PERSPECTIVES

Visualization of peripheral nerve regeneration

Peripheral nerve injuries are often caused by trauma and they may result in a partial or total loss of motor function or sensory perception. After nerve injuries, peripheral axons have the ability to regenerate and reconnect the proximal and distal ends of severed nerve axons if the nerve gap is small. For larger nerve gaps, surgical treatments are often required to repair the injured nerves.

Currently, there are a variety of treatments for repairing the peripheral nerve after injuries, which include transplantation of autologous nerve grafts (autografts)/allografts (Schmidt and Leach, 2003; Muir, 2010), implantation of nerve conduits (Mackinnon and Dellon, 1990a), and cell-based therapies (Frattini et al., 2012; Marconi et al., 2012; Sowa et al., 2012). A summary of treatment options for the regeneration of peripheral nerve injuries is listed in **Table 1**. Autografts are considered as the gold standard in peripheral nerve repair. However, the lack of tissue availability, the limitation of graft material length, and the requirement of a second surgical procedure to remove the graft tissue (Pollard and Fitzpatrick, 1973) are still hurdles. Although the use of allografts can potentially allow functional recovery comparable to that of autografts, the implantation of allografts need immune suppression on patients for at least 18 months (Mackinnon et al., 2001). To overcome the drawbacks of these nerve grafting techniques, the use of artificial nerve conduits is a promising alternative. There are other advantages for using a conduit. For example, a conduit can provide a suitable environment for tissue repair, reduce neuroma and scar formation (Alluin et al., 2009), facilitate the accumulation of high concentration of neurotrophic factors, and guide the regenerated nerve to their own targets. Nerve conduits can be made by materials in two categories: natural materials and synthetic materials. The choice of proper materials can increase the biocompatibility and mechanical tolerance of the nerve conduit. The structure of the conduit (*e.g.* the porosity, permeability, and guidance structure) is also important (Lu et al., 2009; Ni et al., 2010). Moreover, the designed conduits can be incorporated with the functionalized bioactive additives for promoting nerve repair (Ni et al., 2013). Recently, many studies have suggested that the addition of supportive cells in the conduits is a positive strategy for repairing long nerve defects (Sowa et al., 2012). However, the role of supportive cells in the conduit was still unclear during the nerve regeneration process.

Previous studies have already documented the regenerative process and stages occurring within a silicone tube across a 10-mm rat sciatic nerve gap (Williams et al., 1983). Briefly, the nerve regenerative process can be divided into four phases: the fluid phase, the matrix phase, the cellular phase, and then the axonal phase. In the first fluid phase, the tissue fluid originating from the damaged nerve fills the tube within 12 hours. This fluid is filled with neurotrophic factors and extracellular matrix (ECM) precursor molecules. After the fluid phase, acellular fibrin cable forms from the ECM precursor molecules between the proximal and distal stumps within one week. During the second week, this fibrin cable provides a route for Schwann cells, fibroblasts, and endothelial cells to migrate from both nerve stumps. These Schwann cells then proliferate, align, and form the tissue cable for the axonal phase of repair. In the axonal phase, new regenerative axons sprout and are guided by their growth cones to reach both nerve stumps. These regenerative stages could be observed within hollow, impermeable silicone conduits.

Although silicone tubes are chemically stable, elastic, and easy for observation of the nerve sprouting, they are not permeable and biodegradable. A second surgery is required to remove the tubes. Better conduits are made of biodegradable polymers, such as poly(glycolide) (PGA) (Mackinnon and Dellon, 1990b), poly(lactide) (PLA), poly(lactide-co-glycolide) (PLGA), and poly(ε-caprolactone) (PCL). Besides, biodegradable conduits with an asymmetric microporous structure were found to increase the nutrient exchange/waste drainage (Chang and Hsu, 2006) and prevent the cell infiltration (Maquet et al., 2000) which benefit for nerve regeneration (Hsu and Ni, 2009). Moreover, peripheral nerve regeneration can be visualized using imaging techniques, such as magnetic resonance imaging (MRI). MRI techniques can provide images for various tissues in the body due to their different water contents. Image contrast may be weighted to exhibit different anatomical structures or pathologies. The first MRI study on peripheral nerve regenerated in a conduit was reported in 2011 (Hsu et al., 2011). Peripheral nerve regeneration in an asymmetric PLA conduit (20 mm) was monitored for 18 months in a rabbit model by 1.5-Tesla MRI equipment. Based on the MR images, the volume/mass losses of the nerve conduits as well as volume/mass increases in the regenerated nerve were in the opposite trend. The regenerated nerve kept increasing in the diameter while the polymer continued to be degraded *in vivo*. The degradation kinetics of the nerve conduit may be further defined. This study laid the foundation of using the fine resolution of MRI to visualize the sprouting nerve and monitor the regeneration process *in vivo* without invasion.

