Bio-electrospraying and Cell Electrospinning: Progress and Opportunities for Basic Biology and Clinical Sciences

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Engineering of functional tissues is a fascinating and fertile arena of research and development. This flourishing enterprise weaves together many areas of research to tackle the most complex question faced to date, namely how to design and reconstruct a synthetic three-dimensional fully functional tissue on demand. At present our healthcare is under threat by several social and economical issues together with those of a more scientific and clinical nature. One such issue arises from our increasing life expectancy, resulting in an ageing society. This steeply growing ageing society requires functional organotypic tissues on demand for repair, replacement, and rejuvenation (R^3). Several approaches are pioneered and developed to assist conventional tissue/organ transplantation. In this Progress Report, "non-contact jet-based" approaches for engineering functional tissues are introduced and bio-electrosprays and cell electrospinning, i.e., biotechniques that have demonstrated as being benign for directly handling living cells and whole organisms, are highlighted. These biotechniques possess the ability to directly handle heterogeneous cell populations as suspensions with a biopolymer and/or other micro/nanomaterials for directly forming three-dimensional functional living reconstructs. These discoveries and developments have provided a promising biotechnology platform with far-reaching ramifications for a wide range of applications in basic biological laboratories to their utility in the clinic.

1. Introduction

Basic biology has greatly advanced due to major discoveries over the last 60 years or so.[1–3] These findings have significantly contributed to our understanding of many cellular functions/processes (protein synthesis, division, communication, etc.) from a molecular level upwards; therefore, contributing to our increasing know-how on tissue and organ function, on advancing novel cell/molecular-driven therapies, and towards the discovery of new drugs, which are a few examples amongst many. These significant developments have seen a decrease in the need of surgical intervention. Therefore, our life expectancy has considerably increased, while promoting the inevitably increasing demand for repairing, replacing, or rejuvenating tissues and/or organs, much needed by a growing ageing population. At present, transplantation medicine is limited in many ways, including a limited donor pool and the necessity for immune compatibility between donors and recipients and the consequent application of life-long immunosuppresion. Therefore, the concept of engineering tailor-made synthetic organotypic tissues from a molecular level upwards opens several opportunities to clinical medicine. There are several approaches to engineering a synthetic tissue. These could be categorised as a function of their creation process, namely non-jet and non-contact jet-based approaches. The non-jet approaches range from the many manifestations of lithography, which include photolithography, softlithography, and dip-pen lithography. In essence, these techniques are based on the principle of fabricating a predetermined architecture using a mould of some kind. There are several manifestations under each of these fabrication processes to other mixing-based techniques. The reader should note that any modifications required during structural development will require a new mould, therefore, increasing costs. Conversely, non-contact jet-based techniques are those that explore needles and microfluidic networks for handling media, which are not in contact with the substrate, explored for deposition.

In this Progress Report we will briefly introduce the entire non-contact jet-based, direct-cell handling superfamily and, thereafter, discuss recently developed techniques that have swiftly undergone development for directly engineering organotypic tissues. In coda, we will identify and discuss some promising applications evolving from these approaches that have been demonstrated for basic biology and clinical applications.

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2. Non-Contact Jet-Based Direct-Cell Handling Approaches

The reader should note at the onset, that this category arguably could supersede those non-jet based approaches, as these technologies have the capacity to handle cells in any permutation and combination with a biopolymer (matrix), and if required with the addition of other materials (functional molecules) for precision placement (distribution) in three-dimensions. This feature in itself is unique as this would minimise bioreactor time, as the created living entities would have the cells and molecules imbedded within the matrix throughout the formed architecture, thus negating the time required for cell migration to proliferation deep into the generated structures. Furthermore, these technologies are sometimes referred to as additive technologies, as they are able to add materials (spanning structural, functional and biological) when required, rather than being removed when not. In fact removing materials, especially, those of a biological nature may not be an option as such advanced biomaterials, are not recyclable and are substantially costly.

Suwan N. Jayasinghe leads the BioPhysics Group in the Department of Mechanical Engineering at University College London, UK. Jayasinghe's group endeavours to thoroughly understand all the sciences associated with the design and development of synthetic tissues mimicking native tissue. This work endevours to contributes to the development of multicellular microenvironments, undergoing evaluation for repairing, replacing, and rejuvenating damaged and/or ageing tissue/organs. His group also contributes to the study of synthetic reconstructs for the sustained delivery of tailor-made therapeutics as a localised system through the development of active arcitectures containing experimental and/or medical cells and/or genes.

