PERSPECTIVES

Roles of reinforced nerve conduits and low-level laser phototherapy for long gap peripheral nerve repair

Peripheral nerve injuries are common in clinical practice because of traumas such as crushing and sectioning. Lesions of the nerve structure result in lost or diminished sensitivity and/or motor activity in the innervated territory. The degree of lesion depends on the specific nerve involved, the magnitude and type of pressure exerted, and the duration of the compression. The results of such injuries commonly include axonal degeneration and retrograde degeneration of the corresponding neurons in the spinal medulla, followed by very slow regeneration (Rochkind et al., 2001). The adverse effects on the daily activities of patients with a peripheral nerve injury are a determining factor in establishing the goals of early recovery (Rodriguez et al., 2004). The most severe form of nerve damage involves complete transection of the nerve, which results in the loss of sensory and motor function at the site of injury. Although a degree of recovery can be expected in most untreated nerve injuries, the process is slow and often incomplete. Moreover, despite considerable advances in microsurgical techniques, the functional results of peripheral nerve repair remain largely unsatisfactory. The regrowth of nerves across large gaps is particularly challenging, usually requiring a nerve graft to correctly bridge the proximal and distal nerve stumps. At present, nerve autografting is the most common treatment used to repair peripheral nerve defects. However, this recognized "gold standard" technique has a number of inherent disadvantages, such as limited availability of donor tissue (IJkema-Paassen et al., 2004), secondary deformities, potential differences in tissue structure and size (Nichols et al., 2004), and numbness at donor sites (Bini et al., 2004). Although xenografts and allografts have been proposed as alternatives to autografts, the success rate of these techniques remains poor, often resulting in immune rejection. Thus, researchers have invested considerable effort in developing synthetic nerve conduits for the repair of peripheral nerve defects.

Nerve guide conduit

Scientists have developed various non-degradable (Chen et al., 2000) and biodegradable (Wang et al., 2001; Bini et al., 2004; Liu, 2008; Hsu et al., 2011) materials as synthetic nerve conduits, for example, PLGA (Bini et al., 2004) and PLA (Hsu et al., 2011). Of these materials, doctors have widely used non-degradable materials such as silicone rubber in general clinical cases because of its inert and mechanical properties. However, the main disadvantages of non-degradable conduits are that they remain *in situ* as foreign bodies following nerve regeneration and may require removal *via* a second surgery, which could possibly cause damage to the nerve. In contrast, biodegradable materials potentially avoid these problems. Therefore, biodegradable conduits seem a more promising alternative for reconstructing nerve gaps. Nerve guidance channels fabricated out of collagen have already shown rather favorable results in nerve repair (Itoh et al., 2002). However, clinical experience with collagen products has demonstrated that cracks and tears can occur when the suture needle penetrates the conduits. In addition, biodegradable conduits that degrade with time may lose their functionality as a structural cuff. Accordingly, an ideal biodegradable conduit should maintain its structural integrity during the regenerative processes (Yannas and Hill, 2004).

Gelatin is less expensive and much easier to obtain in concentrated solutions than collagen. Moreover, gelatin is a biodegradable polymer with excellent biocompatibility, plasticity, and adhesiveness. However, swelling of the degradable tube walls caused by absorption of body fluids may occur during the nerve regenerative processes. This swelling could occlude the lumen and therefore impair axonal regeneration. In addition, the handling characteristics are unsatisfactory for suturing, and the lumen of gelatin channels may collapse or be obliterated following implantation. Therefore, the use of proper cross-linking agents to modulate the mechanico-chemical characteristics of gelatin is desirable in order to prevent toxicity and generate stable materials for biomedical applications. Various cross-linkers, such as formaldehyde, glutaraldehyde (Chen et al., 2005), genipin (Yang et al., 2010, 2011), and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Chang et al., 2007) have been used to compensate for the disadvantages inherent in gelatin, and to make gelatin nerve substitutes resistant to natural biodegradation following transplantation. Since cross-linked gelatin may have low mechanical strength under physiological conditions, its applications may prove limited.

