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Metal Oxide Surfaces for Enhanced Colorimetric Response in Bioassays

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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 hours). 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.

Graphical abstract

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ITO surfaces offer physically stable platform to enhance colorimetric response of enzymatic assays. D = *o*-phenylenediamine HCl, H_2O_2 = Hydrogen peroxide, HRP = horseradish peroxidase.

Keywords

PMMA; silver nanowires; bioassays; hybrid platforms; microwave; silver thin films

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 metal oxide (ITO) on 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 scanning 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, α -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 indium tin oxide (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 m Ω .cm resistivity at 250 °C was obtained from Millipore Direct Q UV3 system with a 0.22 μm filter. Copy paper (8.5" $\times$ 11") was purchased from Staples (Towson, MD, USA). Scanning Electron Microscope with EDX capability (Phenom XL) was 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 Watts 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 a 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 hours 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 hour. 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 hours).

2.2.2. Surface analysis—To determine whether the PMMA discs were modified to have NH₂ 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 NH₂ 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 large and a 21-well mask was used to introduce the silver thin films with thicknesses 1, 5, and 10 nm onto the surfaces of chemically modified PMMA platforms (i.e., with NH₂ groups). To distinctly delineate the wells, a 21-well silicone isolator was carefully aligned on top of each individual sputtered spot and the platforms were covered with paraffin film and stored in a desiccator until ready for use (Scheme 2).

2.2.4. Preparation of ITO on PET platforms—ITO dots were cut out from the ITO-coated PET films using a 1-hole punch and securely aligned with each of the individual wells of the silicone isolator (Scheme 1). The 21-well silicone isolators with the attached ITO dots were subsequently positioned onto the blank circular PMMA discs.

2.2.5. Deposition of Ag NWs onto paper—The Ag NWs were synthesized using the polyol method as described by Coskun et al. [19]. Following synthesis and purification, Ag NWs were deposited onto paper substrates via the dip-and-dry method. A one-hole punch was used to obtain Ag NW dots which were securely attached to the 21-well silicon isolator and pressed firmly on the circular PMMA discs (Scheme 2).

2.3. Bioassay preparation

2.3.1. Model direct bioassay for b-BSA—We used the procedure described by Mohammed et al. by [20] for colorimetric detection of b-BSA. However, there were modifications to the microwave heating time and different b-BSA concentration ranges

(Scheme 2) were considered. In a typical bioassay, a stock solution of b-BSA (prepared in PBS) at a concentration of 10^{-5} M was prepared. Serial dilutions were then performed to obtain concentrations in the range of 10^{-6} M – 10^{-11} M. A 30 μ L solution of b-BSA of different concentrations (10^{-6} M – 10^{-11} M) was transferred into six different wells on three sets of platforms (i.e., ITO, PMMA modified with NH_2 groups (modified with STFs), and Ag NWs deposited onto paper). The platforms were then either incubated at room temperature for one hour or transferred into the household microwave oven operating at 700 W (power level = 1, duty cycle = 3 sec) for 5 min. The wells were washed using deionized water and left at room temperature to air dry. A 30 μ L 5% (w/v) solution of the blocking agent (BSA) was added to the air-dried wells to prevent nonspecific binding and incubated at room temperature or microwave heated for 5 min. The wells were washed using deionized water and kept out to air dry. A 30 μ L solution of streptavidin conjugated with horseradish peroxidase was added to the wells and incubated for one hour at room temperature or microwave heated for 5 min. The wells were washed with deionized water and left to air dry. A 30 μ L of substrate solution comprised of 4 mg α -phenylenediamine HCl dissolved in 10 mL of sodium phosphate-citric acid buffer, pH = 5 and 4 μ L of hydrogen peroxide was added into the wells and incubated at room temperature or heated in the microwave oven for 5 min. The reaction was stopped by transferring the contents of the wells into HTS wells containing 2 M sulfuric acid and the absorbance of the enzymatic product, diaminophenazine (DAP) was measured at 492 nm using an UV-visible spectrophotometer.

