DESIGN OF A MICROFLUIDIC DEVICE FOR THE CREATION AND PURIFICATION OF POLYMER COATED CELLS

HOW RELEVANT SOCIAL GROUPS DRIVE THE DEVELOPMENT AND EVOLUTION OF IMPLANTABLE TECHNOLOGIES

A Thesis Prospectus
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Bachelor of Science in Biomedical Engineering

By
Cole Latvis

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Team Members: Timothy Boyer and Melody Chiang

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.

ADVISORS
Catherine D. Baritaud, Department of Engineering and Society
Christopher B. Highley, Department of Biomedical Engineering

According to Virani et al. (2020), almost 130 million people live with tissue loss related heart disease and 83 million have suffered from stroke resulting in some degree of physical or mental disabilities (p. e494, p. 337). As poor countries develop and the population continues to age, the prevalence of these problems will inevitably increase. To combat this suffering, it is crucial to develop and incorporate new biomedical technologies into our healthcare system. Stem cell therapy is a promising emerging technology that has the potential to benefit people with a range of aliments, including neurological and cardiac injuries. They can do this by delivering localized and sustained regeneration or replacement of lost tissue (L. Wei, Z. Wei, Jiang, Mohamad, & Yu, 2017, p. 50). For example, in animal trials, administering stem cells shortly after a vessel-obstructing stroke results in far better functional recovery with lower degrees of long-term nervous system damage (Yoo et al., 2008, pp. 390-393).

Unfortunately, despite the promise of innovative healthcare strategies, there is only a trickle of such technologies entering the clinic. The Food and Drug Administration (2020) has only approved eighteen treatments that use cell or gene therapies. The closely coupled technical project and STS research both aim at enabling wider diffusion of these technologies.

A major practical limitation of implantable cell-based therapies is rejection by the host's immune system (Moshayedi & Carmichael, 2013, p. e23863-2). To combat this, the technical project group is developing a device that manipulates micro-level fluid flows to encapsulate individual stem cells in thin biocompatible polymer coats which do not elicit immune responses. In the future, this would allow for stem cell therapies to be more widely available, however, there is often a societal hesitation to trust new therapies involving materials implanted into the body. Sometimes this results from the spread of misinformation, as in the case of the anti-vaccination movement, but most of the time it is a general feeling of being underinformed about

the technologies (Ruffin, 2018). Additionally, there are a plethora of regulatory, legal, manufacturing, and media influences on the acceptance of all kinds of technologies. The STS research aims to create a framework for the successful navigation of our socio-technical world based on the historical development of the cardiac pacemaker, the first advanced implant. This analysis can then be used to help emerging implantable or transplantable technologies achieve widespread acceptance.

Timelines and deliverables have been established for both the technical and STS research projects. For the technical project, a literature review was conducted this September and October, and my group has produced preliminary designs for our device. The month of November will be used to develop skills in computer aided design and computational flow programs. By the end of December, the first iteration of our designs will be fabricated. Over the winter break, alternative designs will be developed and, in the following months, all of our designs will be evaluated during in-lab testing. A journal-style article cataloging our project will be prepared and submitted by May 8, 2021. As for the STS research topic, after this prospectus is submitted on November 2, 2020, an oral presentation of its contents will be given on November 19, 2020. Additionally, the STS qualifying exam will be completed between November 6 and November 10, 2020. Research into the STS topic will be conducted in the spring 2021 semester and culminate in a research article that will be submitted by the end of March 2021.

DESIGN OF A MICROFLUIDIC DEVICE FOR THE CREATION AND PURIFICATION OF POLYMER COATED CELLS

Each year 550,000 Americans develop disabilities due to neurological damage sustained when tissues are deprived of oxygen during a stroke (Benjamin et al., 2019, p. e282; Prencipe et al., 1997, p. 531). While current strategies for treatment, such as chemical and surgical

