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Sett, A., De Jonge, J., Bersis, F., Mastrangeli, M., & French, P. (2025). Development of Sensors and Optical Filters Towards Detecting Tissue Damage During Organ Transplants. In *Proceedings of the 2025 IEEE SENSORS* IEEE. https://doi.org/10.1109/SENSORS59705.2025.11330493

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Development of sensors and optical filters towards detecting tissue damage during organ transplants

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***Abstract*— Detection of tissue damage and monitoring organ health is very crucial during organ transplants and surgeries. Till date there is no report on a single device that can detect oxygen and pH levels simultaneously. Monitoring such biomarkers can accurately indicate the health of the organ during transplants. This paper focuses on the development of a single sensor device that can reversibly and simultaneously respond to changes in pH and oxygen levels. The proposed optical sensor integrates both pH- and oxygen-sensitive probes, and is optimized to achieve minimal cross-sensitivity during simultaneous measurements. The paper also initiates the development of optical filters that can segregate the fluorescence associated with oxygen and pH changes. The two distinct fluorescence signals can then be detected and processed to accurately predict organ health. This advancement would enable accurate detection of chemical correlates of organ or tissue damage, thereby improving efficacy and monitoring during organ transplants.**

Keywords*— *Organ health monitoring, tissue damage, pH sensing, oxygen sensing, Optical filter

I. INTRODUCTION

Organ transplants find significant attention due to several chronic and genetic diseases. An organ that is extracted from the donor must be medically treated and tested before it can be implanted into a patient. These organs may be healthy, damaged or partially-ill, which is difficult to differentiate. Due to lack of information on organ health, many of the organs get discarded. In addition, there is always a shortage in supply of organs compared to the demand. Therefore, it is crucial to monitor organ health and treat the organs of suboptimal function with anti-rejection therapies to restore their functionalities.

The key chemical correlates of organ or tissue damage are pH, oxygen tension, lactate and carbon di-oxide. When a portion of organ or tissue is damaged, there is insufficient supply of blood to those regions, leading to a pathological condition called ischaemia. The lack of blood flow ensures shortage of oxygen in those tissues. Mitochondria, the power house of the cell, requires oxygen to generate energy, which is restricted due to tissue/organ damage. This condition enforces anaerobic glycolysis of the cells. During this phase, lactic acid is produced, which further breaks down into lactate and hydrogen ion. The hydrogen concentration reduces the intracellular pH of the tissue. Moreover, the lack of blood flow allows accumulation of the excreted carbon di-oxide from the cells. Hence, an increase in carbon di-oxide and lactate along with decrease in pH and oxygen tension signifies organ damage.

Here, we mainly focus on the changes in oxygen and pH levels for monitoring organ health. There are several reports that proposes the development of pH and oxygen sensors based on fluorescent particles [1-3]. A similar approach is undertaken in this research to fabricate a bi-functional sensor. The metal or organic complexes are suspended into a variety of matrices for fabrication of pH and oxygen sensors. Silica gels [4], silicone rubbers [5-8], polymers [9-12] and sol-gels [13-19] are common matrices to bind the particles. A single bi-functional sensor that comprises both the pH and oxygen sensitive probes (Figure 1), optimized to minimize cross-sensitivity during simultaneous measurements of pH and oxygen, is presented here for the first time. Both the pH and oxygen sensitive probes are encapsulated in a hydrogel matrix. An initial design of two Fabry-Perot filter is proposed to separate out the oxygen and pH fluorescence for further processing. Pyranine loaded in microbeads are used as pH probes, whereas ruthenium bathophenanthroline are used as oxygen sensitive elements [20]. Both the sensitive probes are mixed at specific ratio to eliminate cross-sensitivity during measurements. Figure 1(a) represents the microscopic image and 1(b) depicts the SEM image of the bifunctional sensor matrix.