2.1. Ink-Jet Printing

This technology is largely driven by either thermal or piezoelectric systems (**Figure 1**).[4] Briefly the thermal system works on the principal of generating heat from a source, directly exposed to media (Figure 1A). The application of direct heat to the liquid causes bubble formation, which results with the accumulated media within the area denoted as "pressurised area" to squeeze a controlled volume of media through a needle for deposition. Soon after this event, the bubble retracts/collapses thus enabling the process to repeat. Similar in some respects the piezoelectric system works on flexing a diaphragm (Figure 1B), which subsequently pushes out a known volume of media through a nozzle for distribution onto a substrate. The process could be repeated as a function of the frequency of the piezoelectric device explored. Ink-jet printing (IJP) has been explored for tissue development by the deposition of cell-bearing droplets onto substrates. Although cells have been deposited from concentrated cell suspensions (10^6 cells mL^{-1}), a majority of cells have undergone shearing within the needle (as needle bores are in the tens of micrometers with the additional squeeze on cells subjected as a result of the concaving nature of the inner bore of the needle exit), therefore increasing cell mortality. Reducing cell mortality by shearing could be achieved by using large bore needles, but limits the achievable resolution, as these cell-bearing droplets (>50 μm) when residues spread on substrates (>100 μm). Therefore the technology is resolution limited.[5,6] These limits truly inhibit IJP in its utility in the biomedical and clinical sciences as the yield of viable post-treated cells is significantly reduced. Adding to this limitation, IJP has not undergone a detailed biological assessment in which the technique's effects on cells have been interrogated biologically from a molecular level upwards. In fact, for ink-jet printing a cell viability of nearly 100% post-treatment has previously been reported. This reported percentage[5,6] of viable cells is met with great scepticism, as the methods explored are not suitable for quantifying such parameters. In addition no (+)ve or (–)ve controls have been investigated in parallel to those studies. Lately, however previously reported cell viability percentages have decreased and it is hoped that in-depth geno- and phenotypic studies will be carried out on IJP cells in comparison to controls for enabling one to truly assess the potential of IJP living cells for the development of tissues.

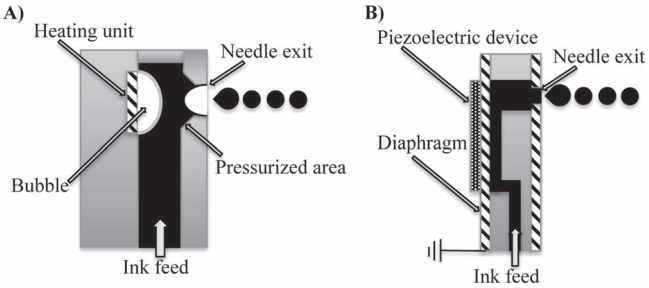

Figure 1. Schematic representations: A) thermal and B) piezoelectric ink-jet printing system.[7] Note that through both systems in practise the generated droplets are twice the diameter of the inner bore diameter of the explored needle. It is also noteworthy to bear in mind that both these systems apply their driving forces directly on the media, which is detrimental when handling sensitive advanced materials such as living cells.

2.2. Laser-Guided Cell Writing

As its name suggests, laser-guided cell writing (LGCW) is a technology driven by finely tuned lasers.[8] There are many manifestations of this approach which explore several different platforms (near surface based cell ejection) from which cells are jetted or handled for deposition onto a substrate. Two prominent laser based cell handling systems, are those where (1) the cells are delivered to the finely tuned lasers at which time they are held by the lasers and later deposited where necessary

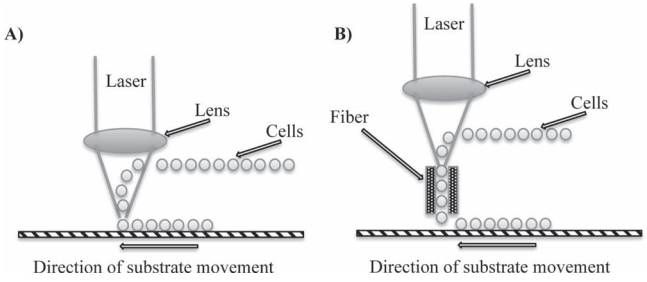

Figure 2. A) Characteristic schematic diagram depicting the fed cells being grabbed by the laser and subsequently being placed on a substrate. B) The scheme illustrates the fed cells being guided through a fibre for placement on a substrate.[9]

onto a substrate (**Figure 2**A). 2) The second similarly is fed with cells but the lasers drive the cells through a fibre for deposition (Figure 2B). LGCW, much like IJP, has not undergone a full biological assessment in which the cell's genetics, genomics to physiological characteristics, have been fully assessed and compared with controls. Thus, there is doubt that LGCW is truly benign for directly handling and distributing cells. That being said, the technology possesses a unique feature, as it is capable of placing single cells with calculated proximities to other deposited cells, thus demonstrating potential for its use in developmental biology studies (cell-to-cell communication, signalling with other cells to their movement in three dimensions).[8] It would be beneficial if one could be provided with process effects on cells (if any), in terms of cell exposure or resident times to lasers to the effects of cellular impact on jetting for deposition on substrates, to name a few.

2.3. Bioprinting and Self-assembly

This is a technology that explores cell-bearing spheres (pelleted cells) placed at critical proximities in a soft matrix to fuse/merge as a result of surface tension between two droplets, subsequently allowing cells to communicate and function for forming networks to living architectures (**Figure 3**). Recently, several cell types have been explored with this approach for tissue fabrication. However, much like both IJP and LGCW, this technology is limited by its inability to handle both a high viscosity matrix with a heterogeneous population of cells for controlled deposition.[10] In this approach the bioink (soft matrix/biopolymer) is deposited first on a substrate following the deposition of cell-laden spheres. The bioink has two major functions namely providing a cell friendly substrate/environment (matrix), while also allowing the deposited cell pellets to maintain their position on deposition (mimicking the function of a mould). Once a layer of bioink and pelleted cells have been deposited, the process is repeated as required till the desired three-dimensional architecture is formed layer-by-layer (Figure 3). Bioprinting has several pre-processing steps together with the required time for layer-by-layer structural fabrication (dependent on architectural complexity) of a given tissue to initiate cellular self-assembly. Assessing cellular damage post-treatment has been carried out to a greater extent, when compared to both IJP and LGCW, by exploring more specific biological assays.

