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Short communication

Motor outcome and allodynia are largely unaffected by novel olfactory ensheathing cell grafts to repair low-thoracic lesion gaps in the adult rat spinal cord

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HIGHLIGHTS

- Chronic motor deficits and allodynia by thoracic rat spinal cord hemisection.
- OEC/collagen graft does not improve spinal trauma-induced motor deficits.
- Allodynia is not affected by this repair strategy of hemisected rat spinal cord.

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ABSTRACT

Olfactory ensheathing cells (OEC) are a promising graftable cell population for improving functional outcomes after experimental spinal cord injury. However only few studies have focused on experimental models with large cavitations, which require bridging substrates to transfer and maintain the donor cells within the lesion site. Here, a state-of-the-art collagen-based multi-channeled three dimensional scaffold was used to deliver olfactory ensheathing cells to 2 mm long unilateral low-thoracic hemisection cavities. For a period of 10 weeks, allodynia of the hindpaws was monitored using the von Frey hair filament test, while an extensive analysis of motor ability was performed with use of the CatWalk gait analysis system and the BBB locomotor scale. No substantial improvement or deterioration of motor functions was induced and there was no effect on lesion-induced allodynia. On the basis of these data, we conclude that relatively large spinal cord lesions with cavitation may present additional hurdles to the therapeutic effect of OEC. Future studies are needed to address the nature that such lesion cavities place on cell grafts.

Spinal cord injury (SCI) has dramatic effects on the individuals concerned as well as on society. Debilitating consequences such as deficits in motor function and development of neuropathic pain are directly related to the disruption of spinal tissue and the cellular and molecular processes induced by trauma [1,2]. Many different experimental approaches have been used to promote tissue repair or plasticity (*e.g.* regeneration of severed axon tracts) [2]. Delivery of axon-growth promoting cells into the injured spinal cord has proven to be a popular approach in experimental medicine, and clinical trials have been initiated. In particular, olfactory ensheathing cells (OEC) have been considered a highly promising population of cells for SCI repair because they support the regeneration of olfactory nerve fibers from the periphery into areas of the central nervous system (CNS) throughout the life-span of mammals [3].

OEC express many neurotrophic factors, and unlike many other types of cells can be applied as autografts, thereby by-passing side-effects of immunosuppression that would be required for allografts or xenografts [4], while requiring minimal pre-grafting *in vitro* manipulations. A main advantage of grafting OEC into lesioned CNS

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is their ability to intermingle with- and migrate among CNS glia, such as astrocytes [5]. Moreover, OEC tend to form and maintain open channel-like structures that form a regenerative substrate, not only in their normal habitat [6], but also when transplanted into small electrolytic lesions of the of the corticospinal tract [7]. Implanting OEC into partial or complete lesions of the rat spinal cord has been found to induce autonomic and voluntary motor improvements [8,9].

However, when considering human SCI with the formation of large cystic cavitation, it is unlikely that grafting donor cells alone will be sufficient to promote repair. The bridging of large cystic cavities requires a physical implant which would act as a carrier for the donor cells and thus be a substrate capable of supporting nerve fiber regeneration. We have previously demonstrated the cytocompatibility of a degradable biomatrix (based on polylactic acids) with OEC [10]. However, implantation of such OEC–polylactide matrices into cavities of the injured rat spinal cord did not lead to clear improvements in functional outcome [11]. A main limitation in the latter approach may have resided in the fact that OEC were cultured on the surface of the matrices before grafting, rather than throughout the matrices' internal 3D micro-architecture.

A major advance in tissue engineering for long distance axon regeneration within the lesioned CNS has been the development of a 3D collagen-based scaffold containing longitudinally orientated micro-channels that demonstrates excellent cytocompatibility with a range of neural cells including dorsal root ganglion neurons, SH-SY5Y neuroblastoma, human neural progenitor-derived astrocytes, rat Schwann cells, rat astrocytes, and rat OEC [12–15]. In the present study, OEC-seeded or non-seeded collagen scaffolds were implanted into unilateral 2 mm resection thoracic spinal cord injuries of the adult rat. This model of SCI was selected because of its relevance to study both motor deficits and symptoms of neuropathic pain [16,17]. Lesioned animals that received no treatment served as controls. Motor outcome and allodynia were investigated over a post-surgical period of ten weeks. Motor performance was studied using the conventional BBB locomotor test and the CatWalk gait analysis system. Allodynia was assessed using the von Frey hair filament test.

Female adult Lewis rats (Charles River, Germany) weighing 185–220 g used for the present study were maintained in accordance with the guidelines of the German animal protection statute and experimental protocols were approved by the German governmental ethical committee. Every attempt was made to minimize the animal number as well as any pain or discomfort. Animals were housed under temperature-controlled conditions at $21 \pm 1\,^{\circ}C$, with a normal 12:12 h light/dark cycle with *ad libitum* access to water. A diet restriction protocol (15 g chow/rat/day) was used during a 2-week pre-operative period of CatWalk training (see below) and once per week (the day preceding post-operative CatWalk testing). During the rest of the study, food was available *ad libitum*.

