CHAPTER 4

Cell Electrospinning and technology transfer from lab to market scale

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Abstract

Tissue engineering provides a unique approach to personalised medicine for the repair and replacement of diseased, damaged or ageing tissues and organs. Tissue engineering methodologies have undergone a rapid evolution over the past decade, however, tissue engineered products have not yet entered the market place. This is something that can be directly attributed to the relatively recent development of approaches such as cell electrospinning, relative to the timescale of the commercialisation pathway for new technologies.

Here, we assess the market potential of cell electrospinning, making comparisons with competing tissue engineering techniques to establish why cell electrospinning is the tissue engineering method of choice for tissue replacement in humans. We propose that the key criteria necessary for the successful application of cell electrospinning in a commercially-relevant context can be distilled into two critical aspects: 1) defining the suitable biopolymers within which to suspend the cells and 2) ensuring that rigorous testing is carried out prior to the replacement of diseases or damaged tissues with engineered structures that are truly equivalent to the native tissues they are intended to replace.

4.1 Introduction

Through advances in clinical medicine, the estimated lifespan of humans has become significantly increased and the causes of morbidity and mortality have shifted substantially over the past 100 years. The leading cause of death in 2015, according to the World Health Organisation was ischaemic heart disease, closely followed by stroke; these two causes of death were responsible for 15m of the 56.4m deaths in 2015. Chronic diseases now cause an increasing number of deaths worldwide and lead to a significantly reduced quality of life. In 2015, non-communicable diseases contributed to between 62% and 89% of deaths in all regions of the world except Africa, where only 33% of deaths were attributed to this cause. In the United States, the leading causes of death in 1900 were infectious diseases with heart disease ranked at number 4, yet in 2014, heart disease was ranked at number 1 and the top three causes of death (heart disease, cancer and chronic lower respiratory disease) accounted for over 50% of all deaths. Over the past 5 years, the leading causes of death in the US have remained relatively constant and it is reasonable to assume that this trend will be maintained in other developed nations. [1-3]

Tissue and organ transplantation technologies provide a means of replacing diseased and dysfunctional tissues and organs, however, perusal of the national transplant activity reports from any country collecting and publishing such data reveals that the number of patients on transplant waiting lists vastly exceeds the number of available donors. Each year, significant numbers of patients die waiting for a suitable donor organ to become available. Cross-species organ transplantation has been considered as a means of improving the number of organs available for transplant, with genetic modification used to overcome the difficulty of human antibody binding to animal antigens, as occurs in the case of xenotransplantation using pig organs. Under these circumstances, a number of different genetic modifications have been employed to prevent recognition of the pig's vascular endothelial galactose oligosaccharide, galactose-α1,3-galactose, by human antibodies and to provide the pig with increased resistance to human complement-mediated injury. The use of animal organs in the clinic is fast approaching the stage of clinical trials with international consensus. [4,5]

Donor availability is not the only limitation that can impact on improving patient quality of life through transplant. It can also be difficult to find a suitable donor match for patients. This can occur, for example, in the case of patients of multiracial heritage who require therapeutic treatments based on stem cells obtained from cord blood, where certain genetic markers are inherited. In such conditions, a donor with similar racial heritage may present a much improved chance of success. Mesenchymal stem cells (MSCs) obtained from cord blood provide a very good example of this. MSCs are of great utility in the replacement of damaged and diseased tissues as a result of their ability to differentiate into a wide variety of tissues and a number of

tissue engineering strategies have been developed using MSC cells. [6] The FDA has approved at least five different stem cell-based medicinal products derived from cord blood. [7]

Stem cells play a central role in regenerative medicine, an emerging branch of medical sciences that enables treatment for patients whose own regenerative systems are unable to restore a lost or reduced functionality. There are six major classes of stem cells that have been identified as having clinical promise for the treatment of defects and the regeneration of tissue. [8] The World Health Organization, amongst others, provides access to a searchable international clinical trials registry platform to identify the clinical trials of stem cell-based therapies that are currently underway. [9] Stem cells coupled with tissue engineering provides a foundation for the provision of therapies for disease and for the repair of damage that cannot be achieved by conventional medicines.

The adoption of tissue engineering strategies to replace diseased or damaged tissues is particularly enticing as this approach is directly aligned with the philosophy of personalised medicine, where bespoke treatments can be created that are directly targeted at an individual's own and unique therapeutic needs. Tissue engineering has the capacity to produce a bespoke replacement tissue using cells in which pre-existing defects have been repaired, which is an extremely powerful and highly individualised approach to the treatment of disease. The generation of replacement tissues for each patient using that patient's own cells bypasses any requirement to identify suitable tissue matches from a donor pool and neatly circumvents the problems of transplant rejection as the patient in question is their own donor, thereby preventing rejection of the tissue to be transplanted. Gene therapy technologies can be included as part of the tissue engineering protocol to rectify any causal mutations encoded in the patient's own DNA prior to construction of the engineered tissues.

