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A mechanical microconnector system for restoration of tissue continuity and long-term drug application into the injured spinal cord

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ABSTRACT

Complete transection of the spinal cord leaves a gap of several mm which fills with fibrous scar tissue. Several approaches in rodent models have used tubes, foams, matrices or tissue implants to bridge this gap. Here, we describe a mechanical microconnector system (mMS) to re-adjust the retracted spinal cord stumps. The mMS is a multi-channel system of polymethylmethacrylate (PMMA), designed to fit into the spinal cord tissue gap after transection, with an outlet tubing system to apply negative pressure to the mMS thus sucking the spinal cord stumps into the honeycomb-structured holes. The stumps adhere to the microstructure of the mMS walls and remain in the mMS after removal of the vacuum. We show that the mMS preserves tissue integrity and allows axonal regrowth at 2, 5 and 19 weeks post lesion with no adverse tissue effects like in-bleeding or cyst formation. Preliminary assessment of locomotor function in the open field suggested beneficial effects of the mMS. Additional inner micro-channels enable local substance delivery into the lesion center via an attached osmotic minipump. We suggest that the mMS is a suitable device to adapt and stabilize the injured spinal cord after surgical resection of scar tissue (e.g., for chronic patients) or traumatic injuries with large tissue and bone damages.

1. Introduction

Spinal cord injury is a devastating event leading to permanent paralysis. Although central nervous system (CNS) neurons possess the intrinsic capacity to regrow after lesion, the regeneration process is stopped at the site of injury. Multiple factors are responsible for the limited growth capacity of CNS axons: (i) molecular inhibitors of axonal growth at the glial and fibrous scar, like Chondroitin sulfate proteoglycans [1], Ephrins [2], Semaphorins [3,4], or the myelin derived inhibitors NOGO [5], MAG and OMgp [6], (ii) intrinsic failure to start a genetic regeneration program [7], (iii) lack of neurotrophic factors [8] or (iv) growth inhibiting events mediated by the inflammatory immune response [9]. Another major factor is the lack of a growth substrate, since, unlike the Band of Büngner (aligned Schwann cells) in the peripheral nervous system, no organized cellular networks form as growth promoting matrix in the CNS after injury. A fibrous scar tissue forms at the spinal lesion site after 7 days, associated with additional growth inhibiting molecules [10,11]. The injured axons show growth cone collapse and stop at the lesion site [12].

For the chronic state, resection of the fibrotic tissue has been considered a possible therapeutic strategy and is currently tested pre-clinically. However, the surgical intervention leaves a tissue gap in the spinal cord and an improvement of functional outcome has thus far not been shown [13]. Moreover, complete spinal cord transection is a widely used model for research on axonal regeneration since it allows unambiguous proof of axon extension into and beyond the site of injury [14]. Several strategies to bridge this spinal cord tissue gap have been described in the literature, like implantation of guiding channels, matrices, foams or tissue grafts [15]. Nevertheless, in-growing axons often remain inside such an implant and do not re-enter the distal intact spinal cord stump. For this reason, a strategy has been developed to re-attach the spinal cord stumps using a highly innovative device, the mechanical microconnector system (mMS). The basic idea follows an established general surgical principle of total removal of avital tissue to allow subsequent tension free precise adaptation of healthy tissue.

Due to the small rat spinal cord dimensions, specific microsystems technology methods were developed to manufacture the mMS in order to fulfill the following specific aims: (i) application of

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homogenous vacuum to suck both spinal cord stumps into the mMS lumen, (ii) fixation of spinal cord tissue in place even after release of vacuum, (iii) availability of continuous drug application into the mMS lumen *via* a system of internal micro-channels.

Regarding material properties, mechanical bridging devices used in spinal cord as described in the literature often contain pores [16–18] in order to allow invasion of cells and exchange of trophic factors and nutrients, e.g., to support grafted cells within the channel. We claim that the latter is not necessary for the mMS since newly formed blood vessels support exchange of nutrients in the mMS lumen. For combinatorial treatments, direct drug application into the lesion site via the mMS is made possible by its incorporated micro-channel system in order to render the device suitable for future clinical use and to reduce the number of surgical interventions for the patient such as additional injections or intrathecal catheter infusions. The latter interventions could lead to additional side effects for the patients including, e.g., compression of the spinal cord [19]. As a test drug for infusion through the mMS, we have applied a human antibody which could be visualized immunohistologically. For future therapeutic use, substances which reduce fibrotic scar formation in and around the mMS, like iron chelators, could be applied in this way [20].