Real-life sandwich bioassay for KI-67 antigen: Scheme 3 summarizes the bioassay steps for Ki-67 antigen. In this regard, mouse monoclonal antibodies (capture antibody) against Ki-67 dilution 1:1000 in a PBS solution was added to wells containing protein A which was incubated overnight and microwave-heated for 5 min. Three consecutive washes followed and the wells were left to dry before adding BSA (1% w/w). This was done to block off the surfaces that unreacted with the capture monoclonal antibodies. After microwave heating the BSA, washing and subsequent drying of the wells, Ki-67 antigen was added. Then, the sample was and microwave-heated after which the antigen was washed off. Once the wells were dry, a secondary antibody; monoclonal mouse antibody against Ki-67 (dilution 1:1000) was added and was again microwave-heated for 5 min. Subsequently, the detection antibody conjugated to HRP (dilution 1:10,000) was added to the wells and microwave heated and wells were washed. Once the wells were dried, the substrate for HRP prepared as outlined in section 2.3.1 was added and the wells were incubated at room temperature for 5 min to develop the color. The enzymatic reaction was stopped by transferring the contents of the wells into HTS wells containing 2 M sulfuric acid and the absorbance of the enzymatic product (DAP) was measured at 492 nm using an UV-visible spectrophotometer. Note: All assay wash steps were performed using PBS (pH = 7.4) and all microwave heating was completed using power level 1 in the microwave settings. Also, the control assay experiments were completed at room temperature with a 1 hour waiting period for each step.

2.4. Data analysis

Each protein concentration was run in triplicates on each of the three platforms and the data analyzed and normalized using SigmaPlot 12.5 software.

3. Results and discussion

3.1. Surface modification of PMMA and paper

Figure 1A shows the contact angles and pictures of a water droplet on chemically modified PMMA platforms. These platforms were silanized and incubated at 125°C for different time periods, i.e., 1, 3, 5, 10, and 16 hours. In the unmodified PMMA surface (no silanization, no baking), a larger water contact angle of 72 ± 2 degrees was observed as compared to the modified PMMA platforms (i.e., water contact angle range 19 ± 2 to 41 ± 10) degrees. It should be noted that the error margin represents standard deviations in water contact angle measurement of five different spots on the modified PMMA surface. The observed changes in the water contact angle provided an evidence for the surface modification of PMMA. Previous reports have shown that the average water contact angle for amine groups are measured to be 33 ± 4 degrees [21]. Subsequently, the PMMA surfaces that were silanized and baked for 1 hour are used in the bioassays described in this work. SEM images of PMMA platforms show that the surface of PMMA platforms appear smooth and roughened before and after chemical surface modification, respectively (Fig. 1B and 1C). Fig. 1D and 1E show SEM images of regular paper and Ag NWs-functionalized paper, respectively. During the collection of SEM images, the electron beam damaged regular paper due to its insulating nature. Paper functionalized with Ag NWs exhibited a dense network of Ag NWs and was not damaged during SEM analysis due to the excellent electrical conductivity of silver [22, 23].

3.2. Colorimetric response from a model bioassay for b-BSA on planar platforms

To evaluate the effect of surface modification of PMMA platforms on bioassay performance, the colorimetric signal from a model bioassay for b-BSA carried out on each of the platforms was measured and compared. Figure 2A (absorbance values for the colored product at 492 nm) and Figure S1 (absorption spectra for the colored product, Supplementary Information) summarize the results of the bioassay carried out on PMMA platforms coated with STFs of thickness 1, 5 and 10 nm. These bioassays were completed at room temperature and using microwave heating. With microwave heating, PMMA platform coated with 10 nm STFs (Figure 1A, upper panel, solid rectangles) showed the highest colorimetric signal output and the lowest level of detection (LLOD) of [b-BSA= 10^{-10} M]. The PMMA platform coated with 1 nm STFs (Figure 1A, upper panel, solid circles) yielded LLOD of [b-BSA= 10^{-9} M] and PMMA platforms with 5 nm STFs produced the lowest signal (Figure 1A, upper panel, inverted solid triangles) and LLOD of [b-BSA= 10^{-7} M]. At room temperature, colorimetric response and LLOD for all the platforms [b-BSA= 10^{-8} M] from bioassays were significantly lower (highest absorbance value was ~ 0.2) as compared to bioassays carried out using microwave heating (highest absorbance value was ~ 0.8).

