QS5

The Effect of Stem Cells and Local Tacrolimus on Neurite Extension

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Purpose: Application of mesenchymal stem cells (MSCs) or tacrolimus (FK506), an FDA approved immunosuppressant, to nerve grafts has been a topic of interest to enhance peripheral nerve regeneration. The aim of this study was to investigate the combined effect of MSCs and local delivery of FK506 on nerve regeneration when applied to nerve autografts and decellularized nerve allografts.

Methods: A three-dimensional (3D) *in vitro* compartmented cell culture system, validated by Tajdaran et al (2019), consisted of rat neonatal dorsal root ganglion (DRG) adjacent to rat nerve autograft or decellularized allograft. This model was used to evaluate regenerating neurites from the DRG into the peripheral nerve scaffold. Nerve autografts and decellularized allografts were augmented with (i) dynamic undifferentiated MSC seeding, (ii) local application of FK506 (100 ng/mL) or (iii) both (N=9/group). Local application was ensured by isolating the central system (i.e. DRG side) from the peripheral system (i.e. nerve graft side), where treatment was applied. After 48-hours of incubation, DRG-nerve graft constructs were collected, fixed, sectioned and stained against neurofilament-160 to measure neurite extension. CD90 staining was used to confirm stem cell characterization.

Results: All grafts treated with MSCs confirmed CD90 expression. Compared to untreated autografts, neurite extension in autografts treated with FK506 and autografts treated with MSCs and FK506 combined were found superior ($P<0.001$ and $P=0.0001$, respectively), and comparable to autografts treated with MSCs ($P=0.12$). Compared to untreated allografts, allografts treated with FK506, and allografts treated with MSCs and FK506 were found superior ($P<0.001$ and $P=0.0001$, respectively), and allografts treated with MSCs were found comparable ($P=0.09$). All autograft groups were found superior compared to their respective allograft treatment group ($P<0.05$). Solely allografts receiving combined treatment were found superior to untreated autografts ($P<0.05$).

Conclusion: MSCs or FK506 treatment improved neurite outgrowth and when combined, this resulted in significant synergistic neurite extension in both autografts and allografts in comparable patterns. **Schematic overview of 3D compartmented cell culture system for isolated evaluation of treatment with MSCs and local FK506 *in vitro*.** A 3.5 mm autograft or allograft with or without undifferentiated MSC seeding is attached to a DRG. DRG-nerve graft constructs are placed through a silicone isolator in the middle of a 24-wells plate to isolate the DRG from the nerve graft. FK506 containing media was added to the nerve graft side.

QS7

Physiologic Signaling of the Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI) During Volitional Behavior

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Purpose: Exoskeletons have become a promising device to restore extremity function to those with limb weakness. However, these devices have not become widely adopted due to the inadequacy of current nerve interfacing methods. The Muscle Cuff Regenerative Peripheral Nerve Interface (MC-RPNI) was developed as a potential solution to this problem. Consisting of a segment of autologous free muscle secured around an intact nerve, the MC-RPNI is able to regenerate and reinnervate, amplifying its contained nerve's signals through generation of EMG signals. These amplified, high-fidelity signals can then be used to intuitively and accurately control exoskeletons. The purpose of this study was to characterize MC-RPNI physiologic signaling during volitional behavior and determine long-term effects on the associated nerve.

Methods: Eighteen rats were randomly assigned to one of three groups: (1) sham surgery/control; (2) nerve