A Combination Method of Tissue Engineered Never Grafts and Partial Artificial Human-Like Tissue for Peripheral Nerve Regeneration

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Abstract— **Peripheral nerve plays a vital role in the communication between brain and body. The regeneration of peripheral nerve has been studied for several years. The traditional strategy is called Tensionless end-to-end repair, which can only function when there is very small gap between the injury and when endoneurium is not damaged. A new emerging method is called Tissue Engineered Never Grafts (TENGs), which can be implanted into a nerve and are hypothesized to repair larger gaps after injury. Given that current methods have yet to achieve complete functional recovery and issues have arisen with biocompatibility, TENGs further are developed by loading other growth factors, like Glial cell line-derived neurotrophic factor (GDNF). Recent results have shown that a sustained release of GDNF in vitro led to an improvement of the nerve regeneration of Schwann cells. However, this is only confirmed in vitro and in an animal model. As for the animal model, the previous way is to use rodent animals, like rat, because rat is small animal and its nerve cell grow faster and it is easy to conduct more experiment. But considering the big difference between rats and humans, the sheep are used for study to more closely replicate the complexity and size of a human patient. Sheep models have been shown to be one of the most relevant animal models for studying nerve regeneration. Although scientists established good results in sheep models, sheep are still different from human beings. To address the problems showed in these studies, we can combine GDNF-TENGs with Partial Artificial Human-Like Tissue (PAHLT), which not only can improve the speed of peripheral nerve cells (PNCs) growth for the introduction of GDNF to TENGs, but also can increase the biocompatibility for the very similar system as human.**

I. INTRODUCTION

Peripheral nerve system plays an essential role in conveying signals between the spinal cord and the rest of the body. It contains three types of cells: neuronal cells, glial cells, and stromal cells [1]. Nerves are comprised of various combinations of motor, sensory, and autonomic neurons. The one that shows major role in the maintenance and function of the peripheral nerves is cell instead of neurons, like Schwann cells which ensheath nerves in a layer of myelin and provide trophic support through the release of important neurotrophies such as Nerve Growth Factor (NGF). [2]. The non-neuronal cells and connective tissues surrounding neuronal axons provide a complex stromal connective tissue scaffold for the nerve and are important in understanding and classifying nerve injuries[1].

There are three structural layers. The deepest one is endoneurium which encases the individual axon directly. Axons are then bundled together by the perineurium, surrounding the endoneurium, to form fascicles. The very outmost connective tissue layer of the nerve is epineurium, which is consists of two parts, epi-fascicular epineurium and epineural epineurium. Epi-fascicular epineurium is dispersed between fascicles while the latter is surrounding the nerve trunk. More advanced, the blood axons needed is provided by microvessels which branch through the nerve according to the structural layers. Epineural vessels are the most susceptible to trauma than other deeper vessels for their outer layer location in nerve[3].

Injuries to the peripheral nerves result in loss of motor, sensory, and autonomic functions based on the different sites [4]. According to the various level and layers, peripheral nerve injuries can be classified, which make the discussion more effective and easier. It is Seddon who was the first to classify nerve injuries into three categories based on the presence of demyelination and the extent of damage to the axons and the connective tissues of the nerve [5]. The mildest form of injury is called neurapraxia[4]. The next level is axonotmesis, and the most severe level is called neurotmesis, which is full transection of the axons and connective tissue layers wherein complete discontinuity of the nerve is observed [5]. Peripheral nerve injury is a common global clinical problem, causing extreme and various challenges to patients, from short-term mild discomfort to long-term impairment [2]. Peripheral nerve system can regenerate after small injuries, but larger injuries may result in Wallerian degeneration in the distal stump and axon degeneration within a small zone distal to the proximal stump[5]. The resulting myelin and axon debris is cleared by macrophages and monocytes after they move into the nerve stumps[5]. However, axon regeneration needs to be activated by Schwann cells, which can proliferate to form Bungner bands and produce neurotrophic factors and extracellular matrix (ECM) molecules [4]. The axon regeneration begins at the proximal stump and continues toward the distal stump[6]. The regeneration process will not stop until the nerve is repaired completely. However, there are some shortcomings, such as the low ability to repair large gap injuries and the poor functional recovery when no intervention is provided, and the regeneration is dependent upon spontaneous peripheral nerve regeneration. Therefore, the medical therapy for peripheral nerve regeneration emerges over the several hundred years.

