Optimization of a Novel Tissue Preservation System for Point-of-Care Storage of Nerve Allografts

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INTRODUCTION: Combat-sustained peripheral nerve injuries often have drastic impacts on military personnel readiness and quality of life, particularly when the injury is over a large segment. Outcomes after contemporary surgical repair and reconstruction techniques for these injuries are inconsistent. A novel method for peripheral nerve reconstruction (PNR) that is being developed through preclinical studies involves polyethylene glycol-mediated nerve fusion (PEGf) which has been shown to provide a level of immune privilege to fresh (viable) nerve allografts such that functional outcomes after PNR are improved. To support this emerging technology, donor peripheral nerve allografts must be preserved using a method that maintains tissue viability during recovery, processing, and storage at point-of-care. One such system that has proven effective for osteochondral allograft transplantation is MOPS® (MTF Biologics), which is currently in widespread clinical use. While designed for OCAs, optimization of the MOPS solution with neurotrophic factors could provide similar success for storage of nerve allografts. The present preclinical study aimed to determine if standard MOPS and/or two optimized MOPS-N solutions could maintain sufficient cell viability (>100 cells/mm2) of rat sciatic nerve allografts for 90 days after recovery.

METHODS: Three different MOPS solutions were prepared: standard MOPS; MOPS-N-GDNF, utilizing glial-derived neurotrophic factor; and MOPS-N-B12, utilizing Vitamin B12. With IACUC approval, rat (n=35) sciatic nerves were aseptically recovered, sectioned as explants, and randomly assigned to solution and storage duration (0-day (n = 5), 30-day (n = 5 in each solution), 60-day (n = 5 in each solution), or 90-day (n = 5) timepoints. Explants were maintained in their assigned solution at room temperature for the assigned duration of storage. At the assigned time point, compound action potentials were assessed in each explant using "Yes/No" criteria. Each allograft was objectively assessed for viable Schwann cell density (VCD) using the live cell stain calcein AM (1 mg/mL) and the dead stain SYTOX Blue (1.25 mM; Thermo Fisher Scientific).

RESULTS: All explants produced compound action potentials through 60 days. At 90-days, 80% of MOPS-B12 and MOPS-GDNF stored allografts had CAPs, compared to only 40% of MOPS stored allografts. Both MOPS-B12 and MOPS-GDNF solutions were associated with maintenance of at least 100 cells/mm^2 at 30- and 60- and 90-days after recovery from the donor. MOPS failed to maintain at least 100 cells/mm^2 in 60% allografts at 90-days, the same allografts that did not have CAPs. There were no significant differences between baseline and 30-day average VCD for any of the solutions (MOPS: 122.97 cells/mm2 to 118.65 cells/mm2, MOPS-N-GDNF: 122.97 cells/mm2 to 118.88 cells/mm2, MOPS-N-B12: 122.97 cells/mm^2 to 123.44 cells/mm^2). There was a significant decrease between the 30-day and 60-day, and 30-day and 90-day VCDs for MOPS (30-day: 118.65 cells/mm^2, 60-day: 107.80 cells/ mm^2, 90-day: 100.21 cells/mm^2) and MOPS-N-GDNF (30 day: 118.88 cells/mm^2, 60-day: 109.71 cells/mm^2, 90-day: 104.09 cells/mm^2. There was a significant decrease in MOPS-N-B12 stored allografts VCD between the 30-day and 90-day timepoint (30-day: 123.44 cells/mm^2, 60-day: 118.75 cells/mm^2, 90-day: 110.34. MOPS-N-B12 had a significantly higher mean VCD) than MOPS and MOPS-N-GDNF at the 60-day and 90-day timepoint.

DISCUSSION: Optimization of MOPS can maintain function (stimulated action potentials) and sufficient cell viability in sciatic nerve allografts for at least 60 days at shelf-stable conditions. As such, MOPS, and the optimized MOPS-N solutions, provide a clinically applicable method to support emerging PEGf technology in improving outcomes after PNR. Ongoing research in our lab is focused on use of MOPS-stored tissues in conjunction with PEGf to implement function- (sensory/motor) and diameter-matched peripheral nerve allograft transplantation to improve functional outcomes after PNR, limiting long-term disability in military and civilian populations.

SIGNIFICANCE/CLINICAL RELEVANCE: The ability of MOPS to store allografts at room temperature allows these allografts to be used in a variety of health care settings, including Role 3 Combat Support Hospitals, bringing the PEGf technology closer to translation into human clinical trials.

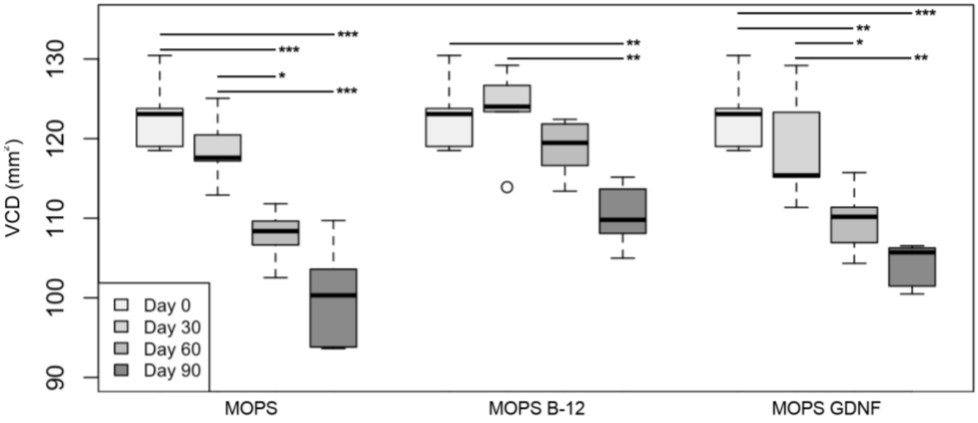

Figure 1: Viable cell density of three MOPS solutions of 90-days. *p <.05, **p <.01, ***p <.001