2. Materials and methods

2.1. mMS designs

The mMS was developed for complete transection injuries in the rat spinal cord. It consisted of two elliptical discs with a thickness of 350 μm and outer diameters of 1.7 mm and 2.7 mm (corresponding to the oval cross section of the thoracic rat spinal cord) constituting the delimiting sidewalls of a microchamber with a surface of 3.6 mm^2. In the present mMS the distance between the two discs was 300 μm adding up to a total thickness of the assembled mMS of 1000 μm. The discs were designed with honeycomb-like holes. Two versions of the honeycomb holes were fabricated. The small honeycomb structure ("SH") consisted of about 55 honeycombs with an inner diameter of 265 μm each. The large honeycomb structure ("LH") consisted of about 15 honeycombs with an inner diameter of 550 μm each. The thickness of the partition walls of the honeycombs was the same in both types of mMS and measured about 20 μm. A photographic picture of the mMS is shown in Fig. 1A. The partition walls of the honeycomb structures were fabricated by an adapted technique (see below) to render the surface of the partition walls to be rough (Fig. 1B), thus allowing the spinal cord stumps to adhere to the wall. This surface modulation should prevent the detachment of the stumps after the vacuum pump is removed.

The mMS was placed between the two stumps of the severed spinal cord (Fig. 1C). Then the mMS chamber was evacuated by a pump. In this respect, three forces have to be taken into consideration: (i) the internal force of the tissue due to its tension, (ii) the adhesive force of the roughened partition walls of the honeycomb structure, (iii) the force applied by the vacuum in the mMS chamber. The vacuum force has to exceed the two other forces, so the tissue is sucked into the honeycomb walls of the system from both sides, rostral and caudal (Fig. 1D). In this way the two stumps of the spinal cord approximate each other to a distance of several micrometers. Now the adhesive force of the honeycomb structures has to exceed the inner force of the tissue to prevent retraction of the two stumps from the mMS when the vacuum in the mMS chamber is released (Fig. 1E). The forces described above are almost impossible to calculate, so *in vitro* experiments were initially performed to identify these parameters empirically. In this way the dimension of the optimal honeycombs diameter was narrowed down to the range of 250 μm–550 μm.

Pharmacological drugs can be infused into the mMS during and after the implantation *via* four micro-channels that lead to different locations in the mMS lumen (1–4, Fig. 1F) to allow a homogenous distribution of the drug within the mMS chamber. Fig. 2A depicts a technical drawing of the mMS. The most important part of the mMS is the honeycomb structure. It provides high mechanical stability with as

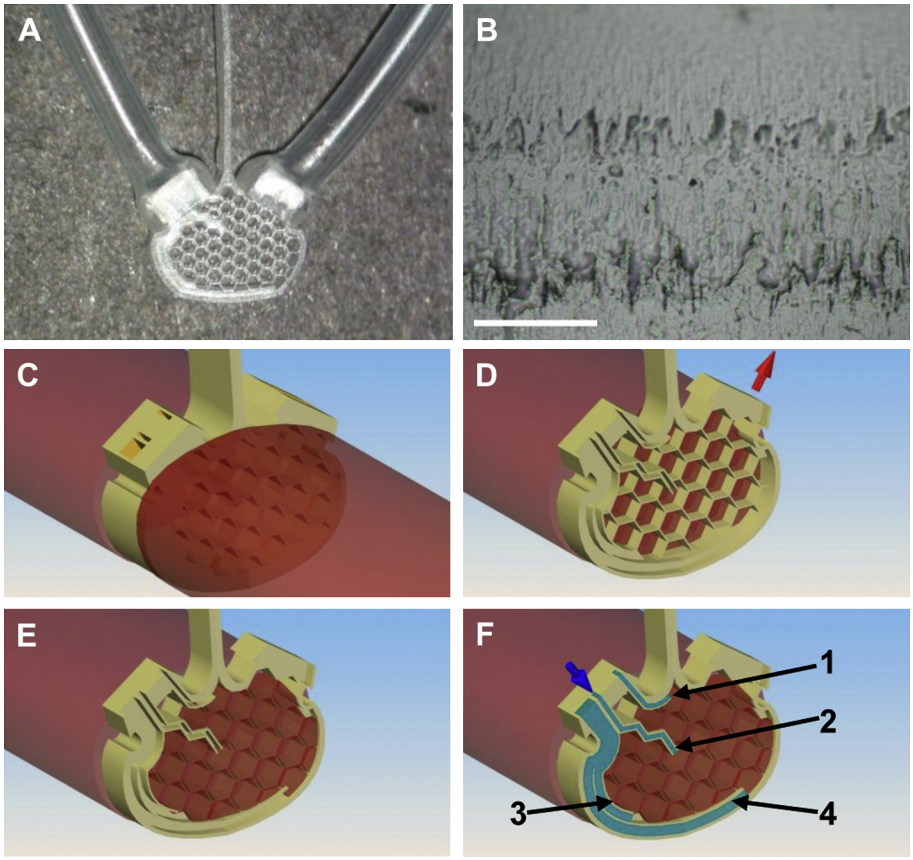

Fig. 1. mMS design and operation principle: (A) photographic picture of the mMS, (B) microstructures of mMS sidewalls, scale bar: 50 μm, (C–F) schematic drawing: (C) implantation of the mMS into the spinal cord lesion, (D) application of vacuum to suck the tissue into the honeycomb structure (red arrow: negative pressure), (E) adhesive force keeps the spinal cord stumps within a distance of several micrometers, (F) distribution of pharmacological substances in the lumen *via* 4 internal micro-channels (black arrows point at micro-channels 1–4). Infusion is depicted by blue arrow. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)