2.4. Aerodynamically Assisted Bio-Jetting and -Threading

Aerodynamically assisted bio-jetting (AABJ) is a technology driven by a pressure (through either a gas or liquid) differential over an exit orifice.[11] The higher pressure within the chamber with respect to the atmosphere, holding within it a needle accommodating the flow of media, is drawn into a liquid filament, subsequently leaving the chamber through an exit orifice (**Figure 4**A). The technology is very versatile, as the number of needles incorporated into the chamber could be varied, thus enabling the direct formation of encapsulations (Figure 4B). The operational parameters are subsequently seen to enable control over droplet break-up and its precision deposition. The technology has undergone complete biological and engineering studies, which have been applied for the handling of

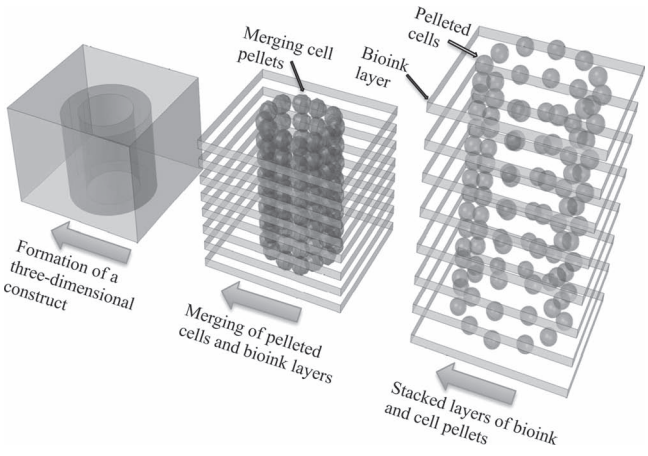

Figure 3. Schematic illustration of the bio-printing and self-assembly process taking form for the generation of a three-dimensional construct coupled with bioreactor time.

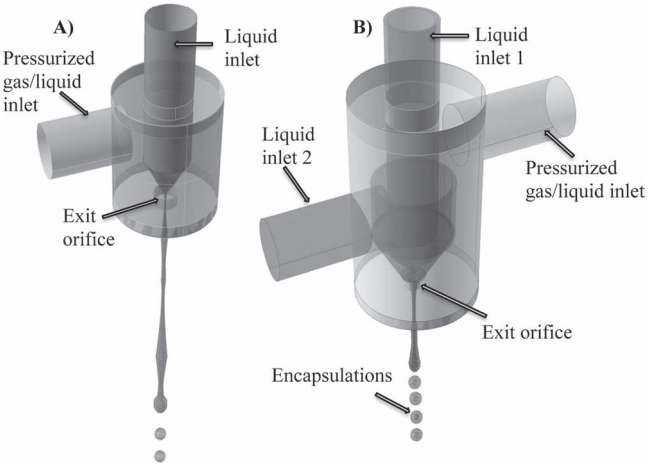

Figure 4. Schematic depictions: A) a single and B) a coaxial or concentric needle AABJ device. Note that driving forces are applied indirectly on the media over the exit orifice, thus being harmless when handling living cells and embryos.

a wide range of cells and whole organisms at very early stages of development.[12,13] These biological investigative studies have been furthered by studies of post-treated cells engrafted into living hosts (mice) that assess biological processes that may have been missed during in vitro studies. These in vivo investigations have included functional studies, which demonstrated spleenic cells homing to the lymph nodes.[14] At present, this technology is undergoing further checks (explored with other animal models) before it enters preclinical studies. There are many variations[15–17] of this process, which allow the technology to generate cell-bearing scaffolds and membranes. Interestingly, generated cell-laden fibre diameters are determined by the cells explored [aerodynamically assisted bio-threading (AABT), pressure-assisted cell spinning (PACS), and pressure-driven cell spinning (PDCS)] unlike in the case of IJP. AABJ is tremendously versatile, as it is able to switch between the generation of cell-bearing droplets and fibres by merely changing the viscosity of the cell-containing suspension, the latter derives scaffolds and membranes formed as a function of collection time during fibre generation.

Although AABJ in its various manifestations should significantly contribute to the development of synthetic tissues and possibly organs, this Progress Report will focus on both bio-electrosprays (BES) and cell electrospinning (CE). In some scenarios, AABJ/AABT may have an advantage over both BES and CE as a result of its driving mechanism, namely pressurised air/liquid.

3. Bio-electrospraying and Cell Electrospinning

Electrospraying and electrospinning work on the principal of exploiting an electric field between two charged electrodes, for drawing a liquid jet which either generates droplets or continuous fibres, respectively.[18,19] The processes work on the principal of charging media flowing within a needle/needles (**Figure 5** A and B), which is subsequently exposed to an electric field brought about by the potential difference between the two electrodes (the charged needle and ground electrode). This external electric field accelerates the charged media exiting the needle towards the grounded electrode. During this acceleration process a cone of liquid is formed at the needle exit from the apex of which a jet is generated. The media properties determine the formation of either droplets or continuous fibres (Figure 5). These technologies have greatly contributed to several fields of research and development. Interestingly both these techniques have been previously shown to possess the capacity to precisely place materials in three dimensions. To this end both electrosprays and electrospinning have been coupled with a point electrode to other ground electrode variations respectively,

