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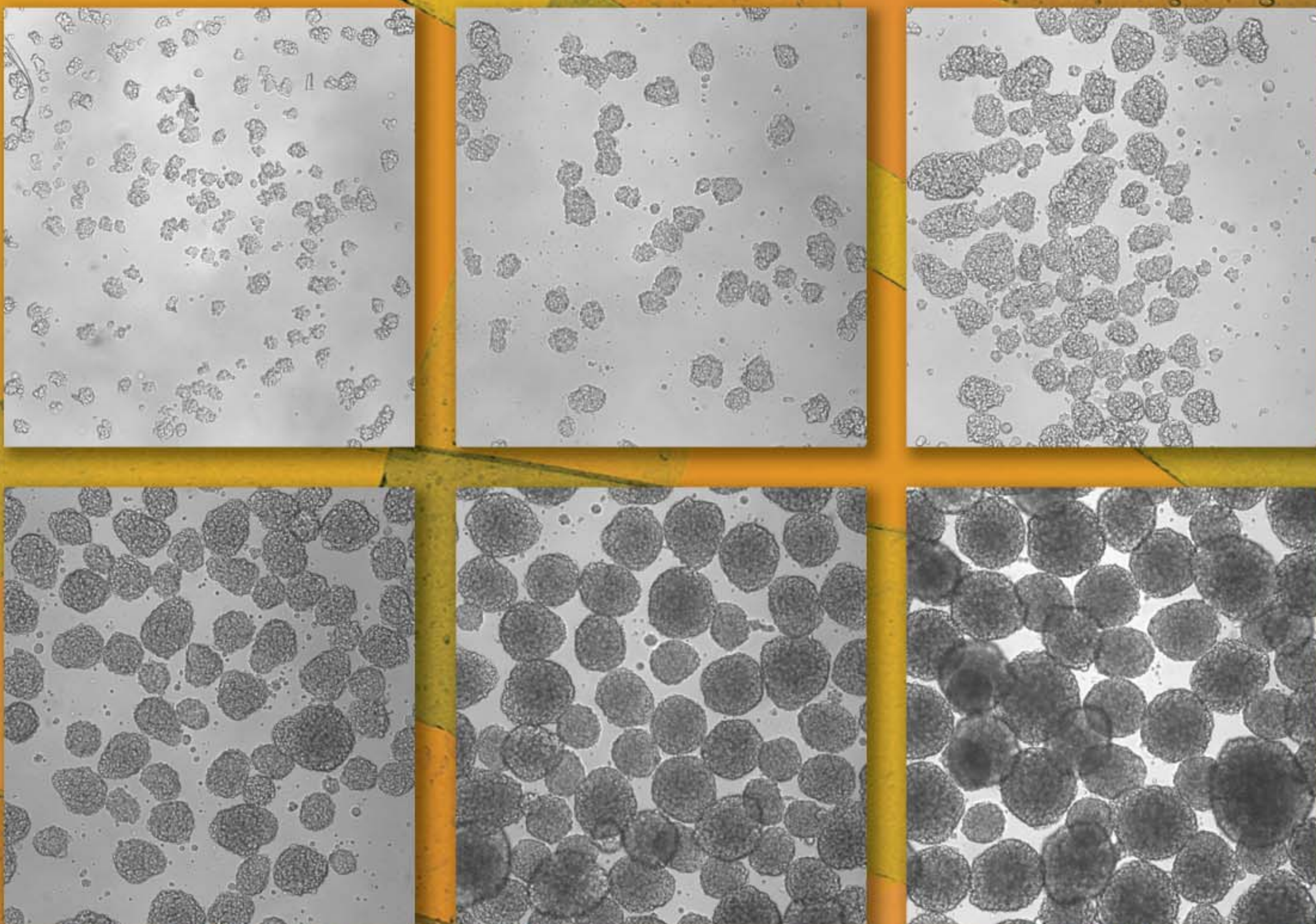

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Bio-Electrosprays and Cell Electrospinning: *Rapidly Emerging Physical Protocols for Potential Life Science Applications*

By SUWAN N. JAYASINGHE

Electrosprays and electrospinning are two interrelated physical phenomena which have been investigated for well over a century. Their similarity is based upon the primary driving mechanism; namely, the applied electric field. However, they have a fundamental difference that distinguishes one from the other: the former generates droplets while the latter forms continuous fibres. These two processing routes have been extensively researched in many areas.

Within the realm of life sciences, these routes have ranged from novel bioanalytical approaches (DNA and biomolecules) to tissue engineering by the formation of scaffolds, which mimic extracellular matrices. Only lately have these methods been explored for the direct process handling of living cells. Hence recently, developmental work has directly linked these techniques to a wide range of biomedical applications.

