Tissue Engineering Approach for Spinal Cord Regeneration

Assigned Tissue: Nerve Tissue

BMEN 480 - 500
Group #4
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Contributions

- **Grant Barkelew:** description of the novel approach and the clinical problem, provided information for the current treatments and translational challenges, formatting and citations, figures 1-4, proofreading the final paper

- **Jose Esparza:** brainstorming, research and literature review, proofread the final paper

- **Nichole Longbottom:** described and found solutions to the translational challenges, gathered information for current treatments and novel approach, proofread the final paper

- **Rachel Rice:** described current treatments, provided information for the testing methods and translational challenges, proofread the final paper

- **Sofia Villanueva:** described the testing methods, provided information for the clinical problem, organized group meetings, proofread the final paper

Spinal Cord Injury Overview & Significance

Spinal cord injury (SCI) is a neurological condition that typically results in a permanent disability. The National SCI statistical center estimates that there are approximately 294,000 people in the United States that suffer from a SCI and that the most common causes of SCIs are vehicle crashes, falls, acts of violence, sports, and medical/surgical errors[1]. SCI outcomes depend on the severity and location of lesions and can include either partial or complete loss of sensory and/or motor function[2]. Most SCIs following traumatic impact cause displaced bone fragments, discs, and/or ligaments to tear into the spinal cord tissue[2]. Each of these exacerbates spinal cord swelling and can progress into more severe inflammation, ischemia, and the development of a cytotoxic microenvironment, causing cell death and damage to spinal cord tissue[3]. However, as long as the SCI doesn't completely sever the spinal cord, there are a variety of promising therapies available.

Aside from the primary complications that occur as a result of an SCI, SCIs can result in secondary complications that affect an individual's quality of life. Secondary complications include the inability to work or function independently and an increased incidence of depression and anxiety[4]. In many instances, individuals who suffer from an SCI will need a primary caregiver to assist them in their daily activities. Individuals who take on the role of a primary caregiver are more likely to experience symptoms of depression, anxiety, and isolation caused by unexpected role changes[5]. Lastly, SCIs cause substantial socioeconomic implications for patients, their primary caregivers, and society[5]. While other therapies attempt to mitigate the effects of an SCI, a tissue engineering approach aimed at restoring spinal cord function addresses primary implications caused by SCIs and indirectly addresses secondary implications by reducing the burden placed on patients, primary caregivers, and society.

Current Treatment Methods for Spinal Cord Injuries

There are no current tissue engineering approaches used by clinicians to treat SCIs. Surgical interventions to alleviate SCI symptoms may involve the removal of bone fragments, foreign objects, and herniated discs that compress the spine[6]. After surgical intervention immediately following trauma and spinal cord stability have been established, clinicians will administer a combination of rehabilitation and pharmacological therapies to treat SCI patients. Physical therapy is a common rehabilitation approach that involves exercise programs to strengthen muscles following a SCI[7]. Physical therapy begins with a position management plan to inhibit muscle tone following a SCI. This involves a physiotherapist positioning a SCI patient to support and align their bodies to prevent pain and stiffness and limit future problems with

movement. They will also administer prolonged manual stretching, serial casting, and weight-bearing techniques to reduce hypertonicity and spasticity and prevent the loss of sarcomeres in immobilized muscles[8]. Muscle vibration massages may also be applied to help SCI patients regain the ability to walk at a limited capacity[9]. Other therapies aimed at improving motor function include wheelchair, treadmill, gait, strength, and cardiovascular training[8].

In addition to rehabilitation therapies, pharmacological treatments are commonly administered by clinicians to treat SCI-related pain. The first-line treatment for SCI pain is the use of anticonvulsants, such as gabapentin and pregabalin, which increase the activity of inhibitory neurotransmitters to decrease the transmissions of nociceptive pain signals[10]. Additionally, opioid analgesics, such as morphine, are among the most effective drug groups used by clinicians to treat SCI pain due to their ability to decrease the release of pain neurotransmitters[11]. To reduce the inflammatory response, clinicians will also administer anti-inflammatory corticosteroids, such as methylprednisolone, to reduce oxidative stress and necrosis[12]. To treat symptoms of depression and pain following a SCI, clinicians may prescribe antidepressants such as amitriptyline or trazodone, which inhibit afferent pain signals by increasing serotonin in the central nervous system[10]. There are many other pharmacological treatments administered by clinicians to treat SCI-related pain on a case-by-case basis.

