Colloids and Surfaces B: Biointerfaces 154 (2017) 331–340

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Metal oxide surfaces for enhanced colorimetric response in bioassays

Enock Bonyi[a], Zeenat Kukoyi[a], Oluseyi Daodu[a], Zainab Boone-Kukoyi[a], Sahin Coskun[b], Husnu Emrah Unalan[b], Kadir Aslan[a,*]

[a] Department of Chemistry, Morgan State University, 1700 East Cold Spring Lane, Baltimore, MD 21251, USA
[b] Department of Metallurgical and Materials Engineering, Middle East Technical University, Ankara 06800, Turkey

ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 10 March 2017
Accepted 14 March 2017
Available online 18 March 2017

Keywords:
PMMA
Silver nanowires
Bioassays
Hybrid platforms
Microwave
Silver island films

ABSTRACT

Physical stability of metal nanoparticle films on planar surfaces can be increased by employing surface modification techniques and/or type of metal nanoparticles. Subsequently, the enzymatic response of colorimetric bioassays can be increased for improved dynamic range for the detection of biomolecules. Using a model bioassay b-BSA, three planar platforms (1) poly (methyl methacrylate) (PMMA) with silver thin films (STFs), (2) silver nanowires (Ag NWs) on paper and (3) indium tin oxide (ITO) on polyethylene terephthalate (PET) were evaluated to investigate the extent of increase in the colorimetric signal. Bioassays for b-BSA and Ki-67 antigen (a real-life bioassay) in buffer were performed using microwave heating (total assay time is 25–30 min) and at room temperature (a control experiment, total assay time is 3 h). Model bioassays showed that STFs were removed from the surface during washing steps and the extent of ITO remained unchanged. The lowest level of detection (LLOD) for b-BSA bioassays were: 10^{-10} M for 10 nm STFs on PMMA and Ag NWs on paper and 10^{-11} M for ITO. Bioassays for Ki-67 antigen yielded a LLOD of $<10^{-9}$ M on ITO platforms, while STFs platforms were deemed unusable due to significant loss of STFs from the surfaces.

1. Introduction

Solid planar surfaces are widely employed in the field of biosensors. Solid planar surfaces, such as ceramics [1], paper [2,3], glass [4] and plastic [5] offer the advantage of facile adsorption/washing steps for the bioassay components. In biosensing applications, plastic, glass, and paper are used predominantly as base support materials due to their practical characteristics (i.e., optical transparency within the visible range: paper is not optically transparent), light weight, manufacturing ease, cost-effectiveness and availability [6]. On the other hand, several drawbacks still exist, mainly the lack of availability of active surface area for the attachment of target molecules of interest, thereby limiting the extent of analyte detection. Subsequently, bioassays performed on traditional planar surfaces suffer from low sensitivity (lowest concentration of analyte detectable).

To address the issue of lower assay sensitivity, current efforts are focused on the optimization of the surface properties of the planar surfaces without compromising the integrity of bulk [7–11]. In this regard, the retention capacity of the surfaces can be increased by the introduction of metal nanoparticles on the surface of platforms by either physical or by chemical adsorption methods [12–14]. For instance, Abel et al. demonstrated a ~4-fold increase in the colorimetric response signal on glass surfaces deposited with plasmonic thin films compared to the blank non-modified glass substrates, a clear indication of retained complexes [9]. Subsequently, Abel et. al., have established that high-throughput screening (HTS) microplates engineered with silver island films (SIFs) exhibited higher sensitivities compared to the unmodified control experiments [15]. In addition, one can further modify the nanoparticles with self-assembled monolayers (SAMs) of alkanethiols to covalently link the bioassay components to the surface [11,16]. However, the use of metal nanoparticles with the planar platforms presents an additional challenge of loss of the nanoparticle films from the surface, and anchor proteins during washing steps of the bioassays, which compromise the efficacy of the bioassays on these platforms [9,17].

In this work, we investigated the stability of silver thin films (thickness = 1, 5 and 10 nm) on chemically modified PMMA, Ag NWs functionalized paper and a metal oxide (indium tin oxide, ITO) on polyethylene terephthalate (PET) using b-BSA protein assay as a model bioassay carried out at room temperature and using microwave heating. Furthermore, we ascertained whether the surface properties of the platforms were affected during the bioassay steps through optical absorbance spectroscopy and scan-

* Corresponding author.
E-mail address: Kadir.Aslan@morgan.edu (K. Aslan).

http://dx.doi.org/10.1016/j.colsurfb.2017.03.030
0927-7765/

ning electron microscopy. The absorbance values for the colored product of the enzymatic reactions were determined using UV–Vis spectrophotometer, where high absorbance units correlated with higher amounts of the captured biomolecule of interest.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA), streptavidin-conjugated horseradish peroxidase (Strep-HRP), biotinylated bovine serum albumin (b-BSA), phosphate buffered saline (PBS) pellets, *o*-phenylenediamine HCl (OPD), sodium phosphate-citric buffer, hydrogen peroxide (30%), sulfuric acid, lithium aluminum hydride powder (reagent grade 95%), 3-aminopropyltrimethoxysilane (APTES) and protein A were purchased from Sigma-Aldrich Inc. (Milwaukee, WI, USA). Ethanol 190 proof, diethyl ether (reagent grade, ACS) and 2-propanol were purchased from Pharmco AAPER. Antigen Ki-67 recombinant protein, Ki-67 polyclonal antibody (HRP-conjugated) and mouse monoclonal antibody to Ki-67 were purchased from MyBioSource (San Diego, CA, USA) and Abcam (Cambridge, MA, USA), respectively.

