

SURFACE MODIFICATION OF PHOTORESIST SU-8 FOR LOW AUTOFLUORESCENCE AND BIOANALYTICAL APPLICATIONS

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ABSTRACT

This paper reports a surface modification of epoxy-based negative photoresist SU-8 for reducing its autofluorescence while enhancing its biofunctionality. By covalently depositing a thin layer of 20 nm Au nanoparticles (AuNPs) onto the SU-8 surface, we found that the AuNPs-coated SU-8 surface is much less fluorescent than the untreated SU-8. Moreover, DNA probes can easily be immobilized on the Au surface and are thermally stable over a wide range of temperature. These improvements will benefit bioanalytical applications such as DNA hybridization and solid-phase PCR (SP-PCR).

KEYWORDS: Photoresist, SU-8, Gold Nanoparticles, Autofluorescence, DNA Detection

INTRODUCTION

The epoxy-based negative photoresist SU-8 (glycidyl ether of bisphenol A) has been extensively used as a structural material for fabrication of numerous biological microelectro-mechanical systems (Bio-MEMS) or lab-on-a-chip (LOC) devices owing to its ease of fabrication, thermal and chemical stability, and optical transparency.^[1, 2] Though useful, the material also has several disadvantageous attributes, i.e. its hydrophobicity and high autofluorescence profile at visible wavelengths. Many Bio-MEMS or LOC devices today use fluorescence detection as the predominant workhorse, therefore the high background fluorescence generated by SU-8 indeed limits its use as a substrate in devices for bioanalytical applications. However, not many studies have been focused on reduction of the high autofluorescence of SU-8 in order to realize its full potential for applications in biomedical diagnostics.

Attempts to develop highly functionalized SU-8 surface having high immobilization efficiency with good accessibility to target DNA or proteins have also been the major concerns.^[3-5] Nucleic acids, antigens or antibodies could be immobilized on the SU-8 surface by physical adsorption, but this method is not favorable in term of stability and accessibility of the biomolecules. Furthermore, bare SU-8 patterned with conventional photolithography techniques does not easily allow covalent immobilization of biomolecules directly on its surface: the SU-8 surface should be modified so as to have at least one desired functional group, i.e. amine, thiol, aldehyde, carboxyl, etc., for anchoring biomolecules by stable covalent bonds. The covalent immobilization may result in better biofunctionality, less non-specific adsorption and higher stability. Nevertheless, the methods usually use strong chemical (acids or bases) or strong plasma treatments, which may damage the adjacent micro- and nanostructures of the LOC devices physically while modifying the area of interest.^[3, 5]

Matrices consisting of both polymers and inorganic materials offer great benefits.^[6] Coupled with the ease of polymer microfabrication processes, the inorganic phase, i.e. planar gold surface or gold nanoparticles (AuNPs), can provide the LOC devices with novel intrinsic properties such as conductivity, optics, or catalysis.^[7] The gold materials are easily coupled with biomolecules to enable potential applications such as ligand carriers or transducing platforms within the fabricated polymer microdevices. In this study, we show that by depositing a thin AuNP layer onto the SU-8 surface, the autofluorescence of the Au-coated SU-8 surface is significantly less than that of bare SU-8. Moreover, DNA probes can easily be immobilized on the Au surface and are thermally stable over a wide range of temperature, thus improving sensitivity of fluorescence-based detections using SU-8 microdevices.

