Electrical Stimulation Chamber for Inducing Osteogenesis in Tissue-Engineered Scaffolds

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INTRODUCTION: Traumatic bone injuries are prevalent and debilitating. A common approach for treating bone injuries involves using bone grafts, which are invasive and may result in infection. Therefore, scientists have been researching ways to synthetically create alternatives to bone grafts, where cryogel scaffolds have proved a promising technology. Cryogels are biocompatible, macroporous, elastic, and mechanically durable polymer scaffolds that have proved beneficial for osteoinduction. Despite the advantages of cryogels for bone formation, traumatic bone injuries can be extensive and complex, requiring additional components to encourage healing. The collagen matrix of bone intrinsically exhibits natural piezoelectric properties, where these electrokinetic properties aid in bone formation and resorption [1]. The effects of physical load on bone growth can be reproduced by external electrical stimulation to perform targeted regeneration of bone tissue. While beneficial, electrical stimulation has historically been underused due to high cost, as well as challenges with efficacy and the mimicking of *in vivo* properties. MXenes are a new group of ceramic materials (i.e., metal-carbides, -nitrides, and -carbonitrides) and have been recognized for their electrical conductivity, hydrophilicity, thermal stability, and tunable structure. Recently, MXenes have been investigated in the realm of bone regeneration where exposure to a pro-regenerative environment shows high cellular compatibility and improved osteogenic differentiation, both *in vitro* and *in vivo* [2,3]. By merging the benefits of electrical stimulation with cryogels, there is the potential to optimize these scaffolds to further mimic the natural biological healing processes and harness electrical stimulation for bone formation. Therefore, the goal of this project is to combine MXene-cryogel scaffolding with electrical stimulation to enhance the bone healing process through the development of an electrical stimulation chamber for *in vitro* stimulation. We hypothesize that incorporating MXene into cryogels will unite the regenerative effects of electrical stimulation with the bone-like structure of the scaffolds. These scaffolds will enhance bone regeneration by inducing osteogenic differentiation and mineralization of mesenchymal stem cells (MSCs).

METHODS: The cryogel scaffolds were prepared from a base chitosan/gelatin (1:4) solution, as previously described [4]. To enhance these scaffolds for electrical stimulation, 70% MXene ink solution was added prior to crosslinking and the combined solution mixed thoroughly. Note that the 70% MXene concentration was chosen based on preliminary research by our group demonstrating cytocompatibility and conductivity. This solution was poured into syringes and crosslinked at subzero temperatures for 18 hours. Cryogel scaffolds formed without MXene solution served as a control. Scanning electron microscopy (SEM; Tescan Vega3) was used to image the scaffolds and to calculate the average pore area (using software developed by our lab group). Samples were compressed at 50% strain (Instron 5544 electronic load frame) to measure mechanical strength (N=10/group). Scaffolds were submerged in phosphate buffered saline (PBS), and their masses were measured over a 24-hour period to quantify swelling capacity (N=10/group). Electrochemical impedance was measured on cylindrical scaffolds (8mm diameter, 10 mm height) soaked in PBS (VersaSTAT3). Following physical characterization, the electrical stimulation chamber was made as described by Leppik et al., with several modifications [5]. Our chamber contrasts Leppik's because it uses more wells, thinner electrodes, and gold-plated copper rather than silver. This new chamber was constructed for a 12-well plate where two platinum wire electrodes (0.36mm diameter) were inserted into each well. Gold-plated copper wire was soldered to the platinum electrodes to connect the twelve wells in parallel. A wire terminal block connecter was glued to the corner of the well plate, and the gold-plated copper wire was connected to the input terminals. Insulated copper wires (two) were connected to the output terminals, and the other ends of the wires were hooked to a power supply. A voltmeter was used to confirm connection between the electrodes in all 12 wells to the power supply. Additionally, aqueous salt solution (e.g., aqueous NaCl) will be added to each well, and the change in pH will be measured after electrical stimulation (2.5V for 1 hour) to ensure that each well was equally stimulated. The MXene cryogels were then stimulated in the electrical stimulation chamber developed in this study at different levels (1V, 1.5V, 2V) for 1hr/day over a period of 3 days. The scaffolds were then characterized post stimulation to assess changes in properties.

