Neurotrophic Factor Gradient Delivery for Migration Guidance of Schwann Cells and Regenerating Axons

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ABSTRACT SUMMARY
A hydrogel-based neurotrophic factor gradient delivery system is developed for the generation and application of neurotrophic factor gradients over multi-centimeter length scale. This delivery platform exhibits tunable release kinetics, and can be used to optimize the guidance of Schwann cell and axon migration in vitro and in vivo following nerve injury. Our preliminary results showed strong dependence of Schwann cell migration guidance on neurotrophic factor gradient characteristics. This study highlights the potential of this gradient delivery platform for screening various neurotrophic factors with controllable gradients and release kinetics.

INTRODUCTION
Current peripheral nerve regeneration research focuses on improving the body’s regeneration capability through modification of the regenerating nerve milieu. The delivery and presentation of biochemical cues is one of the many approaches utilized to enhance the nerve’s regenerative capacity1,2. Our lab has demonstrated that isotropic delivery of neurotrophic factors results in limited functional recovery of nerves due to the lack of directional guidance cue. Anisotropic biochemical cues, such as neurotrophic gradients, provide the necessary directional guidance cue to nerves and enhance their regeneration compared to isotropic systems3. Recently, Mortimer et al. have shown that the ability for nerves to detect and respond to neurotrophic gradients depends significantly on precise control over the presentation of neurotrophic factors to the cells4. In order to maximize the regenerative capacity of this approach, we need to first understand how nerves sense and respond to neurotrophic cues and determine what conditions are optimal for maximizing the speed of regeneration.

Here, we present a novel UV-crosslinkable gelatin hydrogel-based, multi-centimeter gradient generation system that exhibits significant control over gradient characteristics and release kinetics which can be applied to a multitude of neurotrophic/growth factors. By combining our gradient delivery system with a highly quantifiable live cell imaging system, we aim to elucidate the mechanism by which neural cells respond to gradients of various neurotrophic factors (NFs), and to optimize the gradient characteristics for Schwann cell migration and axonal out-growth. This study will be highly valuable to design strategies to promote more robust nerve regeneration in vivo.

EXPERIMENTAL METHODS
Methacrylated gelatin (Gel-MA) and methacrylated heparin was synthesized as previously reported4,5. Gradients of the glial cell-derived neurotrophic factor (GDNF) and nerve growth factor (NGF) were generated using the surface tension driven, single channel microfluidics gradient generation method6. To generate neurotrophic gradient gels, a PDMS channel with a single inlet and outlet was filled with Gel-MA solution. A larger volume of Gel-MA was pipetted onto the outlet, and a smaller volume of Gel-MA containing the NF of interest was pipetted onto the inlet. The difference in surface tension between the inlet and outlet resulted in convectional flow of the NF solution into the channel and establish the NF gradient. The Gel-MA was then exposed to UV to crosslink and immobilize the NF gradients. Heparin-containing hydrogels were prepared by mixing GDNF and methacrylated heparin (Hep-MA) at molar ratios of 1:1 and 1:10 (GDNF:Hep-MA) prior to GDNF gradient generation.

Following gelation, each gel was sectioned into six segments and each segment was placed into separate wells in a 96 well plate. Fifty μL of PBS was added to each well and was refreshed daily for two weeks. GDNF concentration in medium was measured using ELISA.

The in vitro migration studies were conducted by the generation of linear 0–10 μg/mL GDNF and 0–1 μg/mL NGF gradients immobilized in a Gel-MA hydrogel. Aligned 2-μm PCL microfibers were placed on the top of the gel to provide topographical and adhesion cues to the cells. A 300-μm tall PDMS channel is placed over the gel/fiber construct to mimic the cell migration chamber inside a nerve guidance conduit. Immortalized human Schwann cells derived by the Höke lab were then pipetted into the channel and allowed to adhere for three hours prior to monitoring the migration for 15 h using a live cell imaging confocal fluorescence microscope. Images of cells are recorded every 10 minutes and the cell migration is tracked using MetaMORPH (Molecular Devices) and analyzed using MATLAB (MathWorks).

RESULTS AND DISCUSSION
The surface tension-driven gradient generation method is high effective in establishing and mobilizing the gradient of NFs, e.g. GDNF and NGF. It allows for controllable gradient characteristics (length, steepness, concentration range). The growth factor loading is achieved at ambient temperature and in aqueous medium,
which is amenable to the preservation of growth factor bioactivity. In addition, the NF release profile can be tailored through the incorporation of Hep-MA as shown in Figure 1, with increased heparin concentration resulting in faster release of GDNF. The ratio of heparin to GDNF is easily changeable and can be tailored to fit the required rate of release for in vitro or in vivo applications.

The high degree of control over gradient characteristics provides an opportunity to screen the migratory behaviors for neural cell types as a function of gradient profile and explore the mechanism underlying how cells respond to gradient cues. Tailorable release provides an additional level of control over the presentation of neurotrophic cues and allows for better translation to in vivo systems, which require more extended, controlled release compared to in vitro models.

Using this NF gradient delivery hydrogel, we have characterized the migration kinetics of immortalized human Schwann cells (Figure 2) in response to GDNF and NGF gradients. We used a live cell tracking method to track the entire migration path of the cells. By videomicroscopy analysis, we extracted the dynamic information on cell migration, including migration speed, directionality, and persistence. Figure 2 demonstrates that although GDNF may promote a greater percentage of cells to migrate up the gradient, NGF appears to promote higher degree of directional persistence in cell migration, i.e. cells were more persistent on their path of migration. Such information can be utilized to best formulate a set of biochemical cues that elicits the desired response both in vitro and in vivo. Using various combinations of NF-loaded hydrogels and nanofiber matrices, we will also investigate the potential synergy between chemotrophic guidance provided by NF gradients and topographical guidance provided by fibrous scaffold.

CONCLUSION

This study provides a novel, scalable gradient delivery platform for the generation of multi-centimeter neurotrophic factor gradients. By establishing a biomaterials platform that combines tailorable NF gradient characteristics and release profiles, this study can reveal important insight into the effects of biochemical cues on axonal outgrowth and Schwann cell migration following nerve injury, and help to determine the biochemical cue presentation that provides optimal functional recovery.

REFERENCES


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