EXPERIMENTAL

The SU-8 material used in this study is SU-8 barcode microparticles that have been described elsewhere.^[8] The SU-8 encoded microparticles have high multiplexing capability.^[9] In this study, we use them as a typical SU-8 material for surface modification and demonstration of their implementation. As shown in Fig. 1, the SU-8 microparticles are hydroxylated by incubating with 0.1 M ethanolamine/0.5 M sodium phosphate buffer (pH.7) (Step 1). Then, 2% (v/v) solution of aminopropyltriethoxy silane (APTES) in isopropyl alcohol is added to the SU-8 materials for the silanization reaction (Step 2). After the hydroxylation and silanization, a thin layer of 20 nm Au nanoparticles (AuNPs) is covalently deposited onto the SU-8 surface (Step 3). 10 μ M thiolated DNA probes (5' Thiol-TT TTT TTT TTA GGA AGG TGT GGA CGA CGT CAA GTC ATC ATG GCC 3') is then immobilized on the Au surface by incubating overnight (Step 4). The Au-metalized SU-8 surface is characterized using optical microscopy, electron microscopy and elemental analysis (Fig. 2). Hybridization experiments are performed by incubating with the probe's complementary sequence tagged with Cy5 (5' GG CCA TGA TGA CTT GAC GTC GTC CAC ACC TTC CTA AAA AAA AAA Cy5 3') at 42 °C for 1 h. Finally, SP-PCR is performed with 2 ng/ μ L genomic DNA purified from *Campylobacter jejuni*. Forward primer (5 μ M, 5' GCG AAG AAC CTT ACC TGG GCT TGA TA 3')

and reverse primer (20 μ M, 5' Cy5 TC GCG RTA TTG CGT CTC ATT GTA TAT G 3') are used to amplify the 16S ribosomal gene of *C. jejuni* with enzyme activation at 94 $^{\circ}$ C for 5 min followed by 35 cycles with denaturation at 94 $^{\circ}$ C for 15 s, annealing 54 $^{\circ}$ C for 20 sec, and extension at 72 $^{\circ}$ C for 20 sec. The final extension step is carried out at 72 $^{\circ}$ C for 1 min. Fluorescence measurement is carried out by a scanner BioAnalyzer (LaVision BioTec GmbH, Germany) with appropriate settings for the Cy5 fluorophore.

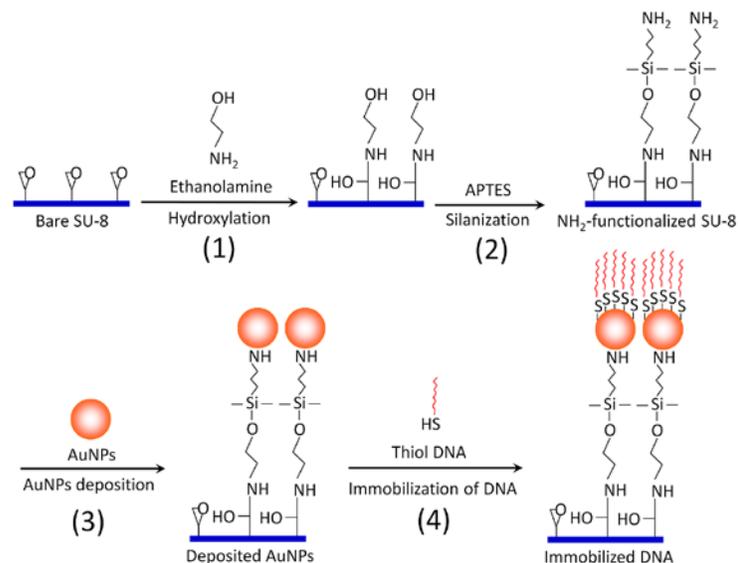


Figure 1: Chemical modification and deposition of 20 nm AuNPs on the SU-8 surface (Step 1-3), the DNA probe is immobilized by thiol chemistry (Step 4).

