Cell Electrospinning: a Unique Biotechnique for Encapsulating Living Organisms for Generating Active Biological Microthreads/Scaffolds

Andrea Townsend-Nicholson[†] and Suwan N. Jayasinghe*[,‡]

Departments of Biochemistry & Molecular Biology and Mechanical Engineering, University College London, London, WC1E 6BT United Kingdom

Received July 5, 2006; Revised Manuscript Received September 2, 2006

Jet-based technologies are increasingly being explored as potential high-throughput and high-resolution methods for the manipulation of biological materials. Previously shown to be of use in generating scaffolds from biocompatible materials, we were interested to explore the possibility of using electrospinning technology for the generation of scaffolds comprised of living cells. For this, it was necessary to identify appropriate parameters under which viable threads containing living cells could be produced. Here, we describe a method of electrospinning that can be used to deposit active biological threads and scaffolds. This has been achieved by use of a coaxial needle arrangement where a concentrated living biosuspension flows through the inner needle and a medical-grade poly(dimethylsiloxane) (PDMS) medium with high viscosity (12 500 mPa s) and low electrical conductivity (10^{-15} S m^{-1}) flows through the outer needle. Using this technique, we have identified the operational conditions under which the finest cell-bearing composite microthreads are formed. Collected cells that have been cultured, postelectrospinning, have been viable and show no evidence of having incurred any cellular damage during the bionanofabrication process. This study demonstrates the feasibility of using coaxial electrospinning technology for biological and biomedical applications requiring the deposition of living cells as composite microthreads for forming active biological scaffolds.

1. Introduction

Recent interest in jet-based technologies has escalated exponentially as these techniques increasingly show great promise for handling materials on a scale ranging from the molecular level, in both the chemical and biological sciences, to micro- and nanosized suspensions used in materials science and engineering. There are a number of different jet-based methodologies capable of handling this diversity of materials, namely, ink-jet printing (IJP),[1] electrospraying,[2] and electrospinning.[3] Ink-jet technology was the first to develop significantly and has now rapidly matured. Having undergone a number of developments that enable it to handle a variety of advanced materials that includes living organisms, this route has been identified as a versatile biotechnique for printing three-dimensional biological architectures of living cells.[4−6] Unfortunately, IJP has limitations on the processability of high-viscosity media with standard needles (generally sized at 30−60 μm) as blockage is usually promoted.[7] IJP is known to form droplets double the size of the needles used, hence on deposition these residues are placed in the hundreds of micrometers as spreading of these low-viscosity liquids takes place.[8,9] These obstacles promote the process fabrication of rather coarse structures. Despite this, ink-jet technology, the most developed jet-based technique, is used by other emerging jet-based approaches as a benchmark.

Electrospraying[10−12] is a jetting approach that is driven solely by electric fields created by an applied potential difference between the jetting needle and a ground electrode. This technology has recently been developed to process living cellular organisms[13] that, when used to pattern active biological architectures, have been shown to survive hostile electric fields of up to 2 kV/mm at a maximum drawing current of 4 mA.[14] However, the constraints that must be met for the maintenance of the physiological properties of the biosuspension are such that it has been difficult to achieve electrospraying in a stable jet mode, principally because viability of the cells requires a high concentration of ions to be present in the medium in which they are electrosprayed.

Nevertheless, we have recently discovered that the use of coaxial bioelectrospraying resolves this difficulty and it is now possible to jet living organisms in stable cone-jet mode with a near monodistribution of droplets.[15] This development will permit material deposition for the creation of two- and three-dimensional active biological structures at a level of resolution hitherto unachieved. Bioelectrosprays are presently being considered for development in conjunction with mass spectrometry as a rapid medical diagnostic tool in the healthcare industry and are also being explored by scientists for applications in developmental biology. In our hands, processed living cells have survived several months and we have been characterizing the biological properties of a newly established cell line, EHDJ/1321N1, to identify whether any long-term changes in the functionality of bioelectrosprayed cells take place, as the successful application of electrospray technology to biological systems is dependent upon the maintenance of a defined cellular phenotype postelectrospraying.

Electrospraying's related technology, electrospinning, has been explored for well over a century[16−18] with applications in both the physical sciences, for the fabrication of nanoscaled mats, and in the life sciences, for the fabrication of controlled

* Corresponding author: Department of Mechanical Engineering, University College London, Gower Street, London, United Kingdom WC1E 6BT; telephone +44 (0) 207-679-2960; fax +44 (0) 207-388-0180; e-mail s.jayasinghe@ucl.ac.uk.

[†] Department of Biochemistry & Molecular Biology.