The two weeks preceding surgery were used for daily training of animals on a 1.5 m long CatWalk runway (Noldus Information Technology, The Netherlands). This training period resulted in rats making consecutive, uninterrupted runs with crossing times between 1.1 and 1.6 s. The methodology of CatWalk training is described extensively elsewhere [11]. During the week before surgery, animals were habituated to the von Frey set-up used to obtain paw withdrawal thresholds to mechanical stimulation. This set-up consisted of Perspex boxes positioned on a wire-mesh surface. Animals were allowed to get used to the apparatus for a minimum of 15 min before testing. The hindpaw withdrawal threshold (PWT) to mechanical stimulation was assessed using von Frey hair filaments of increasing thickness (0.41, 0.69, 1.20 1.40, 2.00, 3.63, 5.50, 11.70, 15.14, and 28.84 g) and the up-down method [18]. Following three pre-operative sessions, baseline PWT values were obtained.

Adult GFP-transgenic Lewis rats were used to obtain OEC as described previously [19]. Primary cultures of OEC, reaching near confluence by 10 days *in vitro* were enriched using magnetic cell sorting (MACS) and the low-affinity nerve growth factor (NGF) p75 receptor for separation. The OEC were seeded onto 2 mm-long cylindrical collagen scaffolds with a diameter of 2 mm (Matricel GmbH, Herzogenrath, Germany) at a number of 400,000 cells in a volume of 20 μL. OEC were allowed to adhere to the scaffold for 30 min before adding growth-factor-containing medium. At 24 h after seeding, the OEC-seeded scaffolds were used for transplantation. Control scaffolds were treated identically but without OEC.

Animals received a subcutaneous injection with Buprenorphine (Temgesic 0.1 mg/kg body weight; Schering-Plough, Utrecht, The Netherlands) 30–60 min before surgery. Anesthesia was induced by 5% isoflurane with air as carrier gas at a flow rate of 250 mL/min using a U-400 anesthesia unit (Agntho's, Lidingö, Sweden) with an open mask system. When the corneal blink reflex and paw withdrawal reflexes were absent, maintenance of anesthesia was set at 2% isoflurane. An ophthalmic ointment was applied to the eyes to prevent drying during surgery. Then, the back of the animal was shaved and disinfected before making a skin incision. A laminectomy of the T10-11 vertebral level was performed, exposing the T13 spinal cord level. The dura was opened gently and four suture stitches (9/0 sutures of monofilament polyamide; 9/0 Ethilon®, Ethicon Inc., Somerville) were positioned at the corners of the opened dural membrane. A right-sided hemisection, about 2.0 mm long, was created using a no. 10 scalpel blade and microscissors. Care was taken to prevent damage to the major dorsal blood vessels or vascular branches. Completeness of hemisection was verified microscopically following aspiration of the lesion site. Animals were divided into three groups at random: (1) receiving no implant ($n = 10$), (2) receiving an empty collagen scaffold ($n = 10$), and (3) receiving an OEC-seeded collagen scaffold ($n = 10$). Dural sutures were used to stabilize the implant after which muscle and skin were closed with 4/0 single suture stitches (4/0 Prolene®, Ethicon Inc., Somerville). Three animals died during surgery due to respiratory depression and four animals showed inappropriate deficits in both hind paws instead of unilateral deficits (no weight support up until four weeks post-surgery) probably due to local bleeding and/or edema. As a consequence, the numbers of animals were 9 control animals without graft, 7 animals receiving empty collagen scaffolds, and 7 animals receiving OEC-seeded collagen scaffold.

During the first week after surgery, BBB scores were obtained at post-operative day (dpo) 1, 3, 5 and 7, and thereafter on a weekly basis. Although both hindpaws were scored in this test, deficits were exclusively detected in the hind paw ipsilateral to the lesion. Weight support was regarded a pre-requisite for reliable testing with the von Frey filaments and this was observed in the hindpaws of all animals from 21 dpo. PWT were obtained at four weeks and at 10 weeks post-surgery. Post-operative CatWalk data were acquired at these same time points (before obtaining PWT to mechanical stimuli).

Behavioral data were analyzed using the SPSS 15.0 software. A general linear model (GLM) with repeated measures (for time) used to analyze overall time, group, and time x group interaction effects (Dunnett's *post hoc* correction with untreated animals as control reference). In case of an overall time difference, an Analysis of Variance (ANOVA) was used to analyze effects at individual time points (Dunnett's *post hoc* correction with pre-operative baseline values as control reference). Data are indicated as mean ± standard error of the mean (SEM). A p-value of 0.05 was considered as the level of statistical significance.

Unilateral hemisection of the low-thoracic spinal cord in rat resulted in onset of hypersensitivity to mechanical stimulation of both hind paws, as reported previously [16]. Untreated animals showed a decrease in PWT to mechanical stimulation of the