Introduction

Methods and protocols are the means by which all biomedically-based assessments are carried out at a pre-clinical stage on biological matter (cells and subcellular-level components).[1-3] One such gold standard is flow cytometry, a widely used fluorescence-activated cell-sorting (FACS) technique.[4,5] Here, hydrodynamics and lasers are being used to make accurate and sensitive measurements of the chemical and physical characteristics of living cells. Cells are hydrodynamically sorted into a stream of labeled single cells which are subsequently passed through an arrangement of lasers that excite the cells. Their response is detected, and the resulting chemical and physical information of each cell is determined and categorised to be alive, necrotic, apoptotic, or simply cellular debris.

Similarly, processing routes such as

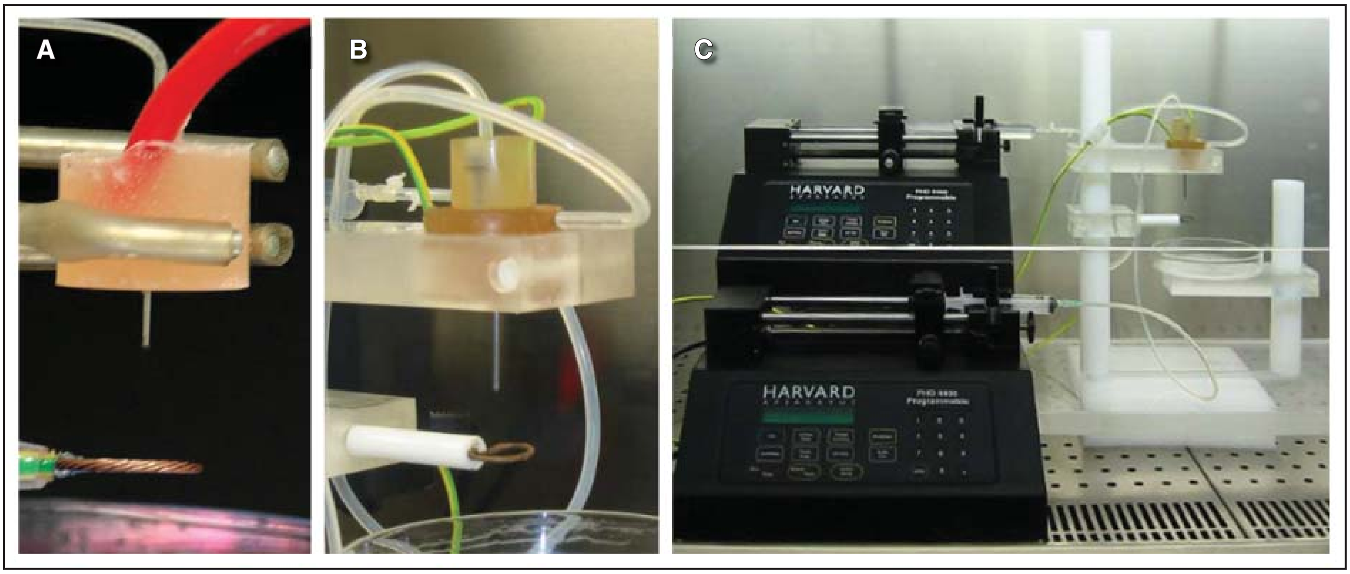

Figure 1. Digital images depicting: A) a single needle bio-electrospray device; B) the coaxial or concentric needle setup explored for both stable bio-electrospraying and cell electrospinning; and C) the bio-electrospray and cell electrospinning equipment arrangement setup in a class II safety cabinet, as explored in investigations.

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soft lithography[6] and inkjet printing[7] are currently being explored for a range of applications within the life sciences. Soft lithography is a process where a stamp having the desired architecture (such as a protrusion) is used in forming an impression on a planar surface. Subsequently, this impression is explored for encouraging the citing of several materials spanning from structural and functional to biological. Structures in the nanometer range (<50 nm), from functional nanoparticulates to biomolecules, have been assembled and studied for promoting the migration to proliferation of cells forming cellular arrays and active cell patterns.[8-10] Recently, this technique has even been explored for assessing subcellular structures.[11]

The inkjet printing technique has been scientifically "retrofitted" to demonstrate an ability to propel a measured quantity of liquid containing living cells for precision deposition on a wide range of substrates, and also fabricate viable biological constructs in multiple and pre-organised architectural dimension.[12-14]

In an advanced patterning technique such as soft lithography, an additional processing stage is involved for forming these cellular arrays, since the process does not handle cells directly. However, the inkjet process can form the desired cellular pattern directly, removing one stage of the processing protocol. Having said that, the inkjet needles limit the number of cells per ml and form active cellular arrays in the several tens of micrometers.[15,16] When jetted with needles sized in the 30-60 μm range, exceeding a limit of ~10^6 cells/ml has been known to promote an increase in cell mortality due to shearing. The cellular concentration in suspension can be raised by increasing the needle bore diameter, resulting in larger cell-bearing deposits and coarser structures.

Therefore, scientists are developing new jetting protocols which will have the best of both worlds. To a great extent, bio-electrosprays (BES) and cell electrospinning (CE) have removed many of the most intriguing obstacles previously encountered. In this review, we will present a technical overview of electrosprays and electrospinning, which forms the platform on which bio-electrosprays and cell electrospinning are based, along with their status to-date. Finally, we will discuss applications where these electrified bio-jets/threads can be explored within the biomedical sciences, extending to an outline of future challenges.

