PERSPECTIVE

Synthetic and nature-derived lipid nanoparticles for neural regeneration

Challenges and opportunities in nerve regeneration: The central nervous system (CNS) has a limited ability to regenerate. Subsequent to spinal injury, glial scar formation, created by fibroblasts, neuroglia, monocytes, and endothelial cells, inhibits regeneration of the injured nerve. The peripheral nervous system (PNS) has a greater regeneration potential than the CNS; however, the current gold standard of treatment for a large nerve defect is still autologous nerve grafts, which require multiple surgeries. For this reason, researchers have been trying to regenerate nervous tissues, including brain, spinal cord, and peripheral nerves, for decades.

Various strategies ranging from biomaterial engineering to cell-based therapy have been explored to repair a damaged neural tissue. For example, physical approaches, including nerve guidance conduits and electrical stimulation, have been applied to facilitate nerve regeneration. Biochemical guidance cues such as neurotrophic factors (which promote the growth and survival of neurons) and adhesion molecules have often been utilized to enhance the regenerative outcomes. However, clinical applications of nerve growth factor (NGF) are still limited due to the deleterious side effects of NGF. Cell-based therapies such as the transplant of mesenchymal stem cells (MSC) or olfactory ensheathing cells have been clinically tested for spinal cord regeneration. However, most reports were case studies intended to evaluate the safety of the procedure, and its efficacy remains to be proved by a large-scale clinical trial (Harrop et al., 2012).

As knowledge and understanding of neurobiology and neuropathology accumulate, more and more intracellular signaling pathways have been identified. These new pathways provide potential molecular targets to enhance intrinsic growth. For example, the regulatory role of microRNAs (miRNAs) in neural development, degeneration, and regeneration is currently being analyzed (Eacker et al., 2009). The use of miRNAs to modulate gene expressions could offer a new treatment for nerve injuries and degeneration. However, challenges remain in effective delivery of biologics, including DNA, RNA, and proteins, to the targeted tissue. First, delivery vehicles must avoid degradation before reaching to the target cell. Additionally, for gene delivery, the cargo must then be transported through the cytoplasm into the nucleus. One avenue of approach to address this issue is the use of lipid-based nanoparticles, as their surface facilitates penetration through cellular membrane. This perspective article discusses the current status of synthetic and nature-derived lipid nanoparticles used with the aim of nerve regeneration.

Lipid-based nanoparticles for nerve regeneration: *Liposomes*: Liposomes are one of the most popular lipid-based materials used for drug and gene delivery. Liposomes have already been clinically used for many applications, such as cancer therapy, nerve block (liposomal bupivacaine), and vaccine. An excellent example of liposome used for nerve regeneration is reported by Popovich et al. (1999). Intravenously injected liposome-encapsulated clodronate to rats, administered after spinal cord injury, was demonstrated to promote axonal regeneration and functional recovery by reducing macrophage infiltration. Cationic liposomes have also been developed for efficient delivery of nucleic acid-based cargos. For example, Lu et al. (2002) delivered glial cell line-derived neurotrophic factor (GDNF) cDNA encapsulated in a cationic liposome to rats after spinal cord injury and observed recovery of locomotive functions. Other genes, including those coding for NGF and vascular endothelial growth factor (VEGF), have been delivered *via* cationic liposomes in rodent models; both have been demonstrated to work for neuroprotection and neurogenesis. In addition, ligands have been conjugated to liposomes for targeted delivery. For example, Pulford et al. (2010) combined small interfering RNA (siRNA) with a cationic liposome conjugated to a rabies virus glycoprotein (RVG) peptide, which specifically binds to a brain tissue, and delivered this complex intravenously into mouse brain cells, in order to decrease the expression of prion proteins. These promising reports suggest that liposomes could be clinically employed for nerve regeneration.

Extracellular vesicles (EVs): As a new potential approach for delivery, the use of extracellular vesicles (EVs) for tissue regeneration is of growing interest. EVs (microvesicles and exosomes) are secreted from many types of cells; they contain functional proteins and genetic material, such as mRNA and miRNA, encapsulated in a lipid bilayer. This lipid bilayer of exosomes can protect RNAs from degradation by RNAse. In the body, EVs work as a vehicle for intercellular communication: in the nervous system, EVs guide axonal development, modulate synaptic activity, and help regenerate peripheral nerve tissue (Lai and Breakefield, 2012).

A number of applications of EVs for regeneration of heart, kidney, liver, and nerve tissue have been conducted recently. Lopez-Verrilli et al. (2013) showed that exosomes derived from primary rat Schwann cells promote dorsal root ganglia (DRG) growth *in vitro* and axonal regeneration *in vivo*. Xin et al. (2013) demonstrated that administration of exosomes derived from MSC to rats following stroke promotes angiogenesis, neurogeneis, neurite remodeling, and functional recovery. Furthermore, they demonstrated that this neurological recovery is promoted by the transfer of miR-133b from MSC to adjacent neurons and astrocytes *via* MSC-derived exosomes.

