AN IMPLANTABLE GLUCOSE SENSOR COMPRISED OF OPTICAL ASSAYS AND A SELF-CLEANING HYDROGEL MEMBRANE

An Undergraduate Research Scholars Thesis

by

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ABSTRACT

An Implantable Glucose Sensor Comprised of Optical Assays and a Self-Cleaning Hydrogel Membrane

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An implantable continuous glucose monitor (iCGM) is necessary for diabetic patients to keep their glucose levels within a safe range. To build a more accessible iCGM with a longer lifetime and cheaper cost, we propose to fabricate an iCGM comprised of an optical glucose sensing assay and a "self-cleaning" hydrogel membrane. Previously, "self-cleaning" hydrogels exhibited exceptionally thin fibrous capsules and rapid healing in biocompatibility studies in vivo. Thus, they are expected to inhibit foreign body responses after implantation, leading to iCGMSs with extended lifetime and higher accuracy. In this study, two cost-effective optical sensing assays were incorporated into the hydrogel membranes. The encapsulation ability of the "self-cleaning" membrane was evaluated, specifically toward small molecules (hydrodynamic radius (D_h) <5nm). The preliminary functionality of the glucose sensors comprised of the hydrogel membranes and optical assays, were also assessed. By successfully encapsulating the optical sensing assay, an iCGM was able to be fabricated as small as a 2 mm x 5 mm cylindrical rod (diameter x length).

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CHAPTER I

INTRODUCTION

As of 2017, 30.3 million citizens of the United States have diabetes mellitus, a disease characterized by an underproduction of insulin, or by insulin resistance of the body[1]. Close regulation of blood glucose levels is vital as extreme deviance from baseline levels can cause heart disease, kidney failure, nerve damage, seizures, or even death in extreme cases. Currently, the gold standard to track a patient's glucose level is the finger prick test[2]. However, it is inconvenient and painful, while generating discrete values only for the test points. Therefore, a continuous glucose monitor (CGM) is necessary to control the glucose changes through time[3].

Some CGMs have been approved by the FDA and released into the market. The major types of these CGMs are based on a transcutaneous electrochemical sensor, for example, the Freestyle Libre® (Abbot)[4]. The Freestyle Libre® is comprised of a hard, transcutaneous sensor probe that is indwelled into the patient's upper arm[5]. Abbot's Freestyle Libre® measures the glucose level in the interstitial fluid and transmit the signal to an outsource device. The hard sensor probe and transcutaneous approach generate an active foreign body response which limits the functionality of the sensors and their lifetime. Thus, these CGMs are susceptible to infections and irritation. They also require frequent replacement of the sensor (3-14 days). Recently, an iCGM was approved by the FDA, Eversense® (Senseonics)[6]. It is comprised of a hydrogel sensor, optical system, and electrical system within a cylindricallyshaped poly(methyl methacrylate) (PMMA) case (18.3 mm x 3.5 mm, length x diameter). To prevent a significant foreign body response, it also includes an anti-inflammatory drug delivery component. The Eversense® has been approved to be subcutaneously implanted 60-90 days in the patient before

replacement with a new sensor. The electrical and optical components are not reusable, which increases the cost. They also increase the size of the implant, leading to a higher foreign body response.

Hydrogels are polymeric networks that imbibe large quantities of water[7]. They are widely used in biomedical engineering due to their excellent biocompatibility. Previously, a thermoresponsive double network hydrogel membrane (DN-25%) was developed as an iCGM sensor membrane[8]. It's comprised of a poly(*N*-isopropylacrylamide-co-2-acrylamido-2-methylpropane sulfonic acid) [P(NIPAAm-*co*-AMPS)] first network, and a PNIPAAm second network. The DN-25% membrane is "self-cleaning". When the temperature fluctuates above/below the hydrogels volume phase transition temperature (VPTT), the membrane deswells/swells, generating a dynamic anti-biofouling process[9]. An *in vivo* biocompatibility study showed that DN-25% has a minor foreign body response compared to a standard biocompatible 10% (w/v) poly(ethylene glycol) (PEG) hydrogel, with thin fibrous encapsulation and a fast healing response[10].