[‡] Department of Mechanical Engineering.

10.1021/bm060649h CCC: $33.50
Published on Web 11/02/2006

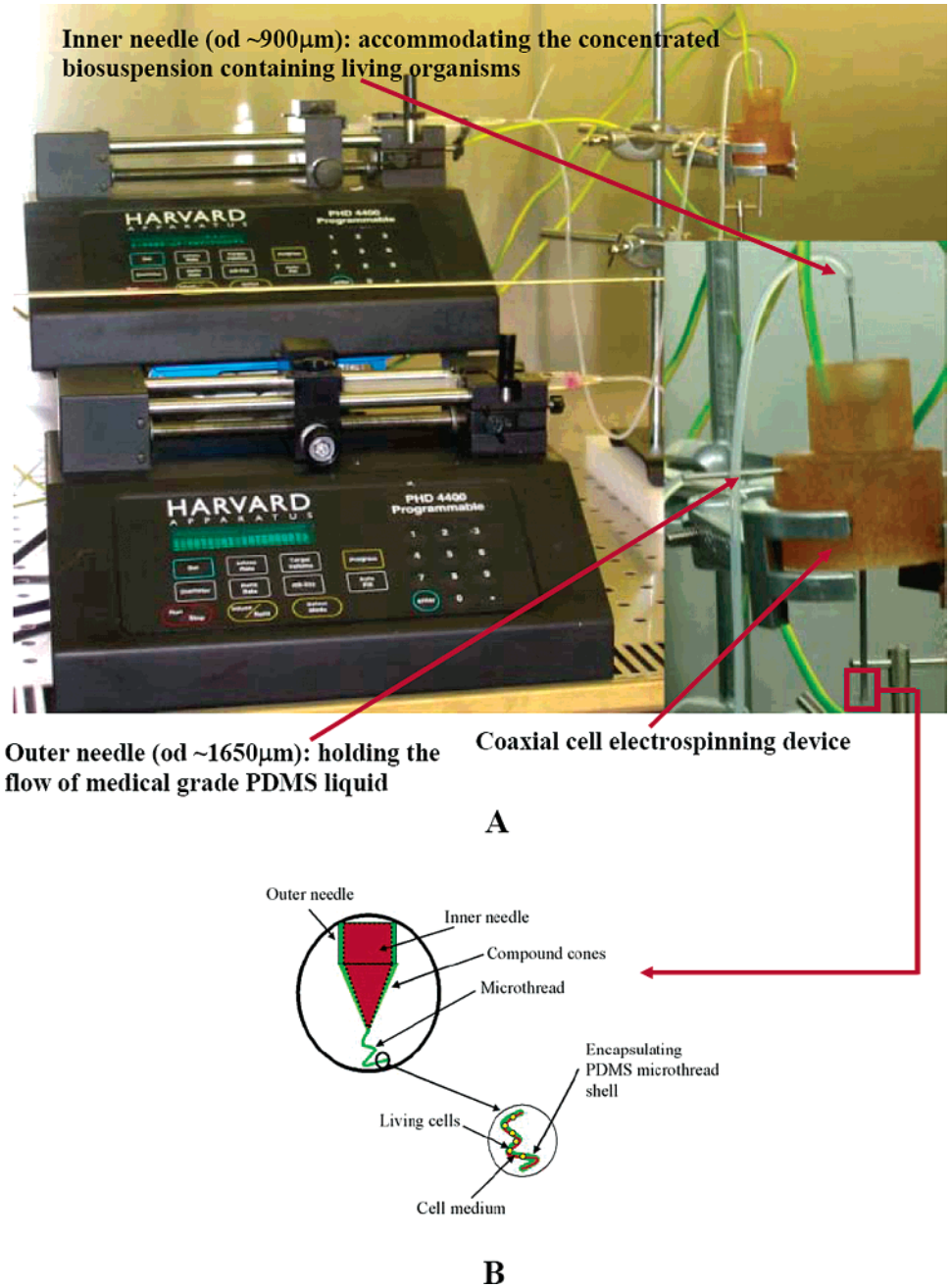

Figure 1. (A) Coaxial cell electrospinning device setup, showing the flow inlets for the biosuspension and PDMS media. (B) A schematic representation of the generated thread.

scaffolds that are frequently used in cell proliferation studies.[19−21] However, previous applications of electrospinning have been used either to form scaffolds from biocompatible materials or for the deposition of nanothreads onto living cells.[22−26] Although, much like electrospray technology, both of these applications are driven by electric fields, the former initiates the generation of droplets while the latter promotes a uniaxial elongating effect on the jet that leads to the formation of micro- to nanosized threads[27] or even multiple threads/jets that have an umbrella-like appearance.[28] A combination of electrospraying and electrospinning has recently been explored to prepare tissue constructs that have been shown to successfully microintegrate.[29]

In this paper, we demonstrate that living organisms can be directly electrospun successfully, as fine composite threads encapsulating the biosuspension containing living cells, using a coaxial needle configuration and a biocompatible polymer. The ability to electrospin biologically active threads and scaffolds of living organisms will be tremendously useful for the development of a whole host of novel bioengineering to medical applications.