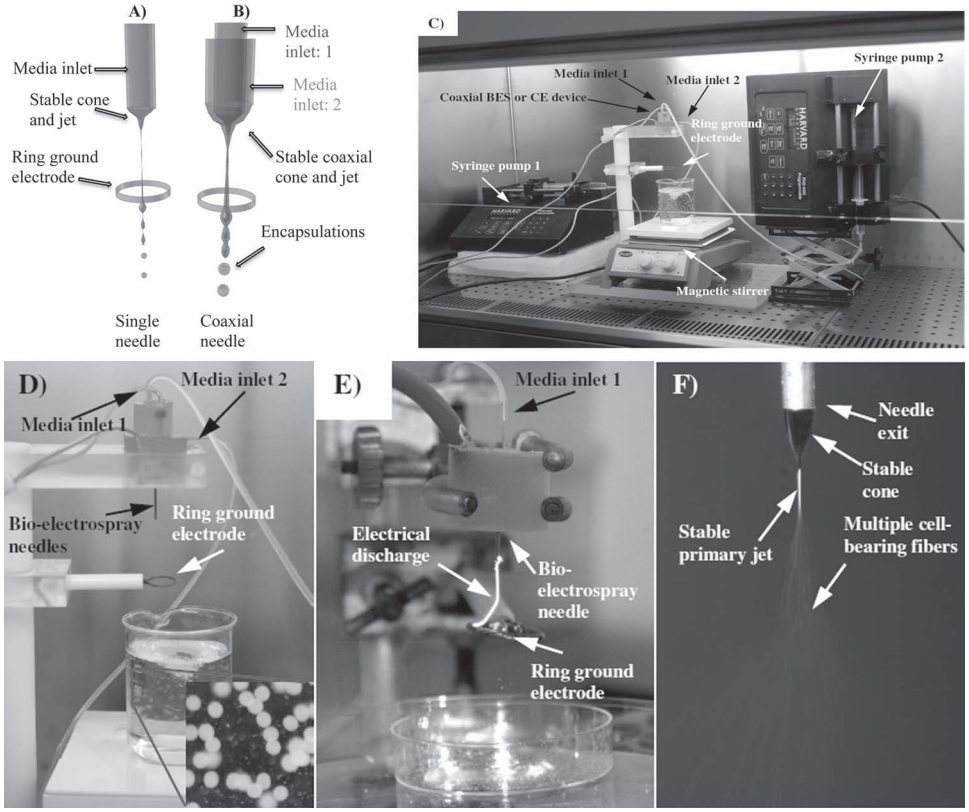

Figure 5. A,B) Representative schematic illustrations of a single and coaxial needle electrospray set-up. C,D) Characteristic digital images of a coaxial bio-electrospray system set-up in a laminar flow safety hood (which could also be used for coaxial cell electrospinning) explored for cell immobilisation. The inset of (D) shows the generated beads. E) Single needle BES configuration in which the voltage has been elevated to ~30 kV, demonstrating the collection of cell-bearing droplets during electrical discharge. F) Cell electrospinning with a modified counter electrode (used also for collecting living fibres/scaffolds). The reader should note that, much like AABJ, the driving forces are applied on the media by the external electric field.

for focussed material placement to the alignment of fibres.[20–26] There are many unique aspects to these jets, some of which are: a) the ability to generate nanomter-sized (<50 nm) droplets and threads, while using needles with inner bore diameters in access of 500 μm; b) the ability to process large densities of materials in suspension; c) the processing of highly viscous liquids (>10 000 mPa s). The reader should note both BES and CE explore applied voltages in the several thousands of volts, but have a correspondingly low current, generally in the order of nA. This, however, is not the case in electroporation[27] a technology explored for gene therapy. Here, the voltage is significantly lower than those explored in both BES and CE but with correspondingly higher currents. The larger currents are required in electroporation for cell permeabilization for introducing a genetic constructed for cell transfection. Although some could argue in disbelief the use of either BES or CE (electric fields driven protocols) in medicine, this is not that farfetched. In fact, electric fields have previously been widely explored in the clinical sciences through a technology referred to as electrosurgery.[28] Therefore, the use of electrosprays and electrospinning for the direct handling of living cells and whole organisms would be a natural progression of these unique technologies.

Electrosprays and electrospinning in the past five years have under gone a rigorous biological, chemical and physical developmental program to assess a) the inertness of these technologies from a molecular level upwards for directly handling cells in many permutations and combinations; b) the ability to use the technology for controlled cell compartmentalisation within droplets or spacing between cells in a fibre; and c) finally the transplantation of those treated cells in a matrix to fully assess the feasibility for engraftment into mouse models.[29,30] These studies have truly put electrosprays and electrospinning of cells (ranging from immortalised, primary to stem cell which included those iPS cells to whole organisms) in the forefront of engineering synthetic organotypic tissues. Thus these technologies are now referred to as bio-electrospraying and cell electrospinning. BES and CE together with AABJ and AABT have been demonstrated as being able to handle heterogeneous cell populations at high cell densities for pinpoint distribution in the three axes. These techniques have also been demonstrated as possessing the ability to eject controlled numbers of cells for forming desired multicellular living architectures. In addition, these techniques have undergone validation for directly handling whole organisms (*Danio rerio, Xenapus tropicalis, Caenorhabditis elegans, Drosophila melanogaster,* and finally *Dictyostelium discoideum*) from a very early development stage; thus, elucidating the capacity of BES and CE to handle complex multicellular dynamically developing organisms, without altering their associated developmental cues or those stages of embryology.[31,32]

In parallel to these in-depth viability studies, BES and CE have been demonstrated as having the capacity to form a wide range of cell-bearing structures, which could have clinical applications (**Figure 6**). These studies have seen the creation of living structural entities that are spherical (beaded) or fibrous from which scaffolds to membranes have been derived.