In addition to the direct administration of EVs, several researchers have tried to use EVs as a delivery vehicle for drugs and genes with the goal of nerve tissue regeneration. For example, Zhuang et al. (2011) treated brain inflammation in mice by encapsulating curcumin, an anti-inflammatory drug, in exosomes by mixing them together and delivering them intranasally. Alvarez-Erviti et al. (2011) fused an RVG peptide to a membrane protein in exosomes through plasmid transfection, and inserted siRNA into the exosomes by electroporation. Once delivered *via* exosome, the siRNA knocked down BACE1 in the brain and thus the expression of β-amyloid 1–42, which plays a key role in the development of Alzheimer's disease. Another group took an alternative approach, and rather than directly encapsulating the drugs or RNAs in exosomes, they used lentivirus to regulate the expression of miR-133b in MSC and its secreted exosomes (Xin et al., 2013). Recently, Smyth et al. (2014) attached a fluorescent molecule to the surface of exosomes and liposomes using click chemistry. Taken together, these reports suggest that EVs can be engineered as carriers for drug and gene delivery.

Targeting lipid nanoparticles to neural tissues: In order to deliver lipid nanoparticles specifically to a neural tissue

rather than the surrounding tissues, careful nanoparticle design is required. Especially for delivery to a brain tissue, lipid nanoparticles need to cross the blood-brain barrier (BBB) and then target the brain tissue. Without targeting, intravenously injected lipid nanoparticles accumulate in the liver, kidney, and spleen (Alvarez-Erviti et al., 2011). Several strategies, such as RVG and RDP peptides, monoclonal antibodies, and coating with polysorbate 80, have been tested. For delivery to the PNS, Lee et al. (2013) modified liposome formulations so that Schwann cells and neurons preferentially endocytose the nanoparticles.

While tissue-specific drug and gene delivery have been demonstrated, cell-specific delivery is still difficult. Xin et al. (2013) reported that, without targeting, the administered exosomes from MSCs were delivered to adjacent neurons and astrocytes. Alvarez-Erviti et al. (2011) demonstrated that RVG peptide-expressing exosomes delivered siRNA not only to neurons but also to microglia, oligodendrocytes, and oligodendrocyte precursors. Monoclonal antibodies are often used for delivery across the BBB, but not for targeting a single cell type. A new strategy targeting a single neural cell type could achieve higher delivery efficiency.

Comparison between synthetic lipid nanoparticles and EVs: Although both synthetic lipid nanoparticles and EVs have shown effective neural delivery, there are a number of significant differences between the two systems. One major difference is the controllability of nanoparticle quality and quantity. The composition and physical properties of synthetic lipid nanoparticles can be easily modified. On the other hand, it is relatively difficult to control surface structures and composition of EVs; only a few groups have engineered EVs by encapsulating genes and drugs and by functionalizing the surface of EVs. Furthermore, the gold standard protocol for isolating EVs with well-defined composition and purity is still lacking (Lotvall et al., 2014). It is relatively simple to generate a large batch of synthetic lipid nanoparticles, while producing a large quantity of EV is tedious and laborious.

Nevertheless, EVs have their own advantages. The cargo of EVs is a mixture of many types of genetic materials and proteins, which may provide synergistic functionality in the body. This complex cargo could be an advantage of EVs over synthetic nanoparticles, because it is virtually impossible to completely mimic the complex functions of EVs by combining all of their genetic and protein components in synthetic nanoparticles. Proteomic and genetic analyses of EVs may lead to find a new material for drug and gene delivery. However, these analyses have not been completed so far, and EV's unknown cargo might induce unexpected adverse effect, such as immune response and apoptosis.

Summary and future perspectives: Delivery of biologics can modulate intracellular pathways and promote cellular and tissue growth. Lipid-based nanoparticles are promising materials for the delivery of drugs and genes with many potential clinical applications. Their lipid bilayer can easily penetrate through physiological barriers and promote delivery to organs or tissues. Liposomes have already been clinically used for many applications and could also be applied in clinical settings towards nerve regeneration. In addition to liposomes, more attention should be given to EVs, which also possess great potential for delivery. Engineering liposomes and EVs, *e.g.*, by mixing with a specific targeting peptide, could enhance delivery efficiency. The study of EVs will not only lead to direct practical uses, but will also provide useful knowledge for the design of new synthetic nanoparticles that may pave the way for more effective nerve regeneration.

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