Screening For Nerve Dieback Compounds in a DRG Explant 3D Hydrogel Culture

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INTRODUCTION: Low back pain (LBP) and knee osteoarthritis (OA) are the leading causes of disability worldwide [1]. These patients show aberrant nerve growth in the intervertebral discs and knee joints, a common pathological feature and source of chronic LBP [2, 3] and knee pain [4]. Current treatments for disc-associated LBP and knee pain do not directly target the nerve fibers in the disc to treat pain and rely on short term pain relief solutions using opioid or NSAID prescriptions which can lead to addiction and severe side effects [5, 6]. **Based on the evidence for innervation in diseased joint tissues and limitations of current treatments, a therapeutic that specifically targets nerve fibers is needed. We hypothesize that retraction of nerve fibers using nerve dieback compounds could alleviate joint-related pain**. Capsaicin, a naturally derived product from chili, is a potent nerve dieback compound when delivered at high concentrations as demonstrated in vitro using dissociated DRG neuron culture [7] and on human skin [8]. Topical capsaicin patches have been FDA-approved as an analgesic to treat diabetic peripheral neuropathy [9] and clinical trials are currently underway to test capsaicin as a treatment for knee OA [10]. The **goal** of this research is to develop a screening platform and identify novel nerve dieback compounds as an alternative therapeutic for joint pain due to innervation. In this work, we cultured DRG explants in 3D hydrogels to mimic physiological properties of tissues and used capsaicin to validate our nerve dieback model. Preliminary work to screen other novel dieback compounds such as lysophosphatidic acid (LPA), a lipid molecule that causes neurite retraction [11], was also performed on the in vitro platform.

METHODS: Hydrogel solutions were made from methacrylated hyaluronic acid (1.25 mg/mL) [12] with collagen type I (4.45 mg/mL), laminin (0.75 mg/mL) and 1X PBS. DRGs from adult male Sprague Dawley rats (12-13 weeks) were surgically removed according to an approved protocol. DRG explants were trimmed and embedded in 250 µL hydrogels that is cross-linked at 37ºC for 30 minutes followed by 90 second UV exposure then maintained in culture with complete media composed of neurobasal-A media with 10% FBS, 1% penicillin-streptomycin, 1% GlutaMax, 2% B-27 and 50 ng/mL Nerve Growth Factor for 14 – 40 days before nerve dieback experiments. Capsaicin was dissolved in 100% ethanol (EtOH) and diluted in complete media to attain a final concentration of 3.79 mM with 0.055% EtOH. Lysophosphatidic acid (LPA) was resuspended by sonication in 1% bovine serum albumin (BSA) in 1X PBS and diluted in complete media to attain a final concentration of 100 µM with 0.2% BSA. Control solutions consisted of media and solvents (BSA or EtOH) without the dieback compound. Capsaicin-induced dieback was performed on 38 DRGs harvested from two adult rats with a total of 17 replicates in control media group and 15 replicates in capsaicin treated group. Preliminary experiment to show LPA-induced dieback was performed on 10 DRGs harvested from one adult rat with 5 replicates in each control media and LPA treated group. DRGs were incubated with treatment solutions for 3 days, and brightfield images were taken pre-treatment and on days 1, 3 and 7 post treatment. Up to 12 continuous neurite paths per DRG at each timepoint were traced and measured using SimpleNeuriteTracer (SNT) plugin in ImageJ. Fraction of neurite length to pre-treat (baseline) was calculated to assess changes in neurite lengths. Statistical analysis using 2-way ANOVA and Tukey's comparison test was used to compare mean fraction of neurite lengths between treatment groups and across time.

RESULTS: We were able to successfully culture adult rat DRG explants with excellent neurite outgrowth in the hydrogel culture platform as seen in the pre-treat images (Figure 1). During capsaicin-induced dieback experiment, DRGs had reduced neurite length over time and this reduction was enhanced in capsaicin-treated DRGs by day 7, which suggest neurites had dieback, although differences between control and capsaicin group did not rise to a level of significance (Figure 1B). DRG neurites in the capsaicin treated group formed varicosities and were discontinuous by day 3 (Figure 1B). Preliminary testing with LPA showed a substantial decrease in neurite length by day 7 and retracted neurite morphology with bulbed ends (Figure 1C). These preliminary results indicate the potential of using adult rat DRG explants cultured in hydrogels as a nerve dieback screening platform.