A Brief History of Electrosprays and Electrospinning

Reportedly, electrosprays evolved from the observations made by English scientist and physician William Gilbert in the 1600s. Gilbert's electrostatic observations disseminated through his famous book, *De Magnete*[17] reporting on how a droplet elongates, forming a cone between a piece of amber and another surface while attached to both surfaces. Subsequently, studies into the behaviour of liquids flowing through conducting capillaries demonstrated great potential with respect to a grounded element. Lord Rayleigh[18,19] investigated this phenomenon and reported on the behaviour of these jets and their breakup. He observed that surface tension played a pivotal role, helping to form the basis for the concept of jet instability, known as the "Rayleigh's instabilities." In the early 1900s, Zeleny[20-22] described these jets and their behaviour on a wide range of liquids. In 1964, Taylor,[23] unlike Lord Rayleigh and Zeleny, focused his attention on the stability of these jets to the continuity, which stemmed from the stable formation of a liquid cone, cusp-to-jet.

This helped launch the mode of jetting widely referred to as the "Taylor cone" or the "cone-jet." Several other scientists around the world have investigated these electrified jets and the generation of droplets mathematically.[24-28] The analytical models have been of great use to the aerosol science community, and those interested in the processing of single-phase, low viscosity media. However, it has only been in the last decade or so that these jets have been implemented in the physical and life sciences (in particular, to those scenarios which involve suspensions).

Closely related to electrosprays, electrospinning dates back to the first patents filed by both Cooley and Morton in 1902.[29,30] Several follow-on patents and scientific papers have been published in this area of research[31-35] but the process was primarily explored for textile applications. Now, unique electrospinning developments can produce fibres that are hollow, fibrous, porous, aligned structures, and fibers containing nanomaterials. Many have been heavily explored as tissue engineering materials.[36-41] Most mathematical electrospray and electrospinning models are for processing single-phase media.

Unlike inkjet printing, electrosprays and electrospinning are capable of handling high viscosity liquids, multiphase in nature (containing either micro/nano materials in suspension at concentrations >40% in weight, having viscosities >>1,000 mPa seconds) through needles with bores sized in the several hundreds of micrometers. Notably, the resulting droplets/continuous threads, with their respective residues, are in the few micrometers, if not in the nanometers (<50 nm), which are generated with ease.[42-45] Having seen the enormous implications for both electrosprays and electrospinning and what they can offer to applications in the real world, the recent discoveries of BES and CE are truly groundbreaking.

Electrosprays: Intricacies and Applications

This is a jetting-to-droplet generation methodology driven by high intensity electric fields. Conducting capillary (usually stainless steel) setups in single or coaxial/concentric configurations are connected to a high voltage power supply and held above a grounded electrode (Figures 1A and 1B). Individual capillaries have an input of media via connected, precision syringe pumps (Figure 1C). The grounded electrode placed below the needle exit(s) could take the geometric variations from a ring, plate-to-point (Figure 2). The potential difference between the conducting needle and the grounded electrode forms an electric field. Subsequently, the charged media exiting the conducting needle enters this

