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PERSPECTIVE

Self-healing hydrogel for tissue repair in the central nervous system

Neurological disorders are diseases of the central and peripheral nervous systems. These disorders include Alzheimer's disease, epilepsy, brain tumor, and cerebrovascular diseases (stroke, migraine and other headache disorders, multiple sclerosis, Parkinson's disease, and neuroinfections). Hundreds of millions of people worldwide are affected by neurological disorders. Approximately 6.2 million people die because of stroke each year; over 80% of deaths take place in low- and middle-income countries. More than 50 million people worldwide have epilepsy. It is estimated that there are globally 35.6 million people with dementia with 7.7 million new cases every year. Alzheimer's disease is the most common cause of dementia and may contribute to 60–70% of cases. The prevalence of migraine is more than 10% worldwide. Therefore, repairing the damaged nervous system is one of the greatest challenges in medicine. Damage to the peripheral nervous system (PNS) can lead to the loss of sensation, motor function, and muscle weakness, but the PNS is capable of significant spontaneous regeneration and in many cases some function can be restored. In contrast, neuronal regeneration following damage to the central nervous system (CNS) is generally unsuccessful and the injuries of CNS can cause permanent paralysis and loss of sensation. Therefore, CNS injuries present significant therapeutic challenges. Current clinical options for CNS disorder treatment, including drug delivery and rehabilitation therapy, are limited and these treatment options do not fully restore the original functions. Recently, cellular therapy has emerged as a treatment option for repair and regeneration of nerve injuries. However, transplantation of stem cells to the injured sites showed poor cell survival and engraftment (Wu et al., 2011). In this regard, the combination of functional biomaterials and cell delivery is a favorable and promising strategy for CNS repair.

Biomaterials used in neural tissue engineering should meet certain criteria. For example, they should have similar mechanical properties to the nerve tissues, be able to transport nutrients and metabolites, exhibit a suitable degradation rate without inflammation occurrence, and integrate well with host tissue. Among all biomaterials, hydrogels are an ideal category of candidates and have been recently utilized in regenerative medicine. Hydrogels can serve as drug delivery platforms for biomolecular therapeutics into the CNS or as scaffolds for delivering cells to repair the injured CNS tissue (Li et al., 2012). On the other hand, the size and shape of the lesion in the CNS can vary widely depending on the site of injury. Therefore, an injectable hydrogel which can fill an irregular void is particularly well suited for treating CNS injury. However, injectable hydrogels still have some drawbacks, in particular, uncontrollable gelation kinetics. Hydrogels with rapid gelation kinetics may result in clogging of the needle during delivery. On the contrary, systems with a slow gelation process may suffer from embedded drug or cell loss or diffusion from the target sites. Compared with the traditional injectable hydrogels, self-healing hydrogels can flow and deform into liquids under excessive strain and recover back into hydrogels when higher strain is removed (Zhang et al., 2011). Because of the above mechanism, a self-healing hydrogel does not show gel fragmentation following the injection and can self-heal into a bulk gel at the target site. This superior characteristics makes self-healing hydrogels more suitable than the traditional injectable hydrogels for tissue engineering applications.

Injectable hydrogels of different formulae have been used in CNS and they can be classified into naturally derived hydrogels and synthetic hydrogels. Natural polymers, such as hyaluronic acid, agarose, collagen, and chitosan, have many advantages such as good biocompatibility. They have already been directly used in clinical applications (*e.g.*, wound sealants) and have available functional groups for further chemical modification. Literatures have indicated that chitooligosaccharide (chitosan degradation products) (Yang et al., 2009) or primary amine groups of chitosan (Ren et al., 2009) can promote neurite outgrowth and neuronal differentiation. On the other hand, the gealtion of chitosan-based hydrogels can be controlled by pH variation. Chitosan hydrogels can also be degraded *via* enzymatic action. Chitosan is thus a suitable material for neural tissue engineering.

The mechanical properties of hydrogels should match those of native tissue to resist *in vivo* forces and to maintain structural integrity. On the other hand, the modulus of hydrogels has influence on cell behaviors (Leipzig and Shoichet, 2009). The adhesion and proliferation of astrocytes were suppressed when cells were cultured on soft substrates. Besides, changing the modulus of hydrogels could direct stem cells into different lineages. For neural stem cells (NSCs), the differentiation into neuronal lineages was favored on soft scaffolds, while the oligodendrocyte differentiation was favored on stiffer scaffolds (Brännvall et al., 2007). It is thus possible to manipulate the cell lineage, proliferation, and growth of NSCs by fine-tuning the mechanical properties of hydrogels. In general, hydrogels with modulus similar to that of the nervous tissue (compressive modulus ~2.0 kPa) (Georges et al., 2006) are ideal for applications in CNS regeneration.

Injectable hydrogels have been used to delivery drug to the CNS. Drug delivery by hydrogels can achieve the sustained release of bioactive molecules and promote the recovery of nerve injuries (Hoare and Kohane, 2008). In spite of the extensive studies on drug delivery by hydrogels, only one study has indicated that self-healing hydrogel as drug delivery system may hold promise for better applications in anti-tumor therapy and not focused on CNS repair. Other than drug, cells embedded in hydrogels are also used to induce neural regeneration. Injectable hydrogels encapsulating cells could promote nerve regeneration in CNS, *e.g.*, spinal cord injury (SCI) or stroke. However, the use of self-healing hydrogels (with or without cells) in CNS repair has not been reported until very recently.