2. Experimental Section

2.1. Preparation of Living Biosuspensions. Cell suspensions were prepared and, when appropriate, labeled with Rhodamine 6G for electrospinning experiments as described previously.[15] The biosuspension was electrospun at a final concentration of 10^6 cells/mL. Electrospinning was carried out within 1 h of cell harvesting.

2.2. Coaxial Electrospinning Equipment Setup. The equipment (Figure 1A) used for electrospinning has been described previously[15] and consisted of a coaxial stainless steel needle arrangement with each needle connected, via medical-grade silicone tubing, to a syringe placed in the cradle of a pump able to deliver flow rates in the range of 10^{-5} to 10^{-20} m^3 s^{-1} (PHD 4400, Harvard Apparatus Ltd., Edenbridge, U.K.).

Table 1. Physical Properties of the Jetting Media Investigated in These Cell Electrospinning Studies

sample	electrical conductivity (S m^{-1})	viscosity (mPa s)	surface tension (mN m^{-1})	density (kg m^{-3})	relative permittivity
PDMS	$\sim 10^{-15}$	12 500	21	970	2.67
biosuspension	$\sim 10^{-3}$	23	53	910	32

Both needles were connected to a high-voltage power supply (FP-30, Glassman Europe Ltd., Tadley, U.K.) capable of delivering a positive/negative voltage of up to 30 kV with a maximum delivered current of 4 mA at a voltage resolution of ±0.1 kV. The inner needle accommodated the flow of the concentrated biosuspension, while the outer needle held the medical-grade poly(dimethylsiloxane) liquid (supplied by Polymer Systems Technology Ltd, High Wycombe, U.K.**)**. The ring-shaped copper ground electrode was held approximately 100 mm in line with the central axes of the coaxial needle arrangement. The experimental setup was housed in a class II laminar flow safety cabinet.

2.3. Operational Guides. To map a parametric window in which the finest possible fibers that still encapsulated living cells would be formed, spinning was carried out for a wide range of applied voltage to flow rate regimes. Initially, the flow rates of the inner and outer needles were set to their minimum and the applied voltage was gradually increased until an external electric field was generated. The flow rates were then increased until stable spinning was achieved. Importantly, it was determined that the flow rate of the biosuspension (inner needle) must not exceed the flow rate of the PDMS medium (outer needle) for encapsulation to take place.[30]

2.4. Collection and Microscopy. Threads were collected for (1) analysis of the electrospun cells and (2) analysis of the electrospun fibers. For analysis of the electrospun cells, threads were collected in growth medium-containing Petri dishes. Aliquots of cells were mixed with Trypan Blue solution (Sigma−Aldrich, U.K.) at a final concentration of 0.2%, and live (clear) and dead (blue) cells were counted within 2 min of sample preparation by use of a hemocytometer. Cell viability was estimated by employing the following formula:

$$\%\ \text{cell viability} = [(\text{unstained living organisms})/(\text{total organisms})] \times 100$$

The mean cell viability of control cells and of cells passed through the electric field was compared by use of an unpaired, two-tailed Student's *t*-test, with $p < 0.05$ indicating statistical significance. Threads collected in Petri dishes were cultured over the course of the following week, when cell confluence was achieved, and the cell attachment, cell growth, and morphological properties were recorded photographically at different time points for evaluation. For analysis of the electrospun fibers, composite threads were collected on nylon membranes placed on top of the ring electrode. Visualization of the collected fibers was facilitated by the conjugation of a fluorescent label to the PDMS medium and by labeling of the living cells with Rhodamine 6G. Standard bright-field and fluorescence microscopy were performed with an Axiovert 25 inverted microscope (Carl Zeiss Europe) together with a Micropublisher 5.0 RTV video camera (QImaging, Canada) and OpenLab 4.0.1 software (Improvision, USA).