interventions to break up blood clots, simply aim to prevent further damage, stem cell therapies have the potential to actually regenerate the lost neurons and thus restore physical function (Prabhakaran, Ruff, & Bernstein, 2015, p. 1451; L. Wei, Z. Wei, Jiang, Mohamad, & Yu, 2017, p. 50). This benefit has been demonstrated in both animal and human models (L. Wei, Z. Wei, Jiang, Mohamad, & Yu, 2017, p. 50; Moshayedi, P., & Carmichael, S. T. (2013), p. e23863-1). However, the success of these trials requires that the stem cells be collected and prepared from the test subject's own body. This is meant to avoid immune responses that would nullify the therapy by destroying foreign stem cells (Marei et al., 2018, p. 5). Since the optimal window for treating many neurological injuries is as little as three hours, performing this time-consuming, expensive, and invasive procedure on a patient-by-patient basis would make stem cell therapy impractical (de Los Ríos la Rosa et al., 2012, p. 1). If foreign cells could be hidden from the patient's immune system, this treatment for neurological injuries would no longer be limited by cell sourcing, meaning that stem cells could be stored at hospitals in advance.

ENCAPSULATING STEM CELLS

It is possible to physically isolate individual foreign particles from the immune system by encapsulating then in thin coats of biocompatible polymer. This technique is called layer-by-layer encapsulation and involves the sequential addition of polymer layers that chemically link to either the particle or the previous polymer layer (Liu et al., 2019, p. 1800939(1)). Through coating stems cells with a biopolymer called hyaluronic acid, Tang, Liao, Tang, Tsai, and Hsieh (2011) demonstrated both increased survivability of foreign stem cells and extended therapeutic benefit. However, layer-by-layer encapsulation is an emerging technique and thus fabrication methods are limited.

The current standard for fabrication, visualized in Figure 1, requires several time-consuming incubation and purification steps, making the process laborious and difficult to scale-up for manufacturing (Liu et al., 2019, p.4). Additionally, this technique relies on centrifugation to separate cells from excess polymer, a process where high G-forces can damage the cells leading to decreased viability (Ferraro et al., 2010, p. 341). Clearly, a more efficient and scalable fabrication technique is needed.

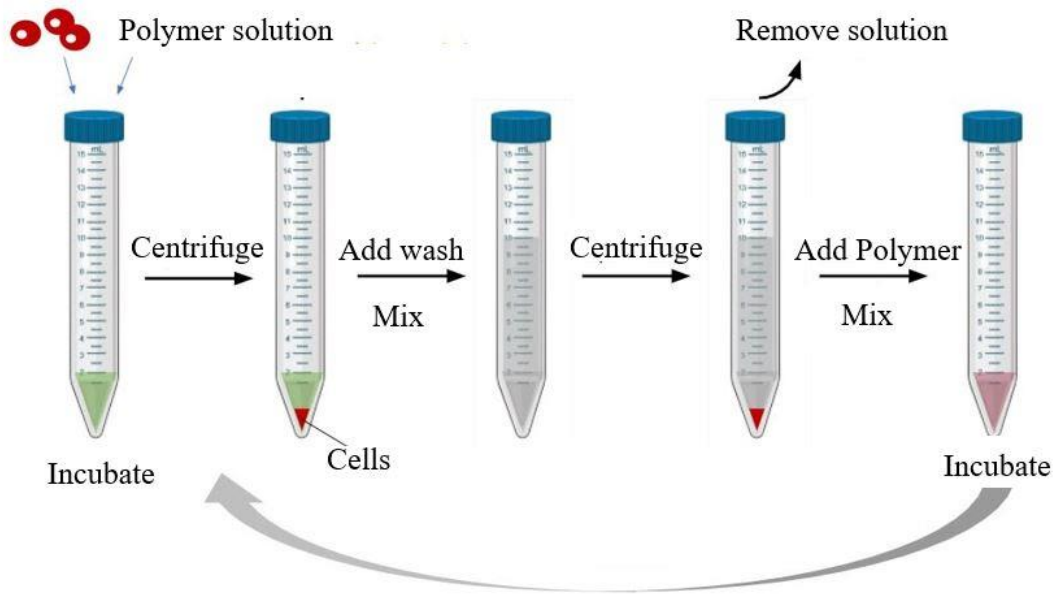

Figure 1: Current layer-by-layer encapsulation procedure. Cells are incubated in a polymer solution to obtain a coat, then centrifuged into a pellet. The polymer solution is replaced with a washing solution and agitated. The cells are then centrifuged again in order to remove the washing solution. This process is repeated until the desired number of layers are applied to the cells. (Adapted by Cole Latvis (2020), from X. Qu, 2020).