PMMA discs (diameter = 5 cm, thickness = 0.2 cm) were purchased from McMaster-Carr (Elmhurst, GA, USA) and 21-well press-to-seal silicone isolators were designed by The Aslan Research Group and were manufactured by Grace Biolabs (Bend, OR, USA). Thermal abuse test chamber with digital temperature controller, heavy duty vacuum pump with exhaust filter, and ITO-coated PET films (height = 0.175 mm, width = 300 mm, length = 1 m, resistivity = 14 Ω/sq), were purchased from MTI Corporation (Richmond, CA, USA).

For the synthesis of Ag NWs, ethylene glycol (EG), polyvinylpyrrolidone (PVP) (MW: 55000), sodium chloride (NaCl) and silver nitrate ($AgNO_3$) were used. All chemicals were purchased from Sigma-Aldrich and used without any purification.

Deionized water with 18.0 mΩ cm resistivity at 25 °C was obtained from Millipore Direct Q UV3 system with a 0.22 μm filter. Copy paper (8.5" × 11") was purchased from Staples (Towson, MD, USA). Scanning Electron Microscope (SEM) with EDX capability (Phenom XL) was purchased from Phenom-World B.V. (Virginia, USA). An EMS 150R S sputter coater and silver targets (diameter = 57 mm) were purchased from Electron Microscopy Sciences (Hatfield, PA, USA). Microwave heating was performed using a commercial 700 W Emerson kitchen microwave. Absorbance was measured using Varian UV–Vis spectrophotometer and plotted using SigmaPlot version 12.5 and water contact angle goniometer was purchased from Kruss Inc (Matthews, NC, USA).

2.2. Methods

2.2.1. Surface modification of PMMA

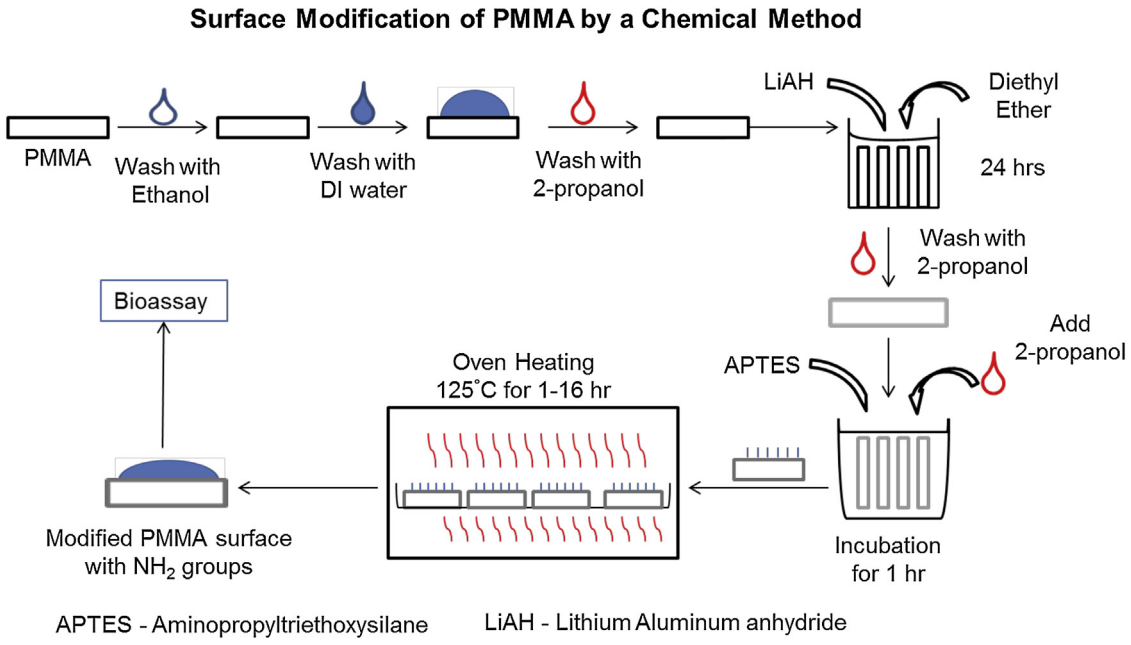
We adopted the chemical modification method for PMMA proposed by Cheng et al. [18] with modifications in the baking time and Scheme 1 gives a summary of surface modification process. The circular PMMA discs were thoroughly cleaned with 2-propanol and rinsed in deionized water and left to air dry. The discs were then immersed in lithium aluminum hydride solution in diethyl ether (16 g of LAH in 100 mL diethyl ether) for 24 h and were kept under constant agitation for chemical reduction to take place. The discs were rinsed in 2-propanol and then immersed into a solution containing APTES in 2-propanol (5% v/v) for 1 h. APTES-modified PMMA discs were then rinsed in 2-propanol by sonication and later cured in a vacuumed thermal abuse test chamber at 125 °C for a range of hours (i.e., 1, 3, 5, 10, and 16 h).

2.2.2. Surface analysis

To determine whether the PMMA discs were modified to have NH_2 groups, water contact angle measurements were performed using water contact angle goniometer. In this regard, 20 μL of deionized water was placed on the chemically modified circular PMMA surfaces and water contact angles recorded. Data obtained were compared with the literature to determine the surfaces that were modified with NH_2 groups. Further, SEM analysis was employed to characterize the surface morphologies of circular PMMA discs before and after the chemical modification.

2.2.3. Deposition of STFs on modified PMMA surfaces

A sputter coater fitted with silver target and a 21-well mask were used to introduce the with thicknesses 1, 5, and 10 nm onto the surfaces of chemically modified PMMA platforms (i.e., with NH_2



Scheme 1. Schematic depiction of procedure for the surface modification of poly(methyl methacrylate) (PMMA) by a chemical method.