3. Results and Discussion

3.1. Coaxial Electrospinning and Operational Guide Generation. Electrospinning via a coaxial needle configuration has been previously carried out with a range of liquids and particulate-based suspensions that have been shown to form micro- to nanosized continuous threads. Liquid−liquid combination studies form fine compound and hollow threads, when liquids are jetted in both the inner and outer needles.[31,32] Previous studies have also shown that when jetting is conducted with a combination of liquid and particulate-based suspensions, threads are formed having particles located on a selected surface (inner or outer) or as a matrix.[27,33] We wished to adapt this technology for the electrospinning of a biosuspension. Having identified that the use of a coaxial needle configuration, with medical-grade PDMS in the outer needle, significantly enhanced jet stability,[15] we applied a similar approach but used a medical-grade PDMS with a viscosity that was an order of magnitude higher than we had used previously, as this viscosity was found to form microthreads via single-needle jetting.[34] These media have also been reported to give rise to jets-on-demand.[35]

Table 2. Permutations of Flow Rates Used in the Generation of a Coaxial Cell Electrospinning Operational Guide

Inner Needle Flow Rate ($m^3 s^{-1}$) \ Outer Needle Flow Rate ($m^3 s^{-1}$)	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	10^{-12}
10^{-12}	A6	B6	C6	D6	E6	F6
10^{-11}	A5	B5	C5	D5	E5	F5
10^{-10}	A4	B4	C4	D4	E4	F4
10^{-9}	A3	B3	C3	D3	E3	F3
10^{-8}	A2	B2	C2	D2	E2	F2
10^{-7}	A1	B1	C1	D1	E1	F1

We began our studies by determining the individual characteristics of the PDMS medium and of the biosuspension that would be likely to have an effect on the electrospinning process, such as electrical conductivity, viscosity, surface tension, density, and relative permittivity. These data were obtained as described previously[13] and are shown in Table 1. From this table, it can be seen that the two media differ in their electrical conductivities and viscosities. Under our experimental conditions, the PDMS medium has an electrical conductivity that is twelve orders of magnitude lower than that of the biosuspension and a viscosity that is three orders of magnitude higher. By having the PDMS medium flowing in the outer needle, the high-conductivity biosuspension is encapsulated within a layer of dielectric medium, which facilitates the drawing of both the inner and outer media as a microthread under stable conditions. The PDMS medium acts as the driving medium.[30]

To map the operational space for stable cell electrospinning, combinations of defined flow rates in both inner and outer needles were tested in a range from 10^{-7} to 10^{-12} m^3 s^{-1} for an applied voltage ranging from 5 to 11 kV (Table 2). As a starting point, the flow rate in each needle was set to 10^{-12} m^3 s^{-1} and was subjected to an electric field by imposing an applied voltage between the needle and the ground electrode. Our choice to limit the flow rate came about because we wished to form threads that were as fine as possible while still encapsulating living cells. All of the combinations of inner and outer needle flow rates shown in Table 2 were investigated. Successful encapsulation of the living organisms and media was achieved at a flow rate of 10^{-10} and 10^{-8} m^3 s^{-1} in the inner and outer needles, respectively, for an applied voltage of ∼9 kV (Figure 2A). At an applied voltage of ∼9.5 kV (corresponding to an electric field strength of 0.09 kV mm^{-1}), the finest threads were formed at flow rate combination E6 (Figure 2B), while the largest threads were fabricated at combination A2 (Figure 2C). At a flow rate of 10^{-12} m^3 s^{-1} in each needle (flow combination