DESIGNING A MICROFLUIDIC DEVICE

The technical project aims to design a device that manipulates micro-level fluid flow to perform layer-by-layer encapsulation of neural stem cells in an automated and high-throughput manner. To this end, our microfluidic device must perform two functions, cell encapsulation and small particle separation. My team consists of undergraduate Biomedical Engineering students

Timothy Boyer, and Melody Chaing, and myself. Assistant Professor of Biomedical Engineering and Chemical Engineering Christopher B. Highley is our primary advisor for this project. Additionally, Biomedical Engineering Ph.D. candidate Xuan Qu is acting as a secondary advisor.

The literature review of previously constructed microfluidic devices revealed that numerous designs were capable of either cell encapsulation or small particle separation, but none were designed to do both. Our final technical article in spring 2021 will provide a detailed discussion of this literature review. Inspired primarily by the work of Mach, Kim, Arshi, Hur, and Carlo (2011), the following preliminary design and method was selected for further investigation.

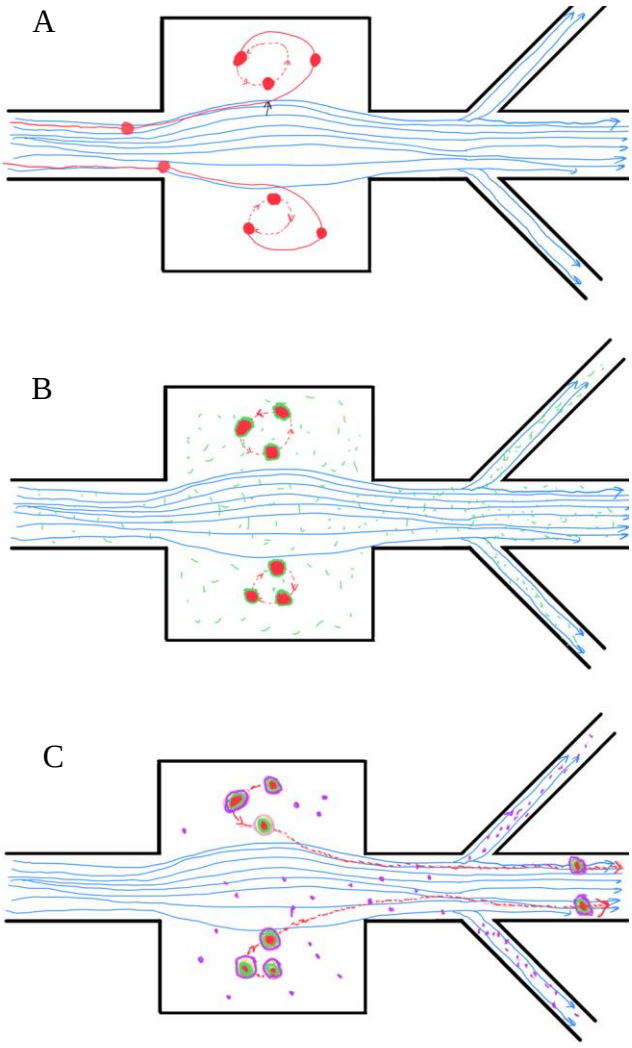

Figure 2: Preliminary entrapment-concentration unit design. (A) Cells become trapped in vortices at high flow rates. (B) Polymer and wash solutions are added sequentially to coat the cells. (C) Lower flow rates release the cells and side channels remove excess polymer and fluid. (Created by Latvis, 2020).

The device contains an array of parallel microchannels. Each channel has a series of the entrapment-concentration units depicted in Figure 2. The sudden expansion of channel width allows large particles, like cells, to become trapped in vortices while smaller particles only transiently pass through (Mach, Kim, Arshi, Hur, and Carlo, 2011, p. 2828). This allows polymer solutions to coat the cells and wash away before the next polymer solution

arrives. Once the encapsulation process is complete, the flow rate can be turned down to release the cells from the vortices. The thinner branches, termed crossflows, serve to both remove excess polymer particles and concentrate cells prior to extraction from the device. Once this device is connected to a series of computer-controlled syringe pumps, polymer and wash solutions can be regulated in order to automate the fabrication of encapsulated stem cells. Further, this method of encapsulation is expected to reduce total fabrication time. Similar devices have reported that particles trapped in vortices require shorter incubation times for surface additions due to increased convection (Mach, Kim, Arshi, Hur, and Carlo, 2011, p. 2832). Although microfluidic methods for cell encapsulation and particle separation have been developed separately, our device would be novel in that it combines aspect of the two methods into a single device for the specific purpose of obtaining encapsulated cells that are purified and concentrated.

