Prospectus

Novel Microfluidic Device for Neural Stem Cell Encapsulation
(Technical Topic)

Evaluating the Success of Medtronic's Infuse Bone Graft
(STS Topic)

By

Timothy Boyer

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On my honor as a University student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments.

Signed: _____Timothy L. Boyer_____

Approved: _____ Date _____
Dr. Benjamin Laugelli, Department of Engineering and Society

Approved: _____ Date _____
Dr. Christopher Highley, Department of Biomedical Engineering and Chemical Engineering

Introduction

The blockage of blood flow in the brain, or ischemic stroke, is an untreatable disease with a high prevalence worldwide. As of today, the regeneration of neural tissue is not possible following a stroke, a disease which was estimated to directly affect 13.7 million people in 2016 (World Stroke Organization, 2016). In 87% of those cases, when a blood vessel in the brain is blocked by a embolus, or blood clot, the surrounding tissue cannot receive proper oxygen supplied by red blood cells and dies. Currently, the most effective treatment is preventative; medical professionals break up the embolus with intravenous fluid. However, this treatment must be done within a few hours and is only given to between 1.8% to 5.2% of stoke patients (de los Ríos la Rosa et al., 2012). Unfortunately for most patients, neurons undergo apoptosis, a type of cell death, following ischemic stroke (Radak et al., 2017).

The potential stem cell therapies have towards advancing tissue engineering is unequivocal. Even before the year 2000, research suggested immature stem cells can differentiate into replacement neurons after an ischemic stroke (Snyder et al., 1997). Over the past decade, criteria essential for successful neural stem cell therapeutics has been established. One of these criteria involves encapsulating neural stem cells in a biocompatible polymeric layer to evade the immune system. To address this problem, my team is developing a novel microfluidic device that encapsulates stem cells and then separates them from excess polymer in an all-in-one process. Utilizing the physics of inertial fluid flow, our microfluidic device will supersede current encapsulation practices by reducing the forces applied to stem cells, the amount of non-encapsulated cells, and the time spent in lab.

Although somewhat counterintuitive, stem cells therapies to treat ischemic stroke requires much more than stem cell research. Despite more than twenty years of research on this

problem, there are still no approved stem cell therapies for stroke. In fact, the discrepancy between any stem cell research and commercially available stem cell products is remarkable. Although more than $10 billion dollars was spent on research and development pertaining to stem cell therapeutics in 2017, only one stem cell company was reported to be in the commercial phase as of March 2018 (Kim, Yu Seon et al., 2019). By illustrating the rise of one of the only financially successful stem cell-utilizing products, Medtronic's Infuse™ Bone Graft, I offer that understanding the social factors involved in stem cell therapeutics development is an important consideration for its nation-wide lack of commercialization.

In effect, knowledge about how stem cell products can be successful in the marketplace combined with microfluidic innovation can be used to better bring therapeutics to industry to treat ischemic stroke and other unmet needs. Developing a stem cell product to effectively treat ischemic stroke is sociotechnical in nature. Using elements of Pinch and Bijker's Social Construction of Technology (SCOT) (Johnson, 2005), I will demonstrate how relevant stakeholders influence stem cell product commercialization through a case study of Medtronic's Infuse™ Bone graft.

Technical Problem

The use of stem cell therapies faces a long road to be clinically relevant in modern medicine. While having the potential to regenerate neurons after ischemic stroke, stem cells must evade the body's immune system. To avoid this, stem cells are encapsulated in multiples layers of different polymers that protect it from immune system rejection (Krishnan et al., 2014). The tissue-engineering Highley Lab at the University of Virginia currently performs encapsulation on neural stems cells by incubating the cells in the desired polymer layer, using a centrifuge to spin

at very high revolutions per minute, and removing the excess waste via aspiration. However, this process can be time consuming, result in the loss of cells due to aspiration, and worst of all alter cell behavior due to excessive rotational forces applied to cells (Ferraro et al., 2011).

The goal of the technical problem is to develop and design a novel, advanced microfluidic device for stem cell encapsulation. This device requires the combination of three major parameters: layer by layer encapsulation, size-exclusion separation of excess polymer, and concentration of resulting polymer for reuse. To do this, we will develop a microfluidic device drawing on current inertial and cross-flow microfluidic research.