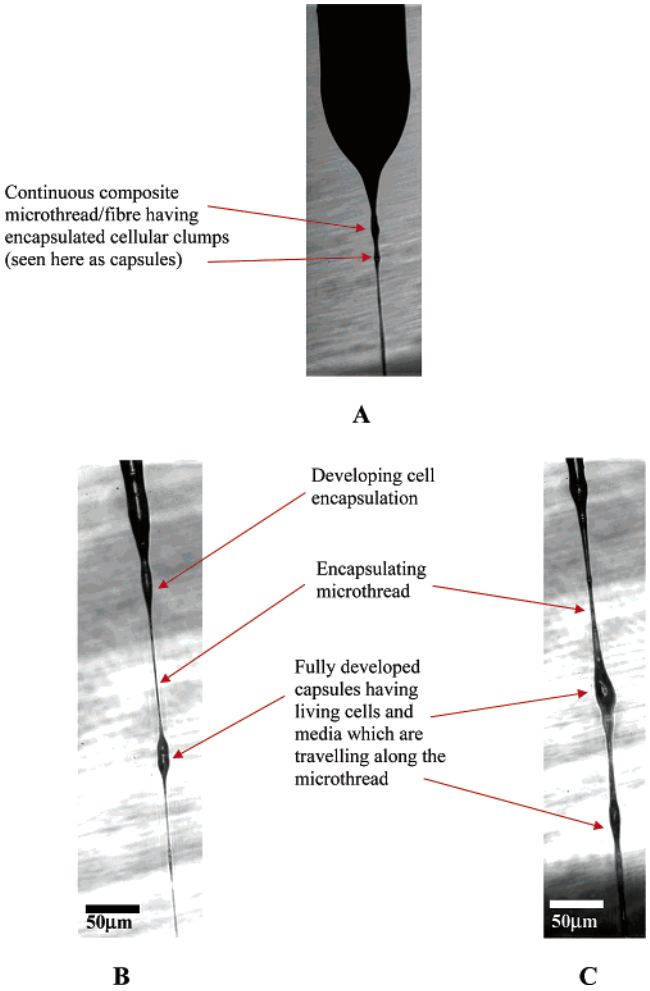

Figure 2. Characteristic high-speed photographs. (A) Stable cell electrospinning, showing capsules generated by cell clumping within the suspension, just below the cone apex. (B) Cell electrospinning of living cells at the flow rate condition "E6". (C) Electrospinning of living organisms using the flow rate condition "A2". The formed fibers near collection show a pellet-like cellular encapsulation seen as a cluster within the biological microthread. Both (B) and (C) were carried out at an applied voltage of ~9.5 kV.

Table 3. Estimated Hydrodynamic and Electrical Relaxation Times for the Jetting Media in These Investigations

medium	hydrodynamic time (s)	electrical relaxation time (s)
PDMS	$(1.6 \times 10^{-3})-16$	23 640
biosuspension	$(9 \times 10^{-3})-90$	2.83×10^{-7}

F6), the biosuspension jetted in the unstable mode and the PDMS medium at the exit of the needle periodically threaded. This was due to the significant difference in their electrical relaxation times ($t_e = \beta\epsilon_0/K$) and in their hydrodynamic times ($t_h = LD^2/Q$), which were determined for flow combinations A2 and E6 (Table 3). It has been shown that, for stable jetting to take place, the hydrodynamic time must be substantially greater than the electrical relaxation time.[36] This allows continuity of the jet, which in our case, leads to the formation of a continuous composite thread. As seen in Table 3, the biosuspension is jetting in unstable mode with $t_h \gg t_e$, while the PDMS medium is undergoing electrically forced jetting/threading[34] with $t_e \gg t_h$. The medical-grade PDMS medium, in contrast to the biosuspension, is able to promote elongation and subsequently microthreads due to its unique properties.

3.2. Microthread/fiber Analysis. The biological microthreads formed during stable electrospinning are shown in situ in Figure 2 and, after collection on nylon membranes, in Figure 3. Both the high-speed photographs and the characteristic fluorescent micrographs of the deposited threads show significant variation in diameters of the thread (Figure 2) and its encapsulations, respectively (Figure 3). Unsurprisingly, the fiber diameter of the deposited threads varied as a function of the flow combination tested at any given applied voltage. At flow combination E6 (Figures 2B and 3A), the finest threads were formed and the smallest encapsulations were seen travelling along the continuous thread. At flow combination A2 (Figures 2C and 3B), the thread was much thicker and was found to be significantly wider. We also observed the biological scaffold formed from these collected threads (Figure 4).

Along the length of collected fibers, randomly placed aggregated cell clumps suspended in media were seen as capsules. We hypothesize that these cell capsules are a function of the biosuspension, which was seen to electrospin cell-bearing threads (Figure 2). Although these encapsulations containing

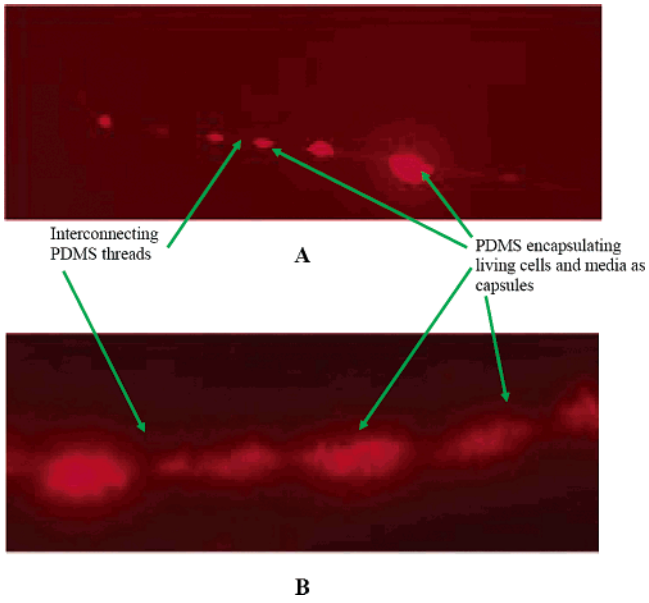

Figure 3. Characteristic fluorescent micrographs showing the variation in fiber diameter that results from cell encapsulations. Experiments were conducted at an applied voltage of ~9.5 kV under flow rate conditions E6 (A) and A2 (B).

