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Versatile PDMS Microfluidic Biosensing Platform for Rapid Detection of Viruses

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Abstract

This project entailed the fabrication of a versatile PDMS (polydimethylsiloxane) microfluidic device to detect viruses and small molecules. Specifically, this device implements surface detection to detect virus secreted glycoproteins and small molecules using a gold biosensor which is integrated into a PDMS mold. The fabrication procedure for this device is innovative, as it significantly simplifies the traditional process of PDMS mold making, and it is versatile in that it can be adapted to fabricate different microfluidic devices that enable several other detection methods. The novel and simplified approach to PDMS mold fabrication developed as a part of this research resulted in 55% reduction in lead time and eliminated the need for a plasma cleaner, decreasing the cost of production by at least $6000. This device can theoretically be used to detect any virus provided that the virus secretes a glycoprotein, and there is a commercially available compound that can be applied to bond that glycoprotein to the gold biosensor. This lab-on-a-chip (LOC) device offers several benefits - versatility in detection of diverse analytes, small sample size requirement, increased efficiency (less time to fabricate the device and quickly scale production to large volumes), lower production cost, device portability in the field, and ease of use for healthcare professionals.

Keywords: Varroa destructor; Apis mellifera; mites; honey bees; essential oils; mist diffusion

1. Introduction

Microfluidics involves the design and study of devices that are used to analyze the properties of fluids, using channels measuring from tens to hundreds of micrometers (Choi, et al., 2013). Microfluidics is not only revolutionizing biological approaches for DNA and enzymatic analysis, but also clinical pathology. Clinical pathology is concerned with the diagnosis of diseases, as well as the detection of toxins or pathogens in the human body.

This project has major applications in clinical pathology, as it introduces a simpler, time-efficient, and cost-efficient procedure to fabricate microfluidic biosensing devices for the rapid detection of viruses and small molecules.

2. Research Questions

A microfluidic chip is a device that enables a tiny amount of liquid to be processed or visualized (Abdelgawad, et al., 2010). Polydimethylsiloxane (PDMS) is commonly used in these devices due to its transparency, unique rheological properties, and

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excellent elasticity that are suitable for constructing microchannels and reaction chambers.

This project aimed to answer three questions:

1. How can a PDMS microfluidic device be fabricated to serve as a versatile biosensing platform to detect viruses and small molecules?
2. How can the PDMS to substrate bonding process be simplified?
    a. Bonding is an essential step during microfluidic device fabrication. After a PDMS mold is made, it must be bonded to a glass slide usually via oxygen plasma bonding. Oxygen plasma bonding requires the use of a plasma cleaner, and the bonding process is error prone. One of the objectives of this project was to simplify this bonding process.
3. How can the process of making PDMS microwells be simplified?
    a. Usually, microwells are made by punching holes into the PDMS mold, but this method requires additional materials and the microwells are harder to replicate.

3. Materials and Methods

This experiment involved a three-part procedure based on prior research and proprietary to Arizona State University (Chan-Park, et al., 2004; Chikkaveeraiah, et al., 2009; Friend and Yeo, 2010; Hardy, et al., 2008; Hongbin, et al., 2009; Natarajan, et al., 2008; Oh, 2008; SadAbadi, et al., 2013; San-Miguel and Lu, 2013; Subramani and Selvaganapathy, 2009; Zaytseya, et al., 2005):

1. Fabrication of gold biosensor device
2. Fabrication of PDMS device (PDMS mold making)
3. Device validation (sensing)

First, simulation and design optimization of the gold biosensor was done using FDTD simulation in LUMERICAL software (Figure 1). The biosensor was then fabricated and involved repetitive sample cleaning, layer depositions, EBL (electron-beam lithography for drawing microstructures), and inspection. The biosensor detects glycoproteins which are found on the surfaces of viruses. Gold (Au) nanoparticles help increase the signal strength during the detection process due to their high surface-to-volume ratio and conductivity. Compatible substances such as streptavidin and biotin are necessary for bonding glycoproteins or small molecules to the biosensor for downstream signaling. Streptavidin is a biotin-binding protein, making streptavidin-biotin interactions useful for bonding molecules in surface detection. Other biotin-binding substances, such as avidin, could also potentially be used as an alternative.

