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Nanosphere lithography (NSL) on Au nanopatterned electrodes for electrochemical DNA detection

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Abstract

In this research, Nanosphere Lithography (NSL) using polystyrene (PS) template with diameter of 500 nm was applied to generate high-ordered pattern of gold nanoporous on ITO on glass substrate for electrochemical DNA detection. AuNPs array was obtained by spin coating and thermal evaporation techniques prior to PS etching and subsequently used to detect hybridization signal of the most distinct SH-tag 16s rRNA gene of *S. aureus*.

1. Introduction

Conventional electrochemical DNA in planar Au electrodes offered limited sensitivity, selectivity, and LOD due to crowding of capture probes on the surface that limits their accessibility by target molecules [1]. Recently, surface nanostructuring has been a solution to this issue because of its increased surface area-to-volume ratio and surface coverage of capture probes, favorable orientation of the immobilized probes, and higher electrocatalytic activity at the surface [2]. AuNPs is excellent for developing affinity based nucleic acid sensors due to its high electrical conductivity, tunable pore morphology, corrosion resistance, biocompatibility, biofouling resistance compatibility with microfabrication processes, and well-studied gold−thiol surface chemistry for conjugating biomolecules[3].

Herein, Au nanopores was produced by spin coating and thermal evaporation following the pattern of PS nanoballs through a simple and inexpensive NSL techniques to test the hybridization of *S. aureus* 16s rRNA, one of the most prevalently isolated bacteria in nosocomial infection [4].

2. Experimental Section

2.1 Au nanopores generation on ITO Electrode

PS mask was prepared by drop casting methods using 2 wt. % of PS. Au nanopores were prepared, first by spin coating of 15 nM of AuNPs (d=13nm) solution (30 s/2000 rpm) while thermal evaporation was with 5 nm Au. After PS removal, the substrate was used in the electrochemical detection of *S. aureus* 16s rRNA (Table 1) (Fig. 1).

2.2 Electrochemical detection of *S. aureus* 16s rRNA hybridization

Cyclic Voltammetry: 0-0.4 V, scan rate: 50 mV/s in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.5 M KCl using three electrodes system.

3. Results and Discussion.

In this work, ~90% of the surface was fully covered by monolayer PS balls (Fig. 2a). Spin-coated AuNPs has the tendency of producing wider frame with smaller pore diameter, i.e ~300 nm (Fig. 2b), while thermally evaporated AuNPs formed nearly dense and packed hexagon array (Fig. 2c) with typical pore size was ~500 nm, similar to that of PS diameter. The thicker frame produced by spin coating is because AuNPs was in the form of solution, therefore, the excess penetration within voids possibly occurred. Whilst, AuNPs nanoisland (5 nm) formed by thermal evaporation tended to elongate due to thermal energy, thus, resulting thinner frame of the hexagons [5].

CV measurement of DNA test implies that smaller pores from spin coated AuNPs provided higher surface area [6] that consequently enhanced peak current of about 86% higher than bare ITO state (Fig 3a), with LOD of 10 pM as compared to AuNPs thermally grown and better sensitivity (Fig 4).

4. Conclusion

Combined techniques of simple and low-cost PS NSL and AuNPs surface modifications have been realized. Au nanopores from spin coating method that is also less expensive with simple instrumentation and preparation substantially reached higher CV profiles than thermal evaporation methods.

Acknowledgement

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References

[1] S. O. Kelley and J. K. Barton, *Science*, vol. 283, no. 5400, pp. 375–381, 1999.
[2] R. Gasparac, B. J. Taft, M. A. Lapierre-Devlin, A. D. Lazareck, J. M. Xu, and S. O. Kelley, *J. Am. Chem. Soc.*, vol. 126, no. 39, pp. 12270–12271, 2004.
[3] P. Daggumati, Z. Matharu, and E. Seker, *Anal. Chem.*, 2015.
[4] M. Otto, *Curr. Top. Microbiol. Immunol.*, vol. 322, no. 7, pp. 207–228, 2008.
[5] S. Szunerits, V. G. Praig, M. Manesse, and R. Boukherroub, *Nanotechnology*, vol. 19, no. 19, p. 195712, 2008.
[6] M. M. Collinson, *ISRN Anal. Chem.*, vol. 2013, pp. 1–21, 2013.

Figures:

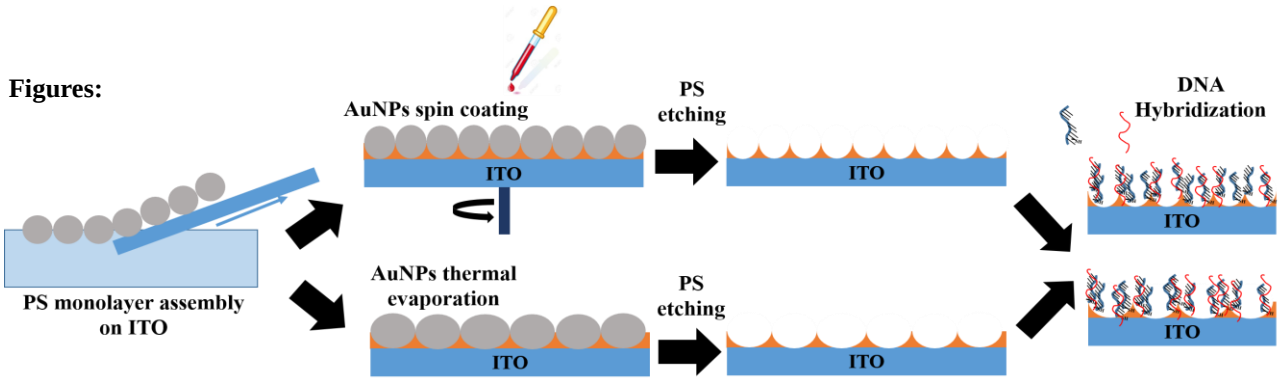

Fig 1. Schematic illustration of NSL, modification of AuNPs and DNA hybridization test

