Soft UV nanoimprint lithography-designed highly sensitive substrates for SERS detection

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Abstract

We report on the use of soft UV nanoimprint lithography (UV-NIL) for the development of reproducible, millimeter-sized, and sensitive substrates for SERS detection. The used geometry for plasmonic nanostructures is the cylinder. Gold nanocylinders (GNCs) showed to be very sensitive and specific sensing surfaces. Indeed, we demonstrated that less than $4 \times 10^6$ avidin molecules were detected and contributed to the surface-enhanced Raman scattering (SERS) signal. Thus, the soft UV-NIL technique allows to obtain quickly very sensitive substrates for SERS biosensing on surfaces of 1 mm².

Keywords: Soft UV-NIL; Nanoimprint; SERS biosensors; Avidin; Biotin

Background

Surface-enhanced Raman scattering (SERS) technique was shown to be a very effective analytical tool for the detection and identification of molecules, thanks to its high sensitivity [1,2]. It has been widely used for ultra-sensitive chemical analysis down to the single molecule sensitivity. Its field of applications is as varied as to include chemical-biochemical analysis, nanostructure characterization as well as biomedical applications [3-7]. Especially in chemistry, SERS is applied for the detection of conformational changes and structural differences regarding preferred orientations of molecules with respect to a metal surface [8]. The facts that SERS gives a specific fingerprint of a molecule and is sensitive to very small molecules make it a good candidate for application in the fields of chemical and biological sensors. The SERS enhancement is due to the localized surface plasmon resonance (LSPR) of the metallic nanostructure. The nanostructured LSPR properties need to be designed and strongly controlled in order to produce highly reproducible active SERS substrates [9,10]. In previous studies, we have discussed the necessity to optimize the size of gold nanocylinders (GNCs) in order to achieve the highest possible SERS enhancement. In addition, we have demonstrated the necessity to optimize the LSPR in the case of each studied molecules [11].

For industrial applications, nanostructured surfaces of at least 1 mm² have to be produced. In the past, electron beam lithography (EBL) has helped us to demonstrate that by optimizing the nanostructure assembly parameter, enhancement factors estimated at $10^5$ to $10^7$ could be obtained for such proteins as bovine serum albumin (BSA) or RNase-A [11]. However, EBL is expensive and time-consuming. Techniques to produce large and organized nanostructured assemblies on transparent substrates have been developed since several years in order to maximize Raman scattering enhancement [12,13]. The most popular is probably the nanosphere lithography (NSL) [14]. The advantage of this technique is to obtain large areas (several mm²) of nanostructures on a substrate. However, the shape and arrangement of the nanostructures are more hardly tuned.

In this article, we propose to use another technique called soft UV-nanoimprint lithography (UV-NIL) [15] in order to fabricate SERS substrates. UV-NIL is biocompatible, since it can be implemented on any flat surface. Another essential advantage is that the samples produced with the same mold are all identical. This is important to guarantee the reproducibility of the results. We have...
The fabrication of gold nanocylinders by UV-NIL

The methods for fabricating gold nanocylinders by UV-NIL are described in this section. The process involves the following steps:

1. **Master Mold Fabrication**: A master mold is fabricated using EBL on PMMA resist combined with reactive ion etching for the pattern transfer into the silicon substrate. The conditions