Tissue engineering methodologies have been undergoing a rapid evolution over the past decade and have clearly moved now from the invention phase into the development phase. Several examples have proved particularly promising and are heading for the clinic. This chapter will describe the use of one such tissue engineering technique, cell electrospinning, for the generation of 3D biological architectures for the replacement of diseased or damaged tissues. Cell electrospinning is explicitly defined here as the generation of cell-laden nanofibres produced using the electrospinning of biosuspensions containing intact cells. Comparisons will be made with competing tissue engineering techniques to establish why cell electrospinning is the tissue engineering method of choice for tissue replacement in humans and key criteria necessary for the successful introduction of this technology in the marketplace will be proposed.

4.2 Tissue Engineering and the Construction of 3D Biological Architectures

Tissue engineering provides a unique approach to personalised medicine for the repair and replacement of diseased, damaged or ageing tissues and organs. This is the primary focus of this chapter. It should be noted, however, that there is a wide variety of different biomedical and bioscience applications of the underpinning technologies used for tissue engineering. The development of prosthetics to replace bone damaged in accidents or malformed during neural crest development is and example. The chromosome 22q11.2 deletion (22qDS) under which DiGeorge and velocardiofacial syndromes fall provides a very good illustration of the benefits of tissue engineering for syndromes that have a range of congenital abnormalities affecting a number of organ systems. [10] Other applications include 3D cell culture and 3D controlled cryopreservation for clinical use. [11] Non-clinical commercial applications involve the use of tissue engineering technologies for cellular agriculture to produce *in vitro* meat and biofabricated leather, oral delivery systems for macromolecular drugs and bioactive agents have been combined with functional feeds in fish and 3D scaffolds for the large scale expansion of anchorage-dependent cells in culture for cell enrichment and drug screening. [12,13]

In order for these potential applications of tissue engineering to come successfully to the market as products, either commercially available to the general public or used clinically, there are a number of defined attributes that the technologies must possess. Biopatterning methodologies must: achieve precision placement of cells within 3D space, be scalable, keep costs to a minimum (by reducing the number of steps, reducing the time needed to fully integrate multiple layers of cells etc.) and be biofriendly, to ensure conservation of the biological properties of the cells being used in the engineering process. The most promising of the techniques currently available are outlined below and the rationale for putting forward cell electrospinning as the method of choice is described.

4.2.1 3D Printing/Biofabrication

3D printing is a technology that essentially stacks layers of 2D material depositions to create a third dimensional z-axis. It has significant cost benefits because it adds materials when required, which prevents the material waste during the forming step that is seen with the other technologies described below. There are a large number of different ways in which these materials can be dispensed or extruded and 3D printing can generate some exquisitely complex and high-definition architectures. 3D printing is an inherently scalable technology, but it is the extrusion methods that present the principal difficulty in the adoption of this methodology as a biopatterning technique because the fine-bore needles used and the driving forces

involved cause significant cellular damage. [14,15] A number of different modifications have been attempted, but none of these currently is able to avoid compromising cellular viability. To date, no material has been identified that would enable cells to be directly handled by 3D printing to create a viable self-standing, fully-cellularised bioarchitecture that requires no additional assistance during the forming stage. [11]

4.2.2 Aerodynamically-Assisted Bio-Jets and Threads

Aerodynamically-assisted jetting and threading uses a pressure field through air flow within a chamber with respect to the surrounding atmosphere to draw liquids through an exit orifice. The nature of what is formed is dependent upon the viscosity of the liquid, producing either droplets (aerodynamically-assisted jets) or continuous threads (aerodynamically-assisted threads). The AAJ/AAT process is similar to that of electrospray/electrospinning, discussed in sections 5.2.3.2 and 5.2.3.3, below, and is scalable, with comparable costs, but is a non-electric field-driven competing technology. Inclusion of cells within the liquid used for AAJ/AAT creates aerodynamically-assisted biojets and biothreads (AABJ, AABT, respectively). These are biofriendly and an effective means by which to produce 3D biological architectures, in particular, those that use highly conducting materials. As they are electric field-driven technologies, bio-electrosprays/cell electrospinning cannot handle highly conducting materials without discharging or damage of the conducting molecules. [11]

4.2.3 Jet-based Techniques

There are a number of jet-based methodologies that are economical, robust and capable of handling materials on the scale required for tissue engineering. These include ink-jet printing, electrospraying and electrospinning.

4.2.3.1 Ink-jet Printing

Ink-jet printing was identified as a versatile biotechnique for printing 3D biological architectures of living cells and was the first jetting technology to develop significantly. Controlled architectures in the millimetre range have been fabricated using ink-jet printing. Scaling-up is feasible through direct-write assembly but has not been demonstrated yet. Ink-jet printers have been used to generate cell-bearing fibres and scaffolds,

however, the size of these fibres is in the hundreds of micrometers, at best, as droplets double the size of the needles can be formed and the spreading of these upon placement limits the resolution of the architectures that can be constructed. There are also limitations on the processing of high-viscosity media due to the 30-60μM size of the needles used, which tends to promote blockage. Cost is not an issue as the resulting architectures do not require significant time in a bioreactor to achieve microintegration, however, cell viability is of concern. One of the fundamental problems with ink-jet printing relates to the fibre-drawing force that is imposed upon the cellular materials as part of the fabrication process. This places significant physical forces upon the cells that may result in shearing or other physical changes. To date, there has been no comprehensive study performed of the short, medium and long-term effects of ink-jet printing on the key biological properties of the cells that would enable these concerns to be assuaged.