While rehabilitation and pharmacological treatments are useful in mitigating some of the primary and secondary complications following a SCI, they also possess many shortcomings. The effectiveness of rehabilitation therapies vary tremendously based on the location and severity of a SCI, physical fitness, the quality of care administered by a physiotherapist, and other factors. Additionally, the restoration of function and reduction of pain are often limited by the ability of the patient's body. Pharmacological treatments can be used to treat SCI-related pain, however, they fail to provide spinal cord tissue regeneration and can lead to unwanted side effects and addiction. The failure of current therapies to completely restore spinal cord tissue and motor function further promotes the need to treat SCIs through a tissue engineering approach.

Tissue Engineering Approach for Spinal Cord Regeneration

Collagen-based scaffolds can be used to support the regeneration and repair of tissue following a SCI. The most dominant forms of collagen between the intervertebral discs in the spinal cord are collagen type I and II. Furthermore, type I collagen was selected as the primary scaffold material because it is found in almost every tissue in the human body and is the easiest/cheapest type of collagen to extract. Collagen is a natural biomaterial that offers many

advantages for spinal cord regeneration including excellent biocompatibility, biodegradability, and porosity[13]. Biocompatibility is an important characteristic for the fabrication of a SCI regenerative scaffold because it ensures that the scaffold performs its intended function without eliciting local or systemic effects and supports appropriate cellular activity within the spinal cord. The porosity of the scaffold plays an important role in promoting cell seeding, distribution, and penetration by increasing the available surface area. The biodegradability of the scaffold will also ensure proper spinal cord tissue remodeling. Other characteristics that make collagen an ideal scaffold for SCI regeneration are its ability to be proteolytically remodeled by cells, minimal inflammatory and antigenic responses, and its ability to promote the migration of neural cells via integrin binding sites[14].

The first step in fabricating a collagen-based scaffold for spinal cord regeneration is the extraction of type I collagen from a host. Although it is possible to isolate type I collagen from human peripheral nerve tissue or human placenta to fabricate a collagen-based scaffold, there are many challenges regarding autologous and allogeneic sources including their cost, availability, regulatory approvals, standardization, regulation, ethical concerns, contamination, the need to find a matching donor, etc. To make the scaffold available to a broader range of SCI patients, type I collagen can be extracted from a rat tail tendon. Since isolation is performed on a xenogeneic source, the isolated collagen would be dissolved in acetic acid, frozen and lyophilized to form a sponge, and then dispersed into acetic acid[15]. Afterward, the sponge would be centrifuged, degassed, sterilized via dialysis, and neutralized to obtain a type I collagen solution[15]. This type I collagen isolation technique is illustrated in *Figure 1*.

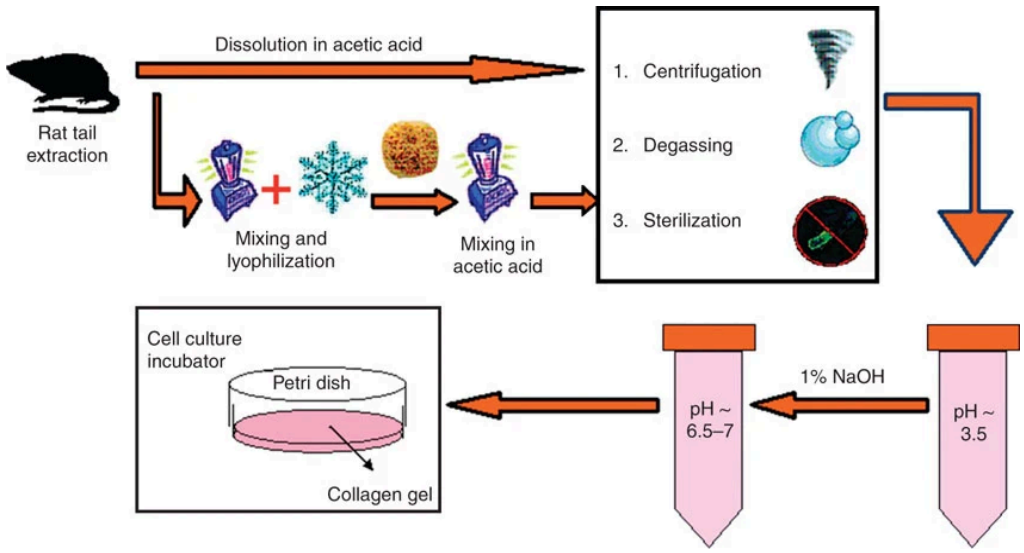


Figure 1. Type I Collagen Isolation Procedure[15]