The next steps for the technical project will be to create 3D models of our design in AutoCAD and learn how to use QuickerSim, a computational fluid dynamics program, to simulate flows through the microchannels. Once we have verified our design, we will work with the Swami Lab to fabricate our device. The exact method of fabrication will be determined once the design is finalized. The microfluidic device will then be connected to a series of syringe pumps and tested for encapsulation efficiency, recovery rate, separation efficiency, and throughput. All required equipment, such as fluorescent microscopes and hemocytometers, are available in the Highley Lab.

By May of 2021, we hope to have created a device that enables high-throughput, efficient encapsulation of neural stem cells for future use in stem cell therapies. Our work and the insights we developed throughout this project will be documented in a journal-style article. While this technical project aims to overcome practical obstacles for transplantable stem cell therapies, an

STS approach is required to understand and navigate the social limitations surrounding this technology. Regulation, flow of information, manufacturing standards, and more are all important considerations, especially as these types of technologies become increasingly complex.

HOW RELEVANT SOCIAL GROUPS DRIVE THE DEVELOPMENT AND EVOLUTION OF IMPLANTABLE TECHNOLOGIES

Beginning with dental implants in the ancient world, humankind has inserted various materials into the body in an effort to replace lost function (Marin, Boschetto, & Pezzotti, 2020, p. 1621). However, it was not until modern times with sophisticated technology and understanding of human physiology that implants became safe and, in many cases, active in supporting the function of normal tissue. The emergence of stem cell technologies has opened a new avenue for implants; one where natural material can be collected, bioengineered, and then implanted into patients to treat cellular level injuries, either through direct replacement or stimulating regeneration of lost tissue. Given the potential benefit of implantable stem cell technologies, it is important to ensure their rapid, but safe development and diffusion in the healthcare system.

There is a network of complications, seen in Figure 3, that can potentially slow the development and diffusion of implantable bioengineered cell technologies. For example, doctors and especially patients often hesitate to accept innovative strategies for addressing old problems. In 2014, Robillard, Roskams-Edris, Kuzelkevic, and Illes conducted a survey on public acceptance of gene therapies, a related technology, and found that over 50% of those surveyed were “most concerned” that they would not have all the necessary information when considering medical applications of gene therapies (p. 744). Further, it has proved difficult to regulate cell-based implants. Those in the industry, such as Sharma, Blank, Patel, and Stein (2013), find the