Many recent studies suggest that cell transplantation may improve peripheral nerve regeneration through neurotrophic factor production and Schwann cell differentiation. However, the role of transplanted cells during peripheral nerve repair is largely unknown. Although histological analyses provide some clues, the migration of transplanted cells during the regenerative process in a conduit is unclear. As mentioned, MRI is a potential tool for visualizing the nerve regeneration. For the purpose, superparamagnetic iron oxide nanoparticles (SPIO NPs) should be used to improve the image contrast (Thanh and Green, 2010). A recent work has shown that the SPIO NP-labeled stem cells in a biodegradable conduit could be monitored by MRI *in vivo* (Tseng and Hsu, 2014). Several studies indicated that the number of SPIO NP-labeled cells detected by 3-Tesla MRI equipment *in vitro* was at least 0.5×10^6 cells (Qin et al., 2012; Li et al., 2013). When stem cells clustered to form aggregates, SPIO could be incorporated into the cellular aggregates (Hsu et al., 2012). The SPIO-labeled mesenchymal stem cell (MSC) aggregates were successfully visualized by 7-Tesla MRI equipment *in vitro* and *in vivo* even when the cell number was much lower than 0.5×10^6 cells.

In a latest study, MSCs in the form of dispersed cells or cellular aggregates were dual-labeled by SPIO and a red fluorescent cell tracker (PKH26). They were then injected into the asymmetric microporous nerve conduits made of PLA and sutured into the defected nerve. These cells were tracked by

Table 1 Treatment options for repairing the peripheral nerve injury

Treatment options	Pros	Cons
Autografts/Allografts transplantation	Gold standard	Lack of tissue availability, immune response
Nerve conduit implantation	Repairing long nerve defects, providing a suitable environment for facilitating tissue formation	Poor functional recovery in a short period of time
Cell-based therapy	Creating a more conductive nerve environment that facilitates cell incorporation within the host regenerative process	Requiring a large amount of transplanted cells due to poor engraftment

Table 2 Imaging techniques for monitoring the peripheral nerve injury/regeneration

Imaging techniques	Pros	Cons
Computed tomography (CT)	Highly detailed and precise in the field of head, lung, and cancer	Limited sensitivity in soft tissue contrast, radiation exposure
Ultrasound (US)	Non ionizing radiation, real-time imaging, inexpensive	Limited resolution for monitoring the axon sprouting
Magnetic resonance imaging (MRI)	Particularly useful for showing soft tissue structures, tracking cellular responses *in vivo*	Expensive, non-urgent conditions

