DUAL-MODE SENSING PLATFORM FOR DETECTION OF INFLAMMATORY BIOMARKER IN MONITORING ORGAN TRANSPLANT REJECTION

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ABSTRACT
Successful graft outcomes in vascularized composite allograft (VCA) transplantation require close monitoring of immunological reactions and potential ischemic complications. Traditional methods rely on centralized core facilities which cause delays in diagnosis. On the other hand, miniaturized sensors offer promise for on-site diagnosis, however, they encounter reliability issues due to small sample volumes and limited sensitivity and dynamic range. In this work, we demonstrate a surgical suture-based dual-mode microcatheter platform capable of simultaneous electrochemical and localized surface plasmon resonance (LSPR)-based quantification of interleukin 6 (IL-6). Realization of the sensor on a suture ensures flexibility and helps in the miniaturization of the platform, whereas dual modalities offer high precision and enhanced accuracy of detection. The developed microcatheter sensor showed a broader dynamic range (0.1 to 1000 pg/mL IL6) with a limit of detection of 0.076 pg/mL (electrochemical) and 0.10 pg/mL (LSPR) for the detection of IL-6, a key inflammation marker. The dual-mode sensor was evaluated in spiked serum samples with co-interferents. The platform holds the potential for precise on-site monitoring post-transplantation.

KEYWORDS
Smart threads, sutures, dual-mode immunosensor, electrochemical sensor, interleukin-6, localized surface plasmon resonance

INTRODUCTION
Vascularized Composite Allotransplantation (VCA) is a promising option for extensive tissue reconstruction. Monitoring graft outcomes necessitates close monitoring of immunological reactions and ischemic progression [1,2]. Excessive immunosuppression may trigger chronic infections and dangerous side effects, while insufficient immunosuppression can result in acute or chronic rejection, jeopardizing graft function. Monitoring these immunological conditions is critical, particularly with emerging tolerance induction protocols or patient transitions from standard therapies [2,3]. While an adequate estimation of graft progression is pivotal; an inadequate biomarker estimation may lead to significant problems such as early rejection or inflammatory conditions, resulting in significant morbidity and mortality. Continuous, real-time monitoring of graft conditions via biomarker concentration monitoring in the vicinity or plasma is imperative. Nevertheless, despite many efforts in this area, achieving this aim remains an unmet challenge. Current clinical rejection confirmation relies on invasive biopsies, which are suboptimal for routine monitoring due to associated risks. Conventional diagnostic platforms for monitoring these target biomarkers in bodily fluids are limited to centralized or clinical laboratories, making timely diagnosis challenging [4].

Point-of-care (POC) and miniaturized biosensing platforms capable of quantifying biomarker levels such as IL-6 offer considerable promise [5-7]. Usually, these platforms use a single modality i.e. either electrochemical or optical as a detection tool. Nevertheless, with a focus on reducing sample volume, the miniaturization of the sensing platform for biomarker detection faces challenges in reliability and precision, particularly with limited sample volumes for accurate quantification of biomarkers [8]. However, with the complementary blend of sensing modalities, it may be possible to achieve higher sensitivity and selectivity, as well as a wider dynamic detection range. This feat is typically beyond the capability of a single sensing modality. Electrochemical and optical sensing have their unique advantages that can be exploited in a single platform by combining them to achieve high sensitivity and dynamic range while improving the reliability of detection. Towards that goal, we propose a dual-mode sensing platform constructed using sutures (thread) and imbibing them with electrochemical and localized surface plasmon resonance (LSPR) modalities for continuous and accurate measurement of IL6 as an inflammatory biomarker.

Thread as a sensing substrate provides higher flexibility, a high surface-area ratio, and capabilities for non-invasive sensing [9,10]. The use of sutures/threads for sensing [9,11] and drug delivery [10] was investigated by our group; however, they were not used with dual-modality to crosstalk. While previous approaches integrating multifunctionality/ multimodal fiber-based platforms are primarily deployed for studying neural activities [12,13], they pose several challenges and limitations in terms of high dynamic range, long-term precise monitoring, and accurate quantification of biomarker levels.