Figure 2B shows the results of the model bioassay for b-BSA carried out on the blank paper and paper functionalized with Ag NWs both at room temperature and in microwave heating. Colorimetric response from bioassays on paper with Ag NWs (i.e., absorbance = 0.4) was increased as compared to blank paper (absorbance = 0.1) using microwave heating. Bioassay carried out using microwave heating on paper functionalized with Ag NWs and blank paper yielded LLOD of [b-BSA= 10^{-11} M] and [b-BSA= 10^{-9} M], respectively (Figure 2B, upper

panel). No marked variations in the colorimetric response from the bioassays carried out on blank paper and paper functionalized with Ag NWs at room temperature environment was observed. LLOD in both platforms was determined to be [b-BSA= 10^{-10} M] (Figure 2B, lower panel).

Figure 2C shows the results of the model bioassay for b-BSA carried out on the ITO films using microwave heating (Figure 3C, upper panel) and at room temperature (Figure 3C, bottom panel). We observed no significant differences in the colorimetric response for the bioassay under the two experimental conditions, however, LLODs of [b-BSA= 10^{-10} M] and [b-BSA= 10^{-8} M] were observed for bioassays carried out using microwave heating and at room temperature, respectively. [Note: The horizontal lines represent the absorbance values of the control experiments and the total assay time was 15 mins and 3 hours under microwave heating and at room temperature, respectively].

To effectively compare the bioassay performance on planar surfaces, colorimetric responses of the model bioassay from STFs on PMMA, Ag NWs on paper, and ITO and their corresponding control experiments were plotted together. Additionally, the real-color pictures of the planar surfaces before and after the bioassay were provided (Figure S2, Supplementary Information). Colorimetric responses at 492 nm from 10 nm STFs on PMMA was the highest (~ 0.8 for 10^{-6} M) among all three planar surfaces, followed by ITO (>0.4 for 10^{-6} M) and Ag NWs on paper (0.4 for 10^{-6} M), as shown in Figure S2A. Background colorimetric responses (control experiments) were similar in all planar surfaces (varied between ~ 0.05 and 0.1), and were 4-order of magnitude less than the absorbance values observed for various concentrations of b-BSA.

In our previous publications related to the use of silver island films in colorimetric bioassays, we noted that a fraction of the colored product remains on the planar surface and/or that silver island films are removed from the surface during washing steps that result in the removal of enzyme from the surface and loss of colorimetric response [9, 17]. To assess whether the above-mentioned observations still occur on the planar surface studies reported here, the optical absorbance spectra of these surfaces were measured and real-color pictures were visually inspected. Figure S2B clearly shows that the colored product (yellow color) is present after the completion of the bioassay on all surfaces. On the other hand, the color of STFs surface is darker in comparison to the other surfaces. This observation can be attributed to marked differences in the initial thickness of the silver films. Subsequently, the absorption spectra of the planar surfaces before and after the completion of the model bioassay were measured to verify and assess the extent of the colored product present on surfaces. Results are shown in Figure 3 and Figures S3–S4 (Supplementary Information). Real-color pictures of ITO after the bioassays (using microwave heating) show yellow color on the surfaces (Figure 3). However, the absorption spectrum of ITO does not change during the implementation of the model bioassay for b-BSA using microwave heating (and at room temperature). For the sake of brevity, we note only the data for the highest (10^{-6} M) and the lowest concentration (10^{-10} M) of b-BSA. These observations imply that the extent of colored product was significantly less than that in the solution, and does not affect the outcome of the colorimetric response of bioassays carried out on ITO surfaces.

