**Abstract**

**Objective:** This article reviews novel restorative therapies for cavernous nerves that may be used to replace resected cavernous nerves at the time of pelvic surgery.

**Methods:** A literature-based presentation (Medline search) on current nerve replacement strategies was conducted with emphasis on neurobiological factors contributing to the restoration of erectile function after cavernous nerve injuries.

**Results:** A promising alternative to autologous nerve grafts for extending the length of successful nerve regeneration are artificial nerve guides. The addition of neurotrophic factors, extracellular matrix components and Schwann cells has been shown to promote cavernous nerve regeneration. Neurotrophic factors can be incorporated in the scaffold or can be supplied by cells seeded into the stroma. The regenerative capacity of these cells can be further enhanced by genetic modification with neurotrophic factor encoding genes.

**Conclusions:** Artificial nerve guides, especially biodegradable ones containing growth-promoting factors or cells, are a promising option for the repair of cavernous nerve lesions.

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**Keywords:** Schwann cells; Nerve regeneration; Erection; Cavernous nerves; Neurotrophic factors; Nerve guides

1. **Introduction**

Preemptive neurovascular bundle resection at the time of radical prostatectomy to achieve complete tumor resection is a common procedure on the basis of highly adverse biopsy and intraoperative findings spurring much interest in nerve replacement strategies. The interest is guided by the premise that nerve grafts may provide a scaffold for autonomic nerve regrowth and reconnection with targets thereby mediating erectile function. While the concept of autologous nerve transplantation is well established, clinical studies using autologous sural nerve grafts to bridge transected cavernous nerves following radical prostatectomy have revealed only modest success [1–5].

Artificial conduits represent an alternative method to nerve autografting providing directional guidance to regenerating axons, preventing axonal sprouting into the surrounding tissue and reducing invasion by connective tissue [6]. The addition of neurotrophic factors, extracellular matrix components and Schwann cells into bioartificial nerve conduits has been shown to promote regeneration [7,8]. Cells are effective and appropriate vehicles for supplying nerve growth promoting factors. Schwann cells were first described by Theodor Schwann (1810–1882) and are the principle glial cells of the peripheral nerve, important for neuronal survival, axonal development and essential for axonal regrowth following injury [9]. After limited injury to
peripheral nerves, Schwann cells form a scaffold to guide regenerating axons, produce extracellular matrix components, secrete neurotrophic factors and remyelinate regenerating axons to reestablish the conductive properties of nerve fibers, ultimately leading to reinnervation and functional recovery. In addition to Schwann cells, olfactory ensheathing cells (OECs) and stem cells are being extensively investigated as transplants to support nerve regeneration [6]. Transfection of these cells to overexpress neurotrophic factors has further expanded their regenerative capacity in the nervous system [10,11]. Recent preclinical work to recover cavernous nerve function and general progress in the neurobiology of peripheral nerve recovery is likely to result in novel restorative therapies to preserve the sexual health of men after radical prostatectomy.

2. Methods

A systematic review of the literature using Medline from January 1988 to March 2005 was conducted. Electronic searches were limited to the German and English language using the keywords Schwann cells, nerve regeneration, erection, cavernous nerves, neurotrophic factors and nerve guides. In addition, abstracts published in the journals *European Urology*, *The Journal of Urology* and *International Journal of Impotence Research* as official proceedings of internationally known scientific societies held in the same period were also assessed. Unpublished information known by the authors which was considered of interest for the readers was also included.