To build a small-sized iCGM sensor, we proposed to fabricate cylindrical DN-25% hydrogel membranes with hollow cavities to incorporate an optical sensing assay. The transportation ability of DN-25% permits diffusion of molecules smaller than their mesh sizes (D_h ~4 nm – 7 nm)[11]. It allows good diffusion of glucose, yet, it also limits the size of molecules that can be housed inside the hydrogel membrane. In this study, one assay incorporated was developed by Dr. Gerard L. Coté[11]. It is comprised of phosphor bound conanavalin A (PEG-ConA-TRITC) and phosphor bound mannotetraose (APTS-MT). The PEG-ConA-TRITC can bind to either APTS-MT or glucose, depending on their concentrations. Förster resonance energy transfer (FRET) happens when APTS-MT binds with PEG-ConA-

TRITC, and the intensity decreases when APTS-MT is replaced by glucose[12]. This assay allows a sensor size 5 mm x 2.5 mm (length x diameter) with good sensitivity. To successfully house APTS-MT (D_h ~3 nm), we utilized a double network hydrogel with controlled mesh size by comb architectures. Their housing capability was evaluated with a long-term leaching study.

Another assay used was developed by Dr. Michael J. McShane[13]. It is comprised of phosphorescent molecules [Pd (II) meso-tetra(4-carboxyphenyl)porphine] (PdTCPP) and the enzyme glucose oxidase (GOx) in alginate microparticles (D ~ 20 μm)[14]. The phosphorescent decay changes with oxygen, which is influenced by glucose oxidation. The alginate microparticles were big enough to be directly incorporated into DN-25%. Their functionality was briefly evaluated.

CHAPTER II

METHODS

Materials

N-isopropylacrylamide (NIPAAm, 97%), 2-acrylamido-2-methylpropane sulfonic acid (AMPS, 97%), *N*-vinylpyrrolidone (NVP, 99%), *N,N'*-methylenebisacrylamide (BIS, 99%), 1-[4-(2-Hydroxyethoxy)-phenyl]-2-hydroxy-2-methyl-1-pro-pane-1-one (Irg 2959), cysteamine hydrochloride (AET, 98%), methacrylic anhydride (MA, 94%), ammonium persulfate (APS, 95%), sodium hydroxide (NaOH, 97%), hydrochloric acid (HCl, 37%), fluorescein isothiocyanate-dextran (FITC-dextran, 4k, 10k, 20k, and 40k g/mol), ethanol (EtOH, ~99%), and acetone (~99.5%) were obtained from Sigma-Aldrich. Potassium hydrogen phosphate (K_2HPO_4, 98%) and potassium dihydrogen phosphate (KH_2PO_4, 98%) were purchased from Alfa Aesar. New-Skin Liquid Bandage was obtained from CVS. Phosphate-buffered saline (PBS) was purchased from Conrning®. Deionized water (DI H_2O) (resistance of 18 MΩ·cm) was utilized for all hydrogel fabrication and all subsequent experiments.

Hydrogel fabrication

Double network (DN-25%) hydrogel (hollow rods)

DN-25% was fabricated as previously reported[15]. The first network precursor solution was made with 75:25 (wt:wt) of NIPAAm:AMPS, 4 wt% of BIS and 8 wt% of Irg 2959. It was sequentially injected into a cylindrical glass mold with a 400 μm stainless steel wire in the middle. The mold was cured under ultraviolet light (UVP UV-Transilluminator, 6 mW cm^{-2}, λ_{peak} = 365 nm) for 30 minutes (15 min/side) while immersing in ice to prevent NIPAAm collapse. The second network precursor solution was synthesized by combining NIPAAm, NVP, BIS, and

Irgacure 2959 in DI. The hollow rods then soaked in the second network for 24 hours and were cured again for 10 minutes (5 min/side) with a 650 µm stainless steel wire.

