Incorporating Fluorescent Nanoprobe Emulsions in Hydrogels for Non-invasive Oxygen Measurement

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Abstract

The Weaver Lab seeks to use siloxane nanoprobes as a means of detecting and monitoring oxygen levels of insulin-secreting cells within a transplanted hydrogel encapsulation device using magnetic resonance imaging (MRI). This method is non-invasive, allowing real-time oxygen detection in live subjects. The siloxane nanoprobe gives off a detectable signal when scanned though an H-MRI technique based off the volume of oxygen in the surrounding area around the nanoprobes within the hydrogel. The insulin-secreting cells are extremely oxygen dependent, which means that cell survivability is directly proportional to the oxygen surrounding the cells. By tracking the oxygen levels within the hydrogel, the Weaver lab has a means of monitoring the cells within the patient.

Background

Diabetes is an ever-increasing issue that affects more and more people each year. The number of people diagnosed with diabetes each year has increased from 108 million in 1980 to 422 million in 2014. In 2016 alone, around 1.6 million deaths were attributed to diabetes while another 2.2 million deaths were caused by high blood glucose in 2012 [1]. The aim of our lab is to develop a new method of type 1 diabetes treatment through cell transplantation. Using hydrogels, we seek to transplant insulin-secreting cells into diabetic patients, which could potentially eliminate complications of diabetes.

Hydrogels are primarily a water-based network of hydrophilic polymers that has a variety of uses in the realm of biomaterials such as mimicking microenvironments or drug delivery. Our hydrogels are a synthetic poly (ethylene glycol) (PEG)-based system used to macroencapsulate cells to protect them from the recipient immune system. Our PEG hydrogels were crosslinked with dithiothreitol (DTT). One problem with macroencapsulation is that the cells we are using are very oxygen dependent and without the proper levels of oxygen will experience hypoxia and die. Therefore, our lab is developing a non-invasive method of detecting the oxygen levels surrounding these transplanted cells. We aim to employ a siloxane nanoprobe to detect the oxygen levels surrounding the cells. Using a special H-MRI technique of detection the nanoprobes will return a signal that corresponds to the level of oxygen in that location. Nanoprobes are already used in a variety of medical applications, such as in the targeting of tumor growths to monitor and enhance MRI contrast [2]. Since cell performance is dependent on oxygen they are surrounded by, this allows us a non-invasive means of detecting and tracking cell performance within a patient.

Methods

The goal of the project was to test nanoprobe retention once they have been incorporated into the hydrogels. The nanoprobes were a nano emulsion comprised of 5% HS-15 surfactant, 40% Polydimethylsiloxane (PDMS), 55% DI water, and 0.1 millimolar of Nile red solution. The nanoprobes were fabricated and emulsified with a sonification process. Nanoprobe solution was encapsulated in three sets of hydrogel at sets of 2%, 4%, and 10% hydrogel to nanoprobe ratios. As well as a control hydrogel comprised of a pure PEG-DTT hydrogel with 0% nanoprobe concentration. The hydrogels were incubated in Phosphate buffered salt (PBS) solution and placed at 37-degree Celsius to simulate internal body temperature. The PBS solution surrounding each hydrogel was extracted and replaced each day for a period of four days which had been determined as an appropriate amount of time to test for a significant level of nanoprobe release from the hydrogels. The samples were collected and tested using a plate reader testing for fluorescent levels within the samples. Fluorescent pictures of the hydrogels were taken as well on the final day of extraction and run through the image analysis program ImageJ to test for fluorescence levels. By utilizing both methods, the appropriate amount of data should be gathered to accurately determine if there was a significant nanoprobe discharge from the hydrogels. Data was compiled over the span of 4 experiments.

Results

The results of the experiment indicated that there was a high immediate release of nanoprobe from the 10%, 4%, and 2% nanoprobe concentrations, followed by a small release across the following days the experiment was recorded. After day 2 each of the fluorescence values fell below the control gel in their levels of detectable fluorescence. This is further supported by the compiled picture data which indicates that there was still a significant amount of detectable fluorescence within the hydrogel images higher than the control fluorescence.