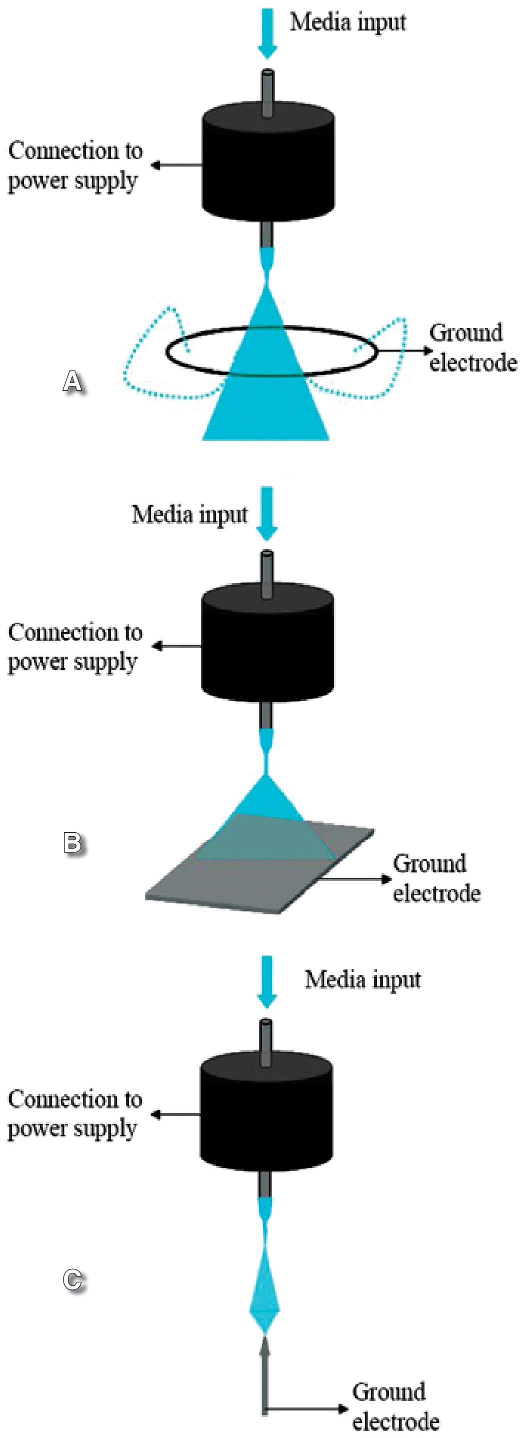

Figure 2. Schematic representations of: A) stable jetting with a ring-shaped ground electrode, exhibiting droplet recirculation; B) jetting in the cone-jet mode for a plate configuration; and C) stable cone-jetting with a point ground electrode which has focused a majority of droplets to the curvature of the pointed end of the electrode.

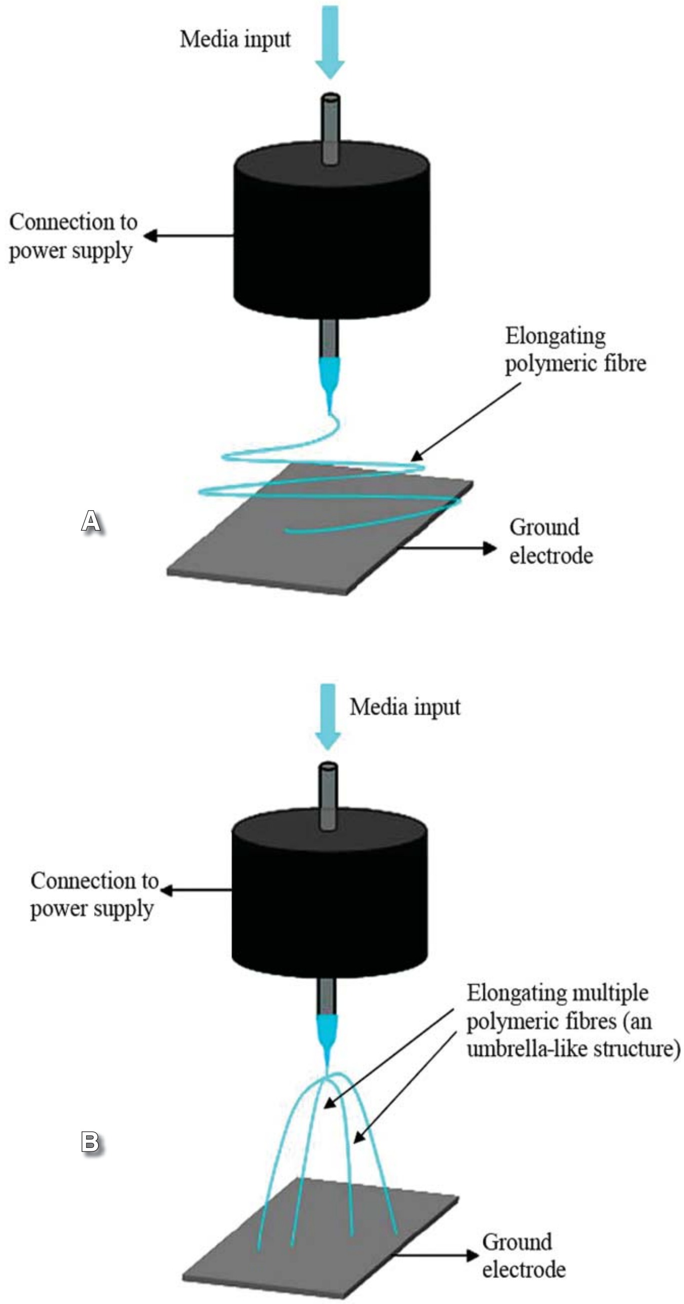

Figure 3. Schematics illustrating stable electrospinning in: A) a single; and B) multiple fibre configurations.

external electrical field, which subjects a combination of forces and stresses on the media droplet in three-dimension. Upon entering the electrical field, the liquid is accelerated towards the ground electrode, promoting the formation of a jet, and undergoes a combination of surface tension effects and Rayleigh's instabilities, fragmenting the jet into individually-charged droplets.

The geometries of these ground electrodes play an important role in determining the shape of the plume-containing droplets (Figure 2). The ring and plate demonstrates the divergence of droplets. The ring and plate directs large droplets to the centre while finer droplets have been found to recirculate on the ring's extremities. The point-shaped ground electrode—unlike both the ring and plate—converge a majority of droplets to the point of this electrode. With the aid of these ground electrode geometries, the plume profiles can be altered, which is a very important detail in specific applications.

