

Fabrication of Microfluidic Biosensor

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Abstract: Biosensors have been developed and used in a wide variety of ways like environmental monitoring of pollutants, detection of biological components in health care management etc. Fabrication of biosensors is not only a labour intensive process but also a very expensive one. The present study aims at developing a rapid, cost and labour effective, multi analytic microfluidic chip that can be used for the detection of markers responsible for any disease condition. One such marker uric acid, that is responsible for Gout a kidney disorder, was detected on the developed platform. The chip can also facilitate multi-analysis of a single sample on a miniaturized platform using flow through systems. The prepared device contains Sodium alginate beads with the entrapped Urease enzyme which detects the uric acid at Micron level. The entire device is established on small chip sized area (2 cm x 3cm) utilizing less volume of sample and reagents. The device is cost effective and rapid in its action.

Key words: Microfluidics, PDMS, Biosensor, Urease.

I. Introduction

Biosensors have been developed and used in a wide variety of analytical settings including biomedical; health care; environmental monitoring; detection of biological, chemical, and toxic agents; physics; research and others. Several enzyme sensors have been developed using electrochemical, acoustic or optical systems. The importance of enzyme-based micro fluidic biosensors has increased considerably during the past decade due to high selectivity of the bio recognition element and the sensitivity of electrochemical signal transduction. Compared with conventional sensing techniques, distinctive advantages of using MFS for biomedicine include ultra-high sensitivity, higher throughput, *in-situ* monitoring and lower cost through cells, to small multi cellular organisms has explosively grown based on the advancement in micro fluidic system. Such technology also has the benefit of scaling the dimensions that enables a range of fundamental features to accompany system miniaturization such as reduced reagent consumption, high temporal resolution due to rapid mixing, high throughput, enhanced analytical performance, less waste, low unit cost, reduced energy consumption, and reduced dimensions when compared to macro scale techniques. There are various materials that can be used for fabrication one of the commonly used material is PDMS, PDMS device fabrication is one of the easiest methods for the rapid prototyping of micro fluidic devices. It is a flexible elastomeric polymer that is an excellent material for micro fluidic device fabrication (6, 5). Considering the above mentioned facts, it would be interesting and innovative to design new platforms. This research thus aims at addressing detection of markers responsible for any disease condition. The developed chip will help detect uric acid in the blood. This will be a basic platform which will involve immobilization of the enzymes in multi-channels and detection of the conversion products by chemical reagents. Microfluidics presents a very cheap, inexpensive and easiest way detecting the two on the same platform making it a multi enzyme micro fluidic based biosensor. Different technologies have been developed in recent years to produce multi analyte sensors (5). The development of large-scale biosensor arrays composed of highly miniaturized signal transducer elements, for example, enables the real-time parallel monitoring of species and is an important driving force in biosensor research. Said this, determination of analytes has been a great challenge.

II. Materials And Methods

1. Extraction of Urease enzyme and its activity.

Materials: 24 hours old grown culture of Urease producing organism (*Staphylococcus aureus* isolated from urinal soil sample) in Urease broth, 50% Acetone.

Method: For extracellular enzyme, 10 ml of the culture was taken into a sterile centrifuge tube and allowed to spin at 4°C at 5000 rpm for 20 minutes. The pellet was later discarded and the supernatant was taken in various ratios (1:2, 1:4, 1:5) with acetone. The mixture was stored in stationary conditions overnight at 4°C. The pellet was separated out by centrifugation at 5000 rpm for 20 minutes at 4°C the supernatant was discarded. To the pellet 10% of glycerol was added to solubilise the proteins and the solution was used for enzyme assay. (14)

2. Immobilization of Urease and Bead assay.

Materials: 2% sodium alginate, 4% Calcium chloride chilled, purified enzyme (1:2), (1:5), (1:10).

Methods: For (1:2) 15 μ l, for (1:5) 6 μ l and for (1:10) 3 μ l of the purified enzyme was added into 1 ml of the 2% Sodium alginate solution each and then using a syringe of 10 ml beads of 1.42 mm³ were prepared in chilled 4% Calcium chloride. The total amount of beads formed from each of the concentration was used for the assay.

Urease Bead assay:

Materials - Urea 10Mm, 0.05 M Tris HCl (pH 7), Enzyme, 1.5 M Trichloroacetic acid, Deionised water, Nessler's reagent were used, Urease immobilized beads.

