Design and Test of a Mechanical System for Electrochemical Oxygen Sensors with *in vivo* Applications

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Abstract – A freestanding micromachined electrochemical oxygen sensor has been developed for the purpose of minimally invasive biomedical applications. When applied to these physiological environments, it is important to understand the physical interactions between this environment and the sensor. These interactions could affect the functionality of the sensor. An anticipated use is applying this sensor within a muscle, which has unknown effects on the sensor. To validate the performance of this sensor in a muscular environment, a mechanical system has been developed that tests the sensor in these conditions using an externally applied force and a muscle phantom. For performance comparison, the sensor was tested in saline before and after experiencing an applied load, that progressively increased, within the phantom. After undergoing testing, this comparison showed no significant difference in current output validating that intramuscular forces have minimal to no significant effect on the performance of these sensors.

I. Introduction

Developing biosensors are useful for limitless medical applications and are prevalent in improving modern medicine. One such improvement can be made in the field of mitochondrial diseases. Primary Mitochondrial Diseases (PMDs) affect the functionality of the mitochondria and prohibit proper consumption of oxygen for energy generation. The primary diagnosis of these diseases involves a muscle biopsy, which is an invasive and painful procedure. In an effort to make this a minimally invasive procedure, among other applications, an alternative is being developed through the use of oxygen sensors [1].

Oxygen sensors are commonly used in a variety of applications with wide commercial success. Despite this, their use in biomedical applications is limited as they are sized too large and are not biocompatible, which is problematic for *in vivo* monitoring. For this purpose, an electrochemical oxygen sensor has been developed that is miniaturized, flexible, biocompatible, and disposable to best suit the needs of this application, shown in Figure 1 [1].

Fig 1. Electrochemical microsensor with SU-8 spacer on the front (left) and PDMS backing on the back (right).

For *in vivo* applications, it is important to understand the mechanical interactions between the tissue environment and these sensors to facilitate insertion into the organism, and to evaluate if the performance of the sensor is affected by these interactions. From this idea, a mechanical set up has been developed that simulates a muscular environment and the acting forces experienced by the sensor within this environment. The main components of this testing system consist of the oxygen sensor being tested, a muscle phantom (a viscoelastic material that mimics muscle tissue), and an applied external force.

II. Background

The oxygen micro-sensor used consists of three electrodes, a liquid electrolyte, and an oxygen permeable membrane [1]. When placed in an oxygen-present

environment, the oxygen within the surrounding medium will diffuse into the sensor through the permeable membrane and into the liquid electrolyte. The oxygen will then diffuse further until it reaches the electrode surface. When the electrodes are supplied with a voltage, the oxygen undergoes a reduction reaction that produces a current through the electrode. This current is proportional to the oxygen concentration as the current directly depends on the amount of oxygen that has diffused into the sensor. Conducting these tests are done with a linear sweep voltammetry (LSV) that uses a potentiostat to supply the outgoing voltage and read the incoming current. In reading these scans, the curve will peak which symbolizes the accurate oxygen concentration. This is due to two factors, the rate of reduction and the rate of diffusion, being equal. Structural components have also been added to the sensor to improve structural integrity that includes a PDMS backing and a SU-8 spacer on the front.

To accurately mimic a muscular environment, an adequate muscle phantom must be selected. Many different materials are used to simulate muscle properties for clinical studies and experiments, such as emulating the conductive property for ultrasonic testing [2-6]. Since the physical interactions of the muscle are being studied, the muscle phantom must have similar mechanical properties to muscle tissue. This can most adequately be quantified through comparing the mechanical modulus (Young's Modulus, Elastic Modulus). The most common materials include uses of gelatin, agar, and PVA [4]. Studies have shown that different concentrations will have different Elastic Modulus proving to be an accommodating way to manipulate and match the modulus of a muscle tissue. Not all muscles have the same physical properties so the modulus that the phantom will be compared to must be of the muscle used in testing trials. This muscle of interest has been determined to be the muscles in the forearm, for human trials, so the mechanical properties must be understood for skeletal muscles [5-7].

In terms of how the sensor would be affected, previous research was referenced to get a sense of values for intramuscular pressure [8-9]. This pressure is dependent on multiple factors, such as movement that induces contraction forces, but the focus is on the pressure in a passive state as the organism is ideally still for the duration of the test. Even so, extreme values are tested to validate performance even under unideal conditions. Translating this pressure into the mechanical system can be done in a variety of ways, so a simplistic design was the aim for convenience and reproducibility.

III. Methods

To select an accurate phantom, the properties of skeletal muscle must first be understood. A study was referenced that related muscle and tendon stiffness to the corresponding Young's Modulus of the gastrocnemius muscle belly and Achilles tendon [5]. A MyotonPRO handheld device and a shear wave ultrasound elastography were used to measure the stiffness and Young's Modulus, respectively, in two healthy participants. This study was selected as it reports a narrower range for the Young's Modulus of the gastrocnemius muscle, which is a skeletal muscle, and can have properties that related to the skeletal muscles in the forearm. The modulus values reported range from about 15-30 kPa for the gastrocnemius muscle.