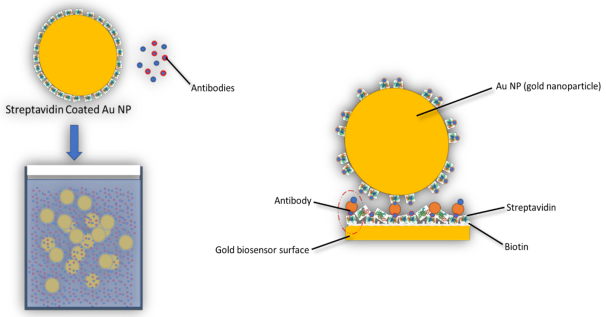

Figure 1: Schematic of the gold NP (nanoparticle)-based biosensor

During the PDMS mold making step, an empty paper bowl was first weighed and PDMS was poured into the bowl. The bowl was weighed again to determine the mass of the PDMS. Using a 1000 µL pipette, a curing agent was added to the bowl (1:10 ratio of curing agent to PDMS) and the mixture was stirred vigorously for 5 minutes. The bowl was placed into a desiccator for ~35 minutes to remove air bubbles from the mixture. A large glass slide was cleaned with acetone, isopropyl alcohol, and DI water and it was placed in an empty petri dish lid. Four 20 mL scintillation vials (lids removed) were obtained (alternatively, glass O-rings may also be used). A new glass slide (76 x 26 x 1mm) was cleaned with acetone, isopropyl alcohol, and DI water. Using a plastic dropper, 2-3 drops of the PDMS was added onto the glass slide. The rim of each vial was lubricated with PDMS and attached to the large glass slide within the petri dish. The vials were bonded to the glass slide by curing the PDMS for 30 minutes at ~90ºC and the degassed PDMS was poured into the petri dish. The PDMS was cured again for 90 minutes at ~90ºC. After the final curing step, the vials were carefully removed to ensure there was no PDMS leakage (Figure 2).

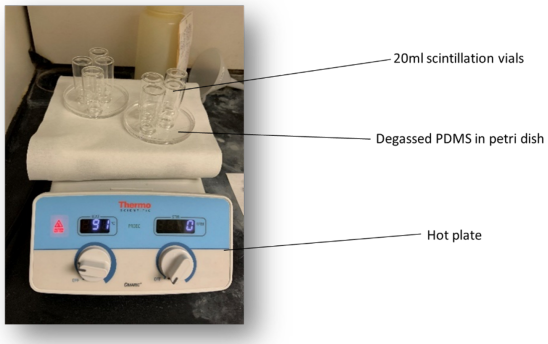

Figure 2: Final curing step for the device

4. Results

The final step was to validate the device capability and sensitivity through a reflection simulation using an Olympus light microscope and spectrometer to determine whether the microstructures within the biosensors were properly functioning (a picture of the final product could not be taken as the laboratory was closed immediately after completion of the experiment due to the ongoing COVID-19 pandemic). Figure 3 shows a schematic of the reflection measurement setup.

The reflection simulation provided quantitative data on each biosensor that was tested to show their respective sensitivities. Reflection measurements for each biosensor were taken in water and thiol to test their detection capability. Water was used as the control substance, while thiolated biomolecules were used as the compatible ligand for testing surface detection. A thiol is classified as any compound containing a sulfhydryl functional group, and it was used in this experiment as it is commonly found in small molecules and proteins. The reflection simulation revealed that the biosensors functioned properly and were able to detect thiolated biomolecules according to the wavelength data. Table 1 displays the quantitative test results.

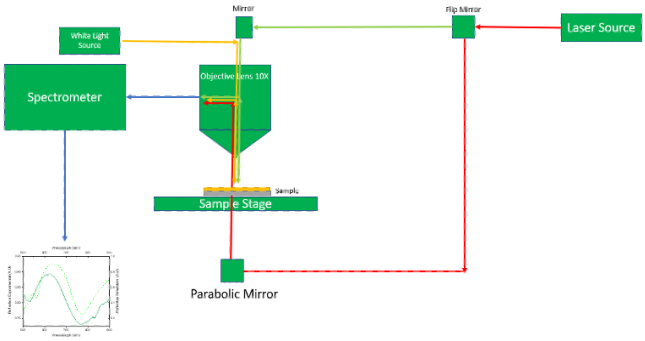

Figure 3. Schematic showing the setup of the experimental spectrum measurement
reflection measurement using the white light source. Measurements were taken using this technique.
alternative reflection measurement technique using laser source and 10x objective lens.
alternative reflection measurement technique using laser source and parabolic mirror.
data from reflection simulation (for all measurement techniques) is retrieved using a spectrometer.

Table 1: Table of reflection wavelengths in nanometers for each biosensor in water (control) and thiol (experimental). These results were received from the reflection measurement analysis.

	Array 1	Array 2	Array 3	Array 4	Array 5	Array 6	Array 7	Array 8		
Water	695.524	722.058	732.123	776.308	693.369	739.514	727.093	778.426		
Thiol	696.627	735.118	737.996	783.265	697.675	742.056	732.45	783.265	**Avg Peak Shift**	**Error**
Peak Shift	1.103	13.06	5.873	6.957	4.306	2.542	5.357	4.839	5.504625	1.26337

Ideally, when measurements are taken in water and thiol, the peak reflection wavelengths for thiol should be higher when compared to water. Furthermore, the wavelength peak shift from water to thiol should be positive for each sample array, indicating a relatively higher reflection wavelength for thiol. As shown in table 1, results from the analysis correctly displayed a positive peak shift for all arrays with an average peak shift of ~5.505 nanometers, thus indicating that the biosensors functioned properly. On a graph, the curve representing the wavelengths when measurements are taken in thiol should be shifted slightly to the right of the curve representing the measurements taken in water. This shift is also indicated numerically in Table 1. Figure 4 provides a visualization of the data shown above.