Electrosprays are also governed by the applied voltage, the flow rate of media to the needle, and its consistency to the media properties: viscosity, electrical conductivity, surface tension, density, and relative permittivity. For a range of permutations and parameter combinations (including the setup itself), the jetting liquid has been observed to present many morphologies, which are referred to as modes of electrosprays.[46-50] There are several jetting modes already well-described in available literature, including: a) cone-jet; b) spindle; c) rim-emission; and d) multi-jet; to name a few. Controllability through stabilisation and continuity in the jet produces near one-sized droplets and residues for the precision drop and placement of these droplets (if studied with the point electrode). Cone-jet mode has proven to be the most desirable.

Several scaling laws have been derived from the investigations of various scientists worldwide, studying the continuity and stability of these jets for single-phase liquids. One such law[48] states that the hydrodynamic time must be greater than the electrical relaxation time in order for the liquid to accelerate between the electrodes and form a continuous jet; subsequently undergoing instabilities and giving rise to the generation of droplets and relics. The mathematical modeling of these vivid jets extends to characterising these "jet break-up systems" categorically into distinct mechanisms.[49] By combining these models together with the properties of the processed, single-phase liquid mediums, and moving on through dimensional analysis, equations have been derived for estimating droplet sizes.[50] Because these equations-to-scaling laws have been well-assessed and explored while processing single-phase media, they have been most useful as an approximation tool in experimentation.

In 2002, the Chemistry Nobel Committee recognised the pioneering efforts of Fenn *et al.* for their discovery of combining electrospray ionisation (ESI) with mass spectrometry (MS), widely referred to as ESI/MS.[51,52] Originally employed for biomolecular recognition, it is now widely used in advanced cancer research.[53]

Electrospray process applications have spanned many sciences such as: a) advanced routes in aerosols; b) agriculture; c) chemical; d) micro/nanoencapsulation; e) drug delivery; f) coating and surface patterning; and g) the fabrication of fibres and three-dimensional structures having unique complexities.[54-76]

Electrospinning: Operation and Applications

Electrospinning and electrosprays share the same driving mechanism, but have fundamental differences. In both protocols, a stable cone is formed through similar parameters, but with electrospinning, the ensuing jet does not undergo break-up-forming droplets. Instead, it undergoes a viscoelastic (or stretching) process which forms a uniaxial, continuous thread. Rheological properties play a major role by causing the jet to elongate into a thread rather than fragmenting. As with electrosprays, electrospinning has been modeled for analysing its behaviour while processing single-phase liquids.[77,78] While one might expect that electrospinning is limited to only one mode, it has been observed forming multiple threads from an emerging single thread (Figure 3).[79,80]

Electrospinning has been exploited in a wide range of research endeavours where nanothreads have been used in forming mats very easily, in a wide range of geometries and sizes down to the meter range.[81-85] Electrospun structural and functional materials have been explored for use in: a) filtration; b) biological scaffolding; c) controlled drug delivery; and d) textile materials.[86-91]

A Research Milestone: Directly Processing Living Cells

The ability to directly explore electrosprays and electrospinning for processing living cells has been demonstrated only recently.[92-95] Initially, unstable jetting conditions (particularly with BES) formed polydispersed droplets and residues.[92,93] Variable cellular suspension properties such as electrical conductivity and viscosity hampered jet continuity and stability.

It was decided that the protocol itself would require further development because the suspension media's high conductivity was crucial to maintaining cell viability. Therefore, the BES needle was modified into a coaxial (or concentric) arrangement to create a stable jetting mode with the added feature of generating a near mono distribution of cell-bearing droplets and residues.[96,97] Medical grade polydimethylsiloxane (PDMS) medium, with a viscosity of ~1,000 mPa and a conductivity of ~10^{-15} S/m, was used to encapsulate the charged, high conductivity/low viscosity cellular suspension as it entered the external electric field. During jetting in the stable mode, a highly concentrated (10^7 cells/ml) cellular suspension was successfully processed by BES.[96,97]

After increasing the outer PDMS viscosity to ~12,500 mPas for CE (with the same cell concentration of 10^7 cells/ml), continuous threads and scaffolds/membranes were formed containing living cells (Figure 4).[94,95]

Exploring the Future: Biomedical Applications for Bio-Electrosprays and Cell Electrospinning

Tissue Engineering and Regeneration

This is becoming a highly explored

area of research due to the increasing demand for fully-functional tissue and organs for repair and replacement. BES and CE protocols could be coupled with a plotting device to fabricate a variety of structured architectures in three-dimension using a range of primary cells. These protocols, coupled with stem cells, have the possibility for directly fabricating *in vivo* unspecialised biological components (depicted in Figure 5). In the future, such regenerated tissues—to possibly even organs—could be used for the discovery of new drugs to therapeutics.

