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Development of a decellularization method to produce nerve allografts using less invasive detergents and hyper/hypotonic solutions

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Summary *Background:* Here, we describe a novel method of processing decellularized nerve grafts using osmotic effects of hypotonic and hypertonic solutions and Triton X-100 (a nonionic detergent) and CHAPS (an amphoteric detergent).
Materials and methods: To evaluate decellularization, the devised method and Hudson's method were compared with respect to remaining cellular components (as assessed by H&E staining and S-100 immunoreactivity) and extracellular matrix structural integrity (as assessed by H&E staining and laminin immunoreactivity) by using rat sciatic nerves. In addition, a 1.5-cm sciatic nerve gap rat model was treated by implanting decellularized nerve grafts prepared using the devised method, Hudson's method, or an autograft to evaluate nerve regeneration. Nerve histomorphometry of distal stumps and wet muscle mass were evaluated at 12 weeks after implantation.
Results: The devised method produced outcomes similar to those of Hudson's method in terms of cellular component removal, but the devised method was significantly better in terms of ECM preservation. Histomorphometric study showed that the devised method produced significantly fewer nerve fiber and axonal densities than autografting, but much more than Hudson's method. The wet muscle mass of the devised method was also significantly lower than that of autografting, but much higher than that of Hudson's method.
Conclusion: The described process for producing decellularized nerve grafts yielded better outcomes with respect to peripheral nerve regeneration than the established ionic

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detergent-based methods in a rat model. This study indicates that decellularized nerve grafts produced in this manner show favorable nerve regeneration used for bridging nerve gaps.

Introduction

In cases of peripheral nerve injury, primary end-to-end anastomosis under tension-free conditions produces best outcomes.[1] However, primary nerve repair is not possible in some complex and delayed cases and autogenous nerve grafting is considered the gold standard for facilitating peripheral nerve bridging.[2] However, autogenous nerve grafts present significant associated problems such as donor site morbidity, lack of sufficient donor tissue, and size mismatches,[1,3] and thus, great interest has been shown in the development of effective alternatives to nerve autografts for the management of peripheral nerve injury.[4] Furthermore, decellularized nerve grafts for peripheral nerve reconstruction have attracted considerable interest recently.[5]

The techniques available for producing acellular nerve conduits are cold preservation,[6] freezing and freeze—thaw,[7] chemical detergent,[8,9] and miscellaneous methods.[10,11] Although many methods have been used to prepare decellularized nerve grafts, all of them aim to eliminate graft antigenicity by completely removing cellular components, such as Schwann cells and myelin, and to enhance regenerative capacity by maintaining the integrities of basal lamina and extracellular matrix (ECM).[4,12] Nevertheless, the detergent-based method is most commonly used to produce decellularized nerve grafts.

Chemical detergents are classified as (1) nonionic detergents, (2) amphoteric detergents, (3) ionic detergents, or (4) cationic detergents according to the charge of the hydrophilic head group.[8] Nonionic and amphoteric detergents maintain better basal lamina integrity, but ionic detergents are better able to remove cells than nonionic or amphoteric detergents.[8] Consequently, the proposed chemical detergent methods usually involve a combination of ionic and amphoteric or nonionic detergents.[9,13—15] However, all these methods have poorer outcomes than autografts when nerve gaps are more than 14 mm.[16]

We hypothesized that inadequate preservation of the ECM structure in detergent-treated acellular nerve grafts contributes to this drawback. Accordingly, we attempted to promote decellularization of nerve tissue by utilizing the osmotic effect[8,10] generated by hypotonic and hypertonic solutions in combination with Triton X-100 (a nonionic detergent) and CHAPS (an amphoteric detergent). We compared our method with an established chemical detergent method developed by Hudson et al.[8] in terms of cellular removal and structural preservation by histologic staining. In addition, to evaluate nerve regeneration, a 1.5-cm sciatic nerve gap was treated by implanting decellularized nerve grafts prepared using the devised method or Hudson's method or by implanting an autograft into a rat model and assessing nerve regeneration by histologic staining and muscle functional recovery by measuring wet muscle masses.

Materials and methods

Animal

All animal procedures were approved by our Institutional Animal Care and Use Committee.

Adult male Sprague-Dawley rats weighing 300—350 g were used as nerve graft recipients and adult male Lewis rats weighing 300—350 g were used as donors. Rats were anesthetized by i.p. injection using a combination of 40 mg/kg of zoletil (Virbac Lab., Corrs, France) and 20 mg/kg of xylazine (Bayer Ltd., Leverkusen, Germany). Animals were euthanized with an intraperitoneal injection of sodium pentobarbital (120 mg/kg).

Preparation of the decellularized nerve graft

All reagents used were purchased from Sigma-Aldrich Chemicals (St. Louis, USA), unless otherwise specified.

Adult male Lewis rats were anesthetized as described above, and both hind limbs were shaved and sterilized using alcohol prep pads and povidone—iodine thrice. Under aseptic conditions, skin was incised using a scalpel and the sciatic nerve was exposed by splitting the thigh muscle. The entire length of the sciatic nerve from the sciatic notch to the distal trifurcation was dissected and harvested, which yielded nerves of ~2 cm in length, and fatty and connective tissues were removed from the nerve epineurium. After harvesting nerves, donor animals were euthanized as described above.

Decellularized nerve grafts were produced using a protocol developed in our laboratory. In all decellularization steps, the nerve tissue and solution were agitated on a vertical rotator at 25 °C. The nerve was placed in a 15-mL conical tube filled with deionized distilled water for 7 h. Then, the nerve was placed in hypertonic sodium chloride solution (1 M) for 15 h. After washing with deionized water, the nerve was transferred to 15-mL conical tube filled with phosphate-buffered saline (PBS) containing 2.5 mM Triton X-100 for 24 h and rinsed for 15 min in PBS. The nerve tissue was placed in a 15-mL conical tube filled with deionized distilled water for 7 h, then placed in hypertonic sodium chloride solution (1 M) for 15 h, washed with deionized water, transferred to 15-mL conical tube filled with PBS solution containing 100 mM CHAPS for 24 h, rinsed with PBS solution (5 × 5 min), and stored in PBS at 4 °C until required.

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