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Click-crosslinked injectable hyaluronic acid hydrogel is safe and biocompatible in the intrathecal space for ultimate use in regenerative strategies of the injured spinal cord

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ABSTRACT

Traumatic spinal cord injury (SCI) causes damage and degeneration at and around the lesion site resulting in a loss of function. SCI presents a complex regenerative problem due to the multiple aspects of growth inhibition and the heterogeneity in size, shape and extent of injury. Currently, there is no widely accepted treatment strategy available and delivering biomolecules to the central nervous system remains a challenge. With a view towards achieving local release, we designed a hydrogel that can be injected into the intrathecal space. Here we describe the synthesis and characterization of a click-crosslinked hyaluronic acid hydrogel and demonstrate controlled *in vitro* release of bioactive brain derived neurotrophic factor. Importantly, we demonstrate that this new hydrogel is both biocompatible in the intrathecal space based on immunohistochemistry of the host tissue response and safe based on behavioral analysis of locomotor function.

1. Introduction

Traumatic spinal cord injury (SCI) causes damage and degeneration to glia, neurons, and axons at the lesion site, often resulting in permanent loss of function below the site of injury [1]. However, SCI rarely results in complete tissue disruption at the lesion site, and in many cases there is some functional preservation in segments below the injury, indicating survival of functional axons across the lesion [2]. Therefore, minimally invasive treatment strategies, which enhance preservation of nervous tissue at the injury site and promote regeneration of lesioned tissue, are attractive for SCI.

While drug delivery-based treatment strategies hold great promise, prolonged systemic delivery often leads to unwanted side effects, and some of the more promising therapeutic proteins degrade quickly when delivered systemically, often not reaching the spinal cord in efficacious concentrations [3]. Moreover, many therapeutic molecules are unable to cross the blood–spinal cord barrier (BSCB), which limits their accumulation in the spinal cord after BSCB repair [4,5]. Strategies that deliver therapeutic molecules locally avoid some of these obstacles. Two techniques have been used to deliver drugs intrathecally to patients: bolus injection, which is simple, results in the drug being dispersed throughout the central nervous system (CNS) by cerebrospinal fluid (CSF) flow [6,7]; and minipump delivery, where the indwelling catheter may either damage the cord or become obstructed or infected [8]. An alternative strategy, which has significant appeal, is local hydrogel injection, where bioactive molecules are dispersed in the hydrogel, thereby localizing the therapeutic molecule to the spinal cord tissue at the site of injection [9,10]. For the latter, injectable hydrogels should be: (1) injectable through a fine gauge needle for minimally invasive insertion; (2) a gel at the injection site to ensure local delivery; (3) degradable/resorbable to avoid a second procedure for biomaterial removal; and (4) bio-inert, eliciting no or minimal toxic or immune response.

Here we describe a hydrogel for intrathecal injection at the site of injury of the spinal cord (see Fig. 1). A small number of injectable hydrogels, categorized as either physical or chemical gels [2], have been used for spinal cord repair, but only a few have been injected intrathecally [9–11]. Some of these hydrogels have proven to be safe and capable of local delivery of therapeutics to the injured spinal cord [9,11]. These physically-crosslinked gels, while obviating the use of potentially cytotoxic crosslinking agents, are often

