Recovery of erectile function comparing autologous nerve grafts, unseeded conduits, Schwann-cell-seeded guidance tubes and GDNF-overexpressing Schwann cell grafts

Florian May1,*,‡, Alexander Buchner1,*, Kaspar Matiasek2, Boris Schlenker1, Christian Stief1 and Norbert Weidner3

ABSTRACT
Dissection of the cavernous nerves during radical prostatectomy for prostate cancer eliminates spontaneous erections. Using the rat as an experimental model, we compared the regenerative capacity of autologous nerve grafts and Schwann-cell-seeded nerve guides. After bilateral excision of cavernous nerve segments, cavernous nerves were reconstructed using unseeded silicon tubes, nerve autografts and silicon tubes seeded with either Glial-cell-line-derived (GDNF)-overexpressing or green fluorescent protein (GFP)-expressing Schwann cells (SCs) (16 study nerves per group). Control groups underwent either a sham operation or bilateral excision of cavernous nerve segments without repair. After 12 weeks erectile function was assessed by neurostimulation and intracavernous pressure (ICP) measurement. The reconstructed nerve segments were excised and histologically analyzed. We demonstrated an intact erectile response upon neurostimulation in 25% (4/16) of autologous nerve grafts, in 50% (8/16) of unseeded tubes, in 75% (12/16) of the Schwann-cell–GFP group and in 93.75% (15/16) of the GDNF group. ICP was significantly increased when comparing the Schwann-cell–GFP group with nerve autografts, unseeded conduits and negative controls (P<0.005). In conclusion, Schwann-cell-seeded scaffolds combined with neurotrophic factors are superior to unseeded tubes and autologous nerve grafts. They present a promising therapeutic approach for the repair of erectile nerve gaps.

KEY WORDS: Erectile dysfunction, Schwann cells, Nerve grafts, GDNF

INTRODUCTION
Neurogenic erectile dysfunction resulting from injured cavernous nerves during surgery still represents a frequent complication after radical prostatectomy for prostate cancer. Whereas current research has focused on pharmacological methods, (e.g. phosphodiesterase type 5 inhibitors) so as to preserve the outgrowth and survival of autonomic nerves including penile erection-inducing autonomic neurons (Palma and Keast, 2006; Laurikainen et al., 2000). Several in vivo studies have demonstrated the ability of the GDNF family to enhance functional repair of injured cavernous nerves after radical prostatectomy (Bella et al., 2007; Kato et al., 2007). Therefore, we chose GDNF for this study.

Following nerve injury, SCs might not release enough neurotrophic factors to preserve neuron survival. As neuronal repair mechanisms might take a longer period of several months, previous work has proposed the delivery of growth factors in peripheral nerve repair (Qin et al., 2016). Numerous investigations have demonstrated that cavernous nerves can be successfully repaired using autologous nerve grafts and artificial conduits. The addition of neurotrophic factors and SCs has been shown to further promote nerve regeneration (Xu et al., 2016; Hood et al., 2009).

We previously demonstrated that conduits seeded with syngenic SCs successfully bridge transected cavernous nerves (May et al., 2004). The regenerative capacity can be enhanced by the genetic modification of SCs to overexpress GDNF (May et al., 2008).

The aim of the current study was to investigate and compare different methods of cavernous nerve grafting. Rat cavernous nerve defects were reconstructed by conduits seeded with GDNF-overexpressing SCs. The functional results were compared with those of silicon tubes filled with GFP-expressing SCs, unseeded tubes and nerve autografts.

RESULTS
Achieving a clear, visible erection with a full increase in shaft length on neurostimulation was interpreted as restored erectile function. While all animals of the sham group revealed an intact erectile response, rats after bilateral nerve resection without interposition grafting (control group) showed no inducible erections, confirming that this animal model is reliable (Fig. 1, Table 1).
Neurostimulation led to full erections in 25% (4/16) of rats with autologous nerve grafts, whereas unseeded tubes restored erection in 50% (8/16) of rats with reconstructed nerves (Fig. 1, Table 1). SC-seeded guidance tubes showed the best results, achieving erections in 94% (15/16) of rats in the GDNF and 75% (12/16) in the GFP group. Intact erectile response promoted by GDNF-transduced grafts was significantly superior to nerve autografts ($P < 0.001$).