Optical absorption spectra of STFs (1, 5 and 10 nm thick) before and after the completion of a model bioassay using low-power microwave heating and at room temperature are given in Figures S3 and S4, respectively. Both Figures S3 and S4 show a broadening and a blue-shift in the spectra for 1 and 5 nm thick STFs after the completion of the bioassay, and the absorption peak for the colored at 495 nm does not appear on both silvered surfaces.

These observations can be directly attributed to the loss of silver from the surface and a change in the dielectric properties of the silver surface due to the absorption of bioassays components [24, 25]. In contrast, 10 nm STFs display marked changes in the absorption spectra after the completion of the bioassay using microwave heating (Figure S3). The absorption spectra for STFs shows the characteristics of a typical thin film before the bioassay: high absorbance values >600 nm and no sharp surface plasmon resonance (SPR) peak for silver around 420 nm [26]. After the completion of the bioassay, SPR peak for silver between 400–450 nm appears and the absorbance values >600 nm are significantly reduced, which implies that STFs are converted into nanoparticle films due to significant loss of silver from the surface when exposed to microwave heating. It is also important to note that absorbance peak for the colored product at 492 nm is detectable on the silver surface, especially for b-BSA concentrations $>10^{-7}$ M (Figure S3).

Figure S4 reports that the loss of silver from 10 nm STFs surface was significantly less for bioassays carried out at room temperature as compared to bioassays completed using microwave heating. These observations can be explained by the interactions of 10 nm STFs with microwaves, where increased coupling of electromagnetic energy with thick metal films are expected when compared to those at 1 and 5 nm STFs. As 10 nm STFs are repeatedly exposed to microwave heating during the execution of bioassays, an electric charge builds up on the surface that results in cracks in the silver films. Also, silver can be washed away during washing steps of the bioassay. Besides, physical damage to STFs by micropipette tips during washing steps can result in the loss of silver from the surface. At room temperature, 10 nm STFs mostly retain the thin film characteristics and the only source for observed changes in the absorption spectra for STFs is the physical damage as mentioned earlier.

3.3. Colorimetric response of real life bioassay on 10 nm STFs and ITO on PET

After establishing that 10 nm STFs and ITO surfaces could enhance the colorimetric response of a model protein bioassay for b-BSA using microwave heating, we employed these two planar surfaces in a real-life bioassay for a biologically relevant antigen (i.e., Ki-67). Ki-67 is a nuclear protein associated with cellular proliferation and prognostic marker in cancer patients [27]. Ki-67 is present in all stages of cell division; S, G1, G2, and M phases, except in Go [28, 29]. Figures 4 and S5 and S6 (Supplementary information) show the colorimetric response for the Ki-67 antigen concentration range of 10^{-7} – 10^{-12} M using microwave heating and at room temperature. Figure 4 reveals that colorimetric response at 492 nm from Ki-67 bioassay on 10 nm STFs were indistinguishable from the background response for both bioassays carried out using microwave heating and at room temperature.

Colorimetric responses from the Ki-67 bioassay carried out on ITO using microwave heating and at room temperature were clearly distinguishable from the background response. The LLOD was determined to be $[Ki-67 = 10^{-10} \text{ M}]$ in both bioassay conditions. It is important to note that the colorimetric response on ITO using microwave heating was significantly larger than those measured after the completion of the bioassay carried out on the identical ITO surface at room temperature (Figure 4B). The observed increase in the colorimetric response from the bioassays carried out using microwave heating can mainly be attributed to the reduction of non-specific interactions of primary and secondary antibodies and HRP-labeled avidin with the ITO surface. Since each bioassay step in the Ki-67 bioassay is completed within 5 min of microwave heating, the occurrence of specific biological recognition events between the antibodies and antigen are more likely than those of non-specific binding of antibodies to the ITO surface. Mass transfer of antibodies towards the surface is increased due to microwave heating [17, 20], and as the antibodies approach the surface specific binding events are more likely to occur between antibodies and antigens that are located ~4 to 16 nm (thickness of protein A and primary antibody and Ki-67) away from the surface and into the solution. In bioassays carried out at room temperature, each step of the bioassays take 1 hour, and subsequently, the extent of non-specific binding of antibodies to the surface can significantly increase (despite the presence of blocking agents on the surface).

