Characterization of hydrogels in a physiologically relevant in vitro model of peripheral pain

Benjamin Gane[1], Evie C. Barnett[1], Rebecca A. Wachs[1]

[1]University of Nebraska-Lincoln, Lincoln NE

bgane2@huskers.unl.edu

INTRODUCTION: Low back pain (LBP) is a prevalent and multifaceted health issue that significantly impacts individuals' quality of life and represents a substantial economic burden on healthcare systems worldwide [1]. It is estimated that about 80% of people will experience LBP at some point in their lives [2]. The understanding of peripheral pain including LBP, characterized by the crosstalk between neurons and immune system presents a complex challenge for researchers to unravel the underlying mechanisms and develop targeted therapeutic interventions [3,4]. To address this challenge, we have developed a physiologically relevant in vitro model for the culture of dorsal root ganglia (DRG) within a hydrogel matrix with three isolated compartments. This model offers both a physiologically relevant anatomical environment for studying the dynamic interactions between neural and immune components and provides a valuable tool for investigating treatment isolation strategies in the context of peripheral pain ultimately leading to improved therapeutic approaches and enhanced patient outcomes. The primary objective of this study is to assess the mechanical and diffusivity properties of the hydrogel used as the matrix for the DRG culture within the multicompartment device.

METHODS: A three-isolated compartment device was modeled using Autodesk Inventor 2022 for computer-aided design (CAD) and fabricated through 3D printing with High Temp V2 resin as previously published [5]. The three isolated compartments allow partition of hydrogels into a center soma compartment connected to two adjacent neurite compartments. Hydrogels were formulated using Type I collagen, laminin, and varying concentrations of methacrylated hyaluronic acid (MAHA) to investigate the effect of MAHA concentration on hydrogel properties. To prepare MAHA solution, 0.6% Irgacure was dissolved in an 80% vol/vol concentrated DMEM/HEPES solution and 20% vol/vol 1X PBS then MAHA was dissolved in Irgacure-DMEM solutions for 72 hours at room temperature with gentle agitation. Neutralized Type I collagen was then combined with each concentration of MAHA, along with 0.75 mg/ml laminin and 1X PBS to form the hydrogel mixture. 65.1 µl and 52.8 µl of hydrogel was added to the center soma compartment and the two neurite compartments and thermally crosslinked for 30 minutes in a 37°C incubator, followed by UV crosslinking for either 90 or 120 seconds with a high-intensity UV lamp (UVP B 100-AP, Analytik). Three final hydrogels formulations were used for the diffusion study (2.0 mg/ml Collagen/1.5 mg/ml MAHA, 2.0 mg/ml Collagen/2.0 mg/ml MAHA and 2.0 mg/ml Collagen/2.5 mg/ml MAHA), with an additional concentration (2.0 mg/ml/3.0 mg/ml MAHA) tested in the rheology study. To evaluate diffusivity of hydrogels and treatment isolation between the soma and neurite compartment, 0.25 mg/ml of fluorescein isothiocyanate (FITC)-dextran of sizes 10 kDa (FD10s-100MG, Sigma-Aldrich) and 150 kDa (46946-100MG-F, Sigma-Aldrich) solutions were used. Ensuring the equal height of solutions in all compartments, 65.16 µl of FITC-dextran solution was added to one neurite compartment and 65.16 µl of 1X PBS was added to the adjacent neurite compartment. 78.12 µl of 1X PBS was added to the soma compartment. To assess diffusion across compartments, 25 µl of solution from each compartment was removed to measure fluorescence intensity (ex 485 nm, em 528 nm) at specific time intervals (0, 1, 24, 48, 72 hours). The rheological properties of the hydrogels (triplicate gels per concentration) were characterized using an Anton Paar MCR 203 (n= 2 for 120 s UV and n=1 for 90 s UV). Hydrogels used for rheology had the same components as those used for diffusion except that there was no laminin. Prism 9 (GraphPad) was used for all statistical analyses. Significant difference was determined using one-way analysis of variance (ANOVA) and Tukey's multiple comparisons tests, with a significance level set at $p < 0.05$ for rheological characteristics.