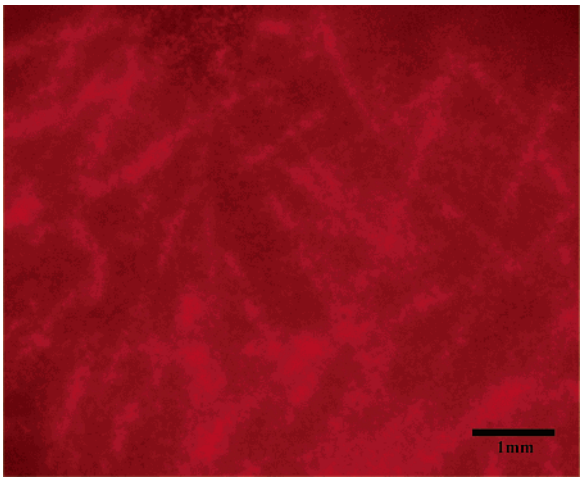

Figure 4. Fluorescent micrograph of a nylon-membrane after collection of a fluorescently labelled electrospun biosuspension. The pattern seen is representative of that obtained in multiple experiments and shows the biologically active scaffold that is comprised of composite threads that contain the living organisms in media.

Figure 5. A characteristic high-speed photograph showing an unstable cell spinning condition, carried out at an applied voltage of ~10.8 kV, where multiple threads, similar to the ramified jets reported in electrospray jetting, are formed from the primary microthread.

living organisms and cell media have not been reported previously, we believe that this may be due to the living cells being comparatively larger in size than the particulate systems that have already been reported. The tendency of cells to clump in the biosuspension[15] and the fact that coaxial electrospinning has never been conducted with the media used in this work may also be contributing factors. In our characterization of the microthreads, we also observed that for any given flow rate combination, the threading process relaxes if the applied voltage is too low. Conversely, if the applied voltage is too high, the composite thread acquires an eccentricity that forms a whipping thread that has secondary threads generated at staggered multiple points along the primary thread (Figure 5). This phenomenon looks very similar to the jet mode that is observed when electrospraying takes place at elevated flow rates and applied voltages, which is known as the "ramified jetting mode".[37]

3.3. Cell Viability. Having identified the parameters necessary for the formation of microthreads under conditions of stable electrospinning, we wished to determine whether these conditions would have any short or long-term effect on the biosuspension. Immediate changes in the biosuspension were evaluated by a cell viability assay. The viability of cells passed through the electric field (67.6% ± 1.9%; $n = 3$) was not statistically significantly different from the viability of control cells (70.6% ± 5.0%, $n = 3$) that were collected at the same flow rate but without an applied voltage ($p = 0.3019$). 1321N1 cells, which have been routinely cultured in the laboratory over a 10-year period, typically give a cell viability of ~65−75% when the cell suspension is prepared in the manner described.[15] To evaluate whether any detectable changes in the morphological properties of the cells took place over a longer period of time, a quantity of electrospun threads containing living cells or of control cells was collected in Petri dishes containing cell growth medium and these cultures were incubated for approximately 1 week. A control of the cell suspension was prepared by plating the cell suspension in growth medium-containing Petri dishes without passage through the apparatus and incubating it for the same period. Upon collection in growth medium, the PDMS encapsulation of electrospun and control cells was lost. The PDMS floated to the top of the growth medium and the cells settled and attached to the bottom of the Petri dish in a manner indistinguishable from that of the cell suspension control. There was no PDMS present in the cell suspension control. Cells were examined for their morphology and for their rate of growth and there was no observable difference in either of these attributes over the period of study, between the control cell cultures (Figure 6A−C) and electrospun cell cultures (Figure 6D−F). Photomicrographs were taken at 55 h (Figure 6A,D), 80 h (Figure 6B,E), and 6 days (Figure 6C,F) following collection. Both control and electrospun cell cultures were identical in appearance with cultured controls of the cell suspension with one exception. A unique aspect that we observed repeatedly during this study, and which we have occasionally seen during electrospraying, is the chainlike structures formed by the collected cells (as seen in Figure 6B,E). This is not a function of the jetting technology (Figure 6B), as we observe the same phenomenon in the control cells (Figure 6E). This phenomenon is not observed in the controls of the cell suspension, leading us to believe that it may be due to the presence of the poly-