II. CURRENT TREATMENTS

Currently, the treatment of choice is meticulous microsurgical repair by tensionless epineurial sutures[7]. When

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peripheral nerve injury makes a real nerve gap where suturing is impossible, interposition of some form of graft between the nerve stumps, that is autologous nerve grafting, remains the gold standard[8]. End-to-end suturing requires nerve segments that are self-donated from another site of the body. Autologous nerve repair requires the sacrifice of the healthy nerve cells and these donor cells are in finite supply. Additionally, clinical treatment is complicated by the fact that the mechanisms behind the process of peripheral nerve regeneration are not yet entirely understood. Thus, the existing levels of therapy should accelerate and improve the regeneration of peripheral nerve cells as the clinical processes are better understood. However, only after the whole process can be completely figured out, the best and most appropriate techniques for the peripheral nerve repair will arise. [9]

After several years development, and for the inherent disadvantages of autologous nerve grafting, the discovery of alternatives to autologous nerve grafts has become a necessity. With the progressive and fast development of regenerative medicine, together with the study of tissue engineering, scientists started to do the research on the combined field of neural and tissue engineering. Based on this new combination and research, tissue engineered nerve grafts (TENGs) have been produced to supplement, and even substitute for autologous nerve grafts. [6] TENGs involve both physiochemical and biological cues, which are provided by a biomaterial- based structure, as well as a multitude of cellular and molecular components. By using this new method, the shortcomings of previous methods may be solved. For the big gap after a severe injury, TENGs can be artificially produced to match the size as the gap, which fits the lack of the nerve properly. TENGs are made of materials other than the pieces of autologous tissue, which enables clinicians to not damage or kill the healthy nerve in another location. Thus, TENGs have become a gold standard for peripheral nerve regeneration.

Research on both the mechanisms of nerve repair and new materials for treatment can only improve and accelerate current treatment strategies. During the recent years, researchers have make great effort on how to improve the function of TENG [10]. The way to improve the result of using TENGs to repair nerve injuries can be classified into two categories. The first strategy is to try to find a most beneficial material to use to make TENGs. Possible materials contain silicone sheet, collagen, vein, free fat graft, vasculature-free pedicled fat flaps, local flaps, omentum, human amniotic membrane (HAM), poly-lactide film, epineural sheath, biodegradable glass fiber wrap (CRG-WRAP), porcine extracellular matrix (PEM), and others [10]. All the materials scientists have used are not guaranteed to improve peripheral nerve regeneration. They are all attempts and are all conducted in vitro based on the hypothesis that they might have a positive impact on nerve cell growth or maturation. Scientists also use different ways to construct biomaterials for peripheral nerve regeneration, such as using electrospinning. [9] Another strategy used to improve the function of TENGs is to load or add the growth factor on it. So far, the most common growth factor used to load in TENGs. TENGs can be split into two classes: 1) neurotrophies, including NGF, brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) and 2) growth factor with neurotrophic actions, including glial cell line-derived neurotrophic factor (GDNF), ciliary neurotrophic factor (CNTF) and fibroblast growth factors (FGFs). [6]