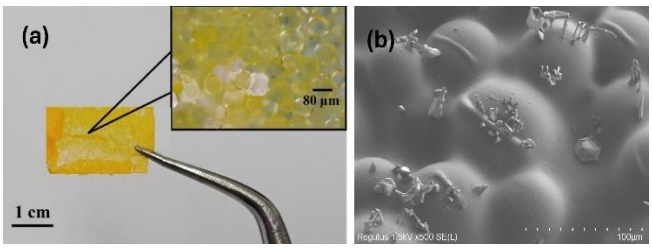


Figure 1:(a) Optical microscopic and (b) SEM image of the sensor comprising of oxygen-sensitive ruthenium and pH-sensitive microbeads

II. EXPERIMENTAL METHODS

Fabrication of a single bi-functional sensor with both pH- and oxygen-sensitive probes was targeted (Figure 2). Initially, 10 g of AmberChrom microbeads were soaked in 10 ml of demineralized (DM) water. 35 mg of 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) was dissolved in 100 ml of DM water and the microbead solution was added to it. The resulting solution was stirred for 15 hours at 400 rpm. The microbeads with HPTS were collected through filtration. 200 mg of the ruthenium complex was dissolved in 5 ml solution of methanol and toluene (4:1). The solution was stirred for 1 hour at 100 rpm to obtain Ru40 solution. The hydrogel matrix was prepared

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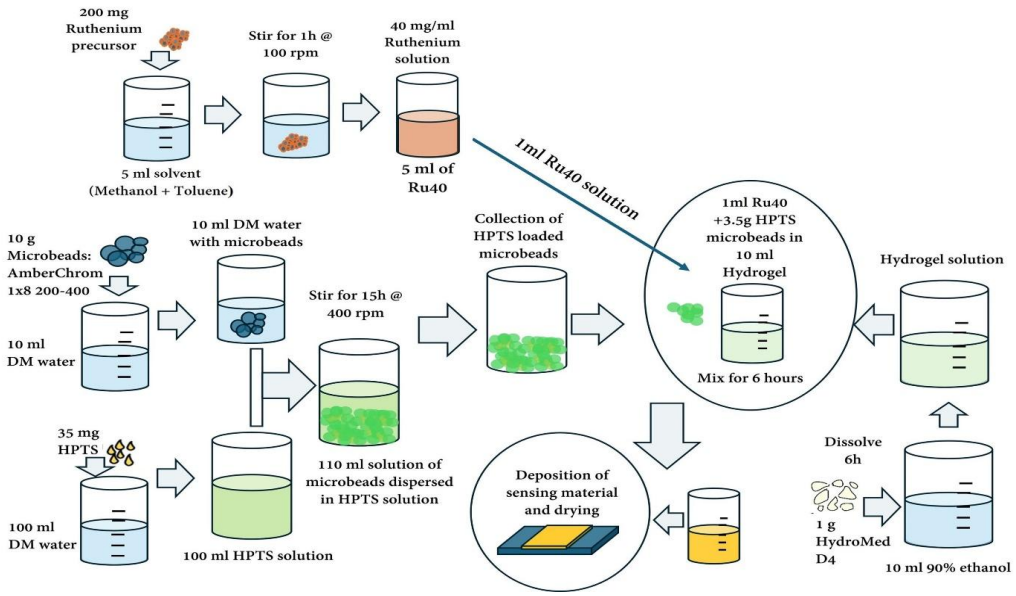


Figure 2: Fabrication steps for developing the bi-functional pH- and oxygen-sensitive hydrogel-based sensor substrate.

by dissolving 1 g of HydroMed D4 precursor to 10 ml of 90% ethanol. The solution was stirred for about 6 hours to get a uniform hydrogel solution. 3.5 g of the HPTS-functionalized microbeads along with 1 ml of Ru40 was added to 10 ml of hydrogel solution. The mixture was stirred for 6 hours to get a well-dispersed mixture of ruthenium and microbeads in the hydrogel. All stirrings were performed at room temperature. The solution was finally drop-casted over a glass slide, followed by drying at room temperature to prepare the bi-functional sensing layer. Concentration of the ruthenium complex was optimized keeping the HPTS concentration fixed. For other concentrations of ruthenium, *i.e.,* 5 mg/ml, 10 mg/ml, 25 mg/ml and 50 mg/ml, severe cross-sensitivity issues were observed. This means that, while measuring the pH of the solution at fixed oxygen levels, the oxygen-sensitive peak (600 nm) undergoes intensity variations. Conversely, while measuring the oxygen levels in the solution at a fixed pH value, the pH-sensitive peak (520 nm) undergoes severe variations in intensity. The effect of cross-sensitivity is highly minimized at the optimized ruthenium concentration of 40 mg/ml in the hydrogel precursor. The effective optimized ratio of ruthenium to HPTS in the sensor is 1:88 by weight ($HPTS_{88}Ru$).