The injectable, self-healing hydrogel was reported for the first time to heal CNS disorders (Tseng et al., 2015). The chitosan-based self-healing hydrogel had a modulus ~1.5 kPa and a damage-healing mechanism to promote the survival and growth of neurosphere-like progenitors, as the reasonable modulus could induce the cell differentiation into neuron-like cells *in vitro*. Besides, choosing a right, fast, and safe preclinical model for the damaged CNS is important. Zebrafish embryos were employed to evaluate the potential of self-healing hyhrogels in CNS repair (Tseng et al., 2015). Zebrafish has emerged as an important vertebrate model for biomedical studies because of the large number of progeny, which greatly facilitates genetic analysis. It is relatively easy to handle and cheap to maintain in comparison to the other vertebrate research models. Besides, the embryo is transparent that facilitates the fluorescent-labeled cells for observation. Because of the above advantages, zebrafish bioassays may be a suitable preclinical model for the damaged CNS.

To evaluate if the chitosan-based self-healing hydrogel with appropriate stiffness (~1.5 kPa) could heal the damaged CNS, the CNS of the developmental zebrafish embryos was destroyed by treating with 2% ethanol. Rescue of the neural deficits by various treatments was evaluated by the in-chorion coiling

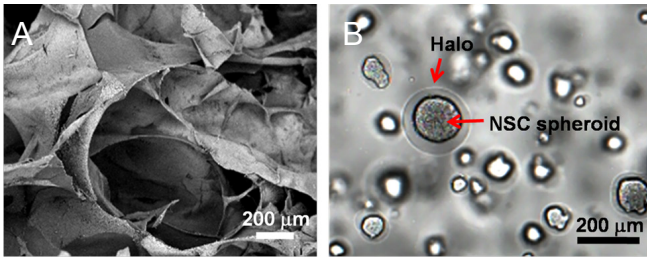

Figure 1 Scanning electron microscope (SEM) images of chitosan-based self-healing hydrogel (A) and the optical image of neural stem cell (NSC) spheroids encapsulated in the hydrogel (B).
The SEM image for lyophilized hydrogel shows open and interconnected porous structure. The average pore size was mainly distributed in the range of 0.2–1.5 μm for the self-healing hydrogel. The NSC spheroids encapsulated in the self-healing hydrogel well maintained their original shape and became greater in size after 3 days. Halo was observed for each spheroid and that may allow more space for spheroid expansion.

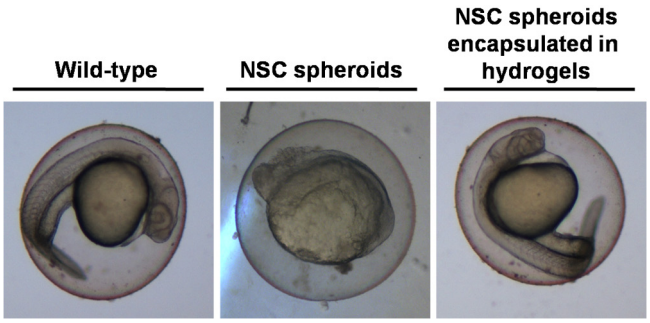

Figure 2 The appearance of zebrafish by neural stem cell (NSC) multicellular aggregate injection.
Embryos were first exposed to ethanol in the medium for 4 hours to induce neural system disorder. The medium was then replaced with fresh normal medium following injection of NSC aggregates. After the treatment, the neural deficit embryos resulted in central nervous system over-growth. Injection of NSC aggregates encapsulated in self-healing hydrogel showed normal development of zebrafish.

contraction (an index of motor function) and hatching rate (an index for CNS function) (%) of the embryos. The values were compared to those of the blank control (without ethanol treatment) to reveal the neural functional rescue by different treatments. Results showed that animals receiving NSC spheroid-laden hydrogel had the highest hatching rate (~73%), followed by those receiving NSC-laden hydrogel (~39%), and then those receiving hydrogel only (~34%). Embryos injected with phosphate-buffered saline and those of the untreated group had the lowest hatching rate. These results suggest that the self-healing hydrogel alone could partially rescue the CNS function probably because of its fast void filling ability. Besides, the self-healing hydrogel had large and interconnected pores, which may facilitate the cell metabolism and cell-cell communication. The proper microenvironment may also account for the proliferation of encapsulated NSCs (**Figure 1**). Chitosan-based self-healing hydrogel encapsulating neurprogenitors could further help to heal the damaged CNS and rescue the otherwise impaired neural function. Interestingly, only injection of NSC spheroids into the neural deficit embryos resulted in CNS over-growth (**Figure 2**). Compared to an earlier study showing that self-healing hydrogels had the ability to control long-term cancer drug delivery (de Las Heras et al., 2005), we hypothesized that the self-healing hydrogel may regulate the release of growth factor produces by NSCs and lead to normal neural development (Tseng et al., 2015).

Using injectable hydrogels, and especially injectable, self-healing hydrogels, for local and controlled cell delivery to CNS is a fast and new expanding discipline of regenerative medicine. A multitude of molecular and NSC therapeutics can be combined with hydrogels to repair injured CNS. In this status review, we have highlighted the chitosan-based self-healing hydrogel with CNS repair efficacy which was supported by the zebrafish model. The chitosan-based self-healing hydrogel is nontoxic, biodegradable, and biocompatible (de Las Heras et al., 2005). NSC spheroids within chitosan-based self-healing hydrogel are able to differentiate into neurons and glial cells as a potential cellular source for neural regeneration. The zebrafish neural injury model may be used to screen potential biomaterials for applications in CNS related diseases. With the recent advances, we expect new smart materials that can respond to changes in external stimuli such as shear, temperature, pH, and specific ligands will be developed in the near future to provide exciting possibilities to repair CNS related diseases in the next ten years.

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