3.4. Evaluation of physical stability of the STFs and ITO on PET during real-life bioassay

To investigate the effect of surface properties of STFs and ITO on the colorimetric response of bioassays as described above, these surfaces were characterized before and after completion of the bioassay. Real-color photographs of ITO after the completion of Ki-67 bioassays using microwave heating and at room temperature show little to no color difference as compared to control. However, they are darker than the ITO surface before their use in the bioassays (Figure 4). Real-color photographs of STFs after the completion of the bioassays show a significant color change on the surface. To investigate the reasons for the above observations, optical absorption spectra of 10 nm STFs and ITO before and after completion of Ki-67 bioassays for 10^{-7} M and 10^{-12} M using microwave heating and at room temperature were measured and are shown in Figure 5. Optical absorbance spectra of STFs after the completion of bioassays using microwave heating and at room temperature (Figure 5A) reveal a significant loss of STFs from the surfaces as evidenced by the decrease in the absorbance values at wavelengths $>600 \text{ nm}$ and the appearance of the SPR peak around 420 nm.

Our earlier observation of indistinguishable colorimetric response at 492 nm from Ki-67 bioassay on 10 nm STFs from the background response can directly be attributed to the loss of silver from the surface. Optical absorbance spectra of ITO after the completion of bioassays using microwave heating and at room temperature (Figure 5A) reveal no loss of ITO from the surface. These observations imply that accurate colorimetric response can be measured from bioassays carried out on ITO. We note that minor differences in the absorption spectra for ITO are due to changes in the dielectric constant of the medium around ITO [30].

To visualize the physical changes described above, SEM images with EDS analysis of STFs and ITO surfaces before and after the completion of the bioassay was collected (Figure 6 and Figure 7). Figure 6 shows that STFs surface appears as a roughened thick film before the Ki-67 bioassay is commenced. After the completion of the Ki-67 bioassay steps, STFs appear as silver island films (i.e., discontinuous silver film), where a significant loss of silver can be visually observed. However, the extent of loss of silver from the STFs surface is also dependent on the concentration of Ki-67 antigen: that is, the loss of silver is higher for $[Ki-67] = 10^{-7}$ M than that observed for $[Ki-67] = 10^{-12}$ M. EDS analysis of the STFs before (Ag: 0.56 ± 0.09 %) and after the completion of the bioassay using microwave heating (Ag: 0.20–0.23 %) and at room temperature (Ag: 0.15–0.28 %) confirmed the significant loss of silver in a quantitative manner (Figure 6-insets).

In contrast to STFs, the surface of ITO after the bioassay was completed using microwave heating appeared to be identical to the ITO surface after the completion of the bioassay at room temperature and before the bioassay (Figure 7). EDS analysis of ITO surfaces agree with the visual evidence that ITO surfaces are physically stable during the execution of the bioassays. Furthermore, no ITO was removed from the surface to affect the outcome to the colorimetric response from the bioassays carried out on ITO. Our laboratory is currently investigating the combined use of Ag NWs and ITO on PET with microwave heating for rapid and sensitive detection of other biologically relevant molecules, and these results will be reported in due course.

