INTRODUCTION

Repairing peripheral nerves following traumas

The standard clinical "gold standard" for bridging nerve gaps is the use of autologous sensory nerve grafts. These grafts are used even though they have many limitations in their ability to promote axon regeneration. They express distinctly different sensory phenotypes from motor nerves, and support the regeneration of their specific axon phenotype [1]. 2. Fewer axons regenerate with increasing gap length [2], being good excellent for gaps of <2 cm [3], limited for grafts >4 cm [3], and no regeneration is seen for gaps >10 cm in length [4]. 3. The number and distance axons regenerate decreases significantly with increasing time between nerve trauma and nerve repair [5]. 4. The extent of neurological recovery decreases with increasing patient age, being best for patients <25 years of age [6].

Other methods tested for bridging nerve gaps that are not as effective as sensory nerve grafts include: acellular nerve grafts [7], empty collagen tubes [8], collagen tubes filled with pure fibrin [9], nerve grafts plus fibrin [10], grafts of CNS tissue [11], Schwann cells conduits filled with dissociated Schwann cells that can be induced to proliferate and increase their release of neurotrophic factors [1], spider silk fibers facilitates Schwann cell proliferation and migration [12], biocompatible non-collagen tubes [13], various forms of conduits containing neurotrophic factors [14], Gore-Tex tubes empty of filled with mesenchymal stem cells [15], synthetic hydrogel tubes [16], conduits made of arteries, veins [17], silicon conduits [18].

Filling conduits with primary Schwann cell-like differentiated bone marrow-derived mesenchymal stem cells (DMSC) or and adipose-derived mesenchymal stem cells (ADSCs) promote axon regeneration [19]. High molecular weight basic fibroblast growth factor support the regeneration of the injured axons across 15 mm long adult sciatic nerve across gaps bridged with silicone tubes filled with Matrigel or a mixture of Schwann cells (SC) and Matrigel [20]. It is proposed that hepatocyte growth factor and insulin-like growth factor-binding protein-1 released from the hESC-MSCs are responsible for promoting the axon regeneration [21].

Nerve allografts promote axon regeneration across nerve gaps up to about 2 cm in length. A alternative material is acellular nerve allografts (ANAs), which induce similar axon regeneration across short gaps [Reid et al., 2013]. But their efficacy depends on their ability to induce host tissue Schwann cell proliferation of [22], and functional recovery decreases as the graft length increases, while the performance of isografts is superior to ANAs at all lengths [22]. This is because long-time denervated host Schwann cells become senescent. However, senescent cells can be induced to proliferate within the central and distal nerve stumps by the addition of neurotrophic factors [22]. Combining adipose-derived stem cells (ADSC) with ANA increases axon regeneration across 10 mm long nerve gaps, compared to axon regeneration induced by ANA alone [23]. This is potentially due to the release by ADSCs of neurotrophic factors.

Tissue-engineered nerve grafts constructed by incorporating autologous bone marrow mesenchymal stem cells (MSCs) into...
a neural scaffold composed of a chitosan conduit inserted with poly(lactic-co-glycolic acid) (PLGA) fibers, induce regeneration across 25-mm-long canine tibial nerve gaps [24]. However, their influence is generally not more extensive than autologous nerve grafts [24], although chitosan/PLGA scaffolds induce axons to regenerate across a 60-mm-long dog sciatic nerve gap [25], and adult neural stem cells (ANSCs) within an autologous venous graft in pig induce axons to regenerate across 30 mm long nerve gaps [26]. This is due to ANSCs increasing CNPase expression, indicating the activation of intrinsic Schwann cells [26].

**Commercial nerve gap conduits**

Commercial processed acellular rat allografts NeuraGen (NG) and AxoGen’s Avance human decellularized allograft [7] have similar efficacy in promoting axon regeneration across nerve gaps. For 14-mm sciatic nerve gaps, the efficacy of isografts is superior to processed allograft, which is more effective than the NeuraGen grafts [7], although, for a 28-mm graft model, isografts are more effective than processed allografts [7]. Since none induces more axon regeneration than autologous sensory nerve grafts [27], sensory nerve grafts remain the "gold standard" for clinical peripheral nerve repair [28].

**Influence of time between nerve lesion and repair on the extent of neurological recovery**

While immediate nerve stump anastomosis leads to almost perfect neurological recovery [29], delays in nerve repairs result in a rapid decrease in recovery, with anastomosis up to 14 days leading to good recovery in only 49% of the patients, and longer delays resulting in limited neurological recovery, but no recovery seen for repairs performed by 10 months [30].