Previous studies (Yang et al., 2010, 2011) developed a biodegradable composite (GGT conduit) consisting of genipin cross-linked gelatin annexed with β-tricalcium phosphate (β-TCP) ceramic particles to enhance the mechanical strength as a nerve guidance channel-material for axon regeneration (**Figure 1**). The results of that study revealed that the TCP ceramic particles provided structural reinforcement to the genipin-cross-linked gelatin (GG) structure. Macroscopic observations show that this study does not observe any unsatisfactory swelling or deformation of the GGT nerve guide conduits. The improvement in the water uptake and swelling ratios may have been attributable to the presence of TCP ceramic particles in the GG matrix. Consequently, the GGT conduits swelled slowly and maintained a lower water uptake and swelling ratios than the GG conduits. Therefore, the hydrated GGT conduits (when grafted *in vivo* to repair nerve defects) did not stenose and collapse to compress the regenerating nerve fiber of the lumen. Mechanical measurements showed that these good mechanical properties, which benefited from the addition of TCP ceramic particles, rendered it possible for the GGT conduit to resist the muscular contraction and keep its cylindrical shape unchanged within a considerable period after implantation into the body. Since the collapse of an unfilled circular conduit is a major block to nerve regeneration in tubulization, the properties of a gelatin tube that can be molded into various configurations and compounded with TCP, can effectively enhance nerve regeneration. Besides, as tricalcium phosphate dissolves during the degradation of GGT, calcium ions could be released from the conduits, and a previous study (Kulbatski et al., 2004) has shown that a post-neuritotomy rise in calcium influx through calcium channels is a necessity for neurite regeneration. In addition, the GGT conduit had the strength necessary to withstand the muscular forces that surrounded it, meaning that a stable support structure for the extended regeneration processes was maintained. These results demonstrate the feasibility of designed GGT conduits in the applications of peripheral nerve repair.

Laser therapy

Clinicians have focused on developing more effective methods to promote nerve regeneration, target organ reinnervation, and restore function at the site of injury. Many physical and neurotrophic factors, as well as pharmaceutical drugs, influence nerve regeneration. Physiotherapy commonly involves the use of thera-

peutic instruments for regenerative purposes (Gigo-Benato et al., 2005). Various forms of external stimulation have been employed to accelerate the process of regeneration, which in turn accelerates functional recovery. Such techniques include electrical (Mendonça et al., 2003), ultrasound (Raso et al., 2005), and low-level laser (LLL) stimuli. Clinical and experimental studies have provided evidence that lasers can increase nerve function, reduce the formation of wounds, increase the metabolic activity of neurons, and enhance myelin production (Bagis et al., 2002). The non-invasive nature of laser phototherapy enables treatment without surgical intervention. LLL therapy began to be used in the regeneration and functional recuperation process of peripheral nerves in the 1970s, and the results obtained so far have been inconsistent. Many animal experiments and clinical studies have indicated that LLL irradiation can attenuate injury, promote repair, and stimulate axonal sprouting and propagation, but its mechanism of action is not well understood (Amat et al., 2006). A review of the literature on phototherapy for peripheral nerve repair found that the use of laser was based on several wavelengths (632–904 nm) (Masoumipoor et al., 2014), lesion types (crushing, neurorrhaphy, and tubulation), sample types, the duration and manner of the emission (Marcolino et al., 2013; Akgul et al., 2014), and the assessment types (such as functional, electrophysiological, and morphometric) (Gigo-Benato et al., 2005).

In many studies, descriptions of the irradiation parameters, such as dose, average power, time, and application methods, have expressly varied, hampering the methodological comprehension required for the reproduction of results and hindering comparisons between studies. Barbosa et al. (2010) sought to analyze the effects of two different GaAlAs laser wavelengths (660 nm and 830 nm) on sciatic nerve regeneration by using the same crushing injuries for a novel comparison of studies reported in the literature. They observed that the 660 nm wavelength treatment group had the best SFI scores on average, indicating that the use of these parameters was more efficient. The possibility that neural tissue is located in more superficial layers may have favored a better response to the shorter wavelength. Data also suggested that 660 nm LLL therapy with low (10 J/cm^2) or moderate (60 J/cm^2) energy densities is able to accelerate neuromuscular recovery after nerve crush injury in rats (Gigo-Benato et al., 2010). Our own previous studies investigated the influence of large-area irradiation using an aluminum-gallium-indium phosphide (AlGaInP) diode laser (660 nm) (Shen et al., 2011) and trigger point therapy using gallium-aluminum-arsenide phosphide (GaAlAsP) laser diodes (660 nm) (Shen et al., 2013a, 2013b) on the neurorehabilitation of transected sciatic nerves in rats after bridging them with the GGT nerve conduit (**Figure 2**). The results for these studies indicated that the GGT/laser system may be very helpful for long-gap nerve regeneration as well as for acceleration of the reinnervation rate of regenerated nerves, which may lead to sufficient morphologic and functional recovery of the peripheral nerve.