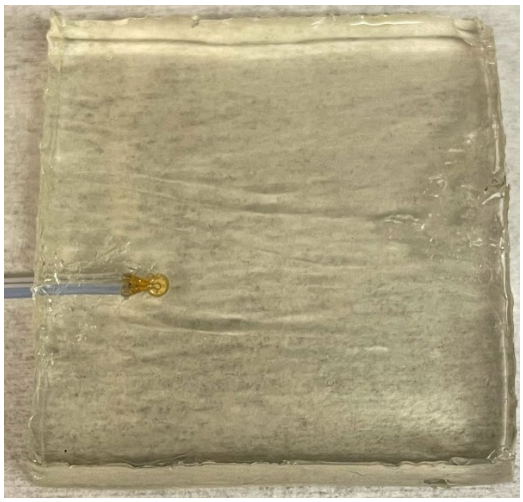


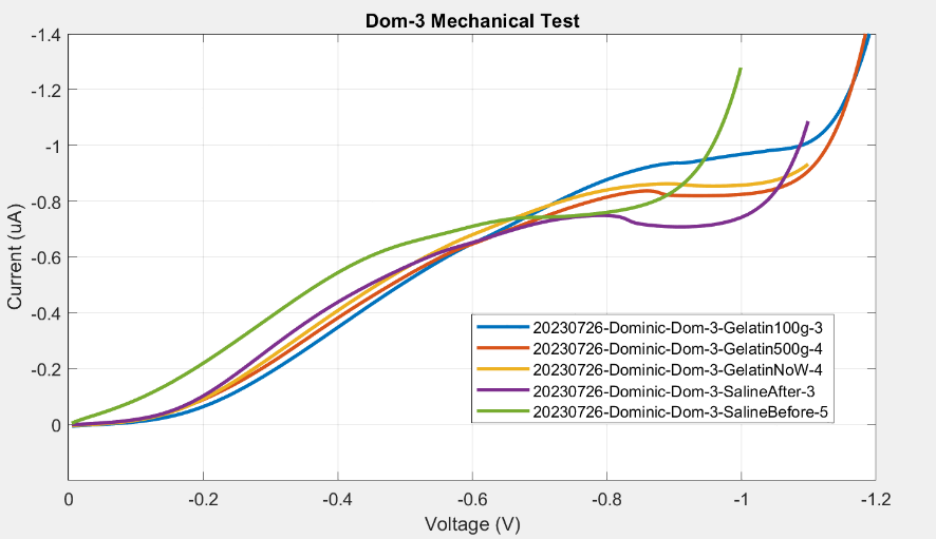
Fig 2. 10 wt% gelatin muscle phantom with inserted microsensor.

Fig 3. Muscle phantom with inserted microsensor and 200g weight applied on top.

Understanding this, a phantom can be selected to best fit the desired properties. It was reported in literature that included recipes for phantoms and the corresponding Elastic Modulus, which is analogous to the Young's Modulus [4]. The three materials and their modulus value range included are Agar with ~102-188 kPa, PVA with ~4-37 kPa, and

Pressure Values	
10g	7.73 mmHg
100g	14.97 mmHg
200g	19.48 mmHg
250g	24.35 mmHg
500g	27.84 mmHg

(a)

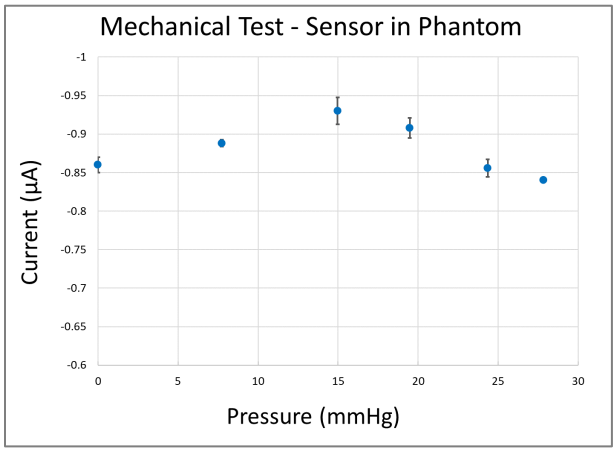


(b)