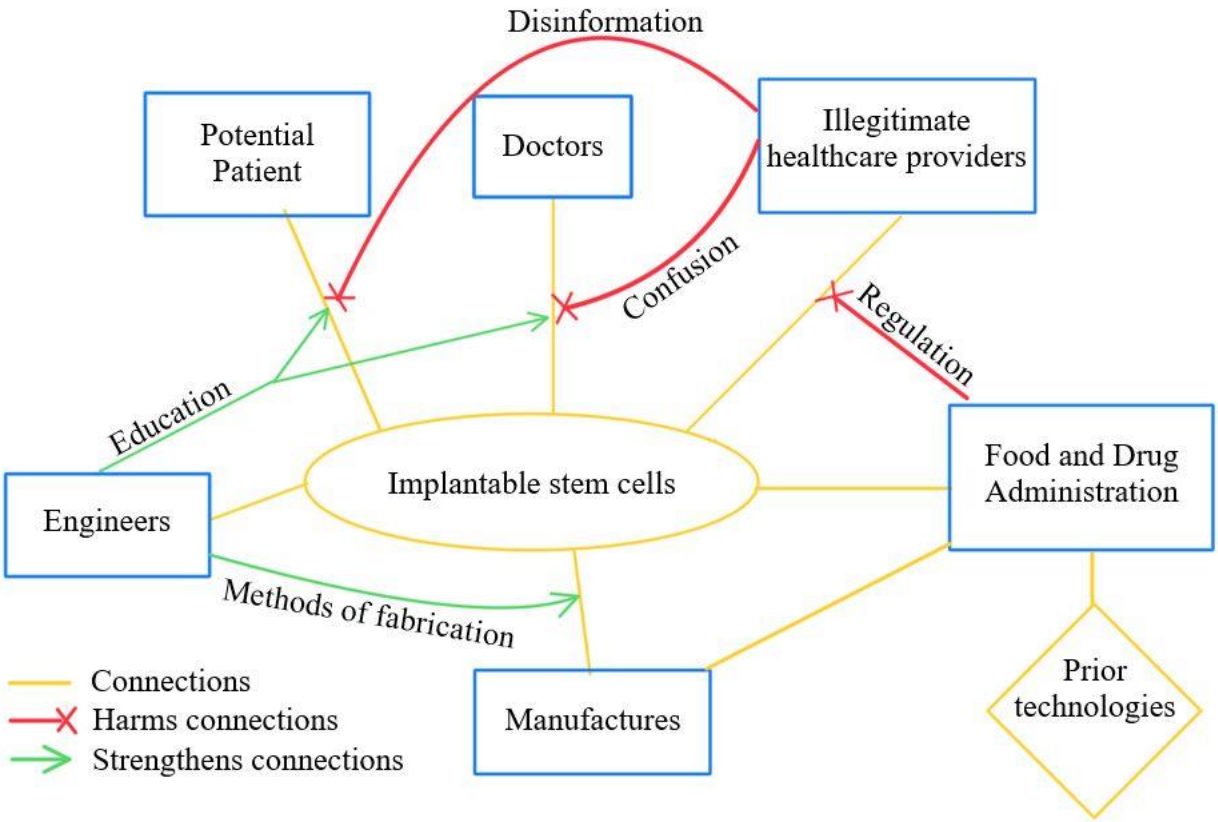

Figure 3: Socio-technical interactions surrounding implantable stem cells: Various social groups interact with emerging stem cell technologies. Many of these groups interact with each other to either strengthen connections, denoted by green arrows, or harm connections, denoted by red arrows ending in a cross. (Created by Latvis, 2020).

existing regulations outdated and cumbersome, while at the same time the Food and Drug Administration (2019) has been forced to issue warnings against illegal, untested, and dangerous stem cell therapies being marketed to the public (p. 107-114). Such issues then feed back into the confusion felt by the potential patient population. In the past, this type of confusion regarding the safety of biomedical technologies has led to the partial rejection of objectively safe and beneficial medications. A major example of this is the modern anti-vaccination movement, which began when Andrew Wakefield published a falsified study claiming the combination measles, mumps, rubella vaccine caused autism and bowel disease. Although the study is now redacted for scientific fraud, the damage is already done (Matthews-King, 2018). Twenty-seven of the fifty states have seen reductions in the number of children being vaccinated (Pilkington,

2019). In addition to factors that are relatively unique to the biomedical field, social influences, such as business interests and manufacturability, also shape the development and diffusion of innovative implantable technologies.

CARDIAC PACEMAKER ACCEPTANCE AS A FRAMEWORK

The cardiac pacemaker is a prominent and widely trusted implantable technology that has successfully navigated the socio-technical challenges facing an advanced implant. The STS research aims to create an outline for the effective development and diffusion of implantable technologies based on the history of the cardiac pacemaker as a model. The first cardiac pacemakers were implanted in 1958 in Sweden and only lasted three hours (Bains, Chatur, Ignaszewski, Ladhar, and Bennett, 2017, p. 23). In the roughly 60 years since, nearly three million people currently live comfortably with pacemaker implants (Wood & Ellenbogen, 2002, p, 2136). To achieve this massive widespread acceptance, the pacemaker needed to overcome many of the same social factors as modern emerging cell-based implants. Understanding how this social arena was navigated could provide vital insights for stem cells to ensure their benefits can become trusted and widespread in the healthcare system. Although pacemakers are an implanted technology and stem cells are technically transplanted from donor to patient, the general goals of both procedures are to restore lost function, and engineered transplant technologies could easily be viewed as the next step in implant technology. To gain this understanding, an Actor-Network Theory perspective will be applied to the history of the cardiac pacemaker.