3. Results

3.1. Neurobiological background

Axotomy or crush of a peripheral nerve leads to degeneration of the distal nerve stump referred to as Wallerian degeneration. During Wallerian degeneration a microenvironment is created that allows successful regrowth of nerve fibers from the proximal nerve segment. Schwann cells respond to loss of axons by down-regulation of myelin gene transcription, dedifferentiation and proliferation. They finally align in tubes (bands of Büngner) and express trophic surface molecules, such as neurotrophic factors, that guide regenerating fibers. The trophic factors are taken up by the growth cones and are retrogradely transported to the nerve cell body to support its survival and improve the axonal regeneration (Fig. 1). Following peripheral nerve injury and axotomy, the retrograde transport of neurotrophic factors from the target tissue is interrupted, leading to neuronal cell death and lack of regeneration. Once neurons have regenerated back to their original target, this process can be stopped indicating the dependence on target-derived neurotrophic factors [12]. Numerous studies in the peripheral nervous system have demonstrated that exogenous supply of trophic factors may further improve axonal regeneration and accelerate target reinnervation [7,13–15].

Several molecules have been described to have neurotrophic effects in the penis [16] including brain-derived neurotrophic factor (BDNF) [17], neurturin [18], immunophilin ligands [19], vascular endothelial growth factor (VEGF) [17,20] and glial cell line-derived neurotrophic factor (GDNF) [21].

Delivery methods for neurotrophic factors range from local injection, targeted administration using biodegradable materials and transfer of genes encoding specific growth-promoting neurotrophic factors. Detailed studies are necessary to determine the most effective delivery mode, the type/combination of growth factors

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Fig. 1. Schwann cells form a mechanical scaffold for axons to grow along and secrete neurotrophic factors expressed on the cell surfaces. The trophic factors are picked up in the growth cones, incorporated in the axon, and are transported retrogradely to the nerve cell body to support neuronal survival and improve the axonal regeneration.
and potential adverse effects before clinical studies can be initiated.

3.2. Autologous nerve grafts

The concept of autologous nerve transplantation is well established and has been applied to treat peripheral nerve, facial nerve and extremity nerve injuries for decades with an excellent safety and efficacy profile [22,23]. There are obviously inherent differences in reinnervation of somatic nerves and autonomic nerves such as the cavernous nerves. While several prior studies have documented regrowth of autonomic nerves such as the phrenic [24] and parasympathetic nerves of the heart [25], clinical trials on cavernous nerve regeneration have revealed only modest success using autologous sural nerve grafts [1–5].

Original preclinical studies by Quinlan et al. [26] and Ball et al. [27] using the genitofemoral nerve as a replacement for resected cavernous nerves introduced the possibility of interposition nerve grafting for the recovery of erectile function after injury to the cavernous nerves. Walsh subsequently investigated this concept in humans and interposed genitofemoral nerve grafts at radical prostatectomy in a small, blinded trial without detecting obvious benefit [3]. In humans, the procedure is technically more difficult, because the cavernous nerve is not a well-defined structure, but consists of multiple nerves, which are dispersed in a plexus surrounded by vessels. Scardino and Kim [1] first evaluated autologous sural nerve grafting for this purpose, proposing that nerve grafting is feasible and offers an opportunity for recovery of erectile function to a better extent than in its absence. Among several reports by this group, they described their extended experience in 28 men undergoing bilateral sural nerve grafting at radical prostatectomy with a mean follow-up of 23 months [2]: Six (26%) of 23 men had spontaneous, medically unassisted erections sufficient for sexual intercourse. Scardino and Kim [4] further explored the application of unilateral sural nerve grafts in men undergoing unilateral excision of the neurovascular bundles showing that graft replacement was superior to a control group without nerve grafting (78% versus 30%) and approximating the results of bilateral nerve-sparing surgery (79%). Anastasiadis et al. [5] described 4 (33%) of 12 men who achieved spontaneous erections sufficient for intercourse after unilateral nerve grafting.