Method: The enzyme activity of the purified Urease enzyme was detected by adding the following reagents in three different concentrations (1:2) (1:5) and (1:10) The reaction was stopped by adding 1.5M TCA. Nessler's reagent was added and tubes. Absorbance was noted at 405 nm. (Kayastha *et al.*, 1995) (Soares J.C 2009)

3. Fabrication of the micro fluidic chip

Materials: Sylgard silicone elastomer (Polydimethyl siloxane PDMS), glass slide (length-six cm and breadth two cm) PVC sheet (length- three cm and breadth two and a half cm), tygon tubing (0.17 mm³), cork borer (1 cm), Agarose 1%, steel wire (1mm³ diameter), aluminium foil, transparency sheet. Oxygen Plasma oven.

Methods: PDMS was prepared by mixing 1:10 ratio (curing agent to base) and centrifuging at 5000rpm for 5 minutes to get rid of the gas bubbles. 1gm Agarose powder was mixed with 99 ml distilled water and was boiled in the microwave for 2-3 minutes till a clear solution was obtained. The agarose solution was poured into a petriplate and was allowed to solidify; accordingly wells were punched using a cork borer of height 2cm to prepare the well chamber. Micro dimensional steel wire was inserted through the four cylindrical assembly of the agarose and the entire assembly was placed over a clean and dry glass slide and also on PVC sheet that was surrounded by a transparency trough. The degassed PDMS was poured over the assembly of agarose with the steel wire and was placed into the hot air over at 40°C for 4 hours for partial curing. The cylindrical agarose well along with the steel wire was removed using a forcep to obtain clear and hollow wells connected with a channel. The PDMS layer was sandwiched using another slide on top with 2 circular wells of diameter 1 cm also at a distance of 1 cm from each other and sealed using oxygen plasma for 5 minutes. Tygon tubing embedded in a PDMS layer was attached to the assembly. The entire set up was allowed to remain for 48 hours before use for studying the flow of the fluids. (5,6)

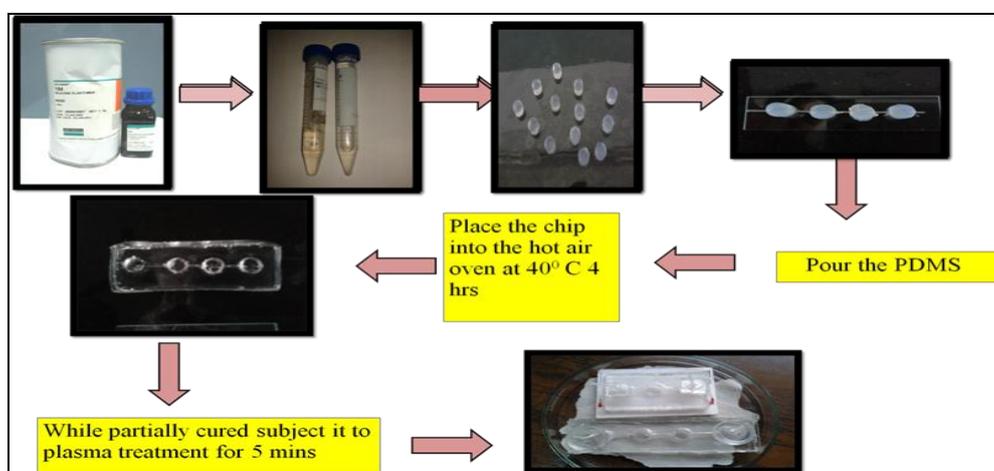


Fig 1 Fabrication of micro fluidic chip.

III. Results And Discussion:

New materials and methods for fabrication micro fluidic systems are needed because etching in silicon and glass is too expensive and time consuming. Some important issues to consider while fabricating are the speed at which the designs can be reduced to working devices and evaluated, design parameters channel size geometry and the availability for required components such as separation, detection or injection.

The Urease enzyme for the study was extracted from the culture of *Staphylococcus aureus*, isolated from urinal soil sample which was further purified using dialysis and acetone precipitation. The enzyme activity was studied using Nessler's reagent for various concentrations i.e. (1:2, 1:4, 1:5) for 24 and 72 hours respectively and the graph (fig 2) represents that the activity of the enzyme increases with the time interval.

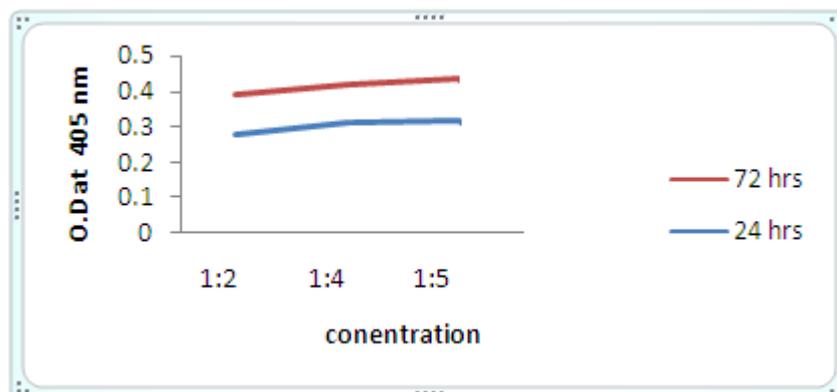


Fig 2. Concentration Vs Various time intervals (hours) for *Staphylococcus* spp

The sodium alginate beads were of diameter 1.42 mm (fig3) with each bead containing 1.2 µl of the purified Urease enzyme. The assay of the Urease immobilized beads was performed for the various concentration and the graph (fig 4) represented 1:2 showing higher activity with distinctive colour change than the other concentrations 1:2 and 1:5.