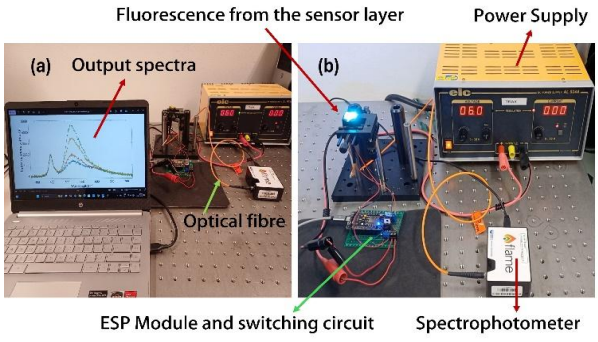


Figure 3: The single-sensor setup for simultaneous optical monitoring of both pH and oxygen changes in tissues.

The hydrogel-based sensor substrate comprising both pH- and oxygen-sensitive probes was illuminated with a 405 nm LED source, and the reflected light was recorded using a spectrophotometer (Figure 3). An ESP module controlled the switching of the LED, which was further controlled by custom MATLAB® scripts.

The sensor was tested for oxygen at a wavelength of 600 nm and for pH at 520 nm, respectively. Figure 4 depicts the envisioned application of the device to monitor organ health in real-time. The single-wavelength excitation to achieve dual-wavelength sensitive emission, independently for pH and oxygen, contributes towards higher accuracy in organ health monitoring.

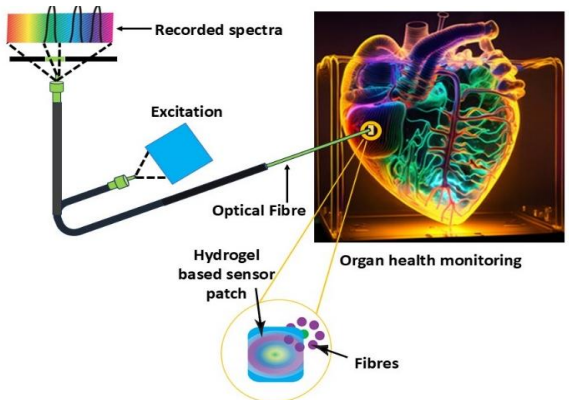


Figure 4: The proposed single-sensor system for monitoring both pH and oxygen concentration as correlates to tissue damage during organ transplants.

III. Results And Discussion

The fabricated sensors were tested initially by illumination with light of both 405 and 450 nm wavelengths. In line with prior literature [21, 22], the pH sensor exhibited good sensitivity for pH variations as indicated by the 520 nm emission peak, and the oxygen sensor demonstrated good

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sensitivity towards oxygen changes at the 600 nm emission peak. The oxygen and pH sensors exhibited better sensitivity when exposed to 405 nm wavelength light compared to 450 nm wavelength. Hence, further experiments were conducted with 405 nm illumination.

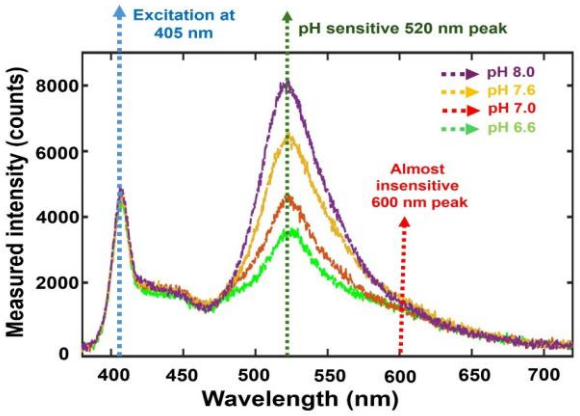


Figure 5: Detection of pH (at fixed 5 mg/ml oxygen level) by $HPTS_{88}Ru$ with insensitive 600 nm peak.

Figure 5 demonstrates the pH sensing characteristics of $HPTS_{88}Ru$. This optimized sensing layer exhibits good pH sensitivity at the 520 nm emission peak and shows almost zero sensitivity at the 600 nm one. The pH sensitivity is calculated to be approximately 75.75% per pH, as per equation (2). The sensor was tested in the pH range of 6.6 to 8, as this is the common pH physiological range for tissues and organs.