While recovery of spontaneous erections in patients after bilateral resection of neurovascular bundles is practically nonexistent, the benefits of the more common unilateral resection and grafting are difficult to document, since patients may recover erectile function when a single nerve is preserved. Advantages of sural nerve grafting include the autogenous basis for the nerve conduit and the favourable outcome achieved in other clinical applications. However, concern persists about the value of sural nerve grafts in the management of patients with localized prostatic carcinoma. Some important issues addressed in a critical review by Walsh [3] are the low probability of recovery of sexual function in patients with extensive extracapsular disease who often require adjuvant therapy which further compromises erectile function; the lack of randomized, controlled studies documenting the efficacy of sural nerve grafting; and the high probability of neurovascular bundle preservation of most patients with localized prostate cancer.

The concept of nerve autografts has several characteristic disadvantages. The donor nerve is usually obtained from nerves which are functionally less important, such as the sural nerve and harvesting the graft causes a sensory deficit at the donor site and the risk of neuroma formation. Moreover, nerve autografts do not offer an optimal environment for the advancing axonal sprouts: Whereas predegenerated nerve grafts [28] and predegenerated nerve transplants in synthetic tubes [29] shorten the delay of axonal elongation and increase axonal regeneration, regeneration fails or is delayed when growing axons encounter the distal nerve segment. Within the degenerated nerve, Schwann cells rapidly respond to loss of axonal contact by down-regulation of myelin genes, dedifferentiation and proliferation. Other molecular changes include upregulation of neurotrophic factors, neural cell adhesion molecules, cytokines and other soluble factors and their corresponding receptors that guide regenerating fibers [9,12]. Although nerve autografts still represent the gold standard to bridge long gaps, injured axons may not necessarily regrow through the nerve transplant, but rather around it, failing to find and reinnervate the original target (Fig. 2A). In this case functional recovery is incomplete or absent.

3.3. Artificial nerve conduits

A promising alternative to nerve autografts for extending the length of successful nerve regeneration are artificial nerve grafts. Artificial nerve grafts are synthetic conduits that bridge the gap between distal and proximal nerve stumps and directs and supports nerve regeneration. This technique has several advantages: it provides a guidance channel and mechanical support for the regenerating fibers, reduces invasion by connective tissue, is easier to perform, and allows the isolated site of repair to be manipulated (Fig. 2B).

Because of its inert and elastic properties, silicone tubing was one of the first and most frequently used
synthetic materials for nerve grafts. Clinical tubulization of regenerating nerves, however, may lead to long-term complications including fibrosis and chronic nerve compression, requiring surgical removal of the conduit. Despite diminishing clinical use, the silicone chamber has been a very useful model for studying nerve regeneration in vitro, allowing spatial and temporal examination of the regeneration process [8].

Bioresorbable grafts are a promising alternative for clinical use, because, after serving as an appropriate scaffold for regeneration, the conduit eventually degrades. Furthermore, bioresorbable grafts can be modified to slowly release growth promoting factors as long as the degradation process continues [30]. The efficacy of a biodegradable copolymer conduit with a collagen sponge was recently demonstrated in a rat model of cavernous nerve injury [31].

For clinical use, various tubes are commercially available. Currently, available tubes include biodegradable ones, such as collagen-based nerve guides [32]. Artificial nerve guides have already been evaluated in clinical studies aiming for peripheral nerve repair. In particular, silicone [33] and polyglycolic acid (PGA) conduits [34] have been used systematically in clinical practice to repair median, ulnar and digital nerves.

3.4. Bioartificial nerve conduits

Tubulization with simple guides usually fails when bridging relatively long gaps of 15 mm in the rat [26] and 30 mm in primates [11]. Because of this limited capacity of artificial guides to support regeneration across long gaps, the introduction of nerve growth-promoting factors or cells into the guides has been investigated extensively. Several studies have shown that the regenerative capacity of artificial conduits can be significantly improved by adding Schwann cells into the guides [9,10].