RESULTS AND DISCUSSION

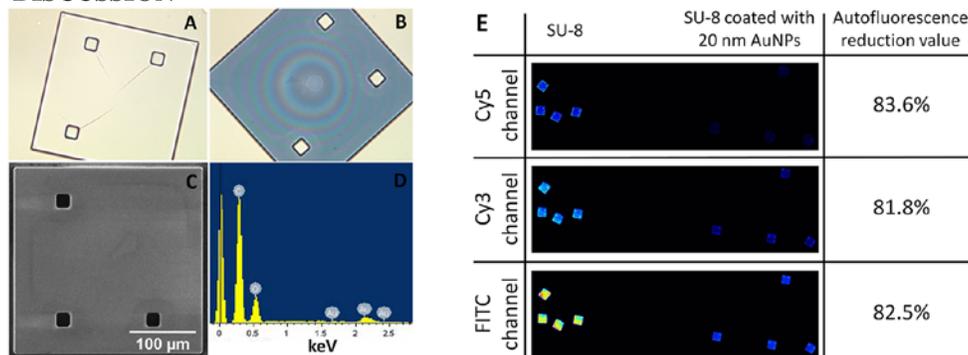


Figure 2: Characterizations of Au-coated SU-8 surface. Optical images of SU-8 surface before and after deposition of 20 nm AuNPs (A and B). C) SEM image of a Au-coated SU-8 microparticle ($L \times W \times H = 250 \times 250 \times 5 \mu\text{m}$). D) Energy Dispersive X-ray analysis (EDX). E) Autofluorescence reduction value of the Au-coated SU-8 surface at 3 channels: Cy3, Cy5, and FITC.

After covalent deposition of the thin layer of AuNPs, the metalized SU-8 surface was characterized using optical microscopy, electron microscopy and elemental analysis (Fig. 2). The results show that AuNPs have been successfully deposited onto the SU-8 surface with high homogeneity. We found that the AuNPs-coated SU-8 surface is as much as 80% less fluorescent than the untreated SU-8 at all 3 widely used fluorescent channels Cy3, Cy5, and FITC (Fig. 2E). This can be explained by the fluorescent quenching properties of AuNPs,^[7] or by physical covering effect. Furthermore, by depositing a thin layer of AuNPs, DNA probes can be easily immobilized on the Au surface through the formation of self-assembled monolayers (SAMs) via thiol chemistry. The immobilization process is simple, less time-consuming and less laborious than other reported chemical modifications,^[3, 4, 10] or UV linking method.^[11] More importantly, we show that the immobilized DNA probes are thermally stable over a wide range of temperatures, more than 65% of the DNA probe molecules remain on the surface without losing their biological function even after extreme temperature treatment e.g., 40 PCR heating/cooling cycles (Fig. 3A). With the lower autofluorescence property, the 45-mer oligonucleotide can be visualized distinctly at 1 nM concentration by hybridizing with its complementary DNA probe immobilized on the AuNPs-coated SU-8, which improves fluorescence measurements by about 2 orders compared to the untreated SU-8 (Fig. 3B). Owing to the benefit of the thermally stable DNA immobilization, we report for the first time that SP-PCR can be now performed on the Au-coated polymer surface (Fig. 4).

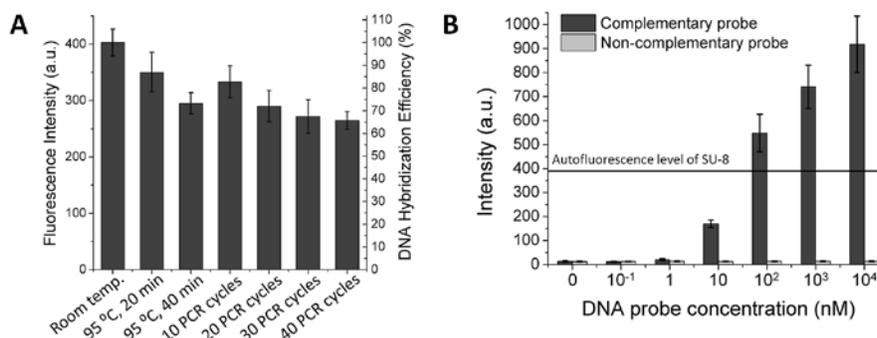


Figure 3: A) Investigation of thermal stability of the immobilized DNA probe. After treated at different temperature conditions, the immobilized DNA probe was hybridized with its Cy5-tagged complementary 45-mer sequence and scanned for the fluorescence intensity. B) Sensitivity of a DNA-DNA hybridization assay on the Au-coated SU-8 surface.