Neurostimulation with measurement of ICP was used to quantify erectile function. GDNF- and GFP–SC-seeded conduits led to the highest increase of this parameter (Fig. 2, Table 2). ICP was significantly increased comparing the GFP group with unseeded tubes ($P = 0.004$), nerve autografts ($P < 0.001$) and negative controls ($P < 0.001$). Both nerve autografts and unseeded conduit rats exhibited a significantly lower ICP increase compared with the GFP group.

Histological analysis of the nerve grafts showed that the regenerated nerves were usually localized in the center of the silicon tube encircled by an acellular substance that filled the area between the regenerated nerve and the inner conduit wall (Fig. 3A,B). Special stains show regenerating nerve fibers including myelinated axons within the entire regenerate (Fig. 3C,D).

**DISCUSSION**

Recovery of cavernous nerve injury following radical prostatectomy for prostate cancer is often poor despite nerve-protecting techniques. Deliberate excision of the neurovascular bundles for oncological reasons also leads to permanent erectile dysfunction. Autologous sural nerve grafts have been used to repair this injury with insufficient results (White and Kim, 2009). Moreover, they are associated with specific morbidity resulting

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**Fig. 1. Recovery of erectile function after bilateral nerve ablation and reconstruction.** At 12 weeks, rats were re-operated and erectile function was evaluated. On direct electrical nerve stimulation, erectile response was analyzed and counted for sham-operated, excision-operated, unseeded tubes, nerve autograft (NA), GDNF-overexpressing-SC-seeded tube (GDNF) and GFP–SC-seeded tube (GFP) treatments.

Table 1. Recovery of erectile function in response to electrical stimulation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Erections (n)</th>
<th>Erections (%)</th>
<th>P-value</th>
</tr>
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<tbody>
<tr>
<td>Sham vs excision</td>
<td>16/0</td>
<td>100/0</td>
<td>0.000</td>
</tr>
<tr>
<td>Sham vs unseeded tubes</td>
<td>16/8</td>
<td>100/50</td>
<td>0.002</td>
</tr>
<tr>
<td>Sham vs nerve autografts</td>
<td>16/4</td>
<td>100/25</td>
<td>0.000</td>
</tr>
<tr>
<td>Sham vs GDNF</td>
<td>16/15</td>
<td>100/93.75</td>
<td>1.000</td>
</tr>
<tr>
<td>Sham vs GFP</td>
<td>16/12</td>
<td>100/75</td>
<td>0.101</td>
</tr>
<tr>
<td>Excision vs unseeded tubes</td>
<td>0/8</td>
<td>0/50</td>
<td>0.002</td>
</tr>
<tr>
<td>Excision vs nerve autografts</td>
<td>0/4</td>
<td>0/25</td>
<td>0.101</td>
</tr>
<tr>
<td>Excision vs GDNF</td>
<td>0/15</td>
<td>0/93.75</td>
<td>0.000</td>
</tr>
<tr>
<td>Excision vs GFP</td>
<td>0/12</td>
<td>0/75</td>
<td>0.000</td>
</tr>
<tr>
<td>Unseeded tubes vs nerve</td>
<td>8/4</td>
<td>50/25</td>
<td>0.273</td>
</tr>
<tr>
<td>autografts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unseeded tubes vs GDNF</td>
<td>8/15</td>
<td>50/93.75</td>
<td>0.015</td>
</tr>
<tr>
<td>Unseeded tubes vs GFP</td>
<td>8/12</td>
<td>50/75</td>
<td>0.273</td>
</tr>
<tr>
<td>Nerve autografts vs GDNF</td>
<td>4/15</td>
<td>25/93.75</td>
<td>0.000</td>
</tr>
<tr>
<td>Nerve autografts vs GFP</td>
<td>4/12</td>
<td>25/75</td>
<td>0.012</td>
</tr>
<tr>
<td>GDNF vs GFP</td>
<td>15/12</td>
<td>93.75/75</td>
<td>0.333</td>
</tr>
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</table>

Using Fisher’s exact test (two-sided) and Bonferroni’s correction for multiple testing. Only $P$-values <0.05/15=0.003 were regarded as significant.
from a second surgical intervention. Artificial nerve grafts might
avoid these deficits.