In this paper, we discuss the scalable fabrication approach for the making of a dual-mode suture-based microcatheter sensing platform for reliable and highly sensitive IL6 sensing. By leveraging the unique characteristics of thread, this platform provides flexibility, easier operation and analysis, while enhancing sensor sensitivity and selectivity. Furthermore, the change in differential pulse voltammetric (DPV) signal for electrochemical readout and plasmon resonance signal for optical readout can be easily acquired using a portable miniaturized wireless potentiostat and portable spectrophotometer. Upon binding the analyte to an immobilized receptor (monoclonal IL6 antibody), the formation of antigen-antibody complex results in a decrease in charge transfer of redox species at the electrode surface. Similarly, antigen-antibody complex results in shifts of resonance wavelength of optical fiber, due to an increase in the size of biomolecule complex. Combined together this platform holds the potential for accurate detection of IL6 in biological medium such as serum or saliva and can be easily deployed for further applications in the biomedical field. To the best of our knowledge, this is the first microcatheter platform with dual modalities (electrochemical and optical) for the detection of IL6.

EXPERIMENTAL METHODS
Design and assembly of dual-mode sensing platform
Figure 1 shows the construction and integration of a microcatheter-based dual-mode sensor for simultaneous electrochemical and LSPR-based monitoring quantification of IL-6 (Fig. 1a-e). The fabrication of the sensing microcatheter platform starts with the insertion of an optical fiber inside a clean and flexible PVC tubing (internal diameter of 0.2 mm). Following this step, three functionalized polysorb™ sutures designated as working, counter, and reference electrodes are uniformly placed at equal distances to

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each other, around optical fiber inserted PVC tubing (Fig. 1a-b). The functionalized sutures were secured in place using biocompatible resin. Subsequently, the whole assembly is enclosed in another PVC tubing with an internal diameter of 0.5 mm (Fig. 1c). All the sensing electrode placements ensure that only the tip of the suture or fiber is available for sensing (Fig. 1d). At the back side of the catheter, all the suture-based electrode was connected to a portable potentiostat and optical fiber was connected to a spectrophotometer.

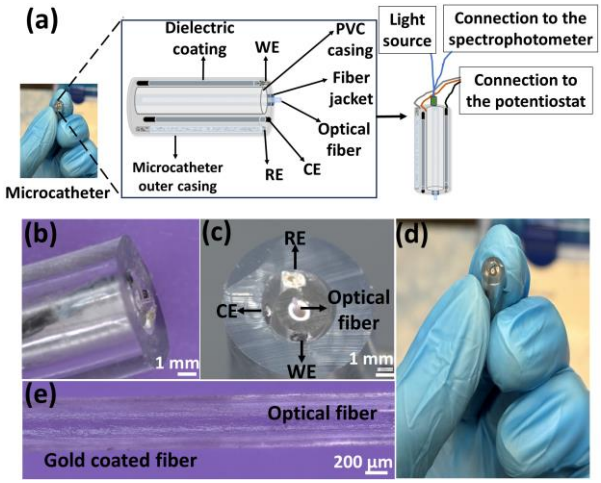

Figure 1: *(a) Microcatheter assembly and internal features; (b) side view; (c) top view; (d) assembled catheter; (e) AuNP coated optical fiber (WE- working; CE- counter; RE- reference electrode.*

Fabrication of electrochemical sensor

Fabrication of the electrochemical sensor (Fig. 2a) starts with coating a conductive-carbon (CC) layer on a polysorb suture to fabricate a working and a counter electrode, and polyvinyl butyrate (PVB) stabilized Ag/AgCl coated suture as reference electrode [9]. Firstly, the CC-suture was cleaned and activated in 0.5 M sulphuric acid (containing 100 mM potassium chloride as a supporting electrolyte) using cyclic voltammetry by sweeping potential from -1.2 to 1.0 V at 100 mV/sec and washed with distilled water (dH_2O). The activated CC-suture underwent Prussian blue (PB) voltammetric electrodeposition, by sweeping potential from -0.30 to 1.0 V at 100 mV/sec. This step is followed by amperometric gold nanoparticles (AuNPs) deposition at 0.40 V for 20 min and washed with dH_2O. This step is followed by thiol-crosslinking chemistry incubating with a freshly made ethanolic solution of 11-mercaptoundecanoic acid (11-MUA, 10 mM) for 8 hrs. Later, a 2 µL of monoclonal IL-6-specific antibody (10 mg/mL) was dropped on the pre-activated 11-MUA/PB/CC-suture in EDC: NHS (3:1 ratio) and incubated overnight at 4°C. The IL6/11-MUA/PB/CC-suture was passivated by incubating in 1.0% bovine serum albumin for 30 min and washed with PBS. A dielectric polymeric layer is coated around the length of the suture to designate only the *tip as a sensing region*. The signal was recorded using a portable and miniaturized potentiostat (Zensor, Thailand).