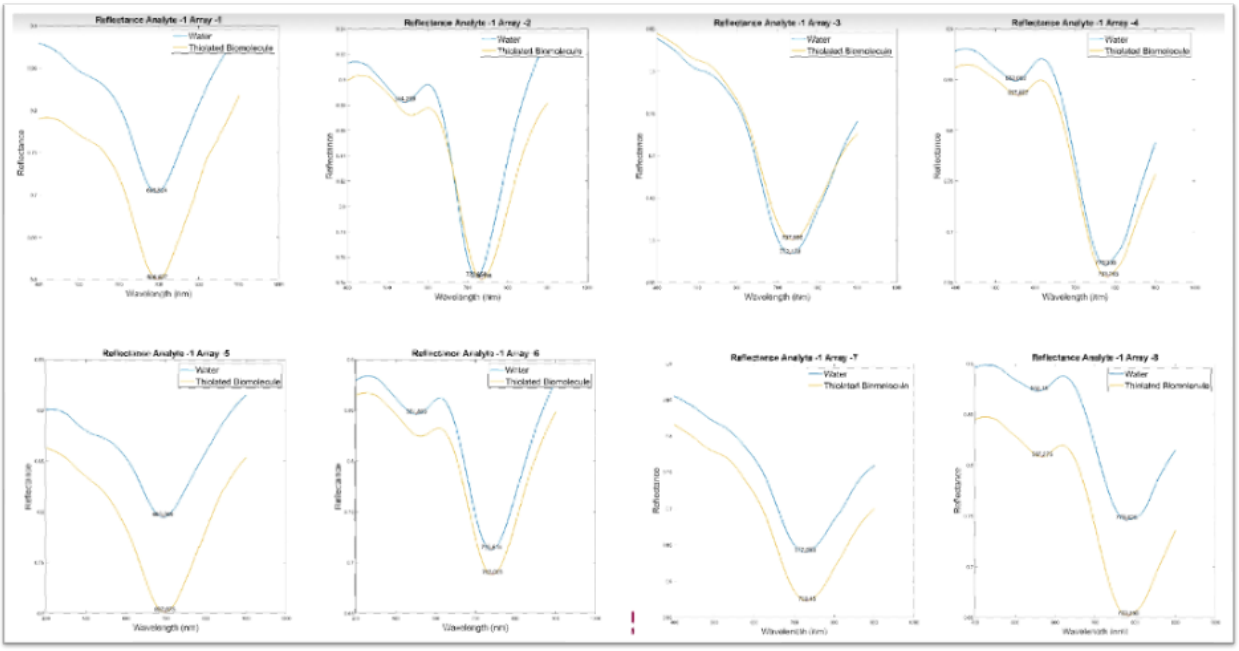

Figure 4: Graphs show the comparison of average peak shifts in wavelength for each biosensor when tested in water (control) and thiol (experimental)

Figure 4 once again shows that all eight of the gold nanoparticle-based biosensors exhibited appropriate reflectance wavelengths for measurements taken in thiol and water. The experimental conditions were identical for all sample arrays, as the sole intention of the reflectance measurement analysis was to validate the biosensors' reliability in detecting compatible biomolecules. As shown, the average peak wavelength for measurements in thiol (represented by the yellow curve) is greater than the average peak wavelength for the measurements in water (represented by the blue curve). These results are consistent for all biosensors that were tested, thus validating their detection capability.

Regarding fabrication process and outcome, the device exhibited no PDMS leakages, and using the newly optimized procedure, two functional PDMS molds were produced within the span of ~5 hours by one experimenter. Traditionally, this process would have taken ~11 hours, indicating that this modified procedure resulted in a 55% increase in efficiency. Based on prior empirical data, this device can detect the Ebola virus, as well as the small molecule cannabidiol (CBD, a natural compound in marijuana).

5. Discussion

This device falls in the category of lab-on-a-chip (LOC) devices that integrate one or several laboratory functions on a single highly miniaturized device. It offers many benefits, such as a simpler and faster fabrication process, higher efficiency in detection, lower cost for production, and small sample size requirement for detecting viruses or small molecules. This device can potentially detect any virus if the virus produces glycoproteins, and there exists a molecule that can indirectly bond the glycoprotein to the gold biosensor. Therefore, this device is theoretically capable of detecting the influenza virus, hepatitis C virus, SARS-CoV, and even the novel COVID-19.

The improvement and production of these microfluidic devices also deliver several marketing and economic benefits. For example, the wide-scale production of these devices can take place with lower

cost requirements: by eliminating the use of a plasma cleaner in the PDMS mold-making process, manufacturers are able to avoid an additional expense of at least $6000. Additionally, the increased versatility associated with this biosensing platform allows it to be easily modified, which could be a potential asset when used in response to outbreaks caused by newly discovered viruses.

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