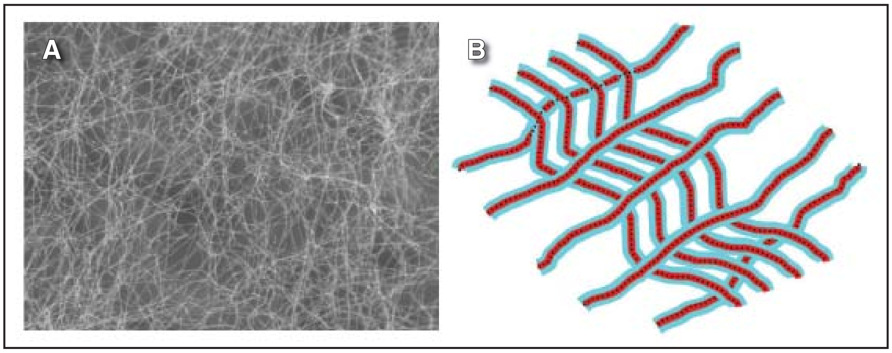

Figure 4. A) Characteristic optical image of a cell-bearing scaffold fabricated by means of coaxial or concentric needle cell electrospinning; and B) a schematic representation of the fabricating process for pre-organised scaffolds (aligned compound or composite threads/scaffolds-to-membranes).

Therapeutic Medicine

For use in a controlled delivery situation or for engineered therapeutics through living cells, appropriate methodology will be addressed by the discovery of encapsulating polymers to act as selected molecular barriers. The required nutrients surrounding the encapsulated cells will be allowed to cross the polymeric barrier while others will be restricted. Cell waste will pass through this polymeric shell while this polymeric system keeps the cells healthy until required, at which time the polymer will degrade and the healthy cells will be delivered for therapeutic purposes.

Developmental work has already demonstrated[100] that, when subjected to these protocols, green florescent protein (GFP)-transfected neonatal cardiac myocytes expressed GFP post-treatment. This opens up another method of therapeutics where the processing cells will undergo gene therapy and be used

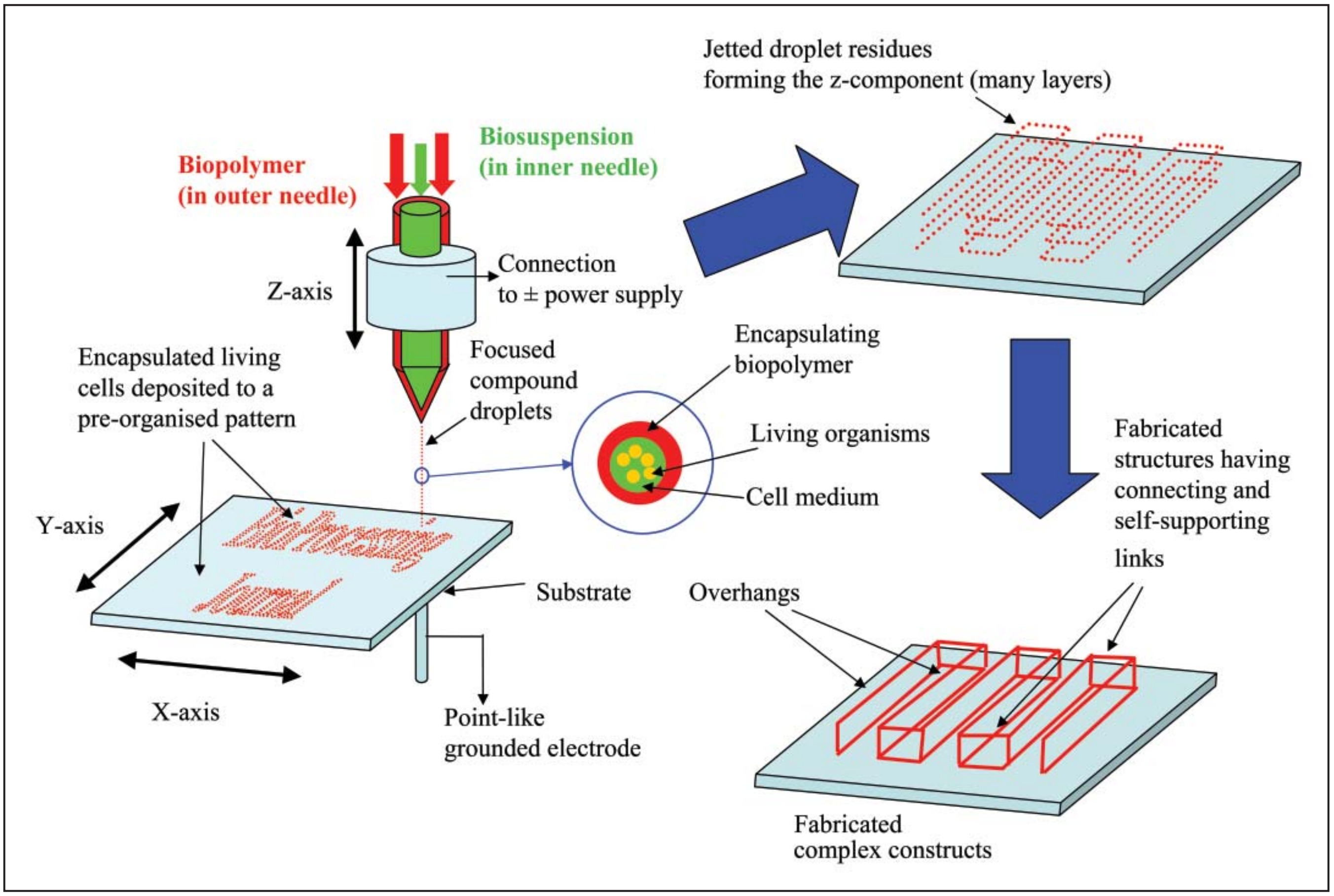

Figure 5. A representative schematic illustration of a three-dimensional bio-microfabrication system. The development of such a bio-protocol could potentially create non-specialized tissues in conjunction with stem cells.

