Unspooling the history of cell electrospinning †

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This opinion article wishes to highlight the thoughts that led to the discovery of cell electrospinning. The author will briefly highlight the advantages this technology has over its competing technologies. In particular demonstrating cell electrospinning living vessel architectures, having all the primary cell types found in native vessels/arteries.

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Introduction

An ageing society sees a rising demand for donor tissues increasing significantly, this rise has caused the loss of life due to waiting times for tissues, to where transplanted donor tissues have been reported to either have deleterious defects to those side effects on patients, brought about by those requiring lifelong immunosuppressants. To avoid these scenarios, fields of research such as tissue engineering and regenerative medicine have promised hope, by the possibility to reconstruct tissues outside the human body. [1] These reconstructed tissues with the patient's own cells are hoped to eliminate rejection and the need for immunosuppressants. Therefore, this endeavour and holy grail spearheads the reconstruction of such tissues which are indistinguishable with the patient's own tissues, therefore transplanted for replacing, repairing, or rejuvenating damaged/ageing tissues/organs. The reader should note well that the author is sceptical with the sometimes-postulated vision/ideas found in the literature, where it is said that reconstructed whole organs could be explored for replacement. The author's reason for this stems from the idea that our organs are accustomed to our individual anatomies and their environments over our lifetime, and therefore replacing an entire organ should only be carried out, if and only if whole organ failure takes place or another critical scenario. Instead, the author believes in patching of existing damaged or dysfunctional organs which in itself is a significant undertaking. This challenge sees the unearthing of competing ideas from the merging of the life sciences with the physical sciences to exploit the established knowledge to facilitate the reconstruction of a three-dimensional tissue.

In this article the author will focus on the many methodologies and ideas which have promised the ability to reconstruct a cell baring three-dimensional tissue for transplantation, in particular focussing on vessel reconstruction. Reconstructing a tissue can be carried out in many ways, some of which are, namely a) the use of a mould, b) a scaffold to, c) the exploitation of additive

manufacturing. [2] The latter approach in the opinion of this author supersedes the first two, as making moulds to suite each patient would be costly, time consuming and a waste of materials. Additionally, if a mould was used with materials mixed with practical quantities of cells, during the formation process (cross linking etc), if not instantaneously formed, cell sedimentation would take place. On the other hand, if the process is instantaneous, then cells would undergo stresses, which would alter/damage them both at a molecular level or even kill the cells. These obstacles are even more pronounced if the requirement is to fabricate a multi-compartmentalised architecture such as a vessel having a given cell type in a given layer or compartment, not to mention the time taken to reconstruct. The second approach, using a scaffold requires cell seeding, which regularly is carried out manually which unfortunately is not uniform and requires the seeded scaffold to be subsequently left in a bioreactor. In this approach the seeded cells in a majority of scenarios have been found to be limited in cellularsing the full depth of the scaffold, to blocking pores which subsequently on transplantation have caused rejection due to foreign body reactions. [3] An interesting parallel challenge that arises during the repeated manually seeding to exposure to bioreactor time, is where sterility issues may arise. Similar to the first approach the second would also have several practical challenges to overcome when required to reconstruct a multicellular architecture (vessel to thick tissue), having compartmentalised multiple cell types. There are many other approaches the author has seen in the literature; however, these are either manifestations/retrofits of the first or second approaches of reconstructing a tissue. Therefore, the most desirable approach to fabricating any cellularised construct, would be where all the constituents found in a native tissue, could be mixed into a single suspension, and deposited when required in a desired pattern for reconstructing a living architecture. Finally forming a fully cellularised architecture requiring less bioreactor time and avoiding issues such as sterility etc.

The additive manufacturing methodologies explored for directly handling cells, range from 3D printing approaches, lab on chip (microfluidics) to electrospinning (electrospraying) or aerodynamically assisted jets/threads. Although 3D printing (also known as bioprinting when handling cells and/or biomaterials) was the first to be applied to this endeavour the technology has limitations which do not afford, its utility in the clinic. This is due to the process directly damaging the handled proteins and/or cells. [4] Similar in some respects microfluidics also has shearing effects as those processed cells as experienced by 3D printing and its manifestations. [4] Unfortunately, these limitations cannot be overcome and therefore these technologies will be seldom in their contribution to the clinic. In fact, these communities have now changed their focus from reconstructing whole tissues/organs for the clinic to constructing small scale tissues for testing drugs etc. However, as processable cell concentrations in multiple cell configurations possess significant issues, for these techniques one would deduce that these approaches have limitations and challenges in this endeavour.