4.2.3.2 Electrospraying

Electrohydrodynamic jetting, also known as electrospraying, has the capacity to jet living cells under hostile electric field conditions without affecting their viability. [16-18] A charged polymeric liquid contained within a conducting needle is placed in an electric field with respect to a grounded or oppositely charged electrode. The potential difference between the electrodes leads to the formation of a liquid cone at the needle exit with a jet emanating from its apex. The breakdown of this jet into a 3D conical spray plume generates droplets. To achieve precision placement of these droplets, the jet must be stable and a Taylor cone formed. Electrospraying is a true 3D printing technology and requires no moulds or other types of support during the forming stage. The inclusion of cells in the polymeric liquid being jetted is known as “bio-electrospraying”.

4.2.3.3 Electrospinning

Electrospinning is the sister technology to electrospraying. Whilst electrospinning promotes the formation of droplets, electrospinning promotes a uniaxial elongating effect on the jet that leads to the formation of a continuously elongating thread from the jet. [19] Electrospinning can be used to generate large quantities of fibres with diameters in the nanometer range, having been spun from single, coaxial or tri-needle systems. [20-22] These fibres are able to form scaffolds and fibres. There is a tremendous capacity to control the alignment of fibres upon deposition, which allows the generation of scaffolds with directionally graded fibres as a function of scaffold depth. Scalability is readily achieved and the costs are, for the most part, determined by the polymer used, as bioreactors are not required to integrate the different cellular layers. With the inclusion

of cells in the polymer, biologically active threads can be produced. This modification of the technique is known as “cell electrospinning”. Cell electrospinning and bio-electrospraying are exceptionally biofriendly processes. The viability and other key biological properties of the cells are maintained during jetting because whilst high voltages (of the order of thousands of volts) are used, it is the current that is the effecting parameter and the currents in cell electrospinning are in the nanoampere range. Cell electrospinning it is currently being developed to create scaffold architectures for a whole host of novel bioengineering and medical applications.

4.2.4 Is Cell Electrospinning Really the Answer?

Scaffolds are an incredibly important consideration in regenerative biology and medicine as they provide the much-needed support that cells require to carry out their normal functions. [11] It has been clearly demonstrated that there are significant effects on the ability of cells to undergo repair, regeneration and rejuvenation and that cells have changes in both phenotype and genotype when they lack a sufficient supporting scaffold. The post-processing coupling of cells with a scaffold fails to create a true 3D, fully-microintegrated scaffold and the generation of a fully functional, cost-effective 3D biological structure enabling an integrated cell-scaffold architecture is both the key goal of tissue engineering strategies and something that is readily achieved through cell electrospinning. [23]

Is cell electrospinning the way to achieve this? Four lines of reasoning suggest that it is a reasonable means by which to do so. Firstly, conventional electrospinning using biopolymer suspensions devoid of cells creates structures with extremely small pores that cells are unable to infiltrate. Ironically, this has found applicability as a means of creating drug-eluting fibres that the smallest self-propelling human cell is unable to pass through, making it a perfect means to serve as an innovative platform technology for HIV-1 protection and sperm inhibition. [11,24] Secondly, it is difficult to produce 3D structures if there is no matrix between the cells to provide the necessary support for such an architecture. Thirdly, the post-processing seeding of scaffolds with cells is problematic. Cells are unable to remodel rigid scaffolds to generate the functional equivalent of an extracellular matrix, whereas they can remodel friendly biopolymers. Finally, with an appropriate experimental design, cell electrospinning is relatively inexpensive as it can be used to create sophisticated 3D scaffolds that do not require extensive periods of time in a bioreactor to ensure microintegration of cells with the scaffold architecture. Cell electrospinning, therefore, can produce structures that mimic native tissues whilst overcoming the limitations of producing integrated cell-scaffold architectures by other means.

4.3 Cell Electrospinning: From Concept To Lab

Whilst electrospinning has been around for over a century, the technique first emerged as an advantageous method, from a commercial standpoint, with Anton Formhals's 1934 patent of a process and apparatus for preparing artificial threads and filaments by passing solubilised materials into an electric field formed between electrodes and collecting the resulting threads on a device in a form that would be of technical use. [25-31] The evolution of "electrospinning" into "cell electrospinning", whereby cell suspensions are used to produce cell-laden fibres and scaffolds, can be traced through a review of publications in the PubMed database, an online search engine used to query the more than 27 million citations for biomedical and bioengineering literature from MEDLINE (the U.S. National Library of Medicine bibliographic database), life science journals and books. Figure 4.1 shows the results, by year of publication, obtained by querying the PubMed database using the search term "cell electrospinning" (including the variant "cell electro spinning"). This is an approach that can prove informative for the identification of points at which significant events impact upon a new technology. Such events may include technological advances, reduction of costs or a widespread adoption of the technology, which contributes significantly to the knowledge base.

From first use of the term in 2001 to 2005, 60 publications using the term "cell electrospinning" are identified. Two thirds of these papers describe the development of biodegradable or biocompatible polymer scaffolds suitable for use in tissue engineering as synthetic matrices to be seeded with cell types of interest or were publications focusing on the production of nanofibres containing biologically active molecules. The remaining third describes the production of nanofibres containing biologically active molecules. None of the work published during this timespan describes the production of cell-laden fibres, which is the definition of cell electrospinning that was presented at the start of this chapter.