DEVELOPING AN ACTOR-NETWORK THEORY MODEL

Actor-Network Theory (ANT) is a theoretical approach developed by Michel Callon, Bruno Latour, and John Law that is used to describe the influences that society and technology

have on each other (Baiocchi, Graizbord, & Rodríguez-Muñiz, 2013, p. 323). It does this by describing various human and non-human influencers called actors and actants, respectively, and organizing them into a dynamic network of interactions with one another (Fioravanti & Velho, 2010, p.2). While this is a descriptive approach, it has the potential for systematically identifying failure points in socio-technical processes.

When ANT models are generated, there can be multiple levels of an overall network. The outermost level is the global network; this encompasses all actors and actants that bear meaningful influence on the development or diffusion of a technology. Subsequent levels can be constructed for actors and actants that more closely interact with the technology. The innermost network includes only those that directly participate in the development and diffusion of the technology. This group exists within what is called a negotiation space where alterations are made to the technology (Law and Callon, 1998, p.289). Figure 4 represents a network of actors and actants generally involved with implantable devices.

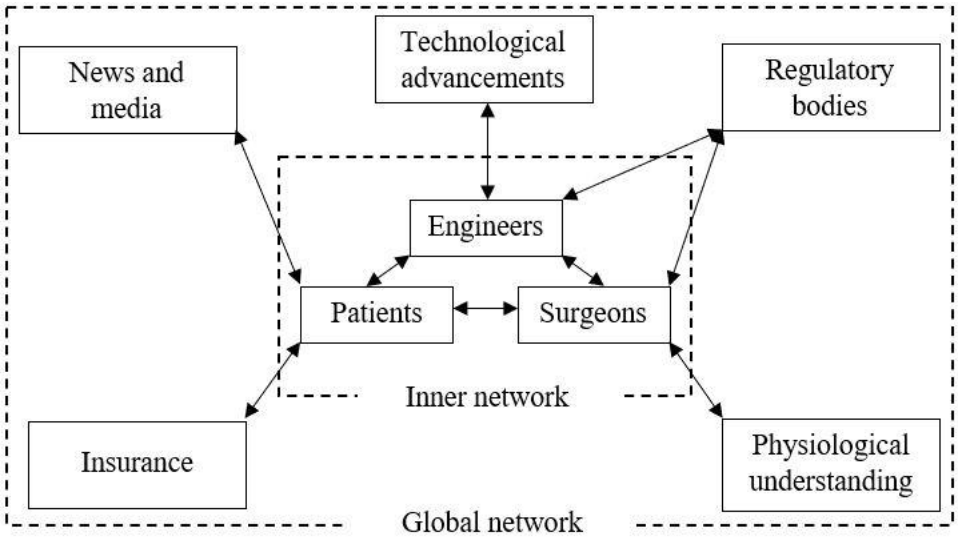

Figure 4: Implantable device Actor-Network Theory mapping. The overall network contains an inner network composed of actors that interact directly with technology. This is part of a larger global network that includes peripheral actors and actants that do not directly interact with the medical device. (Adapted by Cole Latvis (2020) from Downing et al, 2018).

In the inner network, the patients, engineers, and surgeons communicate needs and limitations throughout the lifetime of a device. Actors and actants residing within the global network, such as regulatory bodies, the media, technological advancements, insurance, and physiological understanding, influence those in the inner network, which in turn influences the form, function, and diffusion of the technology. The device is then continually refined based on these direct and indirect influences.

In pursuit of developing a model specifically based on the implantable cardiac pacemaker, the history behind the development and diffusion of the device will be reviewed in order to identify the relevant actors and actants, what connections exist between them, how these connections changed overtime, and how the network developed to allow diffusion of the technology. The search will begin with articles written by cardiologists, electrical engineers, and technology historians. With this background knowledge, evidence will then be collected from legal preceding and personal accounts in newspaper archives of any social controversies surrounding the pacemaker throughout its history. Lastly, academic debates regarding pacemakers will be uncovered through research into the perspectives of scholars at the time.

When this research is complete at the end of March 2021, the insights I have developed will be collected into a research article and presented to my Department of Engineering and Society advisor Professor Catherine Baritaud. By this time, I hope to have developed a framework for successful implementation of implantable devices that can be used to advance the progress of a modern implantable or transplantable technology, such as the encapsulated stem cells central to the technical project.

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