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In vitro analysis of PNIPAAm–PEG, a novel, injectable scaffold for spinal cord repair

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Abstract

Nervous tissue engineering in combination with other therapeutic strategies is an emerging trend for the treatment of different CNS disorders and injuries. We propose to use poly(*N*-isopropylacrylamide)-co-poly(ethylene glycol) (PNIPAAm–PEG) as a minimally invasive, injectable scaffold platform for the repair of spinal cord injury (SCI). The scaffold allows cell attachment, and provides mechanical support and a sustained release of neurotrophins. In order to use PNIPAAm–PEG as an injectable scaffold for treatment of SCI, it must maintain its mass and volume over time in physiological conditions. To provide mechanical support at the injury site, it is also critical that the engineered scaffold matches the compressive modulus of the native neuronal tissue. This study focused on studying the ability of the scaffold to release bioactive neurotrophins and matching the material properties to those of the native neuronal tissue. We found that the release of both BDNF and NT-3 was sustained for up to 4 weeks, with a minimal burst exhibited for both neurotrophins. The bioactivity of the released NT-3 and BDNF was confirmed after 4 weeks. In addition, our results show that the PNIPAAm–PEG scaffold can be designed to match the desired mechanical properties of the native neuronal tissue, with a compressive modulus in the 3–5 kPa range. The scaffold was also compatible with bone marrow stromal cells, allowing their survival and attachment for up to 31 days. These results indicate that PNIPAAm–PEG is a promising multifunctional scaffold for the treatment of SCI.

Keywords: Spinal cord injury; Hydrogel; Injectable; Neurotrophins

1. Introduction

Spinal cord injury (SCI) affects nearly 250,000 Americans, with an estimated additional 10,000 cases per year. The vast majority of these cases are young adults in their early twenties, who face enormous physical challenges with no treatment currently available. In addition, long-term care of these patients creates an overwhelming financial burden on the healthcare system. In order to successfully repair the lost and damaged tissue following SCI and promote functional recovery, a number of problems need to be solved: (i) survival of nervous tissue needs to be increased and lost cells need to be replaced; (ii) the glial scar which is created after the injury and serves as a barrier to axonal growth must be dissolved; (iii) the fluid-filled cyst created at the site of injury must be filled with a matrix that supports axonal growth; (iv) the immune reaction that follows the initial injury and leads to inflammation and secondary injury has to be modulated; and finally, (v) regenerating axons must be guided to their target cells to form functional synapses.

For successful recovery of function, a design must address these problems and creating a permissive environment for axonal growth and repair. A variety of strategies have been proposed to create such an environment, including using trophic factors, cellular transplants or polymeric scaffolds, and combinations of these treatments [1–25]. Tissue engineering is an emerging field in biomaterial research with great therapeutic potential, but the greatest challenge facing this field is to translate engineering approaches to the clinic. There are large numbers of designs proposed

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in current literature that focus on microstructure design of porous scaffolds or channels that must be conditioned ex vivo prior to implantation (reviewed in Ref. [25]). This approach is not only complicated at the engineering level, but also challenging for surgical implantation. The ideal solution would be to create a scaffold that is designed more simply and robustly, and can be easily transplanted and adapted to different injury types.

Common biodegradable materials for scaffold designs are either poly(lactic acid) (PLA), PLA-based copolymers, alginate or collagen [17,24,26–31]. All these materials degrade over time and degradation rates can be adjusted by copolymerization with different polymer blocks. These designs provide only temporary mechanical support to the injury site and do not guarantee injured axons a stable platform for regeneration. If the degrading scaffold loses its stabilization ability before the axons sufficiently regenerated, the injury site will be subject to compressive stresses, leading to more cell death and inflammation [32]. As the scaffold degrades it creates a moving boundary layer between the tissue and biomaterial that can lead to an increased inflammatory response and glial scar formation [33]. Furthermore, if the scaffolds are designed to increase cellular attachment and biocompatibility, then the removal of such a supportive environment for the regenerating tissue could be detrimental.