[Figure 4.1 near here]

Up until this point, the ability to produce cell-laden fibres had been hampered by the fact that the conditions of high viscosity and low conductivity that were needed to achieve the spatial distribution and resolution of which the technology is capable are diametrically opposed to the requirements of low viscosity and high conductivity that are needed for viability of the cells. Initial attempts to microintegrate cells by electrospinning polymer and electrospraying cells encountered difficulties with non-uniformity of cellular integration. [32] These difficulties, however, were overcome by applying the coaxial concentric needle arrangement used to achieve precision placement of electrosprayed cell suspensions. [33] This approach uses

the biopolymer to generate the properties needed for spraying in stable cone-jet mode whilst simultaneously shielding the highly conducting cell suspension from the external electric field. The first scientific paper describing the generation of active biological microthreads/scaffolds from living cells using electrospinning was published in 2006. [34] A sharp increase in the rate of publications using the term "cell electrospinning" ensued, with almost 500 papers published in 2016 - the last complete year for which data are available.

The initial cell electrospinning work had been performed using an immortalised cell line. [34] The technique was then successfully carried out using primary cells, proving the suitability of cell electrospinning for cells of the type used in cell and gene therapy applications. [35] These findings were confirmed the following year, firmly cementing cell electrospinning as a feasible technology with which to address wide variety of biomedical and clinical applications. [36] Cell electrospinning has been used to process a wide variety of intact eukaryotic organisms, including yeast (*Saccharomyces cerevisiae*), nematodes (*Caenorhabditis elegans*), soil-based amoebas (*Dictyostelium discoideum*) and fruit fly (*Drosophila melanogaster*), frog (*Xenopus tropicalis*) and zebrafish (*Danio rario*) embryos, and tissues generated by cell electrospinning have been successfully transplanted into mice, *in vivo*, demonstrating the viability of this technology for the production of engineered tissues that have the biocompatibility needed for successful transplantation into their intended host. These studies revealed the promise of cell electrospinning as a viable technology with significant commercial prospects. [22,37-43]

4.4 Cell Electrospinning: From Lab to Market

At present, there is neither a commercial product on the market nor a medical therapeutic in the clinic that has been derived from cell electrospinning and, to date, only two cell electrospinning patents have been filed. [44,45] Is this really surprising? As Figure 4.1 shows, it is only within the last decade that cell electrospinning has been demonstrated to generate cell-laden fibres at the scale and with the properties needed for the engineering of functional tissues. It is of interest, therefore, to appreciate how the timelines involved in the development of cell electrospinning from concept to commercialisation compare with such timelines for other technologies.

Whilst there is a relatively poor quantity of information from the commercialisation of other technologies with which to compare, when evaluating the elapsed time between the development of a new technology and its commercialisation, studies from the energy sector have shown that innovations to replace existing products have an average time of 29 years from invention to widespread commercialisation. This contrasts

significantly with the 42 year average in this sector that is needed to realise commercialisation for innovation directed at entirely new markets in this sector. Taken in this context, cell electrospinning is not lagging with respect of the time elapsed between invention of the technology and its widespread commercialisation. [46]

Interestingly, cell electrospinning is a technology used for innovations that is directed at entirely new markets. The time elapsed since the major obstacles preventing its use for precision placement were first identified and addressed is of the order of a decade. It is not surprising, therefore, that additional endeavour remains before the commercial advantages envisioned by Formhals can be fully realised. In fact, the timeline from innovation to commercialisation in the energy sector suggests that a further decade or two remains before widespread commercialisation of cell electrospinning applications can be expected, if comparison with the innovation to commercialisation timelines in the energy sector prove to be informative.

Cell electrospinning is a disruptive technology, as it is a technology possessing the capacity to displace existing medical technologies; it disrupts existing pharmaceutical and medical approaches to the treatment of ageing tissues and organs and creates a new market by which this can be achieved. The stumbling blocks currently preventing cell electrospinning products from finding a successful commercial end point can be attributed to two main challenges: overcoming the remaining technical difficulties and the appropriate interrogation of cell electrospun materials prior to use.

4.4.1 Further Technical Refinements

There are at least two technical considerations that may impact upon the commercialisation prospects of cell electrospinning: these are 1) ensuring orientation of the deposited nanofibres and 2) the choice of polymer.

4.4.1.1 Orientating Nanofibre Deposition

One of the significant disadvantages encountered in cell electrospinning occurs when the stacked layers of nanofibre forming the 3D biological architecture under construction cause interference with the electric field, leading to the random deposition of electrospun nanofibres. [47] The use of electric field-focusing plates and rings has removed this interference-based limitation, achieving alignment of the electrospun nanofibre by deflecting the jet. [48-49] This limitation has only been overcome within the past 2-4 years and it is expected that its widespread adoption will dramatically shorten the time remaining until the first commercial

application of cell electrospinning is achieved.