Poly(AMPS) comb (PAMPS-c-X) hydrogel

PAMPS comb hydrogels were fabricated into hollow rods with the same methods as DN-25%. For the first network precursor solution, X mol% of methacrylated AMPS (PAMPS-MA) was added, replacing the same mole of AMPS in the original DN-25% precursor solution (**Table 1**). The second network solution was kept the same. PAMPS-MA was synthesized from Dr. Melissa Grunlan's lab as reported[16].

Table 1: Compositions of all hydrogels tested for fluorescent sensor

	First Precursor Solution			**Second Network Solution**	
Name	**NIPAAm (g)**	**AMPS (g)**	**PAMPS-MA:AMPS**	**NIPAAM (g)**	**NVP (g)**
DN-25%	0.75	0.25	0	6	0.96
PAMPS-c-15% (n=20)	0.75	0.213	15:85	6	0.96
PAMPS-c-50% (n=5)	0.75	0.125	50:50	6	0.96

DN-25% hydrogel with PdTCPP alginate microparticles (slab)

PdTCPP alginate microparticles were obtained from Dr. McShane's lab[13] (~1 mg/30 µL). These microparticles were added into DN-25% first network precursor solution with different ratios (2:1, 4:1, 6:1, 8:1, and 10:1). The solution was then injected into a cuboid glass mold (1 mm in thickness) and cured under UV for 30 minutes (15 min/side). The mold was immersed in ice while curing to prevent NIPAAm collapse. After curing, the hydrogel slab was equilibrated in DI for 48 hours to remove unreacted molecules. Sequentially, it was soaked in the 2nd network for 24 hours and then cured within a cuboid glass mold (1.5 mm in thickness). The

mold was then exposed to UV light for 30 minutes (15 min/side) immersed in ice, and stored in DI after curing.

Characterization

Leaching Study

To evaluate the diffusion properties of the hollow rod membrane, FITC-dextran with determined sizes (M_w ~4k g/mol and 20k g/mol) was encapsulated in their cavity to perform the leaching study[17]. DN-25%, PAMPS-c-50% (n=5), and PAMPS-c-15% (n=20) hollow rods (650 µm x 2 mm, inner diameter x outer diameter) were cut into 5 mm long samples. Next, 1.5 µL of FITC-dextran solution (1 mg/ml) was injected within the cavity. The ends of the rod were sealed by inserting one glass bead (~700 µm in diameter) into each end and applying two layers of New-Skin® liquid bandage. The rods were then immersed in 500 µL of PBS for one week. The accumulative fluorescent intensity of the supernatant was tested every 24 hours with a plate reader (Tecan Infinite M2000PRO). The fluorescence of the solution was measured with an excitation wavelength of 480 nm and emission wavelength at 520 nm[12].

To evaluate the encapsulation capability of the hollow rod membranes towards APTS-MT, leaching studies were performed with 1.5 µL APTS-MT solution (2 µM) with an excitation and emission wavelength of 490 nm and 520 nm respectively[18].

Scanning electron microscopy

A few samples of the PdTCPP microparticles from different batches were vacuum dried in an oven, sputter-coated (Cressington Sputter Coater 108), and analyzed using a scanning electron microscope (NeoScope JCM-500) to characterize particle size distribution. Further research showed that sonication of the microparticles was necessary.

Sensor functionality test

The fluorescent properties of the hydrogel slab with PdTCPP alginate microparticles was characterized with the platereader. The excitation and emission wavelengths were 530 and 720 nm, respectively.