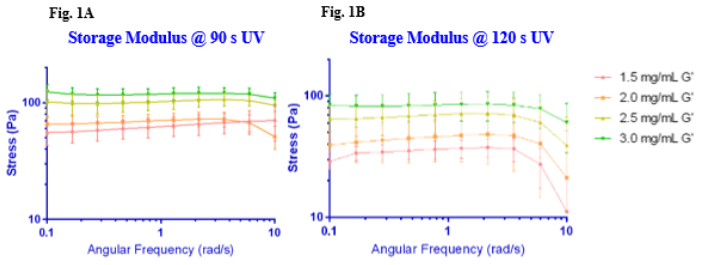

Figure 1: Storage modulus of formulated hydrogels UV crosslinked at **(A) 90** s (n=2) and **(B) 120** s (n=1).

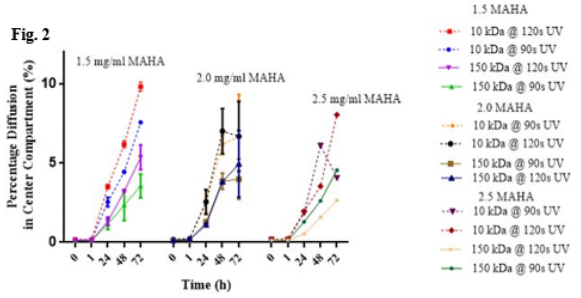

Figure 2: Percentage diffusion of FITC-dextran (10 kDa and 150 kDa) in formulated hydrogels of varying MAHA concentration in the center compartment normalized to baseline within 72 hours. Data of 1.5 mg/ml MAHA and 2.0 mg/ml represent Mean ± SD (n=2).

RESULTS: Rheology results showed that hydrogels crosslinked at a UV time of 90 s had a higher storage modulus than hydrogels UV crosslinked for 120 s (**Fig. 1**). The storage modulus of the hydrogel increased with increasing MAHA concentration (n= 2 for 120 s UV, n=1 for 90 s UV). Tukey test showed a significant difference in storage modulus between the hydrogel after 120 s UV ($p<0.001$) and there was a significant difference in the storage modulus of all hydrogels crosslinked under 90 s UV except between 1.5 mg/ml MAHA and 2.0 mg/ml MAHA ($p = 0.2051$). Hydrogel diffusivity results showed that 10 kDa FITC-dextran diffused across the compartments faster than 150 kDa FITC-dextran (**Fig. 2**). FITC-dextran (10 kDa) diffused fastest in 1.5 mg/ml MAHA at 120 s UV (9.82 ± 0.3%) within 72 hours compared to other gels. Due to irregular readings, 2.5 mg/ml MAHA has one experimental replicate (n=1).

DISCUSSION: Hydrogels with lower MAHA concentrations have lower storage modulus suggesting MAHA plays a role in gel stiffness. FITC-dextran (10 kDa) diffused the fastest in hydrogels crosslinked under longer UV times; therefore, shorter hydrogel UV times may be needed to limit diffusion across compartments. We plan to repeat this experiment (n=3) to obtain more robust data and investigate whether UV time and concentration of MAHA have an effect of the diffusion of FITC-dextran in the in vitro multicompartment device. Additionally, we intend to model the diffusivity computationally. Ongoing work also includes the assessment of DRG neurite outgrowth in varying hydrogel formulations.

SIGNIFICANCE: We believe that characterizing the diffusivity of the hydrogel in our model will ensure its relevance in various applications, including the study of underlying and complex mechanisms (local effect) involved in the development of pain which will help to identify novel therapeutic targets.

REFERENCE: [1] Steingrímsdóttir; 2017. [2] Calvo-Muñoz; 2013. [3] Chen; 2020. [4] Pinho-Ribeiro; 2017. [5] Caparaso; 2023.