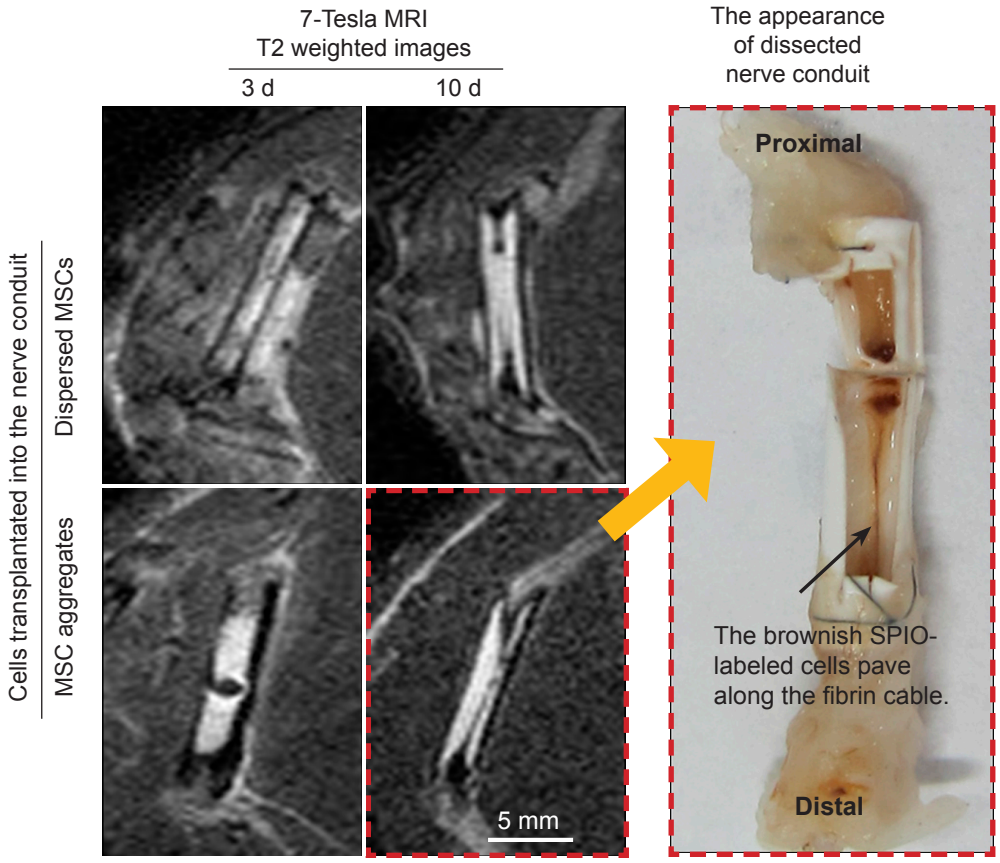

Figure 1 Visualization of the sciatic nerve regeneration process by MRI.
The left four panels are the sagittal views of MR images at 3 and 10 days (d) from animals receiving PLA conduits loaded with either dispersed cells or cellular aggregates of mesenchymal stem cells (MSCs). The right panel shows the appearance after dissecting the conduit that was loaded with MSC aggregates at 10 days post implantation.

7-Tesla MRI equipment *in vivo* (Tseng and Hsu, 2014). MR images were acquired periodically after the surgery. Before nerve sprouting, the dispersed cells were widely distributed inside the conduit after 3 days and migrated to the proximal and distal portions of the injured nerve at 10 days. In contrast, the cellular aggregates were centrally located in the mid-portion of the conduit after 3 days, and a dark signal had connected the proximal and distal ends as early as 10 days. Histological analyses confirmed that SPIO-labeled MSC aggregates bridged the two ends of the conduit (**Figure 1**) in brownish color. The brownish substance was loose and not as organized in the appearance as newly regenerated nerve. The loose substance was most likely the fibrin cable. These observations suggest that stem cell aggregates may pave along the fibrin cable before nerve sprouting. Colocalization of red fluorescence and SPIO in histology further supports that visualization of nerve regeneration and stem cell migration by MRI is feasible. With the help of dual-labeling, the survival rate and differentiation rate of transplanted cells

were estimated. The stem cell aggregates showed a much higher survival rate and differentiation rate. Moreover, nerve regeneration was much faster in this group. Therefore, SPIO cell labeling can be combined with MRI techniques to visualize the cellular and extracellular events that occur during peripheral nerve regeneration in a conduit.

Imaging techniques such as MRI, computed tomography (CT), and ultrasound (Cokluk et al., 2004) are ideal tools for visualizing anatomical structures. The advantages and disadvantages for each technique are summarized in **Table 2**. For neurological studies, MRI and ultrasound have the advantages of providing morphological information on peripheral nerve size and fascicular structure (Sorenson, 2008). CT plays no significant role in visualizing the structure or anatomy of nervous system due to the limited sensitivity in soft tissue contrast and concerns in radiation exposure. In clinical applications, ultrasound is inexpensive, safe, and non-invasive, and is better appreciated by patients in comparison to MRI. Although ultrasound imaging is useful in monitoring nerve anatomy in the region of the trauma and evaluating the peripheral nerve regeneration (Cokluk and Aydin, 2007), the limited resolution does not allow direct observation on the axon sprouting (Kuffler, 2010). Besides, scattering of the ultrasonic wave by the polymeric wall of the conduit significantly blocks the image of newly regenerated nerve inside. On the other hand, MRI has the potential to track cellular responses over time *in vivo* (Shen et al., 2010). A few studies have revealed that the transplanted cells labeled magnetically with either T1-positive or T2-negative contrast agents could be successfully tracked by MRI (Modo et al., 2002; Shen et al., 2009). Also, the homing and engraftment of these cells could be monitored. Taken together, MRI may be a better imaging tool for visualization of the peripheral nerve regeneration process than the other medical imaging platforms.

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