Fabrication of LSPR sensor

Fabrication of the LSPR sensing optic fiber (Fig. 2b) was realized on a glass fiber, which was first cleaned (in acetone) and activated with a piranha solution (H_2SO_4: H_2O_2, 7:3) for 30 min at 85 °C to generate hydroxyl end-terminal. Then, the fiber was modified by incubating in 3-mercaptopropyltriethoxysilane (2 % v/v) prepared in water and methanol mixture (3:1) at pH 4.5 for 30 min. The functionalized fiber was then incubated in AuNPs solution overnight at 4 °C to deposit AuNPs film. The fiber was further modified by incubating in 11 MUA solution for 30 min and followed by antibody immobilization (mAb-IL6) via EDC: NHS chemistry. Later, the antibody-coated surface was passivated by incubating in 0.10 % BSA solution for 30 min and washed with PBS before use. The signal was recorded using a portable and miniaturized spectrophotometer (Ocean Optics, Flame, USA).

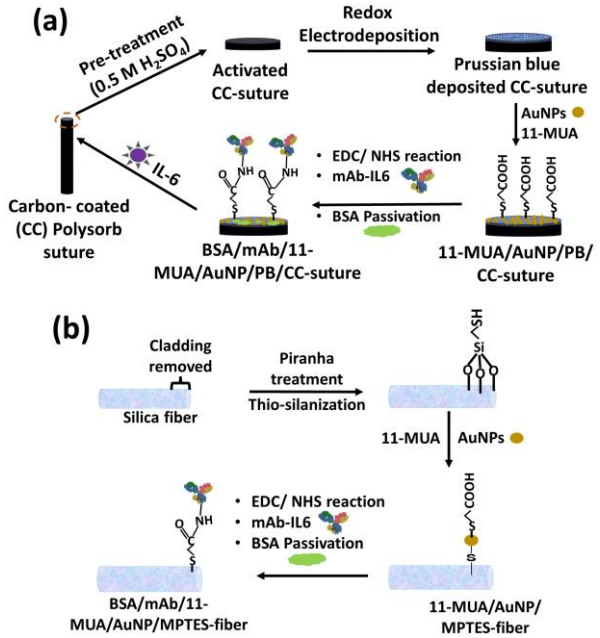

Figure 2*: Step-by-step fabrication of (a) electrochemical; and (b) localized surface plasmon resonance (LSPR) sensor.*

Surface Characterization

The formation and size of AuNPs were determined by the UV absorption spectrum recorded on a UV spectrophotometer (Thermo Fisher, USA) and particle size analyzer (PSA, Thermo Fisher, USA). The surface morphology of the gold (Au) deposited, CC-suture, and PB functionalized suture surface was characterized using scanning electron microscopy (Thermo Fisher, USA). Fig. 1e depicts the optical image of AuNP-coated optical fiber. The synthesized AuNPs used for deposition onto the glass fiber showed

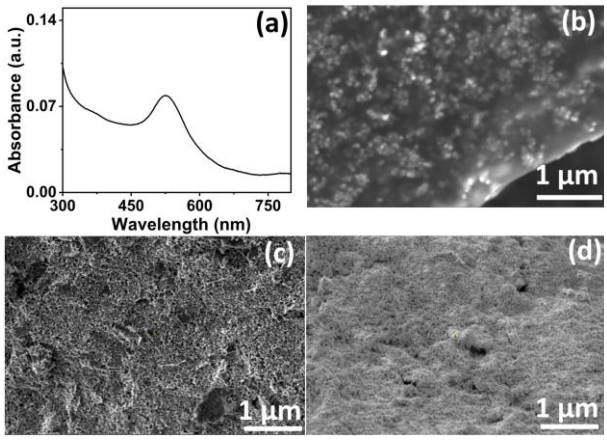

Figure 3:*(a) UV spectrum of gold nanoparticle (AuNPs); SEM images of (b) AuNPs;(c) carbon-coated suture and (c) Prussian blue electrodeposited CC-suture.*

a prominent absorption maximum at 520 nm (Fig. 3a) and an average size of 28 ± 4 nm. From SEM (Fig. 3b), electrodeposited AuNPs exhibited an average size of 30 ± 3 nm, which is similar to calculated from PSA. As depicted in Fig. 3c, the CC-suture showed a rough ridge surface (features of a carbon-coated surface), whereas post electrodeposition of PB, the PB/CC suture had a well-uniform surface with PB coating (Fig. 3d).