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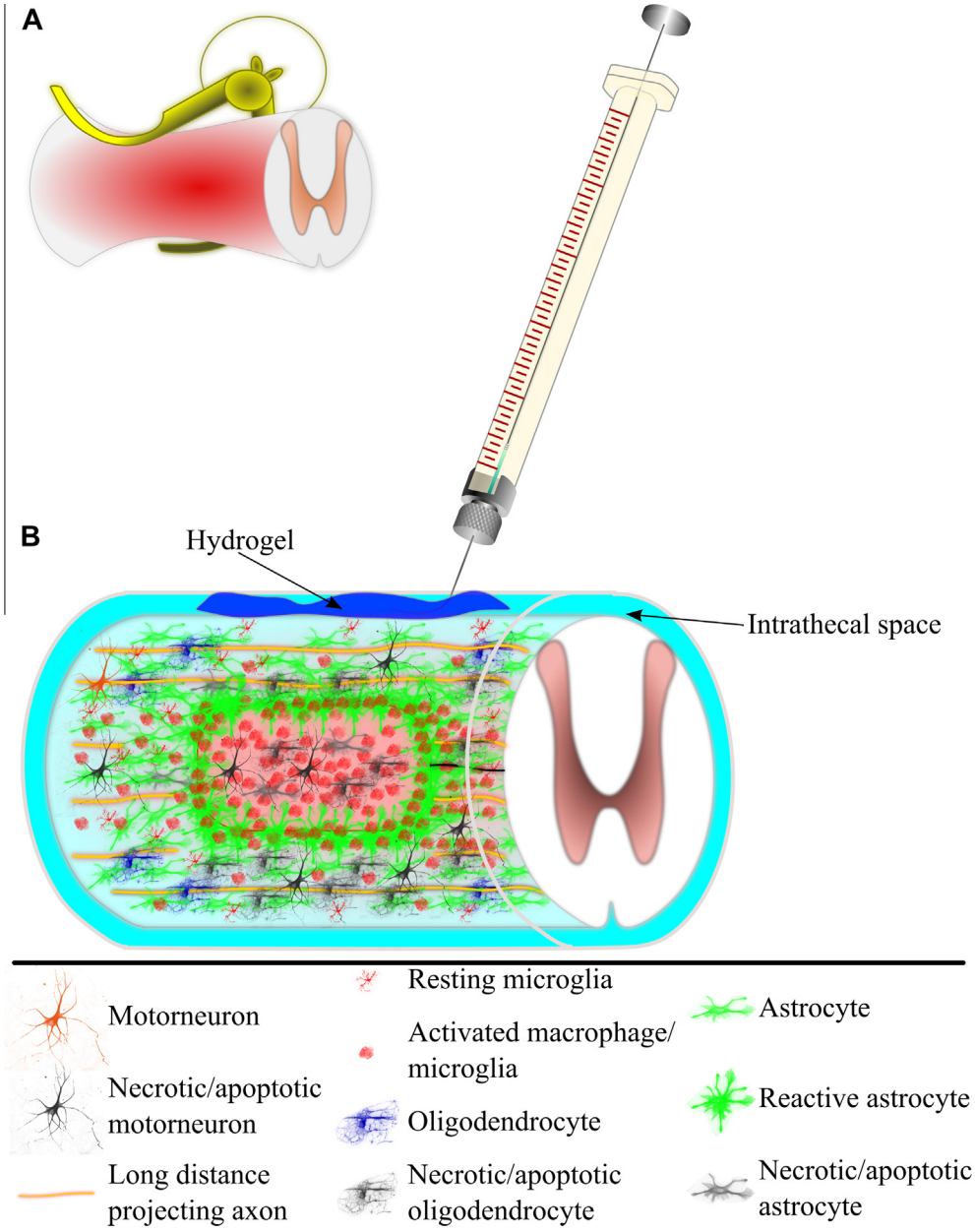

Fig. 1. (A) Schematic of the clip compression injury and (B) the hydrogel injection into the intrathecal space on top of the injury. Injection of the hydrogel into the intrathecal space prevents further damage to the spinal cord (compared to direct injection into the tissue).

stable for days to weeks. Chemically crosslinked hydrogels can be tuned to degrade more slowly, thereby providing a route for prolonged biomolecule delivery to the injured spinal cord [12]. However, chemical gels often require potentially cytotoxic molecules for crosslinking, such as coupling agents, catalysts or photoinitiators, thereby potentially compromising biocompatibility [12,13]. Crosslinking via disulfide bond formation overcomes some of these problems, yet the polymer structure can be negatively impacted and side reactions with native proteins are likely [13,14]. Click-crosslinked hydrogels provide the advantages of more stable gels without the disadvantages of cytotoxic coupling agents and side products [15].

Hyaluronic acid (HA) is a major component of the native extracellular matrix [16], but does not form a gel on its own. By reacting HA-furan with poly(ethylene glycol) (PEG) bis-maleimide, a crosslinked hyaluronic acid (xHA) hydrogel is formed based on the Diels–Alder cycloaddition of the HA-furan and PEG-maleimide [17,18]. Furthermore, HA has been shown to have immunomodulating effects and a hydrogel comprised mainly of HA may be beneficial on its own for tissue regeneration [9,19–21].

We show that this xHA is safe and biocompatible using an experimental animal model of spinal cord injury, and also allows sustained release of bioactive brain-derived neurotrophic factor (BDNF) *in vitro*. To test safety and biocompatibility, the xHA hydrogel was injected intrathecally into both non-injured rats and rats with experimental spinal cord injury using a clinically relevant, moderate clip compression injury model [22,23]. Behavioral and histological analysis demonstrated the safety and biocompatibility of xHA hydrogels, respectively, based on the Basso–Beattie–Bresnahan (BBB) locomotor rating scale [24], and immunostaining of macrophages, microglia, axons and astrocytes. To assess the utility for sustained release of bioactive growth factors, BDNF was encapsulated in poly(lactic-co-glycolic acid) (PLGA) nanoparticles and evaluated for both release, using a BDNF ELISA, and bioactivity