Contrary to both previous methods electrospinning (electrospraying) or aerodynamically assisted jets/threads have been demonstrated to have the ability to handle highly concentrated cell suspensions containing multiple cell types to even whole fertilised embryos without damaging them from a molecular level upwards. [5] Therefore these techniques are the leading approaches for directly handling living cells for reconstruing a tissue. That being said the author will only focus on electrospinning in this article. Briefly, electrospinning is a fibre to scaffold generation approach established for over a century. [6] The scaffolds generated through this approach have been considered for engineering tissues but have not been successful. This is a direct result of the generated scaffolds having very fine pore sizes, thus hindering cell infiltration. The lack of cell infiltration gives rise to foreign body rejection by the host.

Interestingly this limitation has seen the repurposing of these scaffolds as an approach for facilitating fabrics/scaffolds offering dual defence against pregnancy and HIV. [7]

In early to mid 2004 we explored the ability to simultaneously electrospray living cells onto electrospinning scaffolds. Those studies demonstrated that a significant proportion of electrosprayed living cells as droplets were found to bounce off the electrospun scaffolds/mats. In addition, most cells were found to flow off the scaffold rather than be anchored to the scaffold where it was intended to be, on deposition. These observations were not desirable as cells/biomolecules are expensive materials, and wasting such material are not an option. Hence in late 2004 early 2005, we investigated whether electrospinning can directly handle living cells. Our thorough investigations demonstrated that electrospinning is capable of directly processing highly concentrated cell suspensions without damaging cells. Thus, the process was coined as "cell electrospinning". [8] The reader should note that although applied voltages in the range of kV's are applied, the associated currents are in the nanoamperes thus not effecting the cells (operation of which is similar to a taser gun but contrary to the electroporation process). Our initial studies explored a coaxial cell electrospinning needle with medical grade silicone oils. [8] Subsequent studies have seen the use of collagen, gelatine, alginates, pullulan, to many other biopolymers (hydrogel based) including PVA (with additives). Interestingly water soluble PVA unlike the other materials we have explored were found to reduce cells viability. On close examination of the cells within fibres and through the literature we found that PVA inhibits cell attachment and this introduces cells death. [9] Our studies with PVA have been seen to show that we could increase cell adhesion by mixing the PVA with other biological molecules like fibrogen etc. The reason for exploring a wide range of polymers was to allow this approach to enter the laboratory and the clinic, to finally simplify the cell electrospinning process by using a single needle configuration. Cell electrospinning can be carried out in

ambient condition (in a sterile hood) or in a submerged configuration (**Figures 1a and b**). These flexibilities enable this approach to compete in tackling this research obstacle, namely a methodology for directly handling living cells for reconstructing a fully cellularised three-dimensional functional tissue (either a thick flat or vessel like architecture). Throughout these developmental studies we have processed over 600 different human and animal cell types spanning from immortalised, primary to stem cells (also iPS) including whole fertilised embryos. [8] Those processed cells and whole organisms have been directly compared with control cells and organisms, which were indistinguishable. The reader should note that cell electrospinning (like bio-electrospraying) explores large bore needles, at least around 1000um (or bigger) in inner bore diameter, and yet is capable of generating micrometre (when handling cells) and nanometre (when handling biomolecules/nanomaterials) sized composite fibres. Here unlike all 3D printing (or Bioprinting) technologies the opposite of the Barus [10] effect takes place, primarily as the process is driven by an electric field.