A recent work in our group strongly supports this notion showing that silicone guidance tubes filled with primary Schwann cells enhance bilateral cavernous nerve repair far superior to unseeded nerve guides and nerve autografts [35]. Within 12 weeks after bilateral cavernous nerve ablation 90% of animals, which received Schwann cell seeded guidance tubes, regained erectile function as compared to animals receiving nerve autografts (30% recovery rate) and...
unseeded silicone tubes (50% recovery rate). The functional outcome is paralleled by histological analyses (Fig. 4). The results are promising considering a clinical application of Schwann cell seeded guidance tubes to restore erectile function in men following radical prostatectomy.

Ideally, Schwann cells are isolated from the injured individual by a peripheral nerve biopsy, thus allowing an autologous transplantation mode and avoiding graft rejection or long term immunosuppression. Recently, a method has been developed, which allows to isolate autologous Schwann cells from rat peripheral nerve biopsies within 9 days after biopsy employing magnetic-activated cell separation (MACS) of p75 low affinity Nerve Growth Factor (NGF) receptor expressing Schwann cells (Fig. 3) [36]. Ongoing studies investigate the feasibility to isolate Schwann cells from human peripheral nerves using the identical method (personal communication N. Weidner).

Cell therapy can be combined with the application of neurotrophic factors most effectively by genetically modifying cells before transplantation to overexpress therapeutic transgenes [37]. Several studies have shown that Schwann cells can be genetically modified

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Fig. 4. (A–F) Comparison of GDNF-hypersecreting Schwann cell graft (A) versus GFP-transduced Schwann cell graft (C) and unseeded conduit (E) 12 weeks after interposition grafting (assembled photographs from electron micrographs forming a mosaic picture at magnifications of 3400×). At higher magnification (from the boxed area in A) multiple unmyelinated and myelinated sprouts can be detected (B). The histological results clearly demonstrate advanced nerve regeneration within GDNF-transduced Schwann cell seeded nerve guides with larger regenerating nerve fascicles containing abundant unmyelinated and myelinated regenerating axons, sparse extracellular matrix and a tight oligolamellar perineurium. Compared to unseeded nerve guides (E) which show only sparse minifascicles and much undifferentiated matrix (F), GFP-transduced Schwann cell grafts (C) contain large neural areas consisting of multiple regenerating axons (D).
using retroviral vectors to overexpress neurotrophic factors such as nerve growth factor (NGF), neurotrophin-3 (NT-3) or brain derived neurotrophic factor (BDNF) [6,38–40]. The transplantation of neurotrophic factor secreting Schwann cells provides a permissive cellular substrate for nerve regeneration and allows the local delivery of therapeutic transgene products at high concentrations without concomitant systemic side effects.

In a recent study, we demonstrated that GDNF-transduced Schwann cell grafts accelerate the restoration of erectile nerve function compared to Schwann cells expressing the reporter gene green fluorescent protein (GFP) restoring erectile function at 6 weeks after nerve transection in 90% of the grafted animals [41]. The superior functional outcome was paralleled by improved structural regeneration using GDNF-overexpressing Schwann cells. The morphological data provide evidence that the number of regenerated axons at a given time point is enhanced in GDNF-transduced Schwann cell grafts (Fig. 4A and B). Considering that extended denervation of the penis leads to irreversible degeneration of the smooth muscle, the acceleration of nerve repair might dramatically increase the likelihood that regeneration will occur in time to reinnervate an intact corpus cavernosum as a prerequisite for functional recovery in men.

The fact that functional recovery can be achieved within certain time periods call for appropriate measures to shut down the production of therapeutic transgenes such as GDNF once regeneration is completed. As a prerequisite for potential clinical studies, future gene therapy based approaches need to implement regulatable systems (e.g. driven by tetracycline inducible promoters), which have been demonstrated to effectively control transgene expression in vivo [42].

4. Conclusions

Artificial nerve guides, especially biodegradable ones containing growth-promoting factors or cells, are a promising option for the repair of cavernous nerve lesions. Overall, recent advances in cell engineering, gene therapy and biomaterials provide promise that nerve prostheses capable of restoring erectile function in men will become available in the near future.

References