In addition to forming and further carrying out viability tests for assessing cellular viability in a living microenvironment, post-treated cells were explored in mice (**Figure 7**) for completely assessing any cellular aspects that might have been missed out during previous in vitro studies. This level of testing is necessary for advancing these biotechnologies to the clinic (for wound healing or targeted drug delivery, etc.) (Figures 6 and 7).

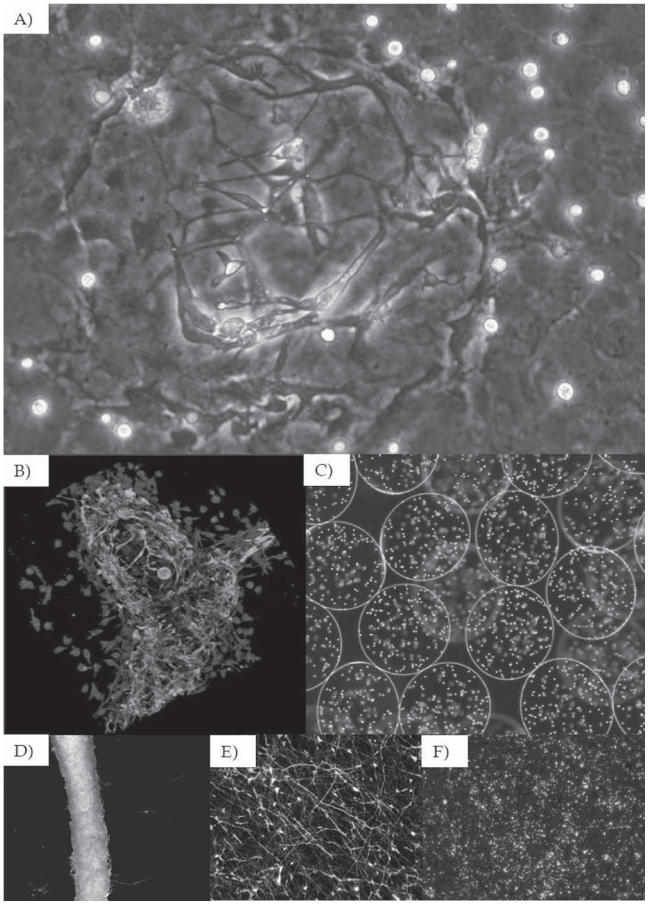

Figure 6. Living structures fabricated using either BES or CE. Panels (A–C) show structures generated through BES,[30,33,36] whereas panels (D–F) were generated through CE.[35,38,39] A) Characteristic optical image illustrating a four cell culture system created in three-dimensions (the image depicts cellular networks in three-dimensions). B) Confocal microscopy image of a three-dimensional culture prepared with the three major cell types of the myocardium (cardiac myocytes, endothelial cells and fibroblasts). C) Immobilised cells as composite living beads; BES has the ability to immobilise any cell permutation and combination with and without other functional materials to form spherical cultures for screening. D) A vessel formed with cells imbedded in the individual fibres. E,F) The fibre configurations which could be altered from containing a single cell to a heterogeneous cell population. Interestingly, the cell populations could be varied as a function of core position in a multicore vessel (gradation as a function of depth from central axis) to the aligning of those cells as a function of their placement within a vessel.

In mouse model-based studies, post-treated cells have been engrafted into mice as both cells alone and cells with a matrix (either as cell-bearing discs or cell-containing scaffolds). These studies have demonstrated the complete inertness of these biotechnologies for forming a wide range of tissues. In addition to those investigative studies, cells with homing capabilities have been put through these techniques and have been transferred into mice, which demonstrated the ability for those cells to home to their expected sites (**Figure 8**).