CHAPTER III

RESULTS AND DISCUSSIONS

Leaching study

For the FRET-based sensing assay, the D_h of PEG-TRITC-ConA is ~30 nm while APTS-MT is only ~3 nm[19]. To evaluate the encapsulation capability of the hollow rod membranes, FITC-dextran (M_w ~4k g/mol) with similar D_h (~3 nm) to APTS-MT was used for the leaching study[17]. As shown in **Figure 2**, over the course of 6 days, a significant amount of 4k FITC-dextran leached out of the hollow DN-25% rod within the first day. Hollow rod membranes with comb architectures [PAMPS-c-50% (n=5) and PAMPS-c-15% (n=20)] showed improved encapsulation. After 24 hours, PAMPS-c-50% (n=5) showed ~75% leaching while PAMPS-c-15% (n=20) showed ~50% leaching. After six days, ~30% of the FITC-dextran 4k remained in the PAMPS-c-15% (n=20) rod.

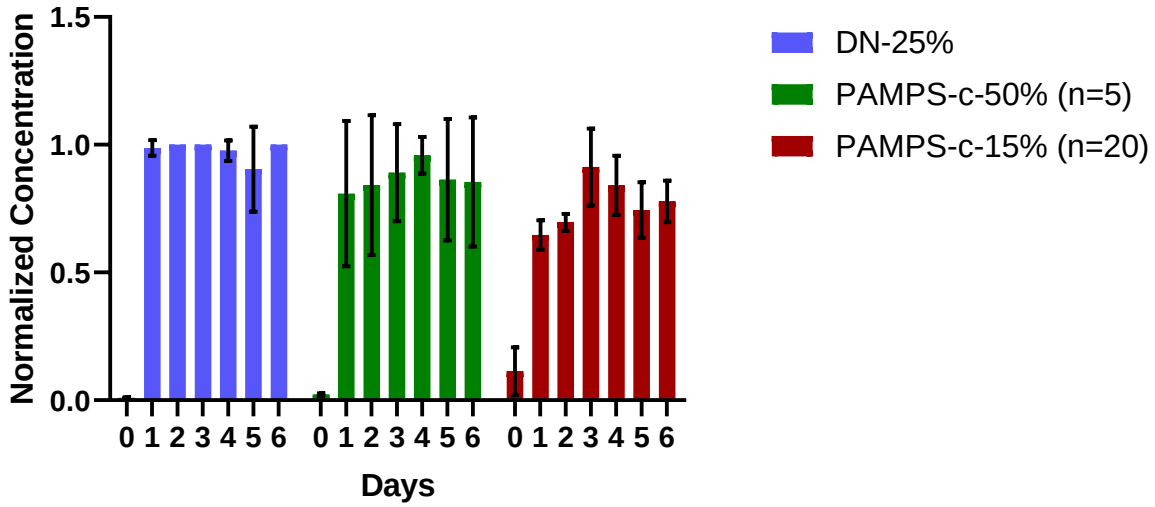


Figure 2: Leaching study performed on DN-25% and PAMPS-comb hydrogel membranes with 4k FITC-dextran

While the results of the leaching tests on the three compositions of hydrogel rods proved inconclusive of encapsulation efficiency, the results of the APTS-MT encapsulation were much more appealing. Seen below in **Figure 3**, over the course of four days, there was substantial leaching of APTS-MT from the DN-25% and PAMPS-c-50% (n=5); however, the PAMPS-c-15% (n=20) rod retained ~60% of the APTS-MT implanted.