III. Current research on my papers

The first paper discussed is titled 'In corporation of double-walled microspheres into polymer nerve guides for the sustained delivery of glial cell line-derived neurotrophic factor'. The aim of this study is to find a biodegradable polymer nerve guide. This kind of polymer nerve guide can locally deliver bioactive neurotrophic factors in physiologically relevant concentrations. This delivery has to continuous across the whole length of the peripheral nerves to cross from the proximal to distal nerve stump. Previous work has demonstrated that loading a neurotrophic factor can improve nerve regeneration in some degree and could potentially be used to solve the present limitations when it comes to a large gap. Glial cell line-derived neurotrophic factor (GDNF) shows promising advantages when applied to nerve regeneration with nerve guides because it can be a promoter in axonal elongation and branching and also promote Schwann cell proliferation and migration. In order to improve the function of the tissue engineered nerve grafts, Kokai et al. introduce a double-walled microsphere delivery system for bioactive GDNF with a sustained release property ¿50 days in vitro. Kokai et al. combined microspheres within degradable poly nerve guide in a reproducible distribution and then inserted this nerve guide in a rat sciatic nerve gap which is across a 1.5 cm defect. This was accomplished in a series of steps. First, Kokai et al. fabricated the double-walled microspheres, followed by testing the release of GDNF from double-walled microspheres. Next, researchers fabricated poly (caprolactone) disks and examined the histological and immunohistochemistry of their fabricated nerve guides. The result showed that there is an increase in the tissue integration in both the proximal and distal segments of the lumen of the nerve guide after 6 weeks implantation. In addition, they also imaged the transverse sections of the distal region of the explanted guides, which indicated the presence of Schwann cells. The migration of Schwann cells to double-walled microspheres suggested that bioactive GDNF was encapsulated and delivered to the internal environment of the nerve guide. The method used to load GDNF with double-walled microsphere, PLGA coated with poly (caprolactone) showed great advantages. As their result shows, after they implanted their system, a greater concentration of intercellular fibers and collagen content can be obviously found. The growth factor GDNF can be well released into their delivery system. [11]

The second article evaluated was 'The use of sheep as a model for studying peripheral nerve regeneration following nerve injury'[12]. A brief review of current literature about

peripheral nerve injury and regeneration highlights that most peripheral nerve regeneration studies are utilize rodents as a model of nerve regeneration and remodeling [12]. Among those, rat models are the most frequently used model for analysis of the cellular and molecular processes of peripheral nerve injuries and axon sprouting[13]. The injuries contain those that isolate all or some of the axons from the cell bodies of the neurons, complete and partial nerve injuries, respectively. The rat is the first most popular and the mouse is the second most popular model [14]. There are four main reasons that scientists use rodent as a model: 1) they are small, 2) their nerves regenerate well, and 3) there are many inbred strains available. The fourth reason stems from the fact that a good in vivo experiment needs to be conducted many times, and even needs to be repeated to confirm results many more times to achieve statistical significance. Rodents are a good model because it is possible to use a large number of rodent animals, allowing experiments to be sufficiently well powered statistically. For now, the rodent nerve has been well studied and is very similar to humans in terms of the major cell types that are important for regeneration [15].

However, there are still some shortcomings when using rodent as model. The peripheral nerve regeneration is much faster in rodent than in human beings. Due to their size, the nerve gap that can be made is too small, which limited the development of the studies of large gaps[12]. In order to overcome the limitation using rodent animal as model, scientists have started using larger animals as the model to conduct the experiments related to peripheral nerve injuries and regeneration. Considering the gap that regenerating axons must move are similar to those in humans, using animal which is as large as human is possible to solve this problem and advance this field of study and produce results that are more convinced and clinically relevant. In this study, the author went over the use of larger animal, what they choose is sheep. As the previous studies showed, sheep is also one of the most related animal models for biomedical experiment and pre-clinical human studies [16]. In this field, the reasons to use sheep are that the nerves in sheep and in humans have the most similar size and the regeneration ability and healing times are the same[12]. Specifically, in this study, the author showed the results from median nerve, radial nerve in forelimbs, and the femoral nerve, sciatic and tibial nerves in hindlimb, the facial nerve, inferior alveolar nerve, and spinal nerves in cranial nerves [12]. The regeneration studies mostly investigated median and facial nerves in sheep models. The regeneration of peripheral nerve can be 5 days to 18 months after different types of treatments. In an experiment, 6 months is the most commonly used study duration. The important thing is the delayed repair in experimental models must be considered because the repair of human nerve injury is always not immediate, which may have a gap in the time between repair and the injury. The studies used sheep as the animal model have used autograft, allograft, as well as tubulization[12]. In summary, there is no effect on animal health and wellness by using sheep as the experimental animal model. The mainly reason is the same size and the same ability to regeneration that sheep has as human[12].

IV. Comparison and discussion of current treatments and new and novel treatments from my primary literature

There are three main methods for the peripheral nerve regeneration based on the mechanism and materials used in the process, which are shown in Table 1. Tensionless end-to-end repair is a traditional strategy. Autologous nerve grafting is a more advanced method, which is like an interim between self-regeneration and xenogenous insert. Tissue engineered nerve grafts is the latest strategy, which then be improved by loading growth factors or by using different way to fabricate and the various materials that can be combined to make the best result.

