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Drug-eluting microfibrous patches for the local delivery of rolipram in spinal cord repair

Timothy L. Downing [a,b,1], Aijun Wang [b,1,2], Zhi-Qiang Yan [c], Yvette Nout [d,3], Andy L. Lee [b], Michael S. Beattie [d], Jacqueline C. Bresnahan [d], Diana L. Farmer [e], Song Li [a,b,*]

[a] UC Berkeley & UCSF Joint Graduate Program in Bioengineering, Berkeley/San Francisco, CA, USA
[b] Department of Bioengineering, University of California, Berkeley, B108A Stanley Hall, Berkeley, CA 94720-1762, USA
[c] Institute of Mechanobiology & Medical Engineering, Shanghai Jiao Tong University, Shanghai, China
[d] Brain and Spinal Injury Center, Department of Neurological Surgery, University of California, San Francisco, USA
[e] Department of Surgery, University of California, Davis, School of Medicine, Sacramento, CA 95817, USA

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ABSTRACT

Spinal cord injury (SCI) remains a major challenge for regenerative medicine. Following SCI, axon growth inhibitors and other inflammatory responses prevent functional recovery. Previous studies have demonstrated that rolipram, an anti-inflammatory and cyclic adenosine monophosphate preserving small molecule, improves spinal cord regeneration when delivered systemically. However, more recent studies showed that rolipram has some adverse effects in spinal cord repair. Here, we developed a drug-delivery platform for the local delivery of rolipram into the spinal cord. The potential of drug-eluting microfibrous patches for continuous delivery of high and low-dose rolipram concentrations was characterized in vitro. Following C5 hemisections, athymic rats were treated with patches loaded with low and high doses of rolipram. In general, animals treated with low-dose rolipram experienced greater functional and anatomical recovery relative to all other groups. Outcomes from the high-dose rolipram treatment were similar to those with no treatment. In addition, high-dose treated animals experienced reduced survival rates suggesting that systemic toxicity was reached. With the ability to control the release of drug dosage locally within the spinal cord, drug-eluting microfibrous patches demonstrate the importance of appropriate local release-kinetics of rolipram, proving their usefulness as a therapeutic platform for the study and repair of SCI.

1. Introduction

Spinal cord repair remains one of the biggest challenges for regenerative medicine. Recent studies have found that spinal cord injury (SCI) is over five times more prevalent than previously estimated, bringing the total number of people living with SCI to over one million in the United States alone [1]. In addition, as palliative care continues to advance, this number will continue to rise.

Severe SCI is marked by a disruption in the ascending and descending axons of the spinal tracts. This disruption prevents vital communication between the brain and other parts of the body. Following injury, neural cells die—namely neurons and oligodendrocytes; molecules that are inhibitory to axon growth are secreted; and reactive astrocytes and infiltrating macrophages facilitate glial scar formation, leading to the physical and chemical impedances of axon growth [2–7]. These events are accompanied by active secondary degeneration of myelin tissues, causing additional losses in neural cell populations at or near the injury site [8,9]. Together, these events cause paralysis and prevent natural recovery. Considering this, a successful regenerative strategy might require a combination of therapies that have independently been shown to mitigate the previously mentioned pathophysiologies. Before such a strategy can become a reality, however, the efficacy and safety of each individual therapy must be ensured.

Previous research has demonstrated the therapeutic utility of small molecule drugs such as rolipram in spinal cord repair [7,10]. With its anti-inflammatory [11,12] and cyclic adenosine monophosphate preserving properties, rolipram has been shown to promote regeneration of new axons [13,14], aid in the preservation of myelinated tissues [15], attenuate acute oligodendrocyte death [16,17], reduce reactive gliosis and subsequent glial scar formation [14], and significantly improve functional recovery [13,14,17,18] after SCI. However, more recent studies have presented data that leave the efficacy and safety of rolipram usage for spinal cord repair in question.