Fig 3. Measurement of the bead

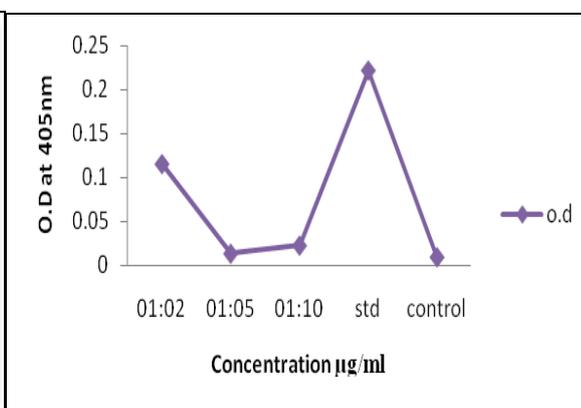


Fig 4. Graph for the immobilised beads

The designed chip had distinct wells of dimensions 7.65mm³ in diameter that enabled to hold 15-20 beads of 1.42 mm³ in diameter with 1.2 micro litre of enzyme that allowed the detection of the compound uric acid in the sample. fig 5 The use of agarose and metal wire to make the wells along with the connected channels paved the way to allow the detection of two analytes at the same time with enough concentration of the enzyme to make the reaction prominent and clearly visible along with an inlet and outlet to allow the entry and exit of the analytes to be tested Also the major advantage of this chip is that it can allow detection of two different compounds at the same time on a single platform. The chip was tested for leakage and was found to be leak proof due to irreversible sealing by exposure to oxygen plasma, Oxygen plasma treatment also led to conversion of the hydrophobic surfaces to hydrophilic making its use versatile as an inert polymer.

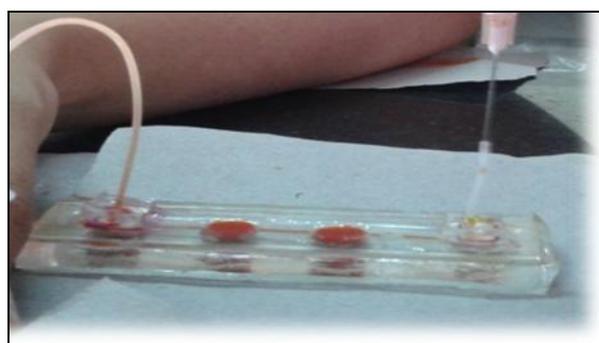


Fig 5 Working micro fluidic chip.

IV. Summary And Conclusion

The studies presented in this paper have demonstrated the successful development of micro fluidic based platform. The material used to prepare the chip PDMS (Polydimethyl silaxone) is an inert polymer and is nearly ubiquitous in micro fluidic devices, being easy to work with, economical, and transparent that aid in the clear visualization of the reaction taking place and thus making detection easy. As there is a lot of progress in the medical diagnostics field to determine the various markers responsible for any disease condition, the prepared chip can be of great importance. This can be employed to determine the uric acid levels in the blood and at the same time also the detection of various other hazardous compounds. The designed chip platform is not only limited to the medical field but can also be used for the detection of industrial contaminants or pollutants of the industrial fields responsible for various types of pollution, contaminants or toxins in the food industry and environment can also be detected. It is based on the principle of bio sensing where in the sensing element can be immobilized into the two wells and the analyte can be allowed to pass through allowing the reaction to take place. The prepared micro fluidic chip is easy to handle portable and cost effective minimising the use of expensive reagents and materials and would be capable of simultaneous detection of any two markers for multiple analysis.

V. Future Prospects

The developed microfluidic chip is a qualitative platform which can be further developed into a quantitative platform by using imaging softwares like Fiji and Image J. The advantage of this platform is that more than 2 analytes can also be analyzed. Also the Lab- on-chip technology can be extrapolated to Lab-on-paper technology making it more cost effective, economical and user friendly.

Acknowledgement

We would like to acknowledge Dr Rajdip Bandopadhaya, Department of Nanotechnology and Colloidal, IIT-Bombay for providing us with the Oxygen plasma oven for this research work

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