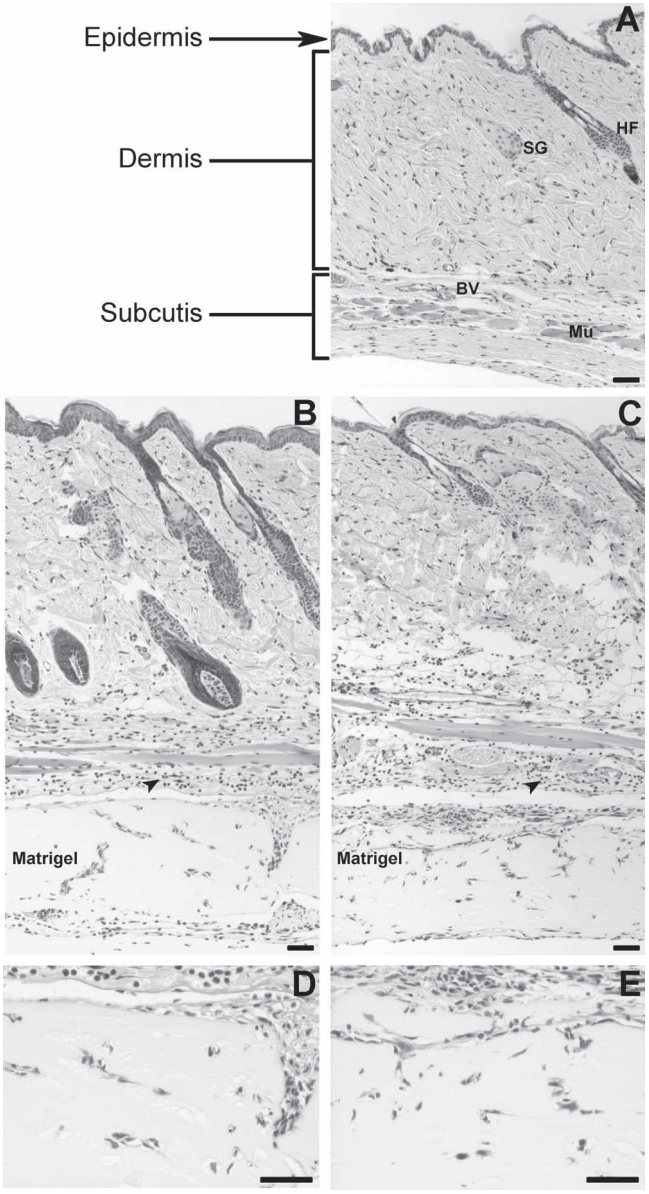

Figure 7. Subcutaneous transplantation of cell-containing microenvironments into mice.[30] Controls (B,D) and bio-electrosprayed (C,E) reconstructs were implanted subcutaneously into the dorsal flank of C57Bl/6J mice. Subsequently the skin was harvested and sectioned for histological analysis after 48 hours. Hematoxylin and eosin staining of control mice skin are shown in (A), demonstrating the normal architectural organisation of the epidermis, dermis and subcutis layers (original magnification ×100). Following engraftment, microenvironments (identified 'Matrigel') were observable in the subcutis layer (B,C; original magnification ×100), with expected occasional inflammatory cells in the surrounding tissue (arrows). Higher-magnification microscopy images of the mouse lung fibroblasts (MLFs) within the Matrigel microenvironment are shown in panels (D) and (E) (original magnification ×200). HF, hair follicle: SG, sebaceous gland; BV. blood vessel; Mu, muscle. Scale bar: 50 µm.

4. Applications

BES and CE have truly demonstrated the ability to directly engineer synthetic fully functional organotypic tissues. Of note, AABJ and AABT are comparable techniques capable of generating similar living structures (either as beads or scaffolds etc) to both BES and CE and could have an advantage over BES and CE in a given instance, in which in vivo cell delivery and therapy may require the need for using a non-electric field driven biotechnology (e.g., for the delivery of stem cells for retinal repair). In our hands these biotechniques are currently undergoing fine tuning and exploration for the following applications with implications to both basic biology and clinical medicine.

4.1. Three-dimensional Cell Cultures to High-Throughput Screening and Drug Development

Both BES and AABJ have the ability to handle highly viscose liquids with and without cells and other materials (micro- and/or nanomaterials). Coupling these biospray systems with a precision three-dimensional plotting device would enable one to directly place biopolymer encapsulated cells of a given type at calculated three-dimensional proximities. Our previous work[30,33] has demonstrated this capacity and great potential for forming controlled cultures with matrigel, which on deposition onto a surface at ~37 °C changes its nature from that of a flowing high viscous media to one that is semi-solid. At present, this biopolymer along with many others (natural and synthetic) are being explored for building three-dimensional cultures for mimicking various tissue/organs (heart, airways/lungs, cartilage etc), which would allow these to be explored as model systems. Such models will enable the study of basic biological processes and behaviour in both functional and diseased tissues. The development of a multineedle system would allow one to conduct high throughput screening studies of a wide range of drugs. This development will also see the reduction in large rodent model-based studies, suggesting the development of a platform biotechnology for carrying out more humane research.[34]

4.2. Development of Synthetic Tailor-Made Tissues

Developing these methodologies to create a three-dimensional multicellular culture[30,33] would allow exploration of this biotechnology for directly creating a synthetic predesigned and determined tissue to address the rising demand for tissues for repair, replacement and rejuvenation. Interestingly, as the technologies have been validated with stem cells (including those iPS cells[35]), this will further widen the many opportunities these biospray systems have on offer for the creation of synthetic fully functional three-dimensional tissues (ranging from dense to hollow re-constructs) generated with both specialised and/or unspecialised cells.

4.3. Personalised Medicine

These techniques have undergone validation with gene therapy,[36] thus opening these biospray technologies as novel cell therapy approaches for combating a wide range of diseases (from autoimmune to cardiac diseases). The exploration

