IN VITRO MODEL EVALUATING NOVEL NERVE ALLOGRAFT SEEDING TECHNIQUES

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INTRODUCTION

- Tissue processing necessary to remove immunogenic components of human nerve allograft also render the graft acellular.[1]
- While seeding the allograft with supportive cells may improve nerve axon regeneration, little has been done to elucidate the best method for physically implanting these cells into the allograft. [6-14]
- **Purpose: To determine the optimal method of seeding nerve allograft with neurosupportive cells.**

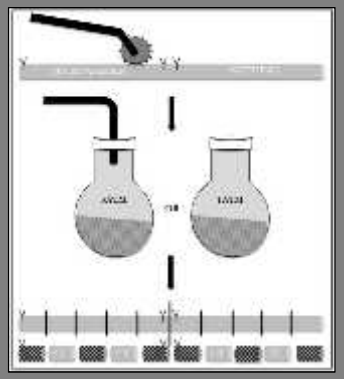
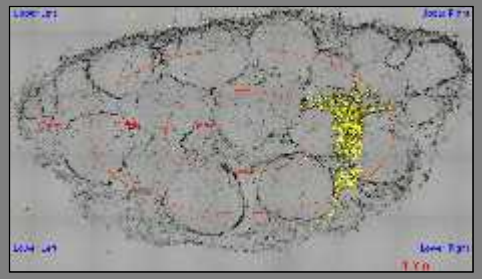


Figure 1

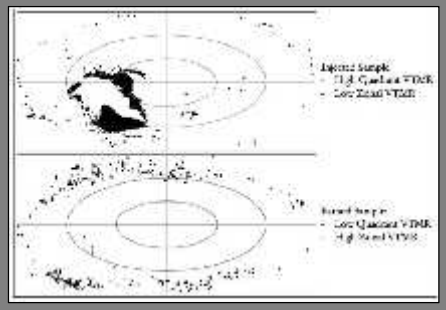
Table 1

METHODS

- Fluorescent beads in solution were utilized to compare "cell" seeding of nerve allografts using hypodermic needle injection vs. soaking allografts in "cell solution" at atmospheric and increased pressure environments with or without first preparing the nerve allograft with a microneedle roller.
- 5mm (13) and 2mm (13) cold processed human acellular nerve allografts of standardized lengths were utilized
- Injected groups were injected according to published techniques.[14]
- Soaked samples were prepared according to **Figure 1**.
- Samples were then cryoembedded and sectioned into 40 micron cross sections, yielding the study groups detailed in **Table 1**
- Confocal laser scanning microscopy was then utilized to capture digital images of cross sections with fluorescence overlay identifying beads
- Binary images were rendered and bead areas were calculated
- Total bead instillation was defined as percent cross-sectional area (CSA) occupied by beads.



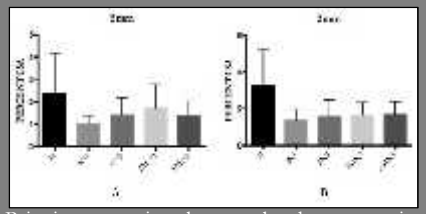
- Percent bead area was calculated for each of three inner – outer zones and 4 quadrants in order to compare uniformity of distribution – as Variance to Mean Ratio



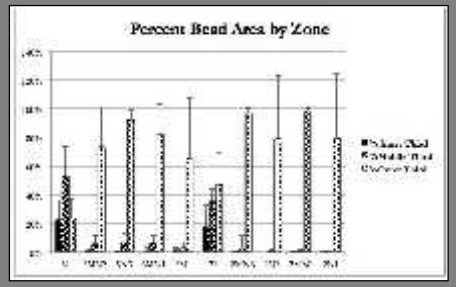
- Cross sectional bead area between two cross sections of the same sample were then compared
- Means for each outcome measure were calculated for each experimental group.
- Mixed model ANOVA followed by Tukey-Kramer post-hoc pairwise comparison was conducted with statistical significance set at $p \leq 0.05$ to assess for differences between groups.

RESULTS

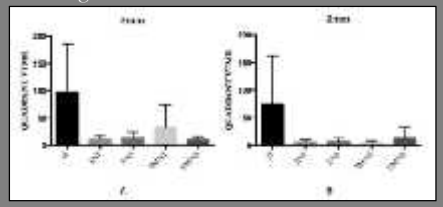
- No consistently significant difference in total bead instillation was observed between experimental groups



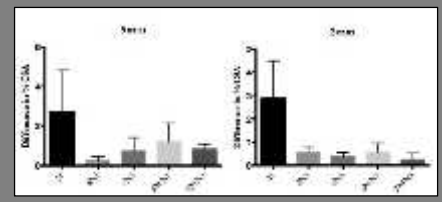
- Pairwise comparison between bead concentrations within the inner, middle, and outer zones revealed statistically significant differences between injection and soaking - which led to higher concentration in the outer third.



- Mean VTMR of bead concentrations within quadrants was significantly lower in soaked samples – indicating more even distribution



- Mean difference in percent CSA between cross sections of the same allograft were significantly lower for soaking compared to injection.



DISCUSSION

- Uniformity of distribution was better for soaking techniques compared to injection, with greater concentration in the periphery.
- Neither microneedle preparation nor pressurization significantly affected bead penetration/distribution.
- Though beads were size and concentration matched to candidate neurosupportive cells in solution, they may not represent an ideal model for cell instillation
- **Conclusion:** The potentially positive neuro-supportive cell survival and proliferation implications of this distribution pattern warrant formal in vivo testing of these seeding methods.

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