4.4.1.2 Choice of Polymer

A wide range of polymers has been used in cell electrospinning, including both natural and synthetic polymers. [47] Many of the cell electrospun tissues under development are intended for use in humans and there has been a great deal of interest in the use of poly(vinyl alcohol) (PVA) as a polymer for cell electrospinning, as this material has been approved by the Food and Drug Administration (FDA) and other regulatory organisations for use in humans. [50] Sodium trimetaphosphate cross-linked PVA-based hydrogels have been shown to possess mechanical properties compatible with circulatory flow, making them of great interest for use in vascular replacements. [51] Unfortunately, PVA has been shown to deter cell attachment, leading to cell death. [52] The poor viability of cells electrospun using PVA solutions has subsequently been confirmed. [53,54]

Effective polymers for cell electrospinning must possess a number of key features, including: 1) the ability to accommodate cells in a manner that provides the functionalities essential to cell viability; 2) the ability to undergo spinning under stable conditions; and, 3) the ability to facilitate some for of solidification or cross-linking that holds cells in place without changing their cellular properties over time. The cell electrospun structures successfully tested *in vivo* were produced using cell-friendly biopolymers, which may suggest that the successful commercial application of cell electrospinning is more likely to be achieved with the use of biopolymers, rather than with chemical polymers. [22,43]

We have explored this possibility further by using a cell electrospinning set up immersed in a cell-friendly media bath (see Figure 4.2a,b) together with a thermosensitive biopolymer to generate a living vessel-like architecture over six inches in length that is comprised of an internal endothelial cell layer surrounded by a smooth muscle cell layer (see Figure 4.3). By using a horizontal rotational mandrel, the length of the tubular vessel-like architecture is defined by the length of the mandrel and its diameter is determined by the bore diameter of the mandrel, making it possible to build bespoke vessels of any defined length and diameter.

[Figure 4.2a,b near here]

[Figure 4.3 near here]

4.4.2 Interrogation of Cell Electrospun Structures

Studies of cell electrospinning have almost always included some measure by which to assess cell viability. Other properties that have been interrogated in processed and unprocessed cells for use as a measure of cellular function post electrospinning/electrospraying, include: phenotypic studies, cell cycle characterisation, determination of the rate of cellular proliferation, change in the rate of apoptosis/necrosis, karyotyping, measurement of the activation of cell surface receptors including G protein-coupled receptors and potassium channels, proteomic and transcriptomic analyses. [49,55]

Interrogations of structures made using cell electrospinning have been confined to a determination of key functional properties at the molecular and cellular levels. To date, there has been no testing of the mechanical properties at the tissue/structural level. For example, the muscle tone of the vessel-like structure shown in Figure 4.3 will need to be interrogated using organ bath studies to determine the pharmacological response to compounds known to elicit contraction and relaxation of vascular smooth muscle, with characterisation of the time course and tension of the muscle tone responses and tests of fluidics with simulated fluids and using blood will provide a richer measure of its functional and mechanical properties. Quantification of the range of values for key properties of engineered tissues, such as the one shown in Figure 4.3, will need to be conducted before being able to conclude that what looks like a tissue actually behaves like a tissue, something that needs to be determined before one can contemplate replacing native tissue with an engineered tissue *in vivo*.

4.4.3 Getting to Market

Taken together, the identification and application of solutions for addressing the dual challenges of providing the additional necessary technical refinements and achieving an effective interrogation of the key properties of structures should rectify the issues that have prevented commercial/clinical applications of this technology from being achieved, to date. Whilst the former has been accomplished, a standardised protocol for the latter remains to be implemented. The patient outcomes from the clinical application of a separate tissue engineering technology have unequivocally demonstrated that it is essential that the functional and mechanical properties of tissue-engineered architectures be shown to lie within the expected parameters of native tissues prior to any clinical use of the materials.

In 2011, the world's first bioengineered tracheae were transplanted in patients. The failure to test that the mechanical and functional properties of engineered trachea were equivalent to those of normal human trachea had a catastrophic outcome for those patients into whom these tissues were grafted. In 2016, a Swedish Public Television (SVT) team was awarded the AAAS Science Journalism Gold Award for Television In-Depth Reporting for their documentary on the patient outcomes of this clinical trial. [56]

As a direct consequence, attention now needs to be placed on defining the engineering parameters needed to ensure that the mechanical and functional properties of engineered tissues are equivalent to those of their native counterparts before commencing any functional replacement *in vivo*. Definition of the required parameters and testing necessary for validation of tissues engineered for therapeutic use will enable the field to successfully move from the functional/mechanical perspective of the engineering question to the translational perspective of bespoke therapeutic treatment in the clinic. As in the case of the tissues engineered for xenotransplantation (please see section 5.1), international consensus will be key in establishing protocols appropriate for defining the clinical suitability of engineered tissues.

4.5 Future Developments & Conclusion

Future developments in the field of cell electrospinning include exploitation of personalised medicine strategies, which includes consideration of the inclusion of experimental and/or medical cell and gene therapy in the tissue engineering workflow to create tissues on demand and to meet patient need. As mentioned previously, this will enable the promise of personalised medicine to be genuinely fulfilled. An area that has not yet been significantly discussed and one that holds great promise for commercialisation is that of the use of engineered tissues to create templates for modelling 3D systems *in vitro*. This is an innovation that will enable the creation of 3D models of human diseases and permit drug and vaccine development and screening. Human tuberculosis is currently being modelled in this way using a bioengineered 3-dimensional model created by bio-electrospraying. Cell electrospinning could be used to do exactly the same. [57] Reduction in animal use is a key strategic priority embedded in all research funding applications in the UK and elsewhere, globally. In the UK, the Animals (Scientific Procedures) Act 1986 embeds the "three R's" (replacement, refinement and reduction), to ensure that animal research is carried out only where no practicable alternative exists. [58] Adoption of 3D models built using cell electrospinning will lead to a reduction in animal studies.