It has also previously been shown that LLL enhances Schwann cell proliferation *in vitro*. Schwann cells myelinate axons of the peripheral nervous system and play a crucial role in post-injury nerve regeneration. They promote neuronal survival, guide axons to their proper targets, and secrete neurotrophic factors that aid axonal elongation (Bhatheja and Field, 2006). Morphological changes in the mitochondria of lymphocytes, as well as in the proliferation of mononuclear cells, have also been observed after radiation with a red laser, and these responses might be beneficial in the process of tissue repair (Gulsoy et al., 2006). The underlying mechanism of phototherapy in nerve regeneration has been proposed in previous *in vitro* studies which showed that phototherapy induced Schwann cell proliferation, as well as massive neurite sprouting and outgrowth in cultured neuronal cells. It has also been suggested that phototherapy may enhance the recovery of neurons by altering the oxidative metabolism of mitochondria (Elles et al., 2003). The same mechanism may guide neuronal growth cones *in vitro*, perhaps through interaction with cytoplasmic proteins and, in particular, by enhancing actin polymerization at the leading edge of the axon (Ehrlicher et al., 2002). One possible molecular explanation is the increase in growth-associated protein-43 (GAP-43) immunoreactivity during the early stages of nerve regeneration proceeding phototherapy (Shin et al., 2003). In summary, all of the aforementioned effects may play a role in accelerating axonal regeneration and preventing the loss of neurons.

Although the preliminary results support the mechanical strength and biocompatibility of the GGT conduit and are encouraging in regards to peripheral nerve regeneration, further studies should attempt to improve the design of GGT nerve guide conduits. Examples of such studies could include an introduction of neurotrophic factors or seeding cells to establish the possibility of using GGT grafts as a suitable alternative to nerve autografts for peripheral nerve regeneration. With regard to clinical applicability, LLL phototherapy makes an important contribution towards the development of a safe and effective strategy for rehabilitating peripheral nerve injuries. Further studies on the use of LLL therapy as a noninvasive treatment modality for various nerve diseases and injuries could pave the way for mainstream acceptance and standardization of this innovative therapy.

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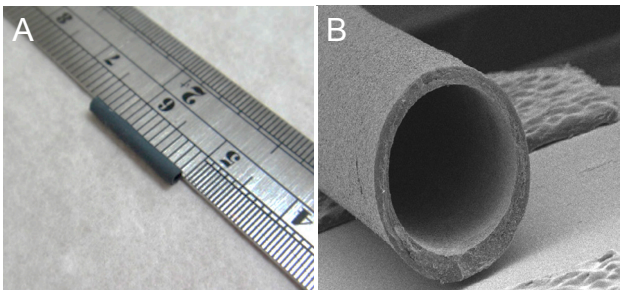

Figure 1 Smacrograph and scanning electron micrograph (SEM) of the genipin-crosslinked gelatin annexed with tricalcium phosphate (GGT) conduit.
(A) The GGT conduit was a hollow tube with a dark bluish appearance. (B) SEM cross-sectional image of the GGT conduit shows that the conduit was concentric and round with a rough compact outer wall surface and a smooth inner lumen.

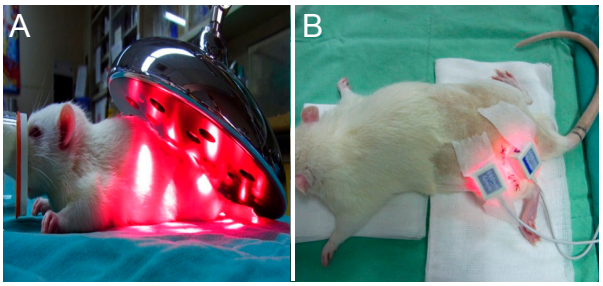

Figure 2 Transected nerve was subjected to a large-area irradiated therapy with the 660-nm aluminum-gallium-indium phosphide (AlGaInP) low-level laser (A) or a transcutaneous trigger point therapy with the 660-nm gallium aluminum arsenide phosphide (GaAlAsP) low-level laser (B).
The diode laser (Megalas®-AM-800, Konftec Co., Taipei, Taiwan, China) used in (A) is a compact multi-cluster laser system for area therapy. It has twenty AlGaInP laser diodes (output power, 50 mW) emitting a continuous 660 nm AlGaInP laser beam capable of irradiating an area of about 314 cm^2. The diode laser (Aculas-AM-100A, Konftec Co.,) used in (B) is a multi-channel LLL system designed for trigger point therapy. This device has five GaAlAsP laser diodes directly taped to the trigger point, with no risk of laser leakage. When set to continuous mode, the laser emits a wavelength of 660 nm at a power of 50 mW with a beam area of 0.1 cm^2.

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