* Corresponding author at: Department of Bioengineering, University of California, Berkeley, B108A Stanley Hall, Berkeley, CA 94720-1762, USA. Tel.: +1 510 666 2799; fax: +1 510 666 3381.
E-mail address: song_li@berkeley.edu (S. Li).
[1] Contributed equally to this work.
[2] Present address: Department of Surgery, University of California, Davis, School of Medicine, Sacramento, CA 95817, USA.
[3] Present address: Department of Animal and Veterinary Sciences, College of Agriculture, California State Polytechnic University, Pomona, CA 91768, USA.

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A study led by Sharp et al. was unable to replicate the beneficial effects observed through the subcutaneous (s.c.) delivery of rolipram in combination with the transplantation of Schwann cells as previously reported by Pearse et al. [19]. A second report also found little added functional recovery from the systemic delivery of rolipram with the transplantation of a glial restricted precursor cell population [20]. Interestingly, the latter report also suggested that the s.c. delivery of rolipram could lead to adverse effects locally within the spinal cord (e.g., increased death of transplanted cells) as well as systemically (e.g., increased body-weight loss and micturition peak frequencies). While others have reported the importance of the local concentration of rolipram within the spinal cord [14], the conflict surrounding rolipram usage for spinal cord repair remains largely unresolved.

Several groups have utilized biodegradable scaffolds for many neural regeneration strategies [21–29]. Previously we have explored the use of natural materials [30] and synthetic scaffolds with and without drug-delivery for spinal cord regeneration [31,32]. Electrospun nanofibrous patches, with rolipram immobilized to the surface via hydrophobic adsorption, showed anatomical as well as functional improvements after a T9–T11 level SCI. The direct combination of rolipram with the fibrous topography of patches was shown to increase axon growth through the membrane and in the lesion, promote angiogenesis through the membrane, and decrease the population of astrocytes and chondroitin sulfate proteoglycans at the lesion [31]. However, with only the adsorption of rolipram onto fibrous patches via hydrophobic interactions, very little control over drug loading and subsequent release-kinetics was achieved. A new platform with greater control over the local delivery of small molecule drugs into the spinal cord would greatly assist in better understanding the effects of rolipram dosage in spinal cord repair, ultimately allowing for the development of more robust therapeutic strategies.

2. Materials and methods

2.1. Microfibrous membrane fabrication

The assembly of drug-eluting microfibrous patches began with the fabrication of biodegradable microfibrous membranes. Electrospinning technology was employed for this purpose as previously described [25,33]. Briefly, a polymer solution composed of 19% (w/v) poly(L-lactide), M.W. 85,000–160,000 (PLLA, Sigma) dissolved in 1-1-1,3-3-3 hexafluoro-2-propanol (Matrix Scientific) was jetted from an electrically charged (12 kV) single-needle spinneret and collected onto a grounded rotating mandrel (800 RPM; 10 cm diameter). The electrospinning distance was fixed at 7 cm. Once formed, membranes where submerged in water at 65 °C and mechanically stretched to further induce fiber alignment. For decontamination, membranes were submerged in 70% ethanol for 30 min and thoroughly washed in sterile PBS.

2.2. The assembly and characterization of drug-eluting microfibrous patches

PRONOVA™ Ultrapure LVM sodium alginate (NovaMatrix™; FMC BioPolymer) was used to form a thin hydrogel layer on top of microfibrous membranes. This hydrogel layer was used as a drug excipient for the local delivery of rolipram into the spinal cord. Removable polystyrene chamber gaskets were modified from Lab-Tek™ II Chamber Slide™ Systems (Thermo Scientific). Chamber gaskets were placed on top of microfibrous membranes and secured, creating a 2 cm by 2 cm well for hydrogel placement (Fig. 1A). Five-hundred microliters of a 2% (w/v) sodium alginate solution, dissolved in deionized (d.i.) water, was added to chamber wells and layered over each membrane surface. In order to obtain a flat and homogenously polymerized hydrogel, this sodium alginate layer was crosslinked using a calcium chloride-rich mist. Mist was produced via ultrasonic vibrations generated by a Nutramist™ 3-head fog module (FutureGarden™). Following crosslinking, newly formed alginate hydrogels were air-dried onto microfibrous membrane surfaces at 37 °C overnight and chamber gaskets were removed. This process helped to slow the elution of loaded drugs during rehydration [34] as well as reduce patch thickness for implantation. The microscale structure of drug-eluting microfibrous patches was resolved under high-powered scanning electron microscopy (SEM). Microfiber diameter typically ranged from 500 nm to 1 μm; however, fiber diameters up to 2 μm were occasionally observed.