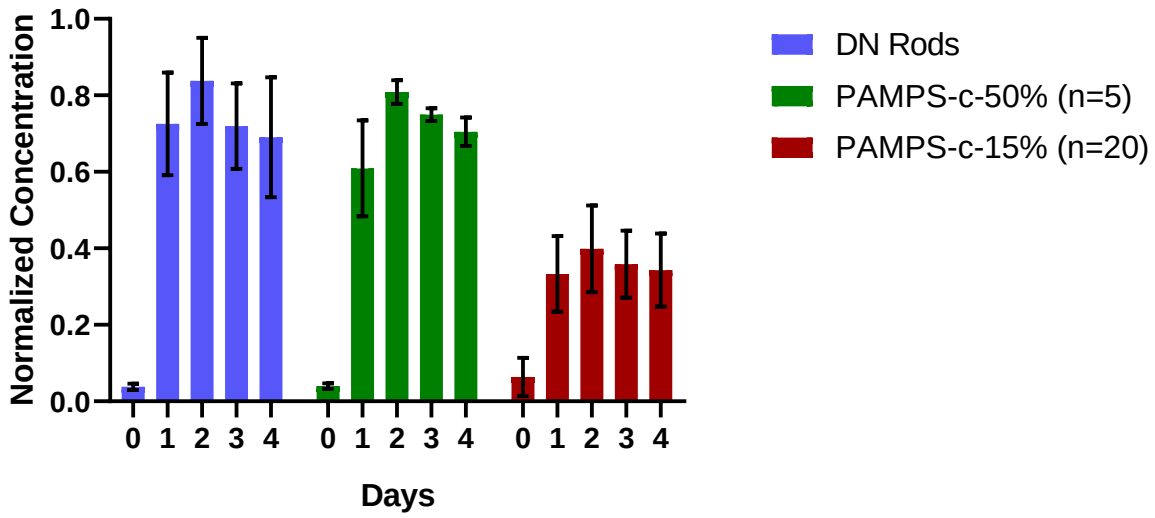


Figure 3: Leaching study performed on DN-25% and PAMPS-comb hydrogel membranes with APTS-MT

This leads to the conclusion that the addition of the comb does, in fact, reduce mesh size of the hydrogel membrane. Further testing and experimentation need to be done to retain ≥95% of APTS-MT implanted before the sensor could be considered for a clinical application, but these results are promising.

Incorporation of microparticles

PdTCPP alginate microparticles analysis

The size of the microparticles developed in Dr. McShane's lab is substantially larger than the mesh size of the DN-25% hydrogel membrane[11]. An original batch of PdTCPP microparticles proved rather challenging to work with, resulting in an SEM image of the

particles being taken. The image of this batch is shown in **Figure 4**. Aggregation of the microparticles had occurred which made the manufacturing process very difficult. A new batch was obtained from Dr. McShane's lab, sonicated to prevent aggregation, and utilized for all further testing.

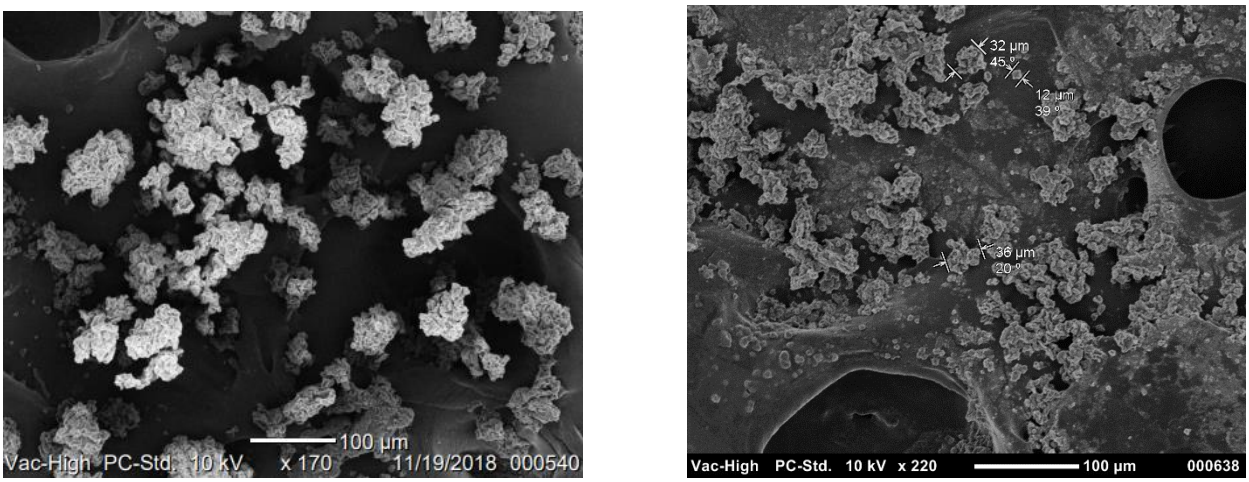


Figure 4: Scanning Electron Microscopy of aggregated PdTCPP microparticles from the first batch before (left) and after (right) sonication. (Scale bars shown for reference)