We therefore looked for alternative nerve-growth-promoting
strategies. Using a reliable animal model that leads to complete loss
of erectile function unless the nerves are reconstructed, we
compared the results achieved by nerve autografts, unseeded
silicon guidance channels, SC-seeded nerve guides and conduits
seeded with SCs overexpressing GDNF. To our knowledge, this is
the first study comparing these modalities for the reconstruction of
erectile nerves. This study shows that SC-seeded nerve guides
effectively restore cavernous nerve gaps in rodents. We demonstrate
that a simple artificial peripheral nerve can be created by placing
SCs, and even neurotrophic-factor-overexpressing SCs, within a
silicon tube to promote the regeneration of cavernous nerves. We
have found that this strategy clearly expands the clinical potential
of unseeded tubes, permitting the repair of the majority of injured
cavernous nerves. Whereas nerve autografts led to the restoration of
erectile function in 25% of grafted nerves, GFP- and GDNF-
transduced SC grafts led to success rates of 75% and 94%,
respectively. ICP measurement supports these findings showing that
the GFP and GDNF group led to the best results, whereas ICP levels
were low for the nerve autograft and unseeded tube groups.

Histological findings confirm recent data of our group showing that
GDNF accelerates cavernous nerve regeneration, enhancing the
number and maturation of regenerated axons (May et al., 2008).

Even unseeded conduits led to better results than nerve autografts,
in which intraneural scarring might inhibit axonal regrowth.
Previous histological findings demonstrated that the architecture
of regenerating nerves within silicon tubes often resembles the intact
axonal structure in contrast to nerve autografts, which showed only
sparse regenerating minifascicles (May et al., 2004). Contrary to
artificial delivery systems, SCs are able to react to changes of their
environment by secretion of multiple growth factors. A major
disadvantage of autologous SCs, however, is the delay caused by
culture and purification of SCs before clinical use.

The unique regenerative capacity of SCs declines after longer
intervals of denervation. The loss of axonal contact during
accomplished by means of micro- or nanostructured conduits effectively controlled before this strategy can be tested in patients. Therefore, animal studies must first provide evidence that dose and timing of neurotrophic factor gene delivery is effective strategy to overcome this functional deficit.

Several studies provide evidence for the successful use of neurotrophic factor gene therapy in humans. Treatment with adenovirus encoding GDNF, BDNF or transforming growth factor β (TGFβ2) significantly prevented the degeneration of facial motor neurons in individuals with facial nerve lesions (Sakamoto et al., 2003). Adenoviral GDNF transfer promoted laryngeal function recovery after recurrent laryngeal nerve injury (Araki et al., 2006) and stereotactic gene delivery for neurotrophic factors was well tolerated in individuals with advanced Alzheimer’s (nerve growth factor; Rafii et al., 2014) and Parkinson’s disease (neurturin; Marks et al., 2016).

There are major limitations in neurotrophic factor gene therapy for peripheral nerve lesions, as it might provoke uncontrolled and misdirected growth of axons, hypersensitivity and neuropathic pain (Hoyng et al., 2015). Therefore, animal studies must first provide evidence that dose and timing of neurotrophic factor gene delivery is effectively controlled before this strategy can be tested in patients with peripheral nerve injuries.

Adequate axonal guidance for injured peripheral nerves could be accomplished by means of micro- or nanostructured conduits combined with cellular delivery of neurotrophic factors. The supportive effect of these cells might prolong the time window for axonal regeneration and improve the rate of functional restoration even in chronic cases.

**MATERIALS AND METHODS**

**In vitro experiments**

Sciatric nerve fragments from adult male Fischer rats were used for isolation and culture of SCs as previously described (May et al., 2004). Vectors encoding the full sequence of rat GDNF were produced as published by Blesch and Tuszynski (2003). Retroviral vectors expressing GDNF derived from Moloney leukemia virus were used for transduction of SCs in *in vitro*. Whereas effective transduction *in vitro* was tested by GDNF-specific ELISA (Promega, Madison, WI, USA), we confirmed *in vivo* GDNF presence by immunohistochemical analysis (May et al., 2008).