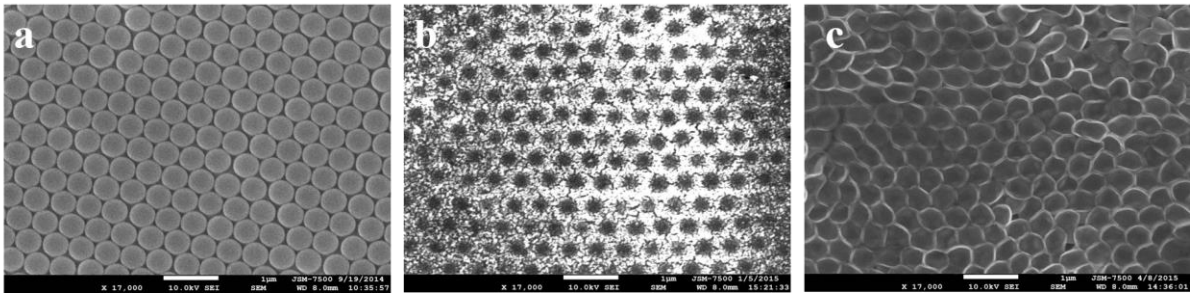

Fig 2. Morphological Characterization of (a) PS assembly on ITO by drop casting methods, (b) gold nanoporous resulted from spin coating, (c) gold nanoporous resulted from thermal evaporation

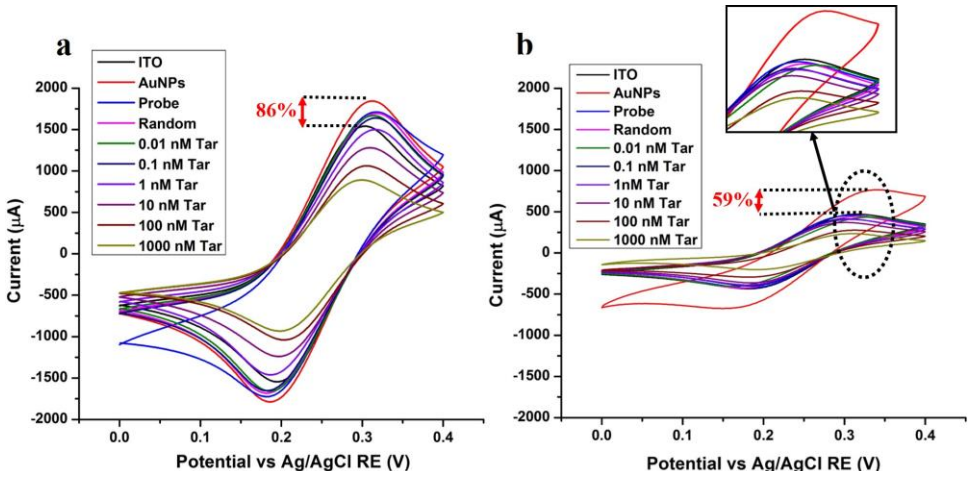

Figure 3. Cyclic Voltammogram (CV) of *S. aureus* 16s rRNA hybridization using (a) gold nanoporous resulted from spin coating, (b) gold nanoporous resulted from thermal evaporation between 0-0.4 V at a scan rate of 50 mV/s in 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and 0.5 M KCl

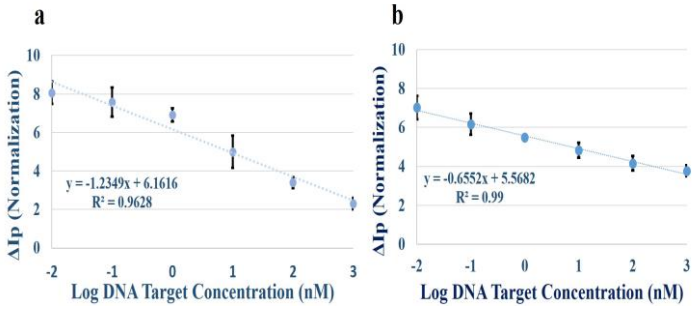

Figure 4. Normalized sensitivity and linearity of peak current (ΔIp) of *S. aureus* 16s rRNA hybridization detection using (a) gold nanoporous resulted from spin coating, (b) gold nanoporous resulted from thermal evaporation

Table. I Synthetic oligonucleotides representing the designed 16S rRNA gene sequence of *S. aureus*

	S. aureus
Probe	5'HS-AAA AAA GTT ATC CCA GTC TTA TAG GTA GGT 3' (30 mer)
Target	5'ACC TAC CTA TAA GAC TGG GAT AAC T 3' (25 mer)
Random Target	5'CGC CGT AAA CGA TGT CGA CT 3' (20 mer)