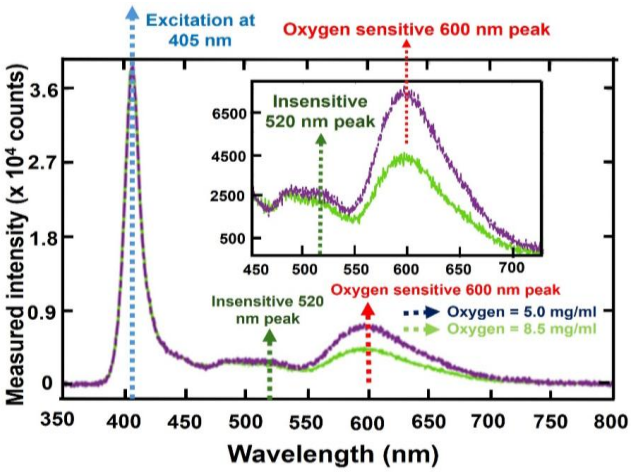


Figure 6: Detection of oxygen levels (at fixed pH value of 7.2) by HPTS88Ru with insensitive 520 nm peak.

Figure 6 illustrates the oxygen sensing characteristics of $HPTS_{88}Ru$. This optimized sensing layer exhibits excellent oxygen sensitivity at 600 nm and demonstrates zero sensitivity at 520 nm. The oxygen sensitivity is calculated to be approximately 20.4% per mg/ml. The insensitive 520 nm peak is of high significance in this study. This result ensures that a single sensor can deliver two independent signal outputs, one for pH and the other for oxygen, respectively, without any evident interference.

A design of two Fabry-Perot filter is proposed to segregate out the two-wavelength specific fluorescence. The pH specific fluorescence at 520 nm is separated out with the help of a seven-layer filter. Three top and bottom layers act as mirrors whereas the middle layer behaves as the resonant cavity. The resonant cavity comprises of 180 nm of SiO_2, whereas the mirror layers alternately contain TiO_2 (45 nm) and SiO_2 (80 nm). The oxygen specific filter was designed with 15 layers having a resonant cavity of 280 nm of SiO_2. The mirror layers contain 50 nm TiO_2 and 90 nm SiO_2 alternately. The simulated transmission spectra at 0° angle of incidence are shown in Figure 7. Further optimization will be carried to have better oxygen filtering characteristics.

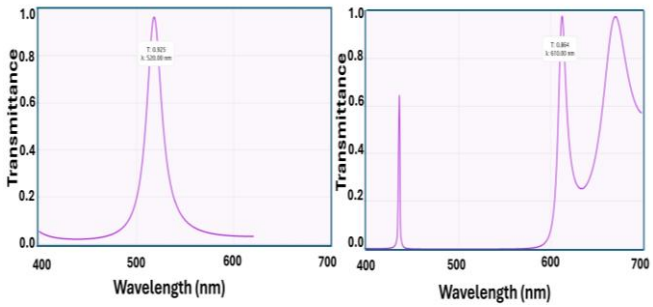


Figure 7: Transmission properties of the simulated pH and oxygen filters

IV. PROGRESS AND FUTURE SCOPE

The sensors were initially tested on a discarded human liver in Erasmus Medical Centre, Netherlands. The sensor signals received were in accordance with the experiments carried out earlier. The oxygen levels altered manually, were detected by the sensor. However, modification on the sensor size and future studies would be carried out soon. Figure 8 depicts the sensor being in contact with the human liver for monitoring the organ biomarkers. The sensors demonstrated good stability for 30 days with 4% and 3.1% standard deviation in the measurements of pH and oxygen respectively.

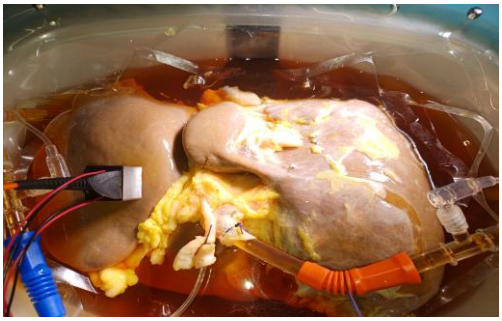


Figure 8: Initial test carried out to detect oxygen levels in human liver

V. CONCLUSION

A single bi-functional optical sensing device was successfully fabricated for application in organ transplants. The sensor can detect changes in the pH and oxygen levels simultaneously, which is reported for the first time. The sensor is optimized meticulously to avoid any cross-sensitivity during pH and oxygen measurements. Two Fabry-Perot filters were simulated for separating the two distinct fluorescence. Moreover, initial tests were carried out on a discarded human liver to determine the sensor efficacy. Hence, the bi-functional sensor has shown great potential for monitoring the health of organs before transplants.

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