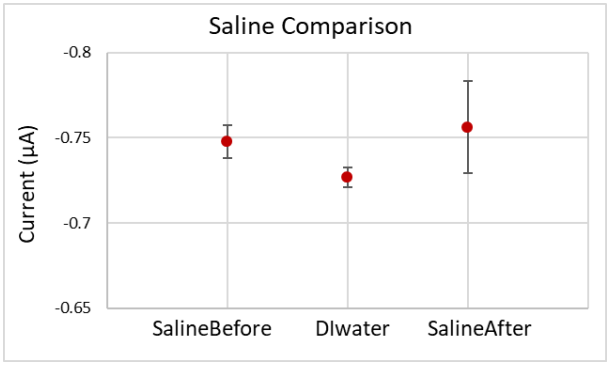
Fig 4. (a) Converted pressure values from the applied gram weights, (b) Current vs. Voltage LSV scan plots with scans from various applied loads and conditions,

Gelatin with ~27-92 kPa. Considering the type of material and the modulus value, the gelatin mixture was selected, as shown in Figure 2, for ease of reproducibility and for the similar modulus to the found skeletal muscle modulus. The phantom mixture used was a 10 wt% mixture that was adapted from the literature. The recipe consisted of 27 g of DI water and 3 g of gelatin powder. The DI water was first heated to 90 °C, then the gelatin powder was added and hand mixed to dissolve the powder in the water. The mixture was then put back onto the hot plate until the mixture appeared clear. The mixture was then poured into a sample box and refrigerated overnight.

In determining how to mimic the acting forces on the sensor and considering intramuscular fluid and muscle fibers, the applied force is best characterized by the intramuscular pressure (IMP). It was reported in literature there was a relation between IMP and strain under passive conditions in the tibilias anterior of the New Zealand White Rabbit [9]. The IMP was measured using a fiber optic pressure transducer while the muscle was passively strained from ~0-0.6. The IMP values ranged from 0-35 mmHg. These values were included in this mechanical system since the tibilias anterior of the New Zealand White Rabbit is a skeletal muscle and is comparative to skeletal muscle of the human body, therefore the intramuscular pressure should be consistent. To emulate this in the mechanical system, various stainless-steel gram cylinder weights were used as the external force as seen in Figure 3 during the mechanical system test. These weights ranged from 10-500 g and each were converted to mmHg, shown in Fig. 4(a) to understand the pressure acting on the sensor.



(a)



(b)

Fig 5. (a) Plot of Current vs. Pressure for phantom scans. The current values were determined by the peaked current value of the curve for each scan under each applied load. (b) Plot of peaked current values under each condition being in saline and DI water before the mechanical system test, and in saline after the mechanical system test.

IV. Results

In Figure 4(b), LSV scans conducted under various conditions are plotted with Current (μA) vs. Pressure (mmHg). Due to the nature of the sensor, the curves of each scan are evaluated at their peak or plateau of the current. From this the scans can be analyzed using these peak currents and compiled into a data set for comparability. The two data sets that were analyzed involved the sensor scans within the mechanical system, and the sensor scans in saline before and after the testing. These sets were plotted with peak current in various conditions in Fig. 5(a) and Fig. 5(b). As seen in Fig. 5(a), there is no a significant peak current difference under different applied conditions. Furthermore, the before and after base saline scans in Fig. 5(b) show little to no variance in peak current. This displays evidence that there is no sufficient change in the performance of the sensor since there is no change before and after undergoing testing in the mechanical system, and that the structural components are effective for in-vivo testing.

V. Conclusion

In developing improved biosensors, it is crucial to understand the role various factors in the intended biomedical applications have on different aspects of the sensor. An electrochemical oxygen microsensor was developed for biomedical applications and includes a PDMS backing and SU-8 spacer to improve the structural quality and performance of the sensor. The research highlighted in this paper was focused on how physical interactions in-vivo environments have on this biosensor and how these interactions affect the performance of this sensor. With the focus on applying this sensor to muscular tissue, a mechanical system was created to replicate the conditions the sensor would face in this environment. This was done by using a muscle phantom that matches the mechanical properties of this in-vivo environment, as well as an applied external load to replicate the internal forces acting on the sensor. The phantom used was a 10 wt% gelatin mixture along with various weights used for the applied loads and combining these components proved to be an effective mechanical testing system that accurately mimicked the intended environment. It was demonstrating through comparing base saline scans before and after undergoing the mechanical system test that there was no significant change in performance. This concludes that the physical interactions experienced by the sensor from an in-vivo environment has no significant effect on the performance of the sensor with the included structural components of a backing and a spacer.

With this mechanical system, further testing can be done on various biosensors to assess their performance in these conditions. The structural components on the oxygen sensor that was used in this test are tedious to fabricate and efforts are being made to develop an easier method with accurate performance, such as dip-coating the sensor in PDMS to fully cover the sensor. This new method can be tested using this mechanical system to validate the performance of the dip-coated sensor. Furthermore, the components of this mechanical system can be modified to suit the anticipated environment such as adjusting the phantom recipe to get different desired properties or to adjust the applied external load to test under more force.

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