Cell electrospinning not only integrates cells and scaffolds at point of delivery, it brings together state of

the art technologies in the life and medical sciences to deliver a platform technology able to contribute the key advances required for the support of human health throughout the ageing process and on a scale that has not previously been achieved. Key considerations needed for the successful application of cell electrospinning in a commercially-relevant context can be reduced to two critical elements: 1) defining the suitable biopolymers within which to suspend the cells and 2) ensuring that rigorous testing is carried out prior to the replacement of diseases or damaged tissues with engineered structures that are truly equivalent to the native tissues they are intended to replace.

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References

1. http://www.who.int/mediacentre/factsheets/fs310/en/
2. https://www.cdc.gov/nchs/data/dvs/lead1900_98.pdf
3. https://www.medicalnewstoday.com/articles/282929.php
4. D.K.C. Cooper, *Proc. (Bayl. Univ. Med. Cent.)*, 2012, **25**(1), 49
5. B. Ekser, P. Li and D.K.C. Cooper, *Curr. Opin. Organ Transplant.*, 2017, **22**(6), 513.
6. C.L. Cetrula, *Stem Cell Rev.*, 2006, **2**(2), 163.
7. A. Rosemann, *J. Cell. Biochem.*, 2014, **115**(12), 2073.
8. R.S. Mahla, *Int. J. Cell Biol.*, 2016, 6940283.
9. http://apps.who.int/trialsearch/
10. A.S. Bassett, D.M. McDonald-McGinn, K. Devriendt, M.C. Digilio, P. Goldenberg, A. Habel, B. Marino, S. Oskarsdottir, N. Philip, K. Sullivan, A. Swillen, J. Vorstman and The International 22q11.2 Deletion Syndrome Consortium, *J. Pediatr.*, 2011, **159**(2), 332.
11. S.N. Jayasinghe, *Adv. Biosys.*, 2017, **1**, 1700067.
12. J. Vein, *US Pat.*, 6 835 390.
13. S. Wadsworth, G. Klaric and S.N. Jayasinghe, *Nor. Patent*, WO2016195509.
14. K. Nair, M. Gandhi, S. Khalil, K.C.Yan, M. Marcolongo, K. Barbee, and W. Sun, *Biotechnol. J.*,

2009, **4**, 1168.
15. G.M. Nishioka, A.A. Markey and C.K. Holloway, *J. Am. Chem. Soc.*, 2004, **126**, 16320.
16. S.N. Jayasinghe, A.N. Quershi and P.A.M. Eagles, *Small*, 2006, **2**, 216.
17. S.N. Jayasinghe, P.A.M. Eagles and A.N. Quershi, *Biotechnol. J.*, 2006, **1**, 86.
18. P.A.M. Eagles, A.N. Quershi and S.N. Jayasinghe, *Biochem. J.*, 2006, **394**, 375.
19. C.A. Bashur, L.A. Dahlgren and A.S. Goldstein, *Biomaterials*, 2006, **27**, 5681.
20. A. Townsend-Nicholson and S.N. Jayasinghe, *Biomacromolecules*, 2006, **7**, 3364.
21. S.N. Jayasinghe, S. Irvine and J.R. McEwan, *Nanomedicine*, 2007, **2**, 555.
22. A.N. Jayasinghe, G. Warnes and C. Scotton, *Macromol. Biosci.*, 2011, **11**, 1364.
23. M.J. Bissell and W.C. Hines, *Nat. Med.*, 2011, **17**, 320.
24. C. Ball, E. Krogstag, T. Chaowanachan and K.A. Woodrow, *PLos One*, 2012, **7**(11), e49792.
25. J.W.G. Rayleigh, *Edinburgh Dublin Philos. Mag. J. Sci.* 1882, **14**, 184.
26. J.F. Cooley, *US Pat., 692 631*, 1902.
27. J. Zeleny, *Phys. Rev.*, 1914, **3**, 69.
28. J. Zeleny, *Proc. Camb. Philos. Soc.*, 1915, **18**, 71.
29. J. Zeleny, *Phys. Rev.*, 1917, **10**, 1.
30. G.I. Taylor, *Proc. R. Soc. A*, 1964, **280**, 383.
31. A. Formhals, *US Pat.*, 1975504
32. J.J. Stankus, J. Guan, K. Fujimoto and W.R. Wagner, *Biomaterials*, 2006, **27**(5), 735.
33. S.N. Jayasinghe and A. Townsend-Nicholson, *Lab Chip.*, 2006, **6**, 1086.
34. A. Townsend-Nicholson and S.N. Jayasinghe, *Biomacromolecules*, 2006, **7**, 3364.
35. S.N. Jayasinghe, S. Irvine and J.R. McEwan, *Nanomedicine*, 2007, **2**(4), 555.
36. J.A. van Aalst, C.R. Reed, L. Han, T. Andrady, M. Hromadka, S. Bernacki, K. Kolappa, J.B. Collins and E.G. Loboa, *Ann. Plast. Surg.*, 2008, **60**(5), 577.
37. D. Greig and S.N. Jayasinghe, *Biomed. Mater.*, 2008, **3**(1), 34125.
38. T. Geach, N. Mongkoldhumrongkul, L.B. Zimmerman and S.N. Jayasinghe, *Analyst*, 2009, **134**, 743.
39. D.W. Clark and S.N. Jayasinghe, *Biomed. Mater.*, 2008, **3**(1), 11001.
40. P. Joly, B. Hennings and S.N. Jayasinghe, *Biomicrofluidics*, 2009, **3**(4), 44107.
41. N. Mongkoldhumrongkul, S.C. Swain, S.N. Jayasinghe and S. Sturzenbaum, *J. R. Soc. Interface*, 2010, **7**, 595.
42. N.K. Pakes, S.N. Jayasinghe and R.S.B. Williams, *J. R. Soc. Interface*, 2011, **8**, 1185.
43. S.L. Sampson, L. Saraiva, K. Gustafsson, S.N. Jaysinghe and B.D. Robertson, *Small*, 2014, **10**(1), 78.
44. L. YuanYuan, J. Chen, X. Ke, L, Shuai, Z. Ting, H. Qingxi, 2015, *China Pat.*, CN104984405 (A)