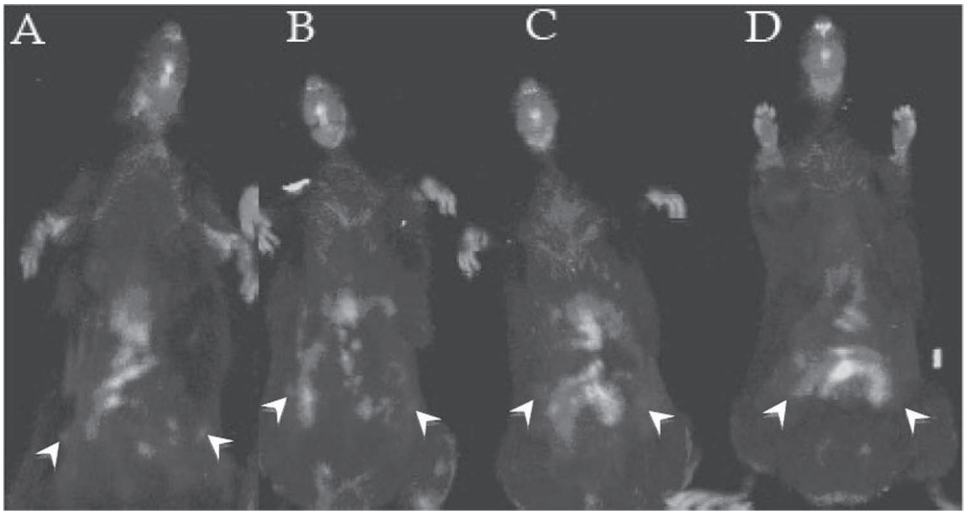

Figure 8. Spleens of C57/BL6 mice were removed post-mortem and the splenocytes separated into three samples groups (controls, BES and AABJ). Following treatment cells were dyed using CellVue Burgundy Dye and injected intravenously into the tail vein of recipient mice. After 24 h, the recipient animals were imaged. Panels depict A) AABJ post-treated cells B) untreated cells C) bio-electrosprayed cells and D) no cells (used for assessing base line fluorescence) transferred to mice. Arrow heads represent the lymph nodes.[14]

of this concept could undergo development as a personalised cell therapy approach. Further advances would see these technologies explored for controlled and targeted delivery of a wide range of experimental and/or medical genes and cells for personalised healthcare.

4.4. Cell Analysis and Advanced Diagnostics

Both these biospray systems possess the ability to eject cell-bearing droplets containing controlled cell numbers.[37] This characteristic could be explored for the deposition of cells as arrays for large scale single cell analysis. One particular aspect our laboratory is currently developing is the coupling of these biospray systems with both nanoparticles and mass spectrometry, which operate at ambient conditions. We hope that we would be able to analyse living cells using mass spectrometry, therefore developing a technique for rapidly assessing large populations of living cells in small volumes. This, we hope, will enable the development of a technology for accurately identifying cellular alterations at a molecular level such as those giving rise to leukaemias and other cancers. Interestingly, in our hands, AABJ is currently undergoing development as a sheathless flow cell, most useful for possibly retrofitting current flow cells in cytometry for analysing the wellbeing of both cells and whole organisms.

4.5. Controlled and Targeted Cell Therapies

Gene therapy has been coupled with these biospray systems.[36] Those studies demonstrated the ability to form multi-structural living reconstructs (such as cell-laden fibres and beads) most useful for combating autoimmune diseases such as diabetes type 1 and arthritis.[36] Interestingly, these cell-laden structural entities allow the sustained delivery of cell/molecular therapies to targeted areas by allowing those generated structures to anchor and localise to those areas requiring therapy.[38,39] Here the explored biopolymers play several crucial roles: 1) they would allow the release and absorbance of controlled molecules from the surrounding environment; 2) enable controlled repair and replacement of the damaged or ageing tissues, and 3) deliver a tailor-made therapeutic payload (molecules tagged genetically into cells by transfection or the release of biomolecules embedded within the polymeric structures) in both a managed and targeted fashion. A further interesting possibility may be one in which enhanced repair or rejuvenation is triggered by exploiting novel biopolymers (thermal or acid driven) that degrade and release therapeutics to the anchored area as natural repair takes its course (this could possibly be achieved by the activation of functional molecules signally to stems cells in that respective tissue/organ).

5. Concluding Remarks

This Progress Report has collated and discussed, in detail, all investigations that have demonstrated evidence that both BES and CE are front running novel techniques for tissue engineering, with promise for utility within the clinic. Although these techniques have been interrogated significantly, more than other technologies described in this report, further testing is currently underway with human samples prior to entering possible clinical investigations. While large-scale preclinical studies are being planned with selected model systems, these biotechniques are undergoing development for the creation of human cultures and as a diagnostic tool for assessing single cells and whole organisms. These cultures will model a wide range of tissue and organ diseases (dermatological, cardiac, cartilage, etc.) at given disease states and see the use of

these cultures for high-throughput drug/vaccine screening and development. Other techniques, also briefly highlighted in this review (AABJ and their manifestations), are undergoing rapid development. Thus, seeing them explored for single-cell and organism diagnostics through AABJ as a sheathless batch processing flow cell technology for cytometry. In coda the authors hope this Progress Report has demonstrated the flexibility in these technologies for their far-reaching ramifications to both basic biology and clinical medicine.