Uniquely, inertial microfluidic devices by themselves allow for both encapsulation and separation. By trapping and focusing particles in vortices, inertial devices have been shown to have the potential to more quickly encapsulate substrates to cell surfaces than standard encapsulation protocols (Mach et al., 2011). Furthermore, inertial methods allow for separation of particles based on size, density, and fluid flow speed (Figure 1A). However, cells must be further concentrated after encapsulation. Thus, our novel device adds a cross-flow microfilter technique (Figure 1B) for concentrating and enhancing the separation of the remaining polymer from neural stem cells. Meanwhile, by concentrating polymers away from fluid solution and cells, the cross-flow microfilters will allow the reuse of those polymers for future encapsulation.

By incorporating inertial incubation chambers in a microfluidic chip along with purifying cross-flow filters instead of using centrifugation, our novel device provides a significant advantage by automating the stem cell encapsulation process. Furthermore, by eliminating intermediate steps, our innovative device significantly reduces the amount of time spent in the lab. If successful, our device will establish a foundation for the creation of similar devices that

accelerate the process of encapsulating stem cells for the treatment of stroke and other pathologies.

To demonstrate our novel microfluidic design is efficacious for advancing the fabrication of stem cell encapsulation processes, we will evaluate encapsulation efficiency, recovery rate, and separation efficiency of the microfluidic device with fluorescent flow cytometry, hemocytometry, fluorescence spectrometry, respectively. Such comparisons will inform further iteration of the device and provide validation of the efficacy of our method compared to current methods.

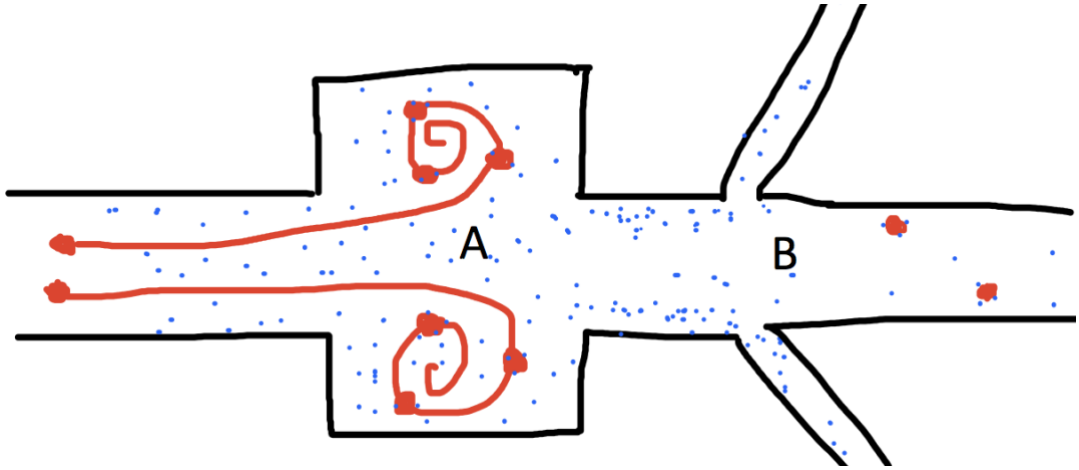

Figure 1: Schematic of proposed microfluidic device. (A) Cells are trapped in microvortices that form when microchannel widths rapidly increase. Under fast fluid flow, cells and polymer particle are trapped in vortices. When fluid flow is slowed, polymer solution leaves first until further slowing when both polymer and cells leave the inertial chamber. (B) For further sorting, smaller particles (polymer) concentrated at the walls, are permitted to flow through the crossflow microchannels while encapsulated cells flow through the main chamber.

STS Problem

Since being approved by the Food and Drug Administration (FDA) in 2002, Medtronic's Infuse™ bone graft has had its share of successes and controversy. In 2011 alone, Medtronic exceeded $750 million in sales for Infuse™, as it was used in 100,000 spinal-fusion procedures (Mauney 2020). As of 2020, Medtronic's product alone dominates with a 90% share in the global bone morphogenic protein market (Mauney 2020). Infuse™ is a synthetic scaffold or supportive material for stem cells to attach to and thrive on in an attempt to rebuild bone. The product uses recombinant human bone morphogenetic protein-2 (rhBMP-2) to attract naturally-occurring stem cells, cause them to differentiate into bone cells, and fuse vertebrae in spinal neurosurgeries (Skovrlj et al., 2014). Medtronic's dominance within the field of spinal fusion is not solely a result of the technological innovation. Through an examination of its development, it would be naïve to suggest the success of Infuse™ is independent of the various relevant stakeholders. I argue that the product's success can be largely attributed to its social influences.