While collagen is an excellent natural biomaterial, it has weak mechanical and structural stability upon the uptake of water. By creating a collagen/chitosan biocomposite, you can improve the mechanical properties of the scaffold without compromising biocompatibility or other favorable characteristics. Chitosan can easily be isolated from crustacean shells. After sterilizing the chitosan, demineralization is the first step in chitosan extraction and can be achieved by treating the crustacean cells with HCl. Afterward, the powdered shells would be deproteinized through NaOH treatment to obtain chitin[16]. Finally, the chitin would be deacetylated with a strong alkali solution of NaOH before being dried to recover the final chitosan product[16]. This chitosan isolation technique is illustrated in *Figure 2*.

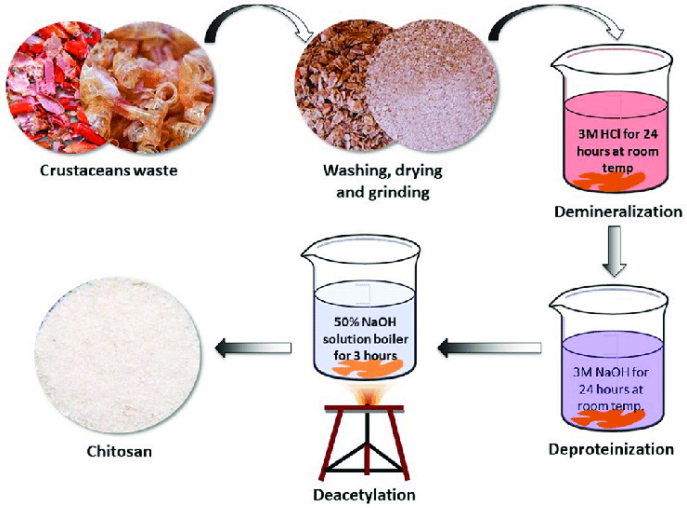


Figure 2. Chitosan Isolation Procedure[16]

3D printing is a promising technique that can be used to manufacture scaffolds for spinal cord regeneration. When compared to traditional manufacturing methods, 3-D printing has control at the micron level and builds layer-by-layer, providing greater control over the shape and morphology of scaffolds. Precision control over the shape and morphology of scaffolds, via 3-D printing, improves the ability to deliver stem cells and can also partially reestablish a permissive microenvironment for axonal regeneration in the spinal cord[17]. Additionally, composite bioinks that support the intrinsic characteristics, biological activity, and mechanical stability of the scaffold can be implemented through a 3-D printing approach to promote biocompatibility. Lastly, 3-D printed scaffolds can mitigate high costs, complexity, and unfavorable immune responses that may arise from traditional methods[18].

After the collagen and chitosan are isolated, the composite bioink can be developed to manufacture the scaffold. First, the chitosan would be dissolved in acetic acid before mixing the collagen into the solution. Afterward, the collagen and chitosan would be centrifuged to obtain a

gelatinized collagen-chitosan material before being incubated overnight to obtain the composite bioink product[17]. The collagen/chitosan bioink would then be loaded into a printer cartridge to perform layer-by-layer printing to obtain the 3-D scaffold structure[17]. Lastly, the scaffold would be freeze-dried, rinsed with deionized water, and sterilized to obtain the final collagen/chitosan scaffold[17].

To repair and restore function in the spinal cord, the collagen/chitosan scaffold can be seeded with stem cells and growth factors to induce axonal regeneration that can reconnect the efferent and afferent spinal cord[19]. While neural stem cells (NSCs) have disadvantages related to cell survivability, the need for purification, and ethical implications, they are easy to harvest and promote remyelination, making them the most ideal candidate to improve neuronal differentiation and spinal cord repair[18]. Nerve growth factors, such as NGF and BDNF, are also important components for maximizing spinal cord regeneration because they promote cell proliferation, migration, differentiation, survival, and the function of NSCs[20]. NSCs can be derived from human embryonic stem cells (hESCs) and seeded onto the scaffold with nerve growth factors to fabricate the final seeded spinal cord regeneration scaffold (*Figure 3*). The final scaffold would then be implanted into the injured spinal cord tissue to treat a SCI.