Some researchers have begun to investigate injectable scaffolds that can fill the site of injury and be delivered using minimally invasive surgery [2]. These scaffolds also have the desirable property of molding to the irregularly shaped injury site. However, most of these scaffolds require gelation (cross-linking) in vivo which could lead to complications from unreacted monomer or excess reactants [2]. In the last several years, temperature-sensitive and in situ-forming hydrogel systems have gained extensive interest for biomedical and pharmaceutical applications. One such polymer is poly(*N*-isopropyl acrylamide), or PNIPAAm. PNIPAAm-based systems have been one of the most commonly used thermosensitive materials for two major reasons: (i) its phase transition conveniently occurs between ambient and body temperature; and (ii) copolymerization of PNIPAAm with different types of monomers can result in materials with a range of different properties [34]. For example, the incorporation of hydrophilic co-monomers tends to increase the swelling capacity of PNIPAAm networks [35,36], allowing them to become more macroporous and thus able to accommodate cells. Cho et al. encapsulated human mesenchymal stem cells in a matrix made from PNIPAAm grafted with chitosan [10]. The cell–thermosensitive gel complex was injected into the submucosal layer of the bladder of a rabbit, resulting in cartilage growth [37]. This study shows that PNIPAAm gels are not only biocompatible, but also good substrates for cell attachment and growth. PNIPAAm-based hydrogels can also be used to immobilize drugs, enzymes, antibodies and other biomolecules, which can then be released in an initial burst followed by drug release from within the matrix via diffusion [38]. The most desirable property of PNIPAAm-based hydrogels is, however, that these gels form in situ and do not require cross-linking in vivo. This eliminates the risk of excess reactants and allows the gels to mold to the irregularly shaped injury site.

Many polymeric scaffolds do not address another key design obstacle—mechanical mismatch. If matrices are not correctly engineered, their use can lead to implant failure [15]. Ozawa et al. [39,40] performed extensive mechanical analysis of the white and gray matter of spinal cord tissue. They found that the compressive modulus of the spinal cord white matter is on the order of 3–5 kPa, providing a baseline magnitude that could be used in mechanical analyses of neural tissue engineered constructs.

Although many different hydrogel-based scaffolds have been evaluated for spinal cord repair, this study is testing a novel injectable polymeric design. We propose that the thermally responsive poly(*N*-isopropyl)-graft-poly(ethylene glycol) (PNIPAAm–PEG) can function as an injectable multifunctional scaffold for tissue engineering applications. Below its lower critical solution temperature (LCST), typically around 29–32 °C, the polymer forms a miscible solution with water, but above this temperature it becomes hydrophobic, separating from water and forming a semi-porous gel. Since the polymeric scaffold is semi-porous, cell can be easily incorporated. Cells and therapeutic factors such as neurotrophins can be mixed with the polymer at room temperature or below and then delivered in a minimally invasive fashion to provide a space-filling multifunctional scaffold that molds itself to the site of injury.

Previously attempted cellular scaffolds have included Schwann cells, olfactory ensheathing cells, fibroblasts, marrow stromal cells and neural precursor cells [13,29,41–45]. Using cellular scaffolds is of great interest because a small number of cells could potentially fill a large "gap". Transplanted cells not only provide matrices for growing axons but may also provide the necessary trophic factors and extracellular cues necessary for axonal regeneration. Cellular scaffolds, although biologically advantageous, do not meet a lot of the biomechanical requirements. Since cellular bridges lack the full design requirements, the combination of a polymer and cell could have advantages.

Marrow stromal cells (MSCs, also known as mesenchymal stem cells) are easily accessible compared to most other proposed transplanted cell lines, and are found largely in the bone marrow. Transplanted MSC in injured mouse models show they are capable of migrating to the injured tissue [46]. Transplanted MSCs have also shown the ability to attract host cells to the transplantation site [47]. It is believed that the greatest benefit of using these transplanted cells is their ability to express and secrete different cytokines and growth factors [22]. The transplantation of these cells into injured rat spinal cords yielded locomotor improvements as measured on the BBB scale, and can be attributed to the release of growth-promoting factors from the MSCs [22].

Since the goal of these studies was to find a scaffold that was best suited for further testing in animal models, it was

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