We used non-biodegradable silastic nerve guides (length, 5 mm; inner diameter, 0.51 mm; outer diameter 0.94 mm) for interposition grafting. The tubes were filled with the GDNF-SC suspension (cell quantity 25,000 cells/ml) as previously described (May et al., 2008).

**Animal experiments**

Forty-eight adult male Fischer 344 rats (250-350 g) were randomized into six groups of eight each (16 study nerves). The bilateral cavernous nerves were transected to create a 5 mm defect, which was immediately reconstructed using unseeded (empty) silicon tubes, nerve autografts, tubes seeded with either GFP- or GDNF-transduced SCs (16 study nerves per group; Table 3). The ipsilateral genitofemoral nerve (7 mm segment) was used for interposition grafting between the transected cavernous nerve ends as previously published (May et al., 2004).

Further animals were either sham-operated or underwent bilateral nerve excision without repair (control groups, 16 study nerves each). The surgical procedures were described previously (May et al., 2004, 2008).

All rats underwent a relaparotomy once after 12 weeks and were euthanized afterwards. Evaluation included neurostimulation of the proximal cavernous nerves over an intact nerve segment and measurement of both intracavernous pressure (ICP) and mean arterial blood pressure (MAP) as described previously (May et al., 2004). All surgical procedures including reexploration and electrostimulation were approved by the local ethics committee and done in full accordance with national and institutional regulations.

**Histological analysis**

The reconstructed nerves were harvested, dissected at mid-regenerate level, fixed and embedded as previously published (May et al., 2008). Semithin sections were used to highlight myelinated fibers (D, black rings) within the minifascicles (framed by red dashed line). A,B,C: Azure II Methylene Blue-Safranin; D: p-phenylenediamine.

**Table 3. Flowchart depicting the design of the study and the different treatment groups**

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>n</th>
<th>Surgical treatment</th>
<th>Evaluation 12 weeks after surgery</th>
</tr>
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<tbody>
<tr>
<td>Unseeded tubes</td>
<td>16</td>
<td>Bilateral cavernous nerve excision and reconstruction</td>
<td>1-3</td>
</tr>
<tr>
<td>Nerve autografts</td>
<td>16</td>
<td>Bilateral cavernous nerve excision and reconstruction</td>
<td>1-3</td>
</tr>
<tr>
<td>GDNF</td>
<td>16</td>
<td>Bilateral cavernous nerve excision and reconstruction</td>
<td>1-3</td>
</tr>
<tr>
<td>GFP</td>
<td>16</td>
<td>Bilateral cavernous nerve excision and reconstruction</td>
<td>1-3</td>
</tr>
<tr>
<td>Sham</td>
<td>16</td>
<td>Sham operation without nerve injury</td>
<td>1-3</td>
</tr>
<tr>
<td>Excision</td>
<td>16</td>
<td>Bilateral nerve excision without reconstruction</td>
<td>1-3</td>
</tr>
</tbody>
</table>

Evaluation parameters: 1, electrical nerve stimulation; 2, intracavernous pressure recording; 3, histological analysis of excised nerve segments.
sections (0.5 µm) were stained with Azure Blue-Safranin and p-phenylenediamine and then analyzed for regenerating axons and the fascicular formation.

Data analysis
Data are presented as mean ± s.e.m. Groups are compared by the chi-square and Fisher exact tests. Intracavernous pressure and systemic blood pressure were analyzed by using nonparametric Kruskal–Wallis ANOVA followed by Bonferroni–Dunn’s test for individual between-group comparisons at the P<0.05 level of significance.

This article is part of a special subject collection ‘Spotlight on Rat: Translational Impact’, guest edited by Tim Altman and Aron Geurts. See related articles in this collection at http://dmm.biologists.org/collection/rat-disease-model.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: F.M., N.W.; Validation: A.B.; Formal analysis: A.B., B.S.; Funding: F.M., N.W.; Writing - original draft preparation: F.M., M.K.; Writing - review and editing: F.M.; Supervision: C.S., N.W.

References