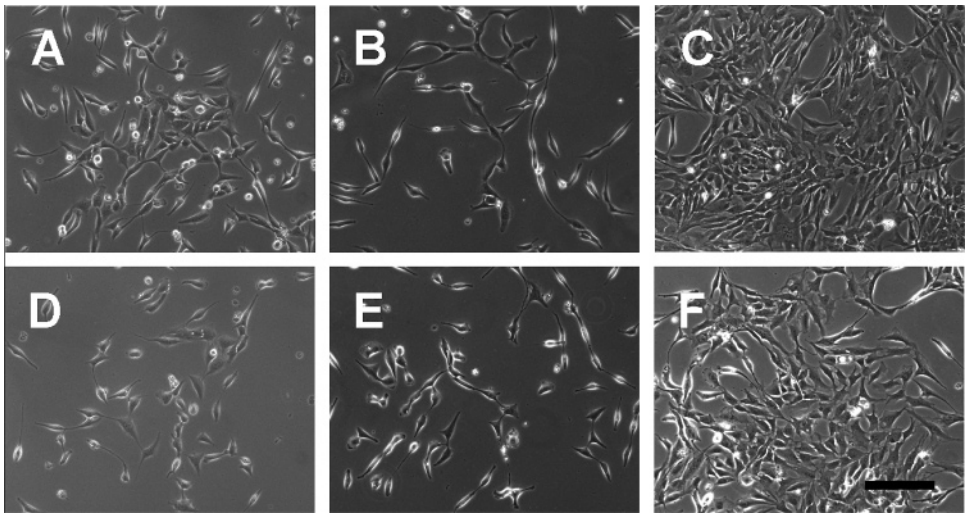

Figure 6. (A-F) Characteristic photomicrographs of collected cells cultured over an incubation period of 9 days. Control cells are shown in panels A, B and C. Electrospun cells are shown in panels D, E and F. Photomicrographs shown here were taken at 55 h (A, D), 80 h (B, E) and at 6 days (C, F) post-cell electrospinning. The scale bar represents 200 μm.

(dimethylsiloxane) used as the driving medium. Further studies of biological activity will be carried out on the electrospun cell lines established from this work (ESPUN/1321N1), and we will include in these an evaluation of the effects of PDMS liquid upon the biological properties of the cell lines used to produce the biosuspensions.

4. Concluding Remarks

Our investigation has shown that it is possible to directly electrospin living organisms under stable threading conditions at a level of resolution that is constrained only by the size of the cells being electrospun. To date, this has not been achieved by any other competing jet-based technology. This has far-reaching implications and will enable significant advances to be made in technologies ranging from tissue engineering to regenerative medicine. We intend to develop this electrospinning technology further, both to achieve the deposition of a controlled number of cells within residue threads and to explore how the technology can be applied to address fundamental questions in cellular and developmental biology. Although we have used poly(dimethylsiloxane) liquid in our experiments, its replacement with different biopolymers is possible, allowing modulation of the structural integrity and half-life of the electrospun threads, which would be spun directly onto a wound to assist in rapid healing.

Acknowledgment. S.N.J. sincerely thanks Ms. Jackie Goertz and Mr. Derek Williams-Wynn of Polymer Systems Technology Ltd, U.K., and Mr. Richard Winn of the Department of Mechanical Engineering for their assistance in the supply of the medical-grade PDMS medium and for support with the construction of the coaxial device used in these investigations, respectively. The Royal Society (U.K.) is gratefully acknowledged for financially supporting these biophysical studies at UCL.