DISCUSSION: Results from capsaicin experiment support prior literature that high concentrations of capsaicin can cause neurite retraction [9]. Additional experiments are underway to verify these observations and determine significance. Preliminary results suggest that LPA could be a viable nerve dieback compound similar to capsaicin. Additional experiments are currently in progress to verify these findings. Future work will include immunocytochemical staining of DRGs with Phalloidin (actin) and primary antibodies anti-neurofilament-H and anti-beta-III-tubulin to assess structural changes in the cytoskeleton after dieback which cannot be seen in brightfield images. BSA and EtOH are needed to properly dissolve LPA and capsaicin, respectively; however, control media groups with solvents (0.2% BSA and 0.055% EtOH) were enough to prevent further neurite growth and cause reduction in neurite length (although not significant) post treatment. Future work will investigate whether these solvent concentrations may be a confounding factor in testing nerve dieback. One limitation of this in vitro platform is the co-treatment of the DRG soma and neurites. To address this concern, our lab is currently developing a fluidically-isolated compartmental device to segregate the media above the DRG soma and neurites in the same hydrogel. Future work will be to optimize the multicompartmental device for screening nerve dieback compounds alongside capsaicin as a positive control.

SIGNIFICANCE/CLINICAL RELEVANCE: The findings of this research will advance the development of pain therapeutics specifically related to joints with aberrant nerve growth and impact the quality of life of the millions of patients suffering from chronic disc-associated LBP and knee OA. This work will also further the scientific understanding behind mechanisms of nerve dieback and neurite retraction.

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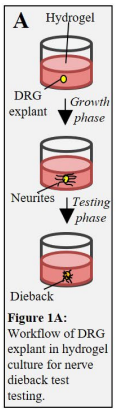

Figure 1A: Workflow of DRG explant in hydrogel culture for nerve dieback test testing.

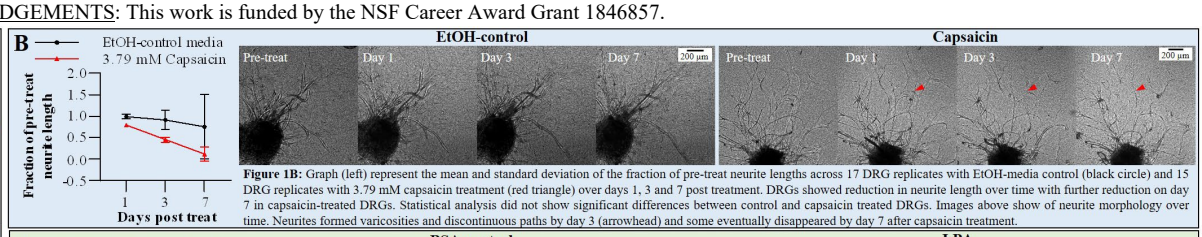

Figure 1B: Graph (left) represent the mean and standard deviation of the fraction of pre-treat neurite lengths across 17 DRG replicates with EtOH-media control (black circle) and 15 DRG replicates with 3.79 mM capsaicin treatment (red triangle) over days 1, 3 and 7 post treatment. DRGs showed reduction in neurite length over time with further reduction on day 7 in capsaicin-treated DRGs. Statistical analysis did not show significant differences between control and capsaicin treated DRGs. Images above show of neurite morphology over time. Neurites formed varicosities and discontinuous paths by day 3 (arrowhead) and some eventually disappeared by day 7 after capsaicin treatment.

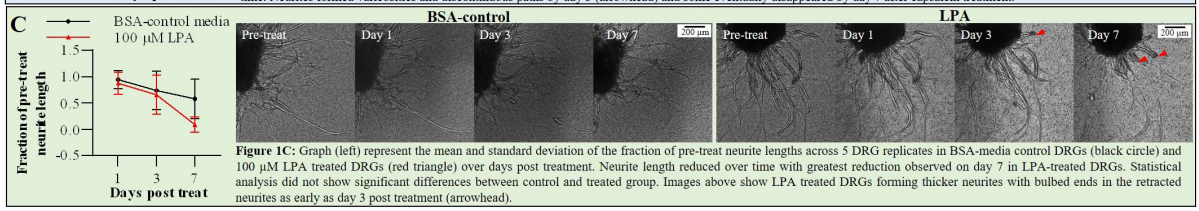

Figure 1C: Graph (left) represent the mean and standard deviation of the fraction of pre-treat neurite lengths across 5 DRG replicates in BSA-media control DRGs (black circle) and 100 µM LPA treated DRGs (red triangle) over days post treatment. Neurite length reduced over time with greatest reduction observed on day 7 in LPA-treated DRGs. Statistical analysis did not show significant differences between control and treated group. Images above show LPA treated DRGs forming thicker neurites with bulbed ends in the retracted neurites as early as day 3 post treatment (arrowhead).

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