WORKING PRINCIPLE'S

The major principle underlines the development of our proposed sensing platform for IL6 detection relies on the universal antigen-antibody complex (IL6-mAb-IL6) formation. Upon IL-6 binding, the IL6-mAb-IL6 complex formation causes a decline in current, which is due to an increase in the charge transfer resistance not allowing redox charge to reach the electrode surface [14]. Similarly, the formation of antigen-antibody complex results in an increase in the resonance wavelength and shifts the spectrum to a higher wavelength (redshifts) [15]. We investigated our hypothesis by measuring the change in the differential pulse voltammetric signal (DPV) for the electrochemical sensor (Fig. 4a) and the change in resonance wavelength for the LSPR sensor (Fig. 4c-d). The sensor performance was verified in both standard phosphate buffer and spiked artificial serum samples.

RESULTS AND DISCUSSION

The analytical performance of the dual-mode sensing platform was evaluated through the detection of various concentrations of IL6 using electrochemical and LSPR measurements. A change in the DPV current for an electrochemical (Fig. 4a) and a change in resonance wavelength for the LSPR (Fig. 4b) sensor. A change in the DPV current signal was observed with an increase in the IL concentration due to hindrance in charge transfer resistance (Fig. 4c). Similarly, a change in resonance wavelength was observed with an increase in IL6 concentration (Fig. 4d). In Fig. 4c, the linear range between 0.10 to 1000 pg/mL of IL6 with $R^2 = 0.9959$ (n=3) for the electrochemical sensor was obtained. The LSPR sensor showed a linear range from 0.10 to 1000 pg/mL of IL6 with $R^2 = 0.9766$ (n=3) Furthermore, this dual-mode sensor limit of detection (LOD) was calculated to be 0.076 pg/mL (for electrochemical) and 0.10 pg/mL (for LSPR) in spiked artificial serum, based on 3S.D./m (where m is the slope of linear regression) Additionally, the platform showed remarkable precision for IL6 monitoring with the highest standard deviation of 4.89% for electrochemical and 5.01% for optical (n=5). We investigated the selectivity of the developed dual-mode sensor for IL6 detection by detecting the change in signal in the presence of co-interferent present in the serum medium. In Fig. 6, none of these interferents significantly affected the change in either the change in DPV current signal (Fig. 5a) or the change in the resonance signal (Fig. 5b), even though all interferents were present at much higher concentrations (10 folds). Consequently, the developed microcatheter dual-mode suture-based sensing platform exhibited high selectivity and negligible cross-reactivity against structural and non-structural potential co-interferents.

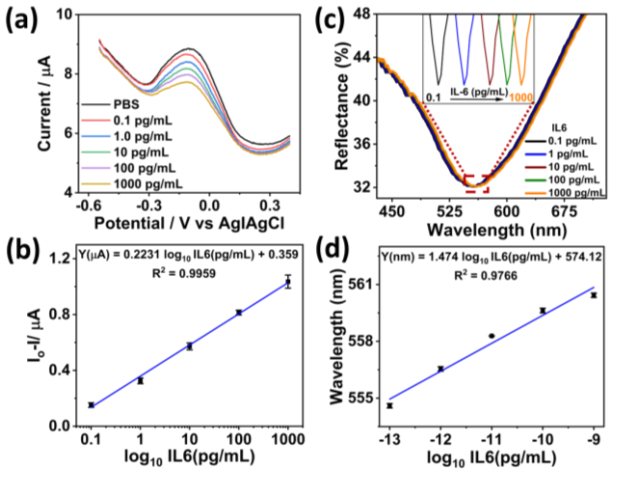

***Figure 4**: (a) DPV curve of electrochemical; (b) Reflection spectra of the LSPR- sensor for different concentrations of IL-6 recorded; (c) Calibration curve shows (c) a change in current, and (d) a change in the wavelength for increasing IL-6 concentration in artificial serum (pH 7.4) (n=3).*