References and Notes

(1) Percin, G.; Khuri-Yakub, B. T. *Rev. Sci. Instrum.* **2003**, *74*, 1120−1127.
(2) Zeleny, J. *Phys. Rev.* **1914**, *3*, 69−91.
(3) Larrondo, L.; Manley, R. *J. Polym. Sci.: Polym. Phys. Ed.* **1981**, *19*, 909−919.
(4) Mironov, V.; Boland, T.; Trusk, T.; Forgacs, G.; Markwald, R. R. *Trends Biotechnol.* **2003**, *21*, 157−161.
(5) Xu, T.; Gregory, C. A.; Molnar, P.; Cui, X.; et al. *Biomaterials* **2006**, *27*, 3580−3588.
(6) Liu Tsang, V.; Bhatia, S. N. *Adv. Drug Delivery Rev.* **2004**, *56*, 1635−1647.
(7) Tay, B. Y.; Evans, J. R. G.; Edirisinghe, M. J. *Int. Mater. Rev.* **2003**, *48*, 341−370.
(8) Zhao, X.; Evans, J. R. G.; Edirisinghe, M. J. *J. Am. Ceram. Soc.* **2002**, *85*, 2113−2115.
(9) Sachlos, E.; Reis, N.; Ainsley, C.; Derby, B.; Czernuszka, J. T. *Biomaterials* **2003**, *24*, 1487−1497.
(10) Taylor, G. I. *Proc. R. Soc. London, A* **1969**, *280*, 453−475.
(11) Hayati, I.; Bailey, A. I.; Tadros, T. F. *Nature* **1986**, *319*, 41−43.
(12) Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. K.; et al. *Science* **1989**, *246*, 64−71.
(13) Jayasinghe, S. N.; Quershi, A. N.; Eagles, P. A. M. *Small* **2006**, *2*, 216−219.
(14) Jayasinghe, S. N.; Eagles, P. A. M.; Quershi, A. N. *Biotechnol. J.* **2006**, *1*, 86−94.
(15) Jayasinghe, S. N.; Townsend-Nicholson, A. *Lab Chip* **2006**, *6*, 1086−1090.
(16) Baumgarten, P. K. *J. Colloid Interface Sci.* **1971**, *36*, 71−79.
(17) Formhals, A. Process and apparatus for preparing artificial threads. U.S. Patent 1,975,504, 1934.
(18) Larrondo, L.; Manley, R. *J. Polym. Sci.: Polym. Phys. Ed.* **1981**, *19*, 933−940.
(19) Su Rho, K.; Jeong, L.; Lee, G.; Seo, B. M.; et al. *Biomaterials* **2006**, *27*, 1452−1461.
(20) Stitzel, J.; Liu, J.; Lee, S. J.; Komura, M.; et al. *Biomaterials* **2006**, *27*, 1088−1094.
(21) Han, D.; Gouma, P. I. *Nanomed.: Nanotechnol., Biol., Med.* **2006**, *2*, 37−41.
(22) Kim, K. H.; Jeong, L.; Park, H. N.; Shin, S. Y.; et al. *J. Biotechnol.* **2005**, *120*, 327−339.
(23) Dalton, P. D.; Klinkhammer, K.; Salber, J.; Klee, D.; et al. *Biomacromolecules* **2006**, *7*, 686−690.
(24) Noh, H. K.; Lee, S. W.; Kim, J.-M.; Oh, J.-E.; Kim, K.-H.; Chung, C.-P.; Choi, S.-C.; Park, W. H.; Min, B.-M. *Biomaterials* **2006**, *27*, 3934−3944.
(25) Li, C.; Vepari, C.; Jin, H.-J.; Kim, H. J.; Kaplan, D. L. *Biomaterials* **2006**, *27*, 3115−3124.
(26) Bashur, C. A.; Dahlgren, L. A.; Goldstein, A. S. *Biomaterials* **2006**, *27*, 5681−5688.
(27) Teo, W. E.; Ramakrishna, S. *Nanotechnology* **2006**, *17*, R89−106.
(28) Shin, M.; Hohman, M. M.; Brenner, M. P.; Rutledge, G. C. *Appl. Phys. Lett.* **2001**, *78*, 1149−1151.
(29) Stankus, J. J.; Guan, J.; Fujimoto, K.; Wagner, W. R. *Biomaterials* **2006**, *27*, 735−744.
(30) Loscertales, I. G.; Barrero, A.; Guerrero, I.; Cortijo, R.; et al. *Science* **2002**, *295*, 1965−1698.
(31) Yu, J. H.; Fridrikh, S. V.; Rutledge, G. C. *Adv. Mater.* **2004**, *16*, 1562−1566.
(32) Loscertales, I. G.; Barrero, A.; Marquez, M.; Spretz, R.; et al. *J. Am. Chem. Soc.* **2004**, *126*, 5376−5377.
(33) Li, D.; McCann, J. T.; Xia, Y. *Small* **2005**, *1*, 83−86.
(34) Jayasinghe, S. N.; Edirisinghe, M. J. *J. Aerosol Sci.* **2004**, *35*, 233−243.
(35) Jayasinghe, S. N.; Edirisinghe, M. J. *Appl. Phys. Lett.* **2004**, *85*, 4243−4245.
(36) Ganan-Calvo, A. M.; Davila, J.; Barrero, A. *J. Aerosol Sci.* **1997**, *28*, 249−275.
(37) Hartman, R. P. A.; Brunner, D. J.; Camelot, D. M. A.; Marijnissen, J. C. M.; Scarlett, B. *J. Aerosol Sci.* **2000**, *31*, 65−95.

BM060649H