**Electrical stimulation**

Electrical stimulation of the central nerve stump induces a 2.3-fold increase in axon sprouting vs. unstimulated control neurons [31], and a 34 -50% increase in the number of neurons that extend axons [32], increases the distance axons regenerate across nerve gaps, and increases the accuracy of sensory vs. motor axon innervation of their appropriate distal nerves [33]. It also induces the Schwann cells to up-regulate their synthesis and release of neurotrophic factors that promote axon regeneration that would otherwise not take place [34]. Part of the influence of electrical stimulation is inducing the stimulated neurons to up-regulate their levels of cyclic-AMP and a number of growth-associated genes [34], with the increased cAMP concentration inducing motor neurons to up regulate their expression and synthesis of the endogenous neurotrophic factor BDNF and its trkB receptor mRNA where the BDNF increases axon regeneration. [35].

**Macrophages**

Macrophages with a pro-healing (M2a and M2c phenotype) vs. pro-inflammatory phenotype combined with polymeric nerve tubes increase the rate of axon across 15 mm rat sciatic nerve gaps [36]. This can be accomplished by the local delivery of either Interferon-gamma (IFN-gamma) or Interleukin-4 (IL-4), within the tubes [36].

**Immunosuppressants**

The administration of FK506, at concentrations far below that required for its immunosuppressant function (1) induces a 2-fold increase in the number of axons that regenerate, (2) increases the speed of axon regeneration across 4 cm gaps, (3) increases the number of myelinated axons by 40%, (4) significantly increases myelin thickness, (5) increases the specificity of sensory and motor target reinnervation, and (6) increases the extent of neurological recovery [37]. FK506 is also effective clinically in inducing more rapid axon regeneration than is seen in control patients [38]. The influences of FK506 can be enhanced if FK506 is administered in conjunction with other factors such as NGF and bFGF [39].

**Novel clinically effective nerve repair techniques**

Based on the successful results from experiments repairing the rat sciatic nerve in which axons regenerated across nerve gaps of 4 cm, we applied for and obtained Institutional Review Board (IRB) approved to perform three different clinical studies. Three novel nerve repair techniques we proposed were to be performed on patients with completely anatomically transected nerves in their extremities, from the wrist to the shoulder, and the ankle to the hip. Any patient who presented who met these criteria was offered the opportunity to participate in the study.

Participation in this study by all individuals required each provide verbal and written consent to an IRB-approved document. The technique, as well as its potential benefits and problems were also verbally described to each patient. Only after they were offered the opportunity to ask any questions they had and had signed their written consent form were the individuals enrolled in the study. Every patient offered the opportunity to participate consented to participate.

Each surgery required about 2 hours and 20 minutes. This is only about 20 minutes longer than the time required to perform the standard peripheral nerve repair technique in which only sensory nerve grafts are used to bridge nerve gaps. Because of the duration of the surgery, all the surgeries were performed under general anesthesia.

Each study required creating a collagen conduit to bridge the nerve gaps. Once the nerve to be repaired was exposed a collagen sheet (Veritas, Synovis Innovations, St. Paul, MN) an FDA-approved product, purchased in a sterile package as a 4x8 cm of bovine pericardium type 1 collagen sheet, was sewn into a tube using the handle of a surgical tool as a template for the desired diameter tube required.

It must be noted that the standard of clinical care technique for repairing peripheral nerves with a gap is to use one or more lengths of the pure sensory sural nerve. The removal a length of sural nerve to use as a graft results in a permanent loss of all sensitivity to the dorsal and lateral aspect of the foot. Although the use of a motor nerve would induce better axon regeneration than that induced by a pure sensory nerve, it is not considered ethical to sacrifice a motor nerve when the permanent neurological loss of the sural nerve results in a minimal neurological adverse consequences.

The collagen tube was either sewn around the sensory nerve grafts (for all patients on whom a sensory nerve graft was used, the nerve was the sural nerve), after they have been secured...
to the central and distal nerve stumps, or the tubes were sewn around a suture and after the tube has been completed the suture was tied to one end of the sural nerve graft and the nerve was pulled through the conduit and then secured to the ends of the nerve to be repaired.

Once the collagen tube and sensory nerve graft were secured in place, the tube was injected through a fine catheter inserted inside the tube from the distal to the central end, and as the catheter was withdrawn the two plungers of a ratio mixing device with one syringe containing platelet-rich plasma (PRP) and the other with thrombin plus calcium, simultaneously pressed mixing the contents of the two tubes. The fibrin polymerized within 20 seconds of being injected. The PRP was obtained by withdrawing 55 cc of whole blood from a peripheral vein of the patient prior to initiating surgery. The whole blood was placed into a Gravitational Platelet Separation (GPS) tube (Biomet), and the tube placed in a GPS centrifuge. During the centrifugation, a floating filter within the tube separated the blood serum, PRP and red blood cells. After their separation the PRP was withdrawn from the tube. When pure fibrin was used TISSEEL fibrin sealant, Baxter) was prepared and when it was use to fill the conduit it was merely injected into the conduit where it polymerized within 20 seconds. When nerve gaps longer than 7.8 cm were to be repaired, two collagen sheets were sewn end to end and cut to the required length of the gap to be repaired.