An SEM image of the new batch of PdTCPP microparticles showed an average diameter of 9-15 µm (**Figure 5**). This micromolecule is much too large to diffuse out through the mesh of the DN hydrogel membrane, but small enough to work with easily. This preliminary data proves promising for incorporation of these microparticles into the DN-25% hydrogel.

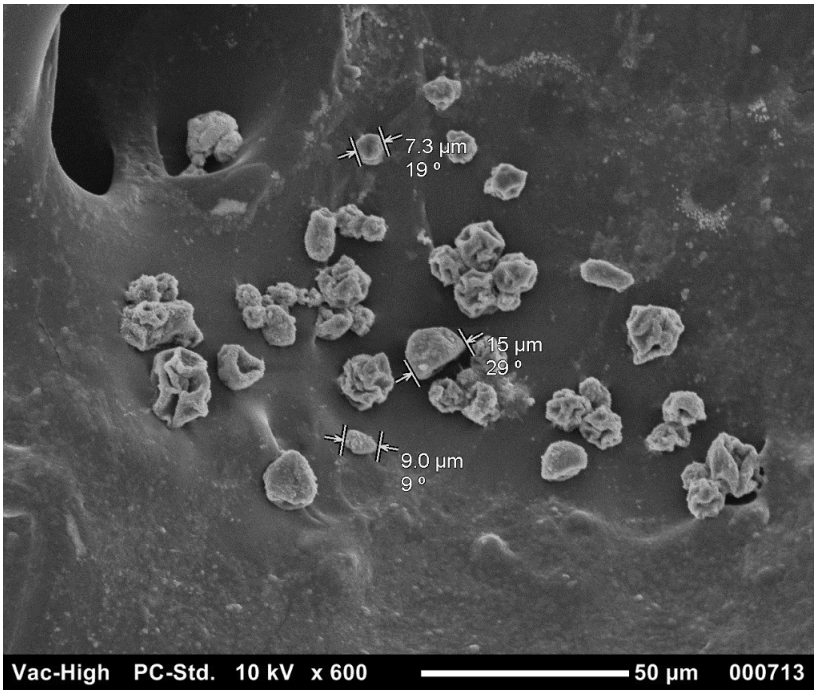


Figure 5. Scanning Electron Microscopy of PdTCPP microparticles from new batch after sonication at a magnification of 600X and a voltage of 10 kV. (Scale bar shown for reference)

The different slabs with the different concentrations are shown below in **Figure 6**.