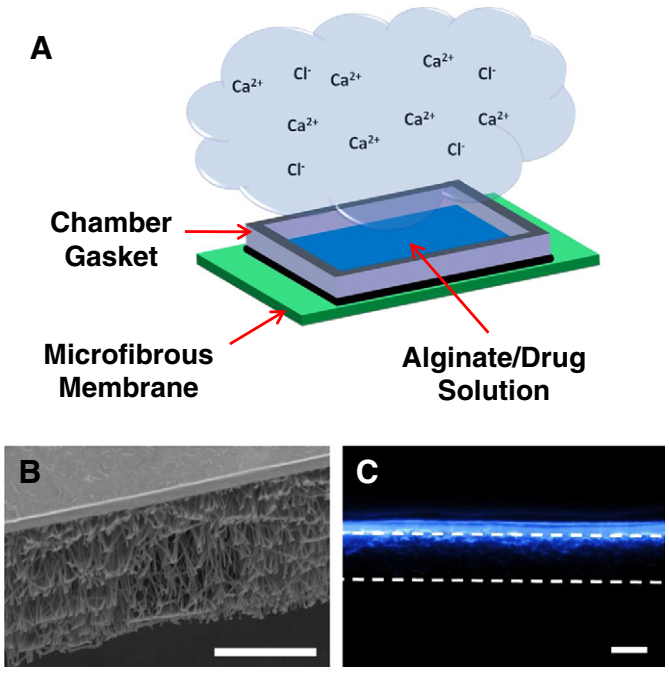

Fig. 1. Assembly and structure of drug-eluting microfibrous patches. (A) A schematic illustrating the assembly of drug-eluting microfibrous patches. Chamber gasket is used to create a well for sodium alginate solution (loaded with drug or empty) placement over the top of PLLA microfibrous membranes. In addition, a novel calcium chloride-rich mist crosslinking mechanism is demonstrated. SEM image reveals (B) the structure of microfibrous membrane and alginate layers within drug-eluting microfibrous patches. (C) Fluorescent micrograph of patch cross-section shows the incorporation of small fluorescent molecules (DAPI) into the alginate layer. Dashed lines indicate microfibrous membrane boundaries. Scale bars = 50 μm.

2.3. Rolipram loading concentration and drug-release profile

Drug-eluting microfibrous patches were loaded with variable concentrations of rolipram. To do so, rolipram was first dissolved in DMSO (0.1 mg/μl) and then added to a larger volume of d.i. water. An equal volume of 4% sodium alginate was then added to rolipram solutions, bringing the final sodium alginate concentration to 2%. Final concentrations of rolipram were 25 (low-dose) and 500 (high-dose) μg/ml. The final amounts of DMSO within hydrogels were ≤0.5% (vol/vol). To minimize the risk of contamination, patches were fabricated under sterile conditions in a laminar airflow hood. In addition, all materials and devices used were decontaminated with 70% ethanol and solutions were sterile-filtered through a 0.2 μm filter prior to use.

To obtain the release profiles of rolipram, drug-eluting microfibrous patches were made by using either 100 or 500 μl of rolipram-loaded sodium alginate. After fabrication, patches were submerged in 0.5 ml of PBS and incubated at 37 °C for up to 14 days. At selected time points (18 h; 1.5, 3, 7, and 14 days) solution was withdrawn and the amount of rolipram in the withdrawn solution was determined via HPLC. From this information the total amount of released rolipram was calculated and release profiles were generated.