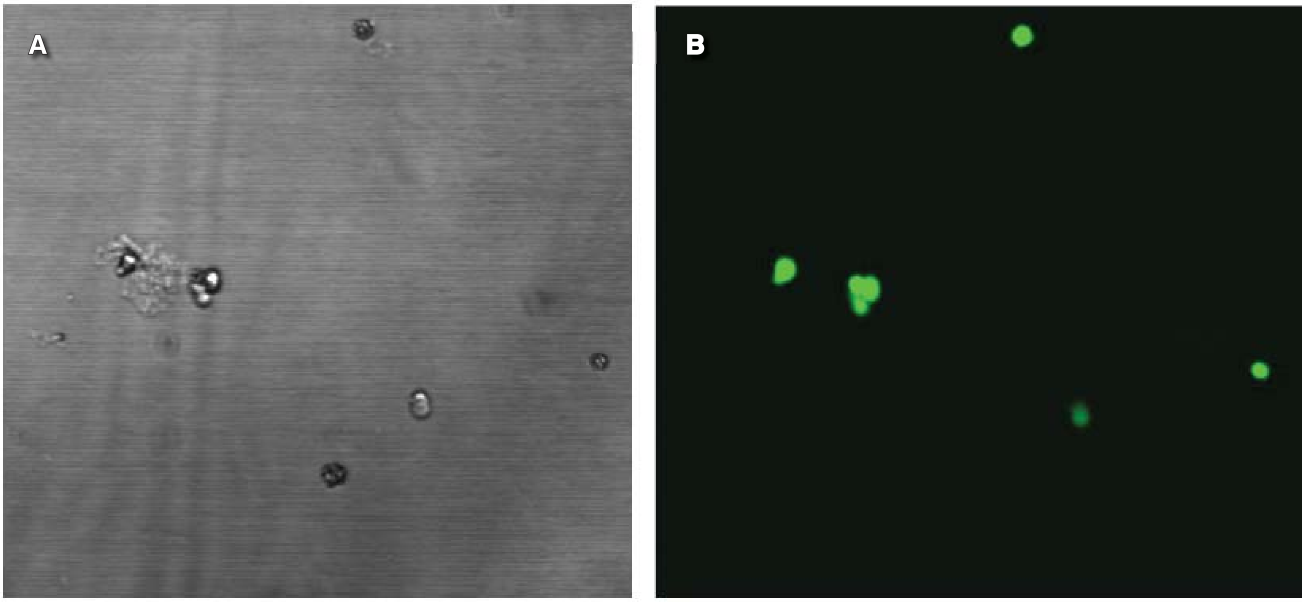

Figure 6. A characteristic: A) optical; and B) florescent image depicting encapsulated GFP-expressing cells generated by way of bio-electrospraying an alginate-cell suspension system.

later for forming tissue. These tissues might be implanted to mechanically engineer a wide range of pathologies through the coupling of gene therapy with these protocols. Explorations with such encapsulation-containing cells are showing great implications ranging from cancer therapy and hormone treatment to the control of diabetes (depicted in Figure 6).[101-103]

Developmental Biology

Coupling these techniques with a plotting device (depicted in Figure 7), several living cells, from mammalian to yeast, could be deposited with precision to study their developmental-to-evolutionary cycles. The deposition of cells at a preset distance apart could help researchers investigate cell-to-cell interactions, an interesting neurobiological/tissue engineering study to explore protocols for constructing tissues-to-neural networks, by having millions of cells in close proximity.

Conclusion

To date, research experiments have demonstrated the ability to produce live, cell-bearing droplets and threads without compromising viability. BES and CE will undergo continued development with the strong possibility of implementation within the life sciences industry. Several immortalised and primary cell types have been explored; the majority of which have been found viable post-jetting, continuing to undergo all expected cellular processes.[99] The post-treated cells from both BES and CE have always been compared with controls (those cells that are passed through the protocol without the application of an applied voltage) to those culture