These techniques were used to induce axon regeneration across nerve gaps up to 16 cm in length, when nerve repairs were performed up to 3.25 years post nerve trauma, and when patients were up to 58 years of age. The fact that the axons had regenerated entirely across the nerve gap was determined by testing for a tingling sensation in response to percussion of the area over the nerve, and the development of sensitivity to temperature, vibration (tuning fork), light touch, pressure, and pain (pin prick), or to movement of muscles innervated by the regenerated axons.

One patient of 24 years of age, with a 5 cm long radial nerve gap repaired 5 months post trauma has his nerve gap bridged with two sensory nerve grafts surrounded by a collagen tube filled with pure fibrin, recovered 100% sensory and motor recovery. The technique using a collagen tube filled with a nerve graft plus PRP 18 nerve gaps, with patients aged 24 to 50 years, with nerve gaps of 2 - 16 cm in length, and with repairs performed 0.5 – 3.25 years post trauma. The technique using a collagen tube filled with only PRP was applied to 8 nerves with patients aged 24 – 50 years of age, nerve gaps of 2 – 16 cm in length, and repairs performed from 0.5 – 3 years post trauma. The patients were accepted as they presented to the emergency room and were composed of only one female.

No patient suffered any adverse events. The only deficit suffered was by those patients whose nerve repairs required the use of a sural nerve graft who lost the sensitivity provided by this nerve. Figure 1 shows a repaired 16 cm long nerve gap bridged with a collagen tube containing a sensory nerve graft and PRP. The axons regenerates entirely through the nerve graft, into the distal nerve and reestablished sensory and motor neurological function (Figure 2). A 16 cm long peripheral nerve gap bridged with a collagen tube filled with autologous PRP. Axons regenerated entirely across the gap, through the distal nerve, and reestablished neurological function. Further studies are required to determine which of these techniques is best and to improve that technique so that it induces more axons to regenerate longer distances induces more reliably neurological recovery.

It has been argued that the purpose of developing a novel nerve gap repair technique is to avoid the need to create a sensory neurological loss by using sural nerve grafts, the combination of the sural nerve graft plus PRP induces more extensive axon regeneration and neurological recovery than using only a sensory nerve graft. Therefore, the sacrifice of the sural nerve function can be well justified. However, the third technique tested showed that the use of a sensory nerve graft is not required to achieve axon regeneration and neurological recovery achieved using sensory nerve grafts alone. However, some surgeons may select to use the "standard of surgical care" in combination with PRP rather than use PRP alone.

One patient who had a nerve gap repaired in the wrist and suffered carpel tunnel syndrome. Because the carpel syndrome surgery was in the same location as the gap repair it was possible to expose the repaired nerve region. The repaired nerve is indistinguishable from the normal nerve. Figure 3 Exposed region of a repaired nerve gap. There are no signs of the collagen tube, but the length of the repaired nerve (parallel to the black line) appears identical to that of the normal nerve. There is some accumulation of connective tissue at the site of the distal nerve graft and distal nerve stump junction, but the axons regenerated entirely across the nerve gap and neurological function was reestablished.

No control nerve repairs were performed on patients as part
of this study. However, this can be justified by the extensive published literature in which the conditions of the patient and nerve wound have been described for nerve gap length, patient age, and time between nerve trauma and nerve repair. These data clearly show that good neurological recovery is only seen for nerve gap <2 cm, for nerve repairs performed <3 months post trauma and for patients under 25 years of age, and that at values longer than these the extent of neurological recovery decreases rapidly. Further, no axon regeneration and neurological recovery has been reported for patients with gap lengths up to 16 cm in length, for nerve repairs performed up to 3.25 years post nerve trauma and for patients up to 56 years of age. Therefore, we relied on histological patient data as controls. Further, based on this we feel it is appropriate to state that these three novel nerve repair techniques induce axon regeneration under conditions where no other technique is effective.

CONCLUSIONS

A variety of techniques induce axons to regenerate across a nerve gap, but none is adequate in promoting large numbers of axons to regenerate across long nerve gaps (>2 cm), when nerve repairs are performed a prolonged times post nerve trauma (<4 months), and for older patients (>25 years of age). Therefore, for large numbers of peripheral nerve trauma patients there is no recovery of neurological function. Although sensory nerve grafts have many limitations in inducing axon regeneration under the conditions of many clinical nerve traumas, as they remain the most effective method of others tested and thus remain the clinical "gold standard" surgical technique for peripheral nerve repairs. This paper presents three simple and novel techniques that have many limitations in inducing axon regeneration under the conditions where no other technique is effective.

REFERENCES