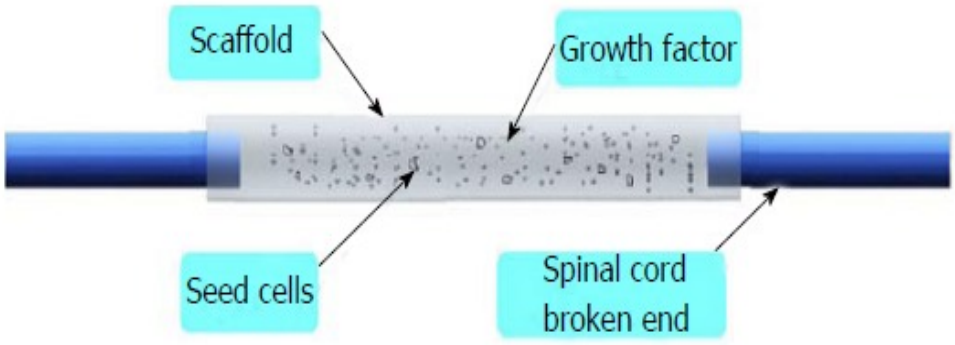


Figure 3. Final Seeded Spinal Cord Regeneration Scaffold Schematic[21]

Testing procedures for our Tissue Engineering Approach for Spinal Cord Regeneration

After 3-D printing and seeding the scaffold with NSCs and nerve growth factors, *in vitro* and *in vivo* testing must be performed to ensure the overall performance of the scaffold for spinal cord regeneration. *In vitro* testing is performed to assess the scaffold cytotoxicity and to make sure that the cells that are seeded onto the scaffold can survive and regenerate spinal cord tissue. The *in vitro* tests that will be performed include a histological analysis, bioreactor assessment, cell viability assay, mechanical testing, and scanning electron microscopy (SEM). These *in vitro* tests will be the first steps to understanding the performance of the scaffold in the

spinal cord microenvironment. The *in vivo* testing will be performed to monitor the dynamic interactions between the living cells, supporting matrix, and surrounding environment.

Histological analysis is the first *in vitro* test that should be performed to ensure the scaffold is decellularized properly. The histological analysis would be performed by suspending a small piece of the scaffold in a petri dish and staining it with Masson's Trichrome. After staining, the concentration of collagen and chitosan would be assessed to ensure the scaffold preserves the collagen and chitosan. Depending on the resulting concentrations of collagen and chitosan, the collagen/chitosan bioink ratio may need to be adjusted. Further confirmation regarding the porosity of the scaffold would be obtained via SEM.

To test cell viability, a cell viability assay would be performed using trypan blue. Trypan blue offers advantages over other stains because of its low-cost rapid protocol[22]. To perform the cell viability assay, the scaffold would be incubated for a short period before being suspended in a petri dish with trypan blue dye. The cells would then be counted using an automatic cell counter to quantify the number of live and dead cells on the scaffold.

After the scaffold has shown successful cell proliferation and viability, mechanical tests must be performed to assess the scaffold's durability. These would be performed before seeding the NSCs and growth factors. Degradation testing would be done by suspending part of the scaffold in an acidic solution to assess the degradation rate. Additionally, a shear test would be performed by anchoring each end of the scaffold and applying a force in the middle to assess whether the scaffold can withstand the shear stresses/strains present in the spinal cord.

Lastly, a bioreactor must be used to allow the NSCs to proliferate on the scaffold before implantation into the spinal cord. The bioreactor would provide information regarding the cell adhesion to the scaffold and the cell survivability. The scaffold would be suspended in a bioreactor for an extended period to mimic a physiological environment that would allow uniform distribution of the NSCs and nerve growth factors and ensure cell survivability and proliferation. The suspended bioreactor schematic is illustrated in *Figure 4*.