45. L. YuanYuan, J. Chen, L. Gang, Y. Hongchen, L. Yi, H. Qingxi, *China Pat.*, CN104862787 (A)
46. http://www.ukerc.ac.uk/asset/ADA12E92-C1DC-4033-8CFA63AC9EA9FE59.6B438A7E-474E-437E-89025FD302A10A7C/
47. R. Vasita and D.S. Katti, *Int. J. Nanomedicine.*, 2006, **1**(1), 15.
48. S.N. Jayasinghe, *Analyst*, 2013, **138**(8), 2201.
49. J. Walser and S.J. Ferguson, *J. Mech. Behav. Biomed. Mater.*, 2016, **58**, 188.
50. K. Saralidze, L.H. Koole and M.L.W. Knetsch, 2010, **3**, 3537.
51. M. Chaouat, C. Le Visage, W.E. Baille, B. Escoubet, F. Chaubet, M.A. Mateescu and D. Letourneur, *Adv. Funct. Mater.*, 2008, **18**, 2855.
52. Y. Liu, N.E. Vrana, P.A. Cahill and G.B. McGuinness, *J. Biomed. Mater. Res. Part B Appl. Biomater.*, 2009, **90**(2), 492.
53. M.F. Canbolat, C. Tang, S.H. Bernacki, B. Pourdeyhimi and S. Khan, *Macromol. Biosci.*, 2011, **11**, 1346.
54. G. Zanatta, D. Steffens, D.I. Braghirolli, R.A. Fernandes, C.A. Netto and P. Pranke, *Braz. J. Med. Biol. Res.*, 2012, **45**(2), 125.
55. A. Eddaoudi, A. Townsend-Nicholson, J.F. Timms, S. Schorge and S.N. Jayasinghe, *Analyst*, 2010, **135**, 2600.
56. https://sjawards.aaas.org/awards/2016-television-depthfeature-reporting-gold
57. L.B.Tezera, M.K. Bielecka, A. Chancellor, M.T. Reichmann, B. Al Shammari, P. Brace, A. Batty, A. Tocheva, S. Jogai, B.G, Marshall, M. Tebruegge, S.N. Jayasinghe, S. Mansour and P.T. Elkington. *eLife*, 2017, **6**, 321283.
58. Great Britain: Home Office, *Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes*, The Stationery Office, 2015.

Figure Captions

Figure 4.1 Number of publications, by year, in the PubMed database containing the term "cell electrospinning". I confirm that this is an original, previously unpublished, figure.

Figure 4.2 Schematic images (A) demonstrating the cell electrospinning equipment arrangement explored for generating cross stitched living scaffolds for fabricating living vessel architectures and (B) a cross-section of the finally generated cell-bearing construct. Reproduced from ref. 48 with permission from the Royal Society of Chemistry.

Figure 4.3 (A) Fluorescent image of a generated living vessel-like architecture. (B) an optical micrograph showing the cross-section. Scale bar in panel (A and B) are ~5mm and 1mm respectively. Panels (C and D) depict fluorescent images of the individual cell constituents within the two-core structure. Panel (E) represents the superimposed fluorescent image of the fabricated architecture. Scale bars in panels (C-E) represent 1mm. Reproduced from ref. 48 with permission from the Royal Society of Chemistry.