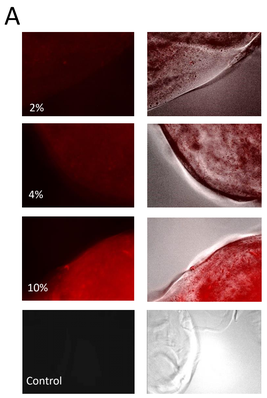

Figure 1. Images were captured on the final day of experiments at the spectrum of 570 nm. Shown in the left column are the fluorescent images. Images shown in the right column are of the stacked fluorescent and transmitted images.

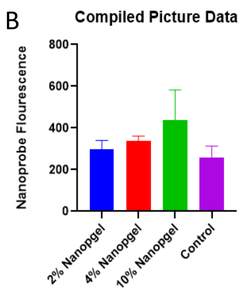

Figure 2. Compiled image data from the processed images taken on the final day of experiments. This graph indicates that each of the images taken had a higher degree of fluorescence than the control gel.

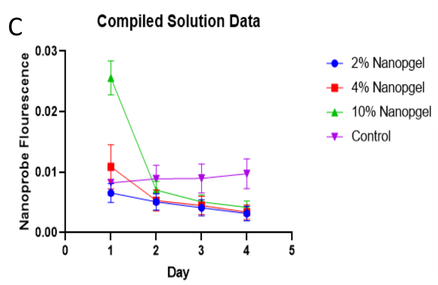

Figure 3. Compiled plate reader data taken over the course of 4 days. This graph indicates that fluorescence dipped below the level of the control solution indicating there was no longer a detectable fluorescence coming from the 2%, 4%, or 10% nanoprobe to DPBS solutions.

Conclusion

According to the data that was gathered by both the image analysis and the plate reader, it can be inferred that there is no significance to the nanoprobe discharge from the hydrogels across all three concentrations. The initial release on the first day is indicative of having excess nanoprobe that failed to be encapsulated in the initial cross-linking process and as a result causes the high release on the first day. This combined with the image analysis data which indicates that there was still a significantly higher fluorescence value than that of the control sample, shows that there the encapsulated nanoprobe successfully remained in the hydrogel.

Future Work

The next step for this project would be to evaluate the nanoprobe signal in gels using H-MRI techniques. The Weaver lab collaborates with the Kodibagkar lab, who performs the MRI tests and calculations. To accomplish this task, we will be working in tandem with the collaborator, and adjusting the gels depending on the results from the different MRI experiments that our collaborator performed. The end goal of the use of these nanoprobes is to be able to accurately and continuously quantify the oxygen levels in a given region of tissue surrounding a hydrogel. This would be invaluable information for finding the best possible transplantation site as well as having a means of noninvasively monitoring the oxygen consumption rates of the cells within a subject.

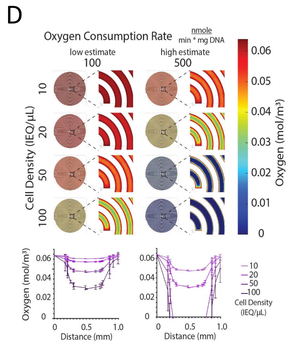

Figure 4. Figures of the oxygen consumption rates given the different cell densities in a given region. The graph on the bottom indicates that the geometry and spacing of the cells correlates to the oxygen consumption rates of the cells. This is an example of the kind of oxygen quantification we aim to achieve in our future experiments.

References

[1] *Diabetes*. https://www.who.int/news-room/fact-sheets/detail/diabetes. Accessed 14 Apr. 2020.
[2] Sun, Conroy, et al. "In Vivo MRI Detection of Gliomas by Chlorotoxin-Conjugated Superparamagnetic Nanoprobes." *Small*, vol. 4, no. 3, 2008, pp. 372–79, doi:10.1002/smll.200700784.

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