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[1] J. D. Watson, F. H. C. Crick, *Nature.* **1953**, *171*, 964.
[2] J. R. Warren, B. J. Marshall, *Lancet* **1983**, *1*, 1273.
[3] A. P. Carter, W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly, V. Ramakrishna, *Nature* **2000**, *407*, 340.
[4] O. A. Basaran, *AIChE J.* **2002**, *48*, 1842.
[5] E. Podolak, *BioTechniques.com* **2010**, "New cell printing approach enables precise design of complex 3-D tissue structures", http://www.biotechniques.com/news/New-cell-printing-approach-enables-precise-design-of-complex-3-D-tissue-structures/biotechniques-198929.html (accessed September 2010).
[6] N. E. Sanjana, S. Fuller, *J. Neurosci. Meth.* **2004**, *136*, 151.
[7] H. P. Le, *J. Imaging Sci. Technol.* **1998**, *42*, 49.
[8] R. K. Pirlo, D. M. D. Dean, D. R. Knapp, B. Z. Gao, *Biotechnol. J.* **2006**, *1*, 1007.
[9] D. J. Odde, M. J. Renn, *Trends Biotechnol.* **1999**, *17*, 385.
[10] K. Jakab, C. Norotte, F. Marga, K. Murphy, G. Vunjak-Novakovic, G. Forgacs, *Biofabrication* **2010**, *2*, 022001.
[11] S. Arumuganathar, S. Irvine, J. R. McEwan, S. N. Jayasinghe, *Biomed. Mater.* **2007**, *2*, 158.
[12] H. Kempski, N. Austin, A. Roe, S. Chatters, S. N. Jayasinghe, *Regener. Med.* **2008**, *3*, 343.
[13] P. Joly, B. H. Jennings, S. N. Jayasinghe, *Biomicrofluidics* **2009**, *3*, 044107.
[14] N. Carter, S. N. Jayasinghe, C. Mauri, *Analyst* **2011**, *136*, 3434.
[15] S. Arumuganathar, S. Irvine, J. R. McEwan, S. N. Jayasinghe, *J. Appl. Polym. Sci.* **2008**, *107*, 1215.
[16] S. Arumuganathar, S. Irvine, J. R. McEwan, S. N. Jayasinghe, *Biomed. Mater.* **2007**, *2*, 211.
[17] S. N. Jayasinghe, N. Suter, *Biomicrofluidics* **2010**, *4*, 014106.
[18] J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong, C. M. Whitehouse, *Science* **1989**, *246*, 64.
[19] N. L. Nerurkar, B. M. Baker, S. Sen, E. E. Wible, D. M. Elliott, R. L. Mauck, *Nat. Mater.* **2009**, **8**, 986.
[20] S. N. Jayasinghe, M. J. Edirisinghe, *J. Mater. Res. Innovat.* **2003**, *7*, 62.
[21] D. Z. Wang, S. N. Jayasinghe, M. J. Edirisinghe, *Rev. Sci. Instrum.* **2005**, *76*, 075105.
[22] A. C. Sullivan, S. N. Jayasinghe, *Biomicrofluidics* **2007**, *1*, 034103.
[23] D. Z. Wang, M. J. Edirisinghe, S. N. Jayasinghe, *J. Am. Ceram. Soc.* **2006**, *89*, 1727.
[24] J.-U. Park, M. Hardy, S. Jun Kang, K. Barton, K. Adair, D. K. Mukhopadhyay, C. Y. Lee, M. S. Strano, A. G. Alleyne, J. G. Georgiadis, P. M. Ferreira, J. A. Rogers, *Nat. Mater.* **2007**, *6*, 782.
[25] M. J. Poellmann, K. L. Barton, S. Mishra, A. J. W. Johnson, *Macromol. Biosci.* **2011**, *11*, 1164.
[26] D. Li, Y. Wang, Y. Xia, *Nano Lett.* **2003**, *3*, 1167.
[27] N. Traitcheva, H. Berg, *Bioelectrochem.* **2010**, *79*, 257.
[28] M. L. Morris, R. D. Tucker, T. H. Baron, L. M. W. K. Song, *Am. J. Gastroenterol.* **2009**, *104*, 1563.
[29] K. Bartolovic, N. Mongkoldhumrongkul, S. N. Waddington, S. N. Jayasinghe, S. J. Howe, *Analyst* **2010**, *135*, 157.
[30] S. N. Jayasinghe, G. Warnes, C. J. Scotton, *Macromol. Biosci.* **2011**, *11*, 1364.
[31] N. Mongkoldhumrongkul, S. C. Swain, S. N. Jayasinghe, S. Stürzenbaum, *J. R. Soci. Interface* **2010**, *7*, 595.
[32] N. K. Pakes, S. N. Jayasinghe, R. B. S. Williams, *J. R. Soci. Interface* **2011**, *8*, 1185.
[33] K.-E. Ng, P. Joly, S. N. Jayasinghe, B. Vernay, R. Knight, S. P. Barry, J. McComick, D. Latchman, A. Stephanou, *Biotechnol. J.* **2011**, *6*, 86.
[34] K. Chapman, S. Robinson, "Challenging the regulatory requirement for acute toxicity studies in the development of new medicines", **2007**; http://www.nc3rs.org.uk/downloaddoc.asp?id=559 (accessed September 2010).
[35] A. Abeyewickreme, A. Kwok, J. R. McEwan, S. N. Jayasinghe, *Integr. Biol.* **2009**, *1*, 260.
[36] E. Ward, E. Chan, K. Gustafsson, S. N. Jayasinghe, *Analyst* **2010**, *135*, 1042.
[37] J. Hong, A. J. deMello, S. N. Jayasinghe, *Biomed. Mater.* **2010**, *5*, 021001.
[38] A. Townsend-Nicholson, S. N. Jayasinghe, *Biomacromolecules* **2006**, *7*, 3364.
[39] S. N. Jayasinghe, S. Irvine, J. R. McEwan, *Nanomedicine* **2007**, *2*, 555.