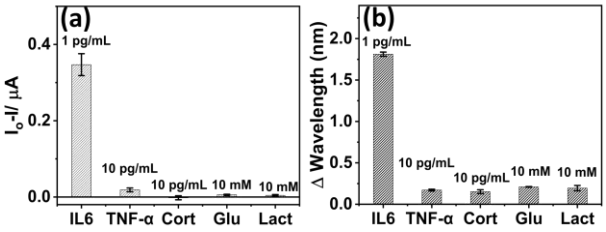

***Figure 5**: Selectivity studies of (a) electrochemical; and (b) LPSR sensor for IL6 detection in the presence of common-interferents in serum sample (n=3). Note: Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL6), cortisol (Cort), glucose (Glu), and lactate (Lact).*

We also validated the reproducibility and repeatability of the platform to ensure its shelf-life and storage. Additionally, the sensor showed remarkable precision for IL6 monitoring with the switching between two different concentrations from 1.0 pg/mL to 100.0 pg/mL in an artificially spiked serum medium (Fig. 6a and 6b). The highest standard deviation was found to be 3.89% (electrochemical) and 4.93% for optical sensors.

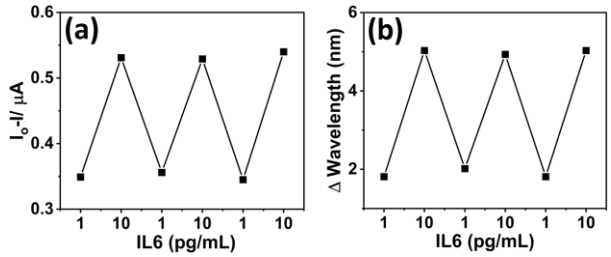

***Figure 6**: Repeatability and stability test of (a) electrochemical; (b) LSPR sensor at 1 pg/mL and 100 pg/mL IL6 spiked in artificial serum medium.*

To validate the developed method for practical applications, we spiked a standard IL6 solution at different concentrations into human serum samples. The blank response was measured in a no-spiked serum medium. The percentage recovery ranged between 97.00 % to 102.0 % with the highest standard deviation of 3.94% and 4.29 % for electrochemical and LSPR sensors, respectively. The obtained results are summarized in Table 1. For practical application, in future studies, the present platform is planned to be deployed for real-time monitoring of IL6 after surgery in animal models. This will be further validated against the gold standard for the same sample to compare the performance. Future studies will use a customized platform with integrated readout, which will allow ease of use and reliable measurements.

Table 1 illustrates the result of the recovery studies for IL6 detection in human control serum samples using a dual-mode microcatheter platform (n = 3).

IL6 added (pg/mL)	EL (IL6 found, pg/mL)	LSPR (IL6 found, pg/mL)	EL (% recovery)	LSPR (% recovery)
0.100	0.099	0.097	99.0	97.0
1.000	1.001	0.998	100.1	99.8
10.00	10.14	10.02	101.4	102.0
100.0	99.65	100.4	99.65	100.4

*EL: Electrochemical Sensor, LSPR: Localized surface plasmon resonance

CONCLUSIONS AND FUTURE WORK

We successfully developed a highly flexible suture-based dual-mode sensing platform in the form of a microcatheter for accurate, reliable, and on-site quantification of IL-6 as an inflammatory biomarker in biological fluids. The platform utilizes dual modality namely electrochemical and localizes surface plasmon resonance or LSPR for sensing, increasing reliability in sensing while improving sensitivity, selectivity, and dynamic range. The performance metric of this dual-mode sensor rivals the best methods employing biopsy and centralized instrumentation for inflammation quantification. The obtained results indicate that this platform provided higher accuracy and precision, and also selectivity for IL6 detection, in the presence of co-interferents. By utilizing this platform, clinicians will be enabled to make more informed decisions regarding immunosuppressive therapy dosing and early intervention strategies, ultimately improving outcomes post-surgeries.

In the future, the work will focus on the deployment of this developed platform for IL-6 monitoring in VCA transplants. Additionally, the device is scalable for the addition of other biomarkers that are important post-VCA surgery such as lactate, proteins, and cytokines.

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