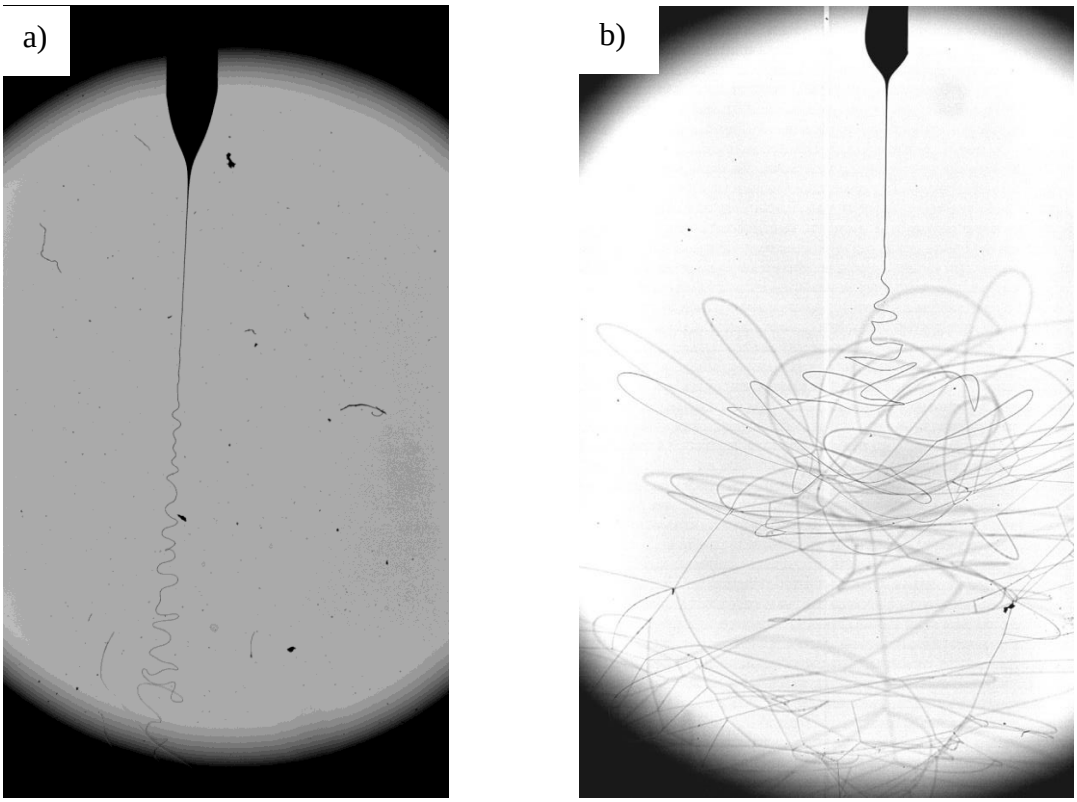

Figure 1. High speed digital images captured at 10000fps a) cell electrospinning in a class II safety cabinet and b) submerged cell electrospinning in a liquid bath. The needles used in these studies have an inner bore diameter of ~1500um.

The cell electrospinning equipment used in these vessel reconstruction studies were carried out using the previously reported equipment set up [8] and a modified version which saw the cell spinning needle(s) submerged. A wide range of applied voltages to flow rate were explored ranging from 5-25kV to 10^{-6}-$10^{-12}$$m^3s^{-1}$respectivly. Although in previous studies multiple cell types have been electrospun simultaneously (mixed and supplied by one syringe) in these studies a given needle accommodated a give cell type mixed in a biopolymer. We explored three cell types in three needles which were individually mixed with the biopolymer. The biopolymers explored in these needles ranges from alginates doped with collagen and other biomolecules to the use of modified pullulan-based biopolymers. The cell types used in these explorative studies were mouse endothelial, smooth muscle, and fibroblasts cells. Each of these cell types were labelled with a dye which enabled easy identification under a fluorescent microscope.

Mouse fibroblasts were labelled with e-GFP-containing fusion construct expressing lenti-vectors at multiplicity of infection (MOI 25) and were mixed in with an alginate-based suspension. The final suspension was at a cellular concentration of 10^7 cells/ml. The suspension was electrospun onto a partially submerged rotating mandrel which was in a calcium chloride bath. The cell electrospinning process was carried out for approximately 30-45 mins and found to form a vessel like architecture. After this time frame we allowed the vessel on the mandrel to further rotate for ~10-15 mins and was later removed from the mandrel using a pair of sterile forceps. Figure 2a represents a fluorescent cross-sectional image of the

cellular dense vessel architecture. Subsequently we labelled the mouse smooth muscle cells with dsTomato-expressing lenti-ventors at a multiplicity of infection 4. The smooth muscle cell suspension was at a similar concentration to the fibroblast density. The smooth muscle cell suspension was electrospun for approximately an hour and found to form a tubular structure. Once the suspension in the syringe had ended the rotating mandrel was stop and the fiberoblast suspension was electrospun on to the smooth muscle bearing vessel on the mandrel. This suspension was electrospun onto the mandrel for a similar time frame and once the syringe contents were empty the mandrel was allowed to rotate for ~10 mins and the resulting architecture was removed as previously and cross-sectioned. Figure 2b shows a fluorescent micrograph of the two-core cellular architecture containing the smooth muscle cells in the inner and the fibroblasts cells in the outer core.