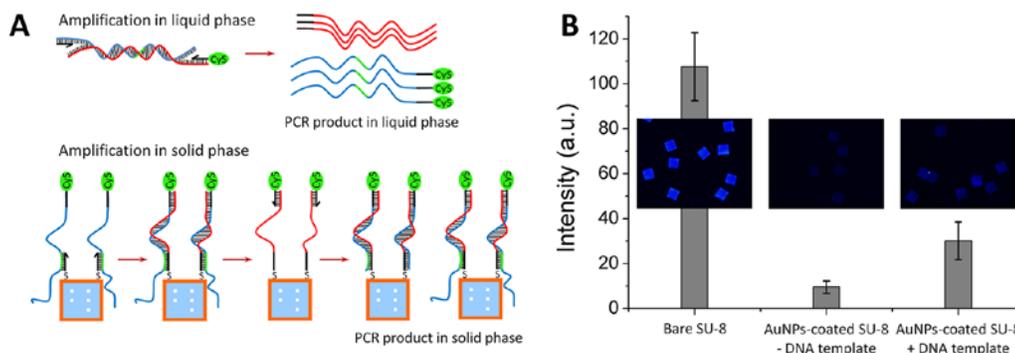


Figure 4: A) Working principle of SP-PCR; B) A preliminary proof-of-principle result of the SP-PCR.

CONCLUSION

This work shows that by depositing a thin AuNP layer onto the SU-8 surface, the autofluorescence of the Au-coated SU-8 surface is significantly less than that of bare SU-8 enabling improved sensitivity of fluorescence-based detections. Moreover, DNA probes can be stably immobilized on the Au-coated surface, which enables bioanalytical applications such as DNA hybridization and SP-PCR. The approach is simple and easily to perform, making it suitable for various Bio-MEMs and LOC devices that use SU-8 as a structural material.

ACKNOWLEDGEMENTS

This work was financially supported by Technical University of Denmark (DTU), Food pathogen project no.8 and grant no.150627 and EU 6th Framework Program OPTOLABCARD (contract no. 016727) and EU 7th Framework LABONFOIL (contract no. 224306).

REFERENCES

1. P. Abgrall, V. Conedera, H. Camon, A. Gue and N. Nguyen, *Electrophoresis*, 2007, **28**, 4539-4551.
2. A. del Campo and C. Greiner, *J Micromech Microengineering*, 2007, **17**, R81-R95.
3. M. Joshi, N. Kale, R. Lal, V. R. Rao and S. Mukherji, *Biosens. Bioelectron.*, 2007, **22**, 2429-2435.
4. D. Sethi, A. Kumar, R. P. Gandhi, P. Kumar and K. C. Gupta, *Bioconjug. Chem.*, 2010, **21**, 1703-1708.
5. S. L. Tao, K. Popat and T. A. Desai, *Nature Protocols*, 2006, **1**, 3153-3158.
6. P. C. Gach, C. E. Sims and N. L. Allbritton, *Biomaterials*, 2010, **31**, 8810-8817.
7. B. Dubertret, M. Calame and A. Libchaber, *Nat. Biotechnol.*, 2001, **19**, 365-370.
8. G. R. Broder, R. T. Ranasinghe, J. K. She, S. Banu, S. W. Birtwell, G. Cavalli, G. S. Galitonov, D. Holmes, H. F. P. Martins, K. F. MacDonald, C. Neylon, N. Zheludev, P. L. Roach and H. Morgan, *Anal. Chem.*, 2008, **80**, 1902-1909.
9. S. Birtwell and H. Morgan, *Integrative Biology*, 2009, **1**, 345-362.
10. R. Marie, S. Schmid, A. Johansson, L. E. Ejsing, M. Nordstrom, D. Hafliker, C. B. V. Christensen, A. Boisen and M. Dufva, *Biosens. Bioelectron.*, 2006, **21**, 1327-1332.
11. H. Gudnason, M. Dufva, D. D. Bang and A. Wolff, *BioTechniques*, 2008, **45**, 261-271.

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