Even before Infuse™, in some of the first studies evaluating the efficacy of rhBMP-2, research was funded by Medtronic (Boden et al., 2000). For the next six years after FDA approval in 2002, there were at least 38 reports of negative side effects of Infuse™ including infection, scar formation, allergic reactions, and life threatening swelling in the neck (Skovrlj et al., 2014). After the FDA mandated further testing on rhBMP-2 in 2008, Medtronic was accused of bribing and heavily influencing doctors to use Infuse™ despite negative consequences in some cases (Armstrong et al., 2008). In 2011, an analysis of the safety and effectiveness of rhBMP-2 at Yale was sponsored with $2.5 million by Medtronic. Even though it was found in 2013 that Infuse™ did not provide a significant advantage over other bone graft products (Simmonds et al., 2013), Infuse™ is still used today.

I argue that it is not the innovate aspect of Infuse™ that has made it the most economically successful spinal fusion biomaterial, but it has thrived due to Medtronic's strong influence towards research, clinical trials, and doctors. Due to the clandestine nature of bribery and intellectual property, these factors may often be overlooked. Additionally, as suggested by Hollister, 2009, the different agendas of stakeholders in industry versus those in academia inherently allows products developed in industry to be much more commercially available than those driven in academia. Specifically, an academic approach to innovative therapeutics rarely focuses on whether or not their devices will pass FDA regulation, and there is not a significant enough advantage for researchers to perfect a technique or process for eventual FDA approval (Hollister, 2009). While direct stem cell therapies for spinal fusion would be a class III device, the Infuse™ technology is class II because it uses the body's natural stem cells as opposed to injecting stem cells. Class II devices cost less and require much less testing, and it is clear that it is in Medtronic's best interest to shape a technology that meets FDA's class II requirements as opposed to class III. As a consequence of understanding the success of Infuse™, I intend to illustrate that for stem cell therapies researched in an academic setting to be clinically relevant, their designs must consider the stakeholders: the influence of doctors, those who fund research, and the FDA like Infuse™ has done.

To frame my argument, I will use Pinch and Bijker's Social Construction of Technology (SCOT) which seeks to illustrate that the adoption of a technology is not necessarily one that performs the best objectively, but is the one the that is most socially accepted (Johnson, 2005). I use the notions of relevant social groups and interpretive flexibility, the idea that the interpretations of artifices are different for different stakeholders, to argue that the aforementioned social groups view the development of Infuse™ differently based on their

respective goals and agendas. I will support my claim by analyzing the direct funding sources and legal proceedings pertaining to Infuse™. Using data such as those presented by Kim et al. 2019, I will evaluate the degree to which the rhBMP-2 research behind Infuse™ received funding from those involved in industry at their early research stages. Moreover, I will further assess how the design and application of Infuse™ was changed to match FDA regulation and the impact bribing doctors may have played in the product's success. In effect, my project aims to show the influence social factors have towards the development of successful stem cell therapies.

Conclusion

Even though research focused on repairing the damage ischemic stroke does to cells is extraordinarily funded, there is still a gap between research and a clinical solution as there are no stem cell therapies for ischemic stroke. One of the reasons stem cell therapies are lacking is because they must evade the immune system effectively. To address this problem, we are developing a novel inertial and crossflow microfluidic device to encapsulate stem cells in a polymer layer, making them biocompatible. However, development and use of this device is not enough for successful proliferation of much needed stem cell therapies. Illustrated with a case study of Medtronic's Infuse™, I will draw upon the STS framework of Social Construction of Technology to demonstrate the importance of industry leaders, patients, doctors, FDA regulators, etc. play in the development and success of Infuse™. My technical project offers a direct advancement towards the success of the development of stem cell therapeutics for ischemic stroke. Simultaneously, my STS projects offers a new perspective as to why there is a gap between academic research and fully commercialized products. Combined, my projects aim to

illustrate how the acceptance of stem cell therapies for ischemic stroke is dependent on research that is clinically-minded.

Word Count: 1848 (Figure Caption and In-Text Citations Excluded)

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