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Nano-structured arrays for multiplex analyses and Lab-on-a-Chip applications

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ABSTRACT

Nanospheres lithographic (NSL) method has been used to fabricate nano-structured arrays (NAs) of hexagonally close-packed gold (Au) using polystyrene beads [PS, diameter ~300 nm] as mask. The developed NA was incorporated with a customized and cheap microfluidics system to demonstrate its applicability as an alternative easy and efficient platform for multiplex analysis and Lab-on-a-Chip applications. The chip functionality was demonstrated with horseradish peroxidase (HRP) and anti-HRP antibody as model for recognition system. The enzyme-linked immunosorbent assay (ELISA) performed on fabricated protein biochip had a detection limit 100 pg/mL for HRP. The antibody chip was also checked for the shelf-life and it was found that these chips could be stored for 50 days when stored at 4 °C without any significant loss of activity. Therefore, NAs based protein biochip with the correct microfluidics could find huge potential application in diagnostics and biosensing technology.

1. Introduction

An orderly arrangement of nanomaterials on the surface and their size controls the performance of the system depending on the application [1,2]. In this context, the fabrication of periodically arranged nano-structured arrays (NAs) of different materials is crucial and ability of generating desired functionality has been of considerable interest [1–4]. Nanosphere lithography (NSL), among the various techniques such as nano-imprinting lithography [5], hole mask colloidal lithography [6] and electron beam and focused-ion beam lithography [7], has been used to fabricate patterns with excellent size, shape, and spacing control. In addition, NSL is a versatile, high-throughput, and cost-effective technique.

NAs of desired material could be fabricated with NSL technique, wherein primary step is an appropriate choice of deposition mask that governs the shape and order of the developed NAs. Polystyrene (PS) bead-based deposition masks are commonly used for NAs development in NSL [1–3]. Therefore, a deposition mask can be developed by employing a coating of PS beads of appropriate size onto the desired solid support viz. glass and plastics. Further, spatially arranged patterns of the deposited material can be obtained by removing the polystyrene mask [1]. Depending on the nature of the deposited material these NAs may be employed for optical, electrical, magnetic and molecular properties, have got applications in sensors [8–10], optical and opto-electronic [3,11], photovoltaic devices [12] along with magnetic data storage memory component designing [13,14]. In spite of mentioned significant applications, the application of NSL based NAs as effective electroactive surfaces for biosensor development is not yet well explored. The current generation of biosensor, there is considerable scope for fabricate nano-structured supported platform to achieve high sensitivity, ultra low detection limit, multiplexing capability, low cost, portability and easy operation [15,16]. Among these properties, multiplex application of protein detection is urgently required to detect ultra low concentration of proteins with high sensitivity in clinical diagnosis for human healthcare [17]. Some of these challenges could be achieved using NSL based efficient highly electro-active and sensitive platforms that can either be used as fabricated or in conjunction with microfluidics channel systems for the development of biosensors. In addition, due to the two dimensional electro-actively ordered array formation and ability to oriented immobilization of biomolecules (enzyme, DNA, antibody, microbial cells), these may also be employed for the development of cheap alternative microarray-based immunosensor to perform multiplexed diagnostics along with high sensitivity.

There are several diagnostics approaches such as, microtiter plate-based assays, and microarrays. These approaches have certain associated drawbacks such as cumbersome antibody immobilization procedures and antibody functionality related issues.

In this manuscript we are addressing the problems associated with the pre-existing approaches by employing NAs with a simple model of microfluidics system to demonstrate the use of NAs as an