Figures

Figure 4.1

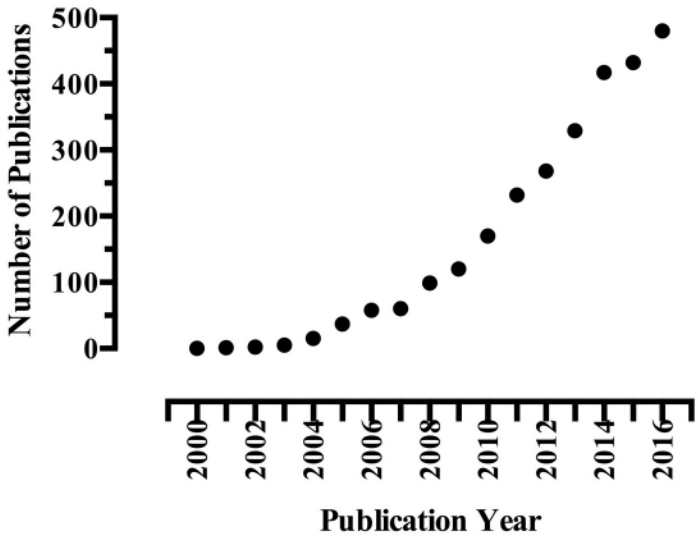

Figure 4.2

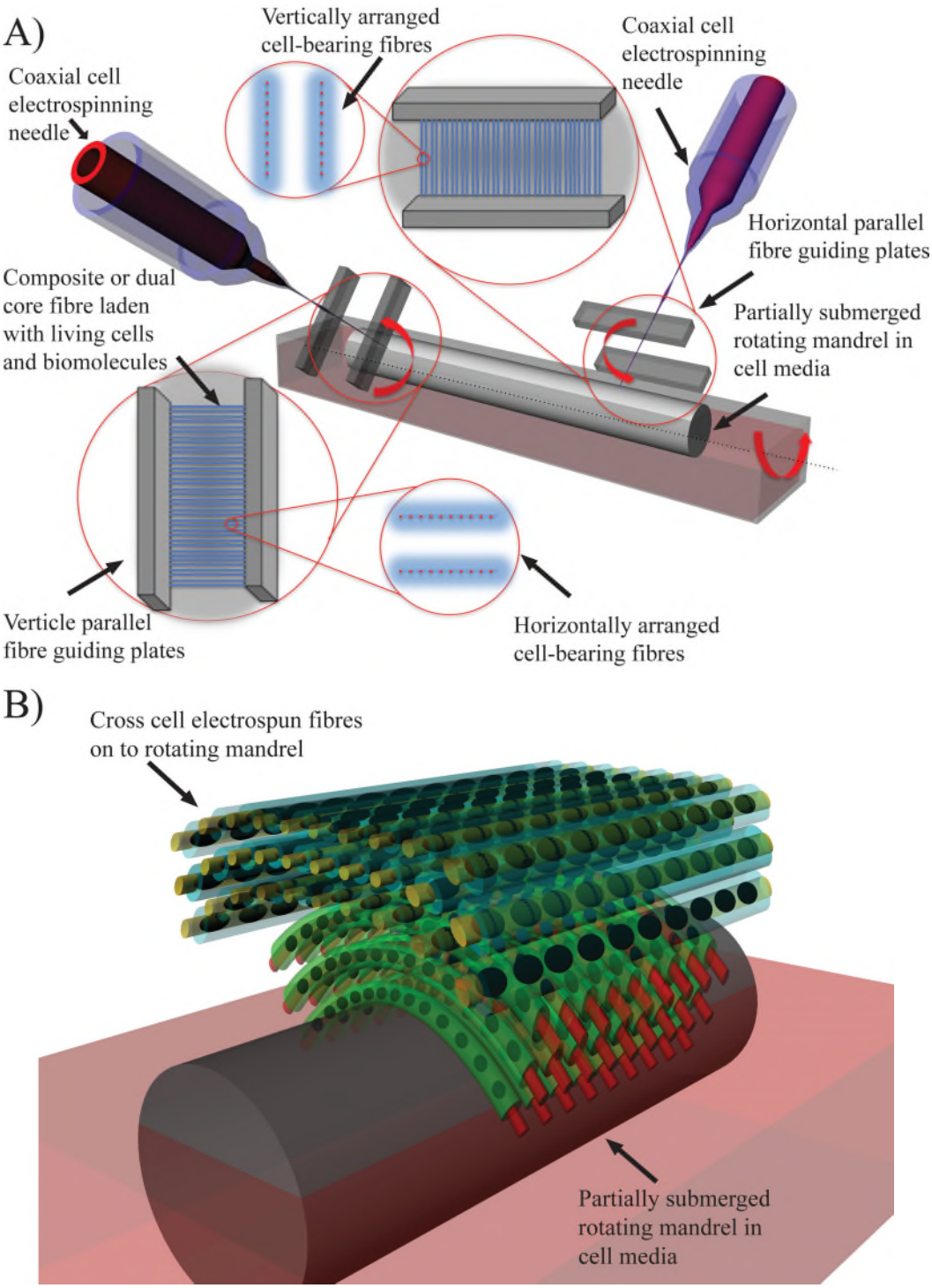
A)
Vertically arranged cell-bearing fibres
Coaxial cell electrospinning needle
Coaxial cell electrospinning needle
Horizontal parallel fibre guiding plates
Composite or dual core fibre laden with living cells and biomolecules
Partially submerged rotating mandrel in cell media
Verticle parallel fibre guiding plates
Horizontally arranged cell-bearing fibres
B)
Cross cell electrospun fibres on to rotating mandrel
Partially submerged rotating mandrel in cell media

Figure 4.3