As for the animal testing, there are also some choices to examine or test the grafts that fabricated in lab and the regeneration process, as shown in Table 2. Rat is the most common animal that can be used in lab almost for every experiment that need to do the animal test. Other rodents such as mouse and rabbit are also the popular animals among scientists. [**?**] With the development of the techniques of peripheral nerve regeneration, it is not enough to use rodent as the animal model. Thus, the new one has come out. That is sheep. For recent several years, scientists have examined the pros and cons using sheep as the animal model in peripheral nerve regeneration studies.

V. Conclusion, future work, new idea-Creativity section

Peripheral nerve system plays an important role in the connection of brain and the rest body. Peripheral nerve injury is a common clinical issue and challenge to human health and can have a major impact on the cost of patient care over the duration of their life. The injury in peripheral nerve can result from natural disasters, industrial injuries, traffic jams, war wounds and even some system diseases[17]. After peripheral nerve injuries, patients may experience motor and/or sensory disease or impairment. Carpel tunnel syndrome is such a disease that caused by compression of the median nerve as it travels through the wrist at the carpel tunnel. It causes pain, numbness, and even some other severe chrome symptoms if it cannot recovery well and most of the time it cannot without any assistant. Thus, it is very important to study the regeneration of peripheral nerve system. Given the effort of scientists and clinicians, there are several therapies on the market and under development [17]. Almost all of these strategies have shown good results [17].

However, there is still a need for refinement and improvement. For example, we can use the new strategy to construct the nerve grafts and can combine molecular targeting strategies. As mentioned before, the present strategies are on the level of improving and accelerating the regeneration of nerve. The next step cannot only be limited in enhancing axonal regeneration. Scientists, engineers, and clinicians should focus more on targeting the Schwann cells in the distal stump and

TABLE I

PROS AND CONS OF DIFFERENT METHODS

	Advantages	Disadvantages
Tensionless end-to-end repair (traditional strategy)	No insert. Self-recovery.	Slow regeneration Cannotrepair the large gap of the injure Incomplete recovery.
Autologous nerve grafting	Can use to repair larger gap in nerve injury. Self-donor nerve will not cause biocompatibilityproblem.	Slow regeneration. Sacrifice of healthy and well nerve cells. Thepiece of self-donor nerve cell is infinite.
Tissue engineered nerve grafts (TENGs) (relatively newstrategy)	Can use to repair larger gap in nerve injury. Various and almost infinite material can beused to fabricate TENGs. Can be loaded into some growth factor. Easier to be feasible and conducted. Flexibleto produce.	The xenogenous tissue implantation. Potential to cause transplant rejection. Decompositionproblem of grafts.

TABLE II

PROS AND CONS OF DIFFERENT MODELS

	Advantages	Disadvantages
Tensionless end-to-end repair (traditional strategy)	No insert. Self-recovery.	Slow regeneration Cannotrepair the large gap of the injure Incomplete recovery.
Autologous nerve grafting	Can use to repair larger gap in nerve injury. Self-donor nerve will not cause biocompatibilityproblem.	Slow regeneration. Sacrifice of healthy and well nerve cells. Thepiece of self-donor nerve cell is infinite.
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the molecular reactions that drive Schwann cell behavior. After the deeper understanding of the structural anatomy and the extracellular matrix of the peripheral nerve, and the mechanism of its regeneration, the design and the fabrication methods should be greatly renewed and improved.

Based on the use of TENGs in lab, the limitations are obvious [10]. The composed of a neural scaffold alone without any biochemical components and cannot be used in clinic because of the presence of various barriers, like biocompatibility, the growth of nerve cell into TENGs, the decomposition of TENGs, etc[10]. And using an animal as the model to conduct the experiment may be not the same as in human. Thus, I hypothesize that investigators should first load GDNF into TENGs, and then fabricate a partial artificial human-like tissue (PAHLT), which can imitate the environment and the activities of part of the peripheral nerve where it is injured. Then, I hypothesize that these GDNF-TENGs should be implanted into PAHLT. Because PAHLT is produced as the same as the injured part of body, and because TENGs are combined with the growth factor GDNF, I hypothesize that the previous problem will be overcome. The future research should also focus on such method.

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