2002;81:57–64.
- [9] Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. *Biomaterials* 2000;21:2475–90.
- [10] Loscertales IG, Barrero A, Guerrero I, Cortijo R, Marquez M, Gañán-Calvo AM. Micro/nano encapsulation via electrified co-axial liquid jets. *Science* 2002;295:1695–8.
- [11] Tsang VL, Bhatia SN. Three-dimensional tissue fabrication. *Adv Drug Del Rev* 2004;56:1635–47.
- [12] Dees JR, Spruiell JE. Structure development during melt spinning of linear polyethylene fibers. *J Appl Polym Sci* 1974;18:1053–78.
- [13] Vozzi G, Previti A, De Rossi D, Ahluwalia A. Fabrication of PLGA scaffolds using soft lithography and microsyringe deposition. *Tissue Eng* 2003;8:2533–40.
- [14] Zein I, Hutmacher DW, Tan KC, Teoh SH. Fused deposition modeling of novel scaffold architectures for tissue engineering applications. *Biomaterials* 2002;23:1169–85.
- [15] Vail NK, Barlow JW, Beaman JJ, Marcus HL, Bourell DL. Development of a poly(methyl methacrylate-co-*n*-butyl methacrylate) copolymer binder system. *J Appl Polym Sci* 1994;52:789–812.
- [16] Cooke MN, Fisher JP, Dean D, Rinnac C, Mikos AG. Use of stereolithography to manufacture critical-sized 3D biodegradable scaffolds for bone ingrowth. *J Biomed Mater Res B* 2002;64:65–9.
- [17] Wu BM, Borland SW, Giordano RA, Cima LG, Sachs EM, Cima MJ. Solid free-form fabrication of drug delivery devices. *J Controlled Release* 1996;40:77–87.
- [18] Karp JM, Rzeszutek K, Shoichet MS, Davies JE. Fabrication of precise cylindrical three-dimensional tissue engineering scaffolds for in vitro and in vivo bone engineering applications. *J Craniofacial Surg* 2003;14:317–23.
- [19] Fong H, Reneker DH. Electrospinning and the formation of nanofibers. In: Salem DR, Sussman MV, editors. Structure formation in polymeric fibers. Munich: Hauser; 2000. p. 225–46.
- [20] Huang Z-M, Zhang Y-Z, Kotaki M, Ramakrishna S. A review on polymer nanofibers by electrospinning and their applications in nanocomposites. *Composites Sci Tech* 2003;63:2223–53.
- [21] Frenot A, Chronakis IS. Polymer nanofibers assembled by electrospinning. *Curr Opin Colloid Interface Sci* 2003;8:64–75.
- [22] Doshi J, Reneker DH. Electrospinning process and applications of electrospun fibers. *J Electrostatics* 1995;35:151–60.
- [23] Matthews JA, Wnek GE, Simpson DG, Bowlin GL. Electrospinning of collagen nanofibers. *Biomacromolecules* 2002;3:232–8.
- [24] Deitzel JM, Kleinmeyer J, Harris D, Beck Tan NC. The effect of processing variables on the morphology of electrospun nanofibers and textiles. *Polymer* 2001;42:261–72.
- [25] Kenawy E-R, Layman JM, Watkins JR, Bowlin GL, Matthews JA, Simpson DG, Wnek GE. Electrospinning of poly(ethylene-co-vinyl alcohol) fibers. *Biomaterials* 2003;24:907–13.
- [26] Mu L, Feng SS. A novel controlled release formulation for the anticancer drug paclitaxel (Taxol®): PLGA nanoparticles containing vitamin E TPGS. *J Controlled Release* 2002;80:33–48.
- [27] Craparo EF, Cavallaro G, Bondi ML, Giammona G. Preparation of polymeric nanoparticles by photo-crosslinking of an acryloylated polyaspartamide in w/o microemulsion. *Macromol Chem Phys* 2004;205:1955–64.
- [28] Zhang G, Niu A, Peng S, Jiang M, Tu Y, Li M, Wu C. Formation of novel polymeric nanoparticles. *Acc Chem Res* 2001;34:249–56.