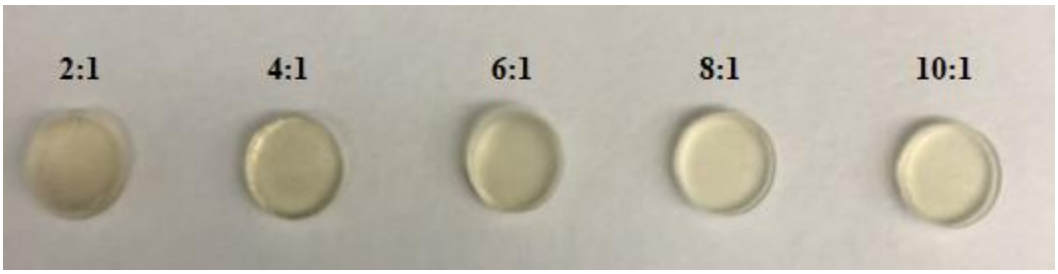
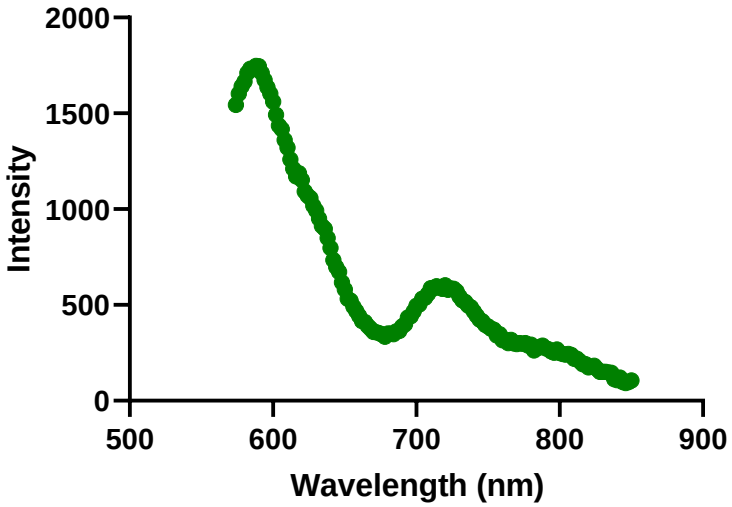


Figure 6: PdTCPP microparticles within DN-25% hydrogel membrane sensor at different concentrations

As the concentration of the PdTCPP microparticles decreases, the fluorescent intensity of the sensor decreases. Mechanical robustness increases though, as the gel is resembling more of the pure DN-25% hydrogel[20]. These two characteristics culminated in the decision to explore the 4:1, 6:1, and 8:1 concentrations.

While incorporation of the microparticles into the DN hydrogel is feasible and straightforward, retaining functionality of the sensor proved to be challenging. **Figure 7** shows a

peak in fluorescent intensity at 720 nm which is indicative of the functionality of the



microparticles.

Figure 7: Fluorescent scan of PdTCPP Alginate Sensor prior to implantation within the DN-25% hydrogel membrane

However, upon incorporating the microparticles with the double network hydrogel membrane, the 720 nm peak shifts to 800 nm (**Figure 8**).

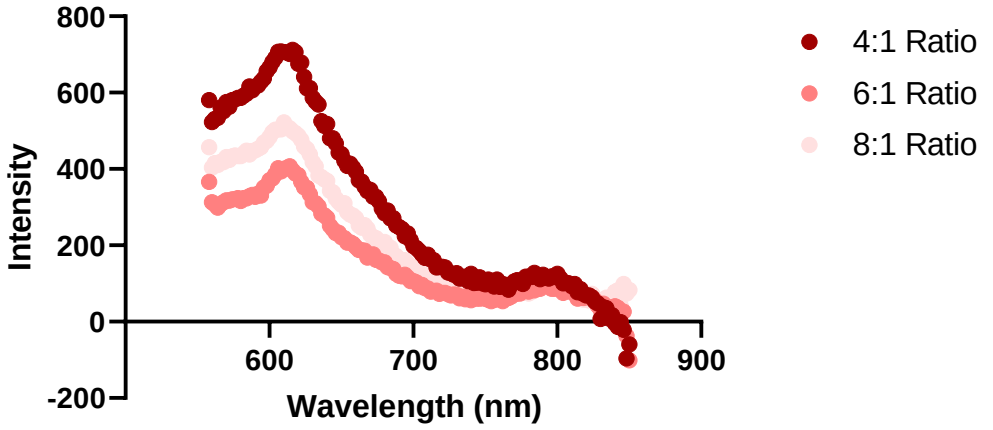


Figure 8: Fluorescent scan of PdTCPP Alginate-DN-25% Sensor after implantation within the DN-25% hydrogel membrane

A few possible solutions arose as to why the functionality of the sensor was being altered, and additional research is necessary to determine what the cause is. Further testing needs to be

done on the signal-to-noise ratio (SNR) of the PdTCPP DN-25% composition to determine whether this shift and reduction in intensity of the peak will prove detrimental to the sensor as a whole.