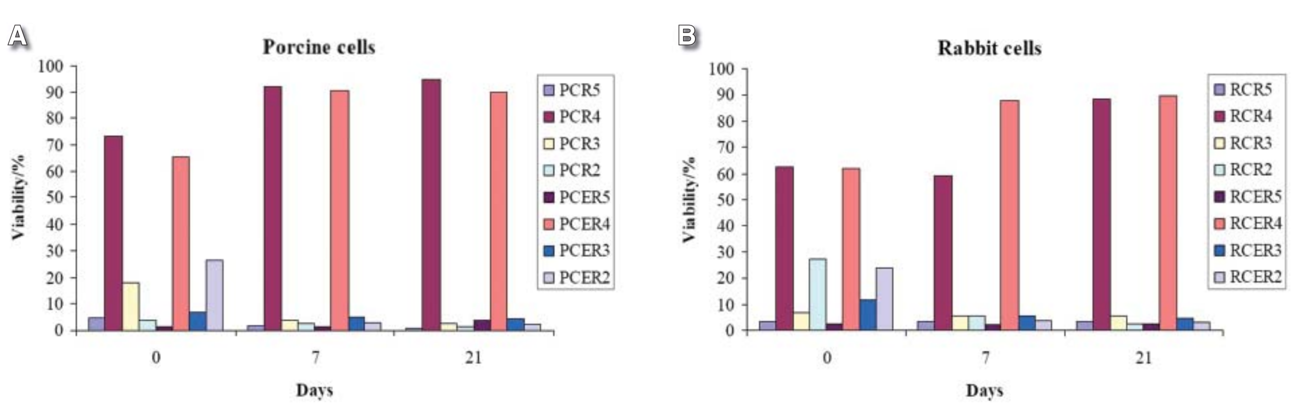

Figure 7. Representative viability data on cell electrospinning with highly concentrated cellular suspensions (10^7 cells/ml) containing: A) porcine aortic cells; and B) rabbit vascular smooth muscle cells. (Please note: The prefixes P and R represent porcine and rabbit cells, while C and CE stand for control and cell electrospun. R5, R4, R3 and R2 represent necrotic [dead], alive, and apoptotic [programmed cell death] cells to those cellular debris respectively, as analysed by flow cytometry.)

controls (untreated cells). These biomedical protocols explored for assessing viability of post-jetted and threaded cells have matured from trypan blue staining to flow cytometry analysis. The latter being a biomedical gold standard, it demonstrated the large population of cells viable from either protocol (Figures 7 and 8).

Developmental work is now in pursuit of discovering the effects these protocols may have at the internal and external subcellular level. It is well known that damaged subcellular components can cause changes which negatively affect fabricated tissues. Research is now ongoing to study the post-treated cells at the DNA level for stresses to components such as chromosomes and nuclei.

Initial studies with BES and CE explored γ histone 2AX labeling and Western blotting, which showed that the post-processed cells (in this study, primary neonatal cardiac myocytes[100]) failed to exhibit any incurred cell stresses and demonstrated no damage to cell DNA. Vigorous studies are taking place with gene expression and karyotyping on the post-treated cells (in comparison to controls) for assessing effects on these components as a function of time. Once these investigations have successfully concluded, studies on the many applications these jets and threads have for the biomedical sciences will commence.

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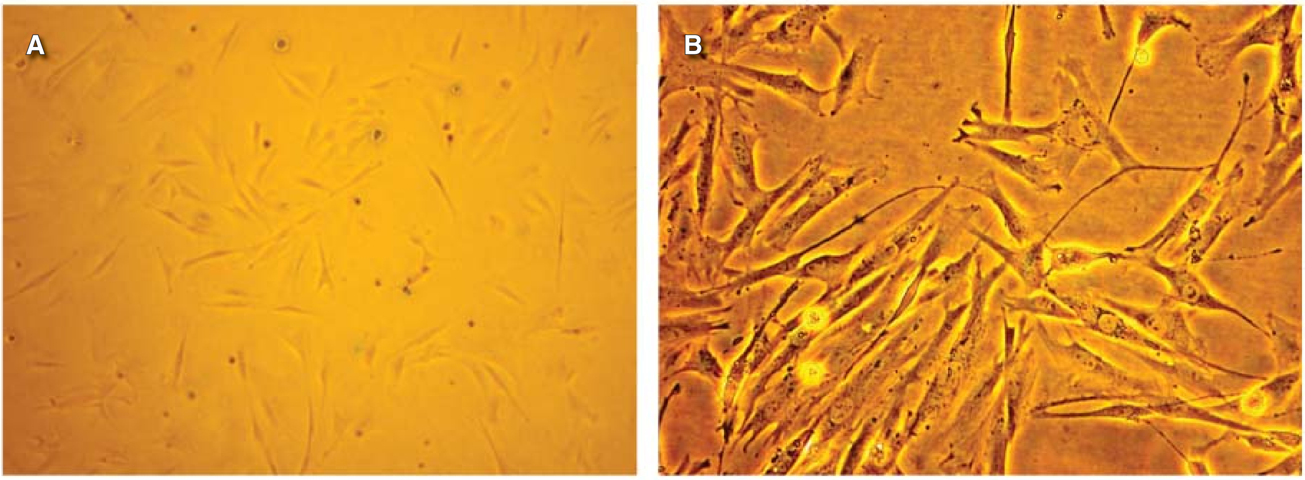

Figure 8. Characteristic optical micrographs: A) low magnification of post-spun cells; and B) high magnification of post-threaded cells; both after a period of three weeks.

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