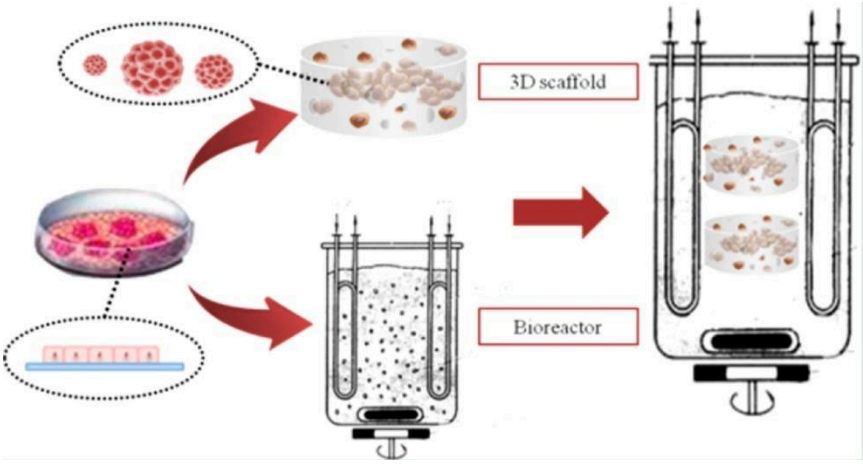


Figure 4. Suspended Bioreactor Schematic[23]

Following *in vitro* testing, *in vivo* testing would be performed to monitor the scaffold's performance in a living organism. This would involve implanting the scaffold in a SCI rat model to watch the progression of the scaffold in the spinal cord. *In vivo* monitoring is critical for obtaining information regarding the cell viability, cell function, and structural integrity of the scaffold. After implanting the scaffold in the rat, bioluminescence imaging would be performed to assess cell migration at the implant site using a luciferase enzyme. Over several months, the rats will be monitored continuously to assess cell migration, the foreign body response, and the performance of the scaffold. Additional imaging techniques such as positron emission tomography (PET) scans and nuclear magnetic resonance (NMR) spectroscopy would be performed occasionally to gain additional spatial information regarding the scaffold's performance and the immune response using radionuclide probes and natural probes, respectively. After a few months, the rats would be sacrificed and immunohistochemistry would be performed to fully understand the extent of the foreign body response. Design modifications would be made depending on the host immune response and overall performance of the scaffold. After successful *in vivo* testing, the scaffold would be implanted in humans to undergo clinical trials.

Challenges for Translating the Scaffold in a Clinical Setting

There are several translational challenges with implanting the 3-D printed scaffold into the human body. The main translational challenges that must be mitigated include the cost, standardization, regulatory approvals, and long-term effects of the scaffold. Along with these translational challenges, additional considerations need to be made regarding the surgical procedure required to integrate the scaffold in the human body. Ensuring the scaffold can be

implanted into a SCI patient will require a significant amount of precision and the ability to perform the surgical procedure will vary on a case-by-case basis.

The foreign body response (FBR) is a long-term translational challenge that involves the formation of a fibrous capsule around the scaffold. Long-term clinical observations of scaffolds implanted in the spinal cord of SCI patients support the feasibility of this approach because no serious adverse events were observed[24]. However, *In vivo* studies in animal models used immunohistochemistry to depict the presence of a fibrous encapsulation, months after implantation[25]. To combat the FBR, surface modifications can be performed based on the proteins that adhere to the scaffold. An important consideration for surface modifications is that they can alter the biocompatibility and functionality of the scaffold and that further testing may need to be performed to mitigate the FBR and accommodate these concerns.

The cost of implementing this approach is another translational challenge. Many of the costs to perform this approach are unavoidable. The isolation of NSCs from human embryos is expensive because their availability is limited and there are heavy regulatory approvals defined by the FDA that drive costs even further. It would also be expensive to train surgeons to perform this procedure on the spinal cord. However, certain steps can be taken to reduce the cost of this approach as much as possible. The cost can be reduced slightly by performing low-cost 3D printing, isolating collagen and chitosan from xenogeneic sources, and performing low-cost *in vitro* and *in vivo* tests, when possible.

Scaling up the production and standardization of the scaffolds would be an issue because 3D printing can take a significant amount of time. Additionally, the viability of the stem cells that are being seeded on the scaffold and the distribution of the cells on the scaffold would be hard to reproduce at a large scale. This process can be considered a ‘cook to order’ process because each patient’s need is distinct. The location of the spinal cord injury, the age and gender of the patient, and the severity of the injury are all factors that have to be considered. The viability of the cells that are being seeded onto the scaffolding will also be an issue for those that are older or those that have compromised stem cells. Scaling up the production and ensuring sufficient cell viability can be slightly improved by placing multiple scaffolds in a suspended bioreactor simultaneously. The scaffolds could also be produced on a larger scale if multiple stem cell sources are used to derive the NSCs to seed the scaffolds.

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