RESULTS SECTION: MXene cryogels were initially characterized to assess the effects of the additive on physical properties. The addition of MXene resulted in larger pores than control cryogels. The average pore area for the MXene cryogels was 2096.28 μm^2, while the average pore area for the control cryogels was 1888.72 μm^2 (Figure 1). There was no significant difference in the mechanical integrity of the MXene incorporated cryogels and the controls, as indicated by their average Young's moduli. The MXene cryogels had increased swell capacity compared to control, where the MXene scaffold swelled to over 2000% of its dry mass, while the control cryogel only swelled to approximately 1750%. The average resistance of the MXene scaffold was slightly over 100 ohms, while the average resistance of the control was 4436.610 ohms, indicating that MXene significantly increased the conductivity of the scaffold (Figure 2). The electrical stimulation chamber allowed for equal and simultaneous electrical excitation within all twelve wells, determined by the change in pH of aqueous salt solution being the same across each well. Figure 3 shows a two-dimensional drawing of the final design setup. Analysis of the effects of various stimulation parameters on MXene cryogel properties is ongoing.

DISCUSSION: Incorporating 70% MXene in the cryogels did not compromise the advantageous properties of the scaffolds (i.e., pore size, swelling, and mechanics). In fact, the addition of MXene visibly increased the pore sizes and swell kinetics of the cryogels while providing them with conductive properties that has the potential to aid in mineralization and osteogenic differentiation. The electrical stimulation chamber was successfully constructed and tested. Future work will evaluate the mineralization and osteogenic differentiation potential of electrically stimulated cryogels (+/- MXene).

SIGNIFICANCE/CLINICAL RELEVANCE: This MXene technology has the potential to be used to replace bone grafts in treating traumatic bone injuries. The work in this abstract provides preliminary characterization of the benefits of MXene cryogels and, with additional cell and animal studies, could provide a means of mimicking the natural biological healing processes necessary for bone regeneration.

REFERENCES: 1. Khare, D., et al. *Biomaterials* (2020); 2. Zhang, F., et al. *International Journal of Nanomedicine* (2019); 3. Fu, Y., et al. Materials Science and Engineering (2021); 4. Kathuria, N., et al. *Acta Biomaterialia* (2009); 5. Leppik, L., et al. *Bioengineering* (2019)

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IMAGES AND TABLES:

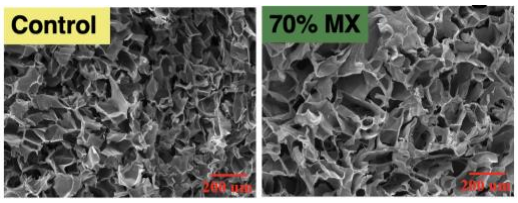

Figure 1: 200x SEM image of both cryogels.

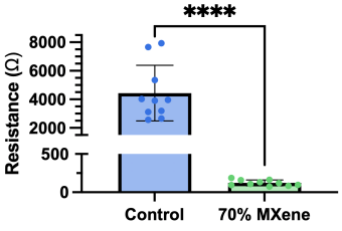

Figure 2: Electrochemical impedance of both cryogels.

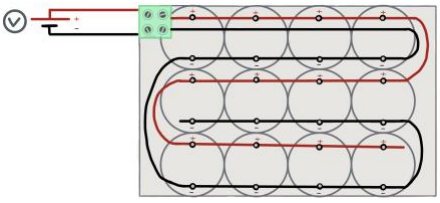

Figure 3: 2D drawing of electrical stimulation chamber.