4. Conclusions

We have evaluated the physical stability of STFs on chemically modified PMMA, Ag NWs on paper and ITO on PET using a model bioassay carried out at room temperature and using microwave heating to speed up the bioassay steps. SEM and EDS analysis of STFs showed that a significant loss of silver from the PMMA surfaces, despite their prior chemical modification with NH_2 groups. The extent of loss of silver from the surfaces was pronounced on 10 nm STFs when exposed to microwave heating. A ~7-fold increase in the colorimetric response from a model bioassay for b-BSA performed on modified PMMA with 10 nm STF using microwave heating was observed as compared to the colorimetric signal produced from the same platform at room temperature. On 10 nm STFs with microwave heating a LLOD of $[b-BSA] > 10^{-10}$ M was observed. Colorimetric response and LLOD for all the platforms $[b-BSA = 10^{-8}$ M] from bioassays carried out at room temperature were significantly lower (absorbance of ~0.2) as compared to bioassays carried out using microwave heating (absorbance of ~0.8). Ag NWs on paper platforms yielded similar results as compared to STFs on PMMA. However, due to the fragile nature of paper that results in physical damage during repeated wash steps deemed these surfaces unreliable for bioassays. An insignificant loss of ITO from PET was observed during the execution of the bioassays, where a LLOD of $[b-BSA] > 10^{-11}$ M was measured. On a real-life assay for Ki-67 antigen, the colorimetric response from bioassay carried out on ITO using microwave heating and at room temperature was clearly distinguishable from the background response and the LLOD was determined to be $[Ki-67 = 10^{-10}$ M].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- Metal oxide surfaces are evaluated for use in colorimetric bioassays
- Colorimetric response was enhanced ~7-fold in a model bioassay for b-BSA on metal surfaces
- Combined use of metal surfaces and microwave heating speeds up the bioassay steps
- ITO surfaces are deemed to be best surface for real-life bioassays

Figure 1.

Water contact angles for chemically modified PMMA surfaces and paper. (A). Scanning electron microscopy (SEM) images for (B) unmodified or blank PMMA (C) chemically modified PMMA, (D) blank paper and (E) silver nanowires functionalized on paper. The error margin represents standard deviation in water contact angle measurements for five different spots on the PMMA surface. Scale bar = 15 μm .

Model Bioassay for b-BSA: Colorimetric Response

Model Bioassay for b-BSA: Absorbance of ITO Platforms

Figure 3.

Optical absorbance spectra of ITO platforms before, during and after model protein bioassay was carried out using (A) microwave (MW) heating and (B) at room temperature (RT).

Concentrations of b-BSA in each well are as follows: 1: 10^{-6} M, 2: 10^{-7} M, 3: 10^{-8} M, 4: 10^{-9} M, 5: 10^{-10} M, 6: 10^{-11} M, B: No b-BSA. Each experiment was repeated three times, and average values were presented.

Figure 4.

Colorimetric response for Ki-67 bioassay on (A) chemically modified PMMA with 10 nm STFs, (B) ITO using MW heating and RT, a control experiment. The error bar represents standard deviation in absorbance of enzymatic product for three experimental trials.

Figure 5.

Optical absorbance spectra of (A) STFs and (B) ITO before and after the completion of Ki 67 bioassay using MW heating and RT (a control experiment). Concentrations of b-BSA in each well are as follows: 1: 10⁻⁶ M, 2: 10⁻⁷ M, 3: 10⁻⁸ M, 4: 10⁻⁹ M, 5: 10⁻¹⁰ M, 6: 10⁻¹¹ M, B: No b-BSA. Each experiment was repeated three times and average values were presented.

Figure 6.

SEM images of PMMA platforms with 10 nm STFs before and after the completion of a real-life bioassay, Ki-67 using MW heating and RT, a control experiment. Elemental analysis values are reported as the average values from 5 different locations on the samples. Scale bar = 10 μ m.

Figure 7.

SEM images of ITO platforms before and after the completion of a real-life bioassay, Ki-67 using MW heating and RT, a control experiment. Elemental analysis values are reported as the average values from 5 different locations on the samples. Scale bar = 10 μ m.

Surface Modification of PMMA by a Chemical Method

Scheme 1.

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

Scheme 2.

Schematic depiction of a model bioassay for b-BSA carried out on indium tin oxide (ITO) on PET, silver thin films (STFs) on PMMA and silver nanowires (Ag NWs) functionalized on paper platforms. Five different control bioassays are also carried out to investigate the effectiveness of the three platforms studied here. All control experiments were carried out using microwave (MW) heating and at room temperature (RT). Total bioassay time: MW = 25 mins and RT = 3 hrs.

Scheme 3.

Schematic depiction of a real-life bioassay Ki-67 carried out on indium tin oxide (ITO) on PET, and chemically modified PMMA with 10 nm STFs. All control experiments were performed using microwave heating and at RT. Total bioassay time: MW = 30 mins and RT = 4.5 hr.