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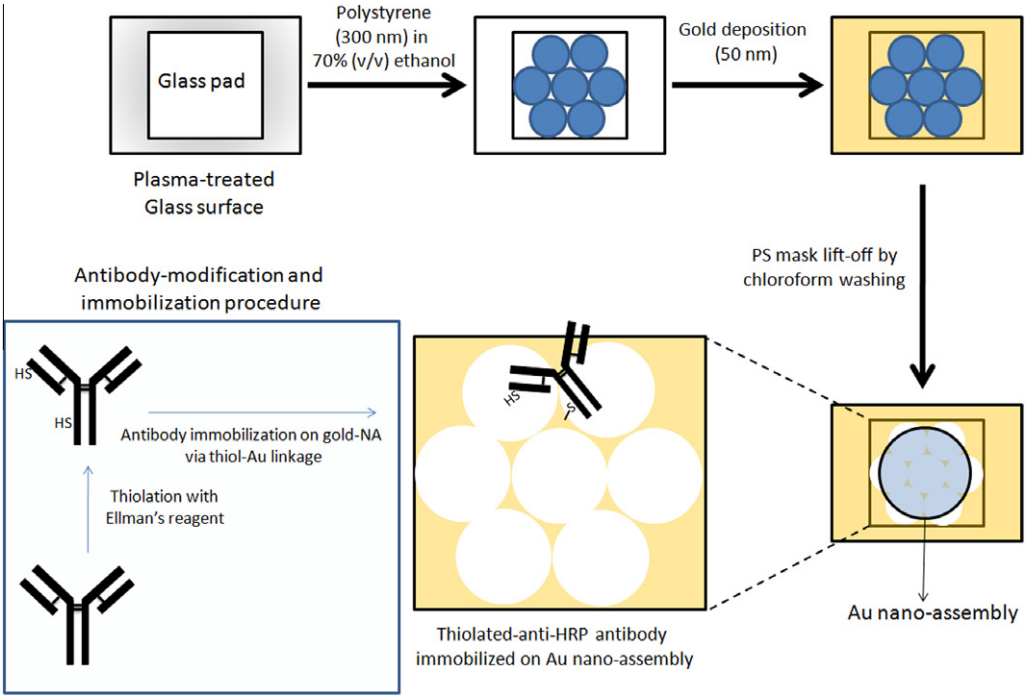

Fig. 1A. Schematic representation of the methodology for nano array (NA) development using nano sphere lithography (NSL). Antibody activation by thiolating them using Ellman's reagent and subsequently their immobilization on the NAs is also depicted.

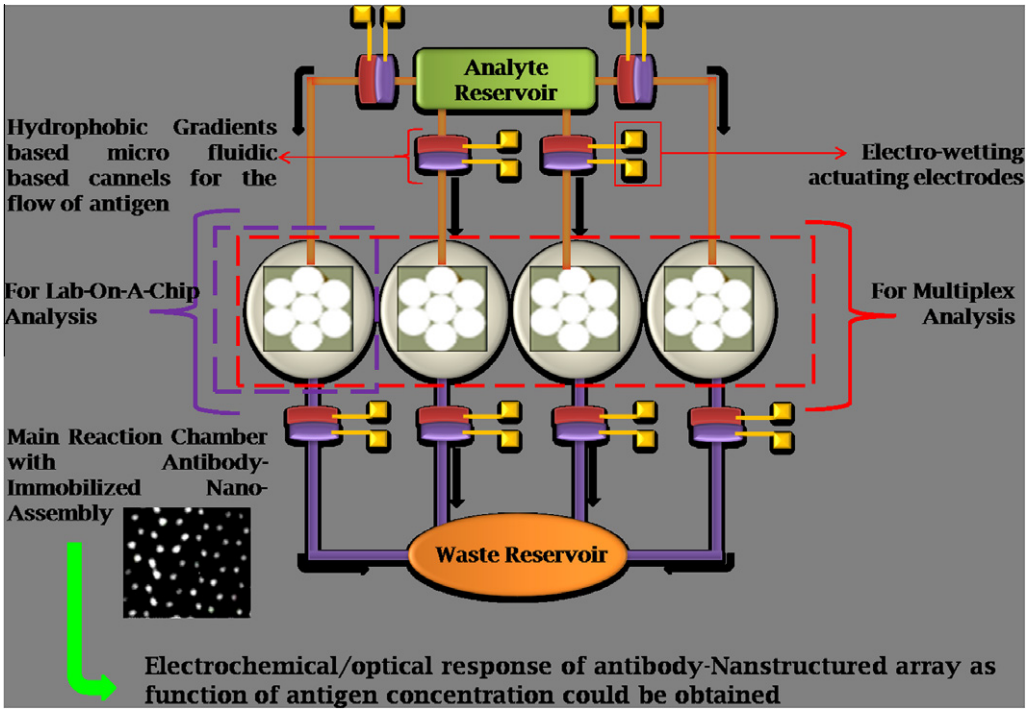

Fig. 1B. The set-up of micro-injection-based microfluidics system employed in conjunction with the developed NA. The developed system could be employed for Lab-on-a-Chip and multiplex analyses.

alternative platform for the development of a Lab-on-a-Chip prototype. We have employed NSL technique to fabricate hexagonally packed nano-structured arrays of Au by using polystyrene bead as a mask. The immunoassay application was demonstrated on the developed NAs by immobilizing anti-horseradish peroxidase (HRP) antibody and performing a concentration dependent colorimetric immunoassay for the recognition of HRP. However, further improvements in the design of microfluidics system must be performed accordingly, for incorporating this platform to develop immunoassays.