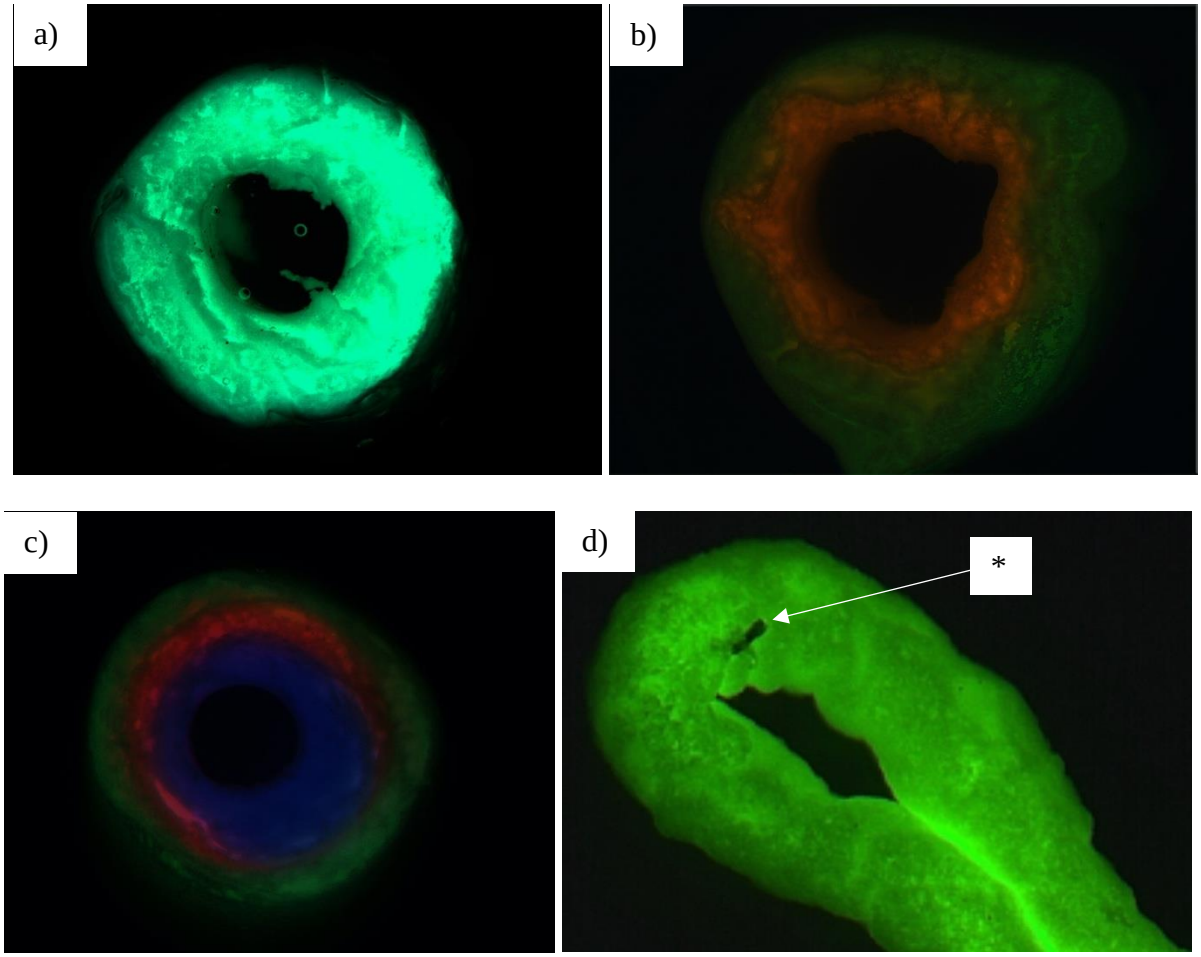

Figure 2. A representative cross-sectional fluorescent image of the a) fibroblasts rich vessel, b) two-core vessel architecture containing smooth muscle cells in the inner core and the outer core accommodating the fibroblast cells. Panels c) represents a characteristic fluorescent image of a cross-section of the three-core architecture containing endothelial cells in the inner core (blue), the smooth muscle cells in the intermediate layer (red) and in the outer core the fibroblasts (green). Panel d) demonstrates the three-core vessel as removed from the mandrel. The "*" indicates the sensor we inserted during the reconstruction process for the possibility of measuring a wide range of vessel flow characteristics (to others such as pressure, vessel flexibility etc).