CHAPTER IV

CONCLUSION

Conclusion

Implantation of a liquid glucose sensing assay within a thermoresponsive "self-cleaning" hydrogel membrane has the potential to create an iCGM for diabetes management.

Leaching studies have shown that the addition of a PAMPS-comb architecture has successfully reduced the mesh size of the previously proven DN-25% hydrogel. These results lead to believe that the concentration and comb length may be modified, yielding the perfect mesh size to allow for glucose diffusion while retaining the slightly larger APTS-MT molecule. Further studies will explore different concentrations and length of the PAMPS-comb monomer, while also trying to find different encapsulation methods that may prove more effective than the current standard.

Research into the incorporation of glucose sensing microparticles alleviates the issue of the sensing assay leaching out through the hydrogel membrane. These phosphorescent-based sensors have their emission altered after implantation within the hydrogel; however, an optical analyzing system that is able to develop a large enough SNR may be able to yield a blood glucose concentration still. Additional testing of functionality needs to be performed by exposing the microparticle hydrogel sensor to different concentrations of oxygen to determine whether a substantial signal can still be received. Final *in vitro* testing will include incorporating the GOx enzyme and exposing the entire sensing system to a solution of glucose, looking to ensure the enzyme is successfully depleting the oxygen present, and the sensor is outputting a proper signal to indicate glucose concentration.

Future Work

Another avenue of future research is the use of PD (II) meso-tetra(sulfophenyl) tetrabenzoporphyrin sodium salt (S-PdBP) as the microparticle instead of PdTCPP[21]. The new microparticles allow for a red light to be used for excitation of the sensor, which penetrates through the skin deep enough to reach the sensor, allowing for clinical application[22]. Some preliminary testing was done using S-PdBP microparticles (1.83 mg/30 µL). **Figure 9** below shows the S-PdBP 6:1 hydrogel composition using the same synthesis procedure as the PdTCPP alginate sensor, as well as a hollow rod with the microparticle solution within (2:1 S-PdBP:DN-25% 1st network). Incorporation of the microparticles into either the cavity of a hollow rod or the entirety of a solid rod is the ultimate goal as it allows implantation of the rod using a needle instead of a surgical procedure.

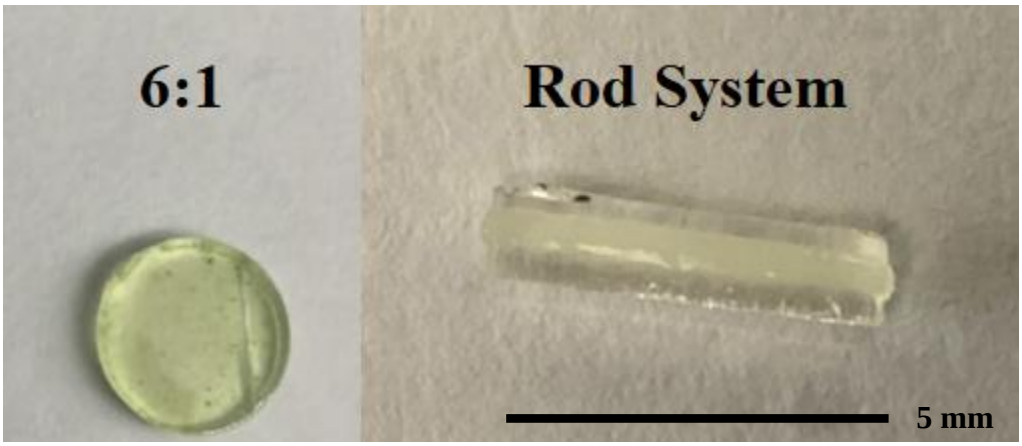


Figure 9: S-PdBP Alginate Sensor within DN-25% hydrogel membrane sensor and S-PdBP microparticle injected within a DN-25% hydrogel hollow rod

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