As the intentions were to reconstruct a vessel/artery like architecture we harvested the mouse endothelial cells and labelled them with Hoechst 33342. As with the fibroblasts and the smooth muscle cells, a suspension as a concentration of ~10^7 cells/ml was prepared and electrospun for ~ 1hour. Similarly, once the endothelial cell suspension syringe was empty, we stopped the rotating mandrel and initiated the cell electrospinning of the smooth muscle cells. Once this suspension was completely spun onto the endothelial bearing vessel like architecture. the fibroblast suspension was electrospun for around the same time. After approximately 3hrs, the vessel like construct was removed, cross sectioned and imaged using fluorescence. Figure 2c-d) demonstrates a representative image of the three-core vessel architecture containing the individually compartmentalised cell types as a three-core vessel architecture.

Through these first studies we came to appreciate that both the single and two-core architectures post fabrication were found to be very soft in texture. These architectures could easily be reenforced with the spinning of intermitted stability enhancing polymers which would assist in strengthening such architectures. We are currently in the process of carrying this work out so that we would be able to pressure (burst) test such architectures prior to them being transplanted into mouse models. Those studies will also investigate the ability to fabricate human scale vessels which are over 6ft in length.

These studies demonstrate cell electrospinning's ability to directly reconstruct fully cellularized three-dimensional vessels/arteries. Note these vessel/artery architectures have not been exposed to any bioreactor time. We are currently in the process of studying these architectures and their ability to handle flow pressures both with and without exposure to bioreactor time, which in the latter case would allow the cells to remodel the architecture.

Several other aspects of these architectures are currently being studied and will soon be entering in-vivo mouse studies.

The author envisages such fully cellularised architectures would be most useful for human transplantation to the modelling of a wide range of disease (both human and animal). Other applications could see such reconstructs being used for screening, developing and delivering drugs to moving closer to personalised medicine, by developing disease specific designer therapeutic payloads

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References

1. Buehring G.C. (1990). Culture of mammary epithelial cells from Bovine milk. Journal of Dairy Science. 73, 956-963.
2. Weinberg C.B., Bell E. (1986). A blood vessel model constructed from collagen and cultured vascular cells. Science. 231, 397-400; Vaz C.M., van Tuijl S., Bouten C.V.C, Baaijens F.P.T (2005). Design of scaffolds for blood vessel tissue engineering using a multi-layering electrospinning technique. Acta Biomaterialia, 1, 575-582; Franz S., Rammelt S., Scharnweber D., Simon J.C. (2011). Immune responses to implants – A review of the implications for the design of immunomodulatory biomaterials. Biomaterials. 32, 6692-6709.
3. Cao, H., Mchugh, K., Chew, S.Y. and Anderson, J.M. (2010). The topographical effect of electrospun nanofibrous scaffolds on the in vivo and in vitro foreign body reaction. J. Biomed. Mater. Res., 93A: 1151-1159.
4. Nishioka G.M., Markey A.A., Holloway C.K., (2004). Protein Damage in Drop-on-Demand Printers, Journal of the American Chemical Society 126 16320-16321; Ning L., Betancourt N., Schreyer D.J., Chen X. (2018). Characterization of Cell Damage and Proliferative Ability during and after Bioprinting. ACS Biomaterials Science & Engineering 4, 3906-3918; Regmi, S., Fu, A. Luo, K. (2017). High Shear Stresses under Exercise Condition Destroy Circulating Tumor Cells in a Microfluidic System. Sci Rep 7, 39975.
5. Jayasinghe S.N. (2013). Cell electrospinning: a novel tool for functionalising fibres, scaffolds and membranes with living cells and other advanced materials for regenerative biology and medicine_Analyst, 138, 2215-2223; Jayasinghe S.N. (2011). Bio-electrosprays: from bio-analytics to a generic tool for the health sciences. Analyst, 136, 878-890.
6. Cooley, J. F., US Patent 692,631, 1902; Morton, W. J., US Patent 705,691, 1902.

7. Ball C, Krogstad E, Chaowanachan T, Woodrow KA (2012) Drug-Eluting Fibers for HIV-1 Inhibition and Contraception. PLOS ONE 7(11): e49792.
8. Townsend-Nicholson A, Jayasinghe SN. (2006). Cell electrospinning: a unique biotechnique for encapsulating living organisms for generating active biological microthreads/scaffolds. Biomacromolecules. 7, 3364-3369.
9. Liu Y, Vrana NE, Cahill PA, McGuinness GB. (2009). Physically crosslinked composite hydrogels of PVA with natural macromolecules: structure, mechanical properties, and endothelial cell compatibility. J Biomed Mater Res B Appl Biomater. 90, 492-502.
10. Guillet, J., Seriai, M. (1991). Quantitative evaluation of extrudate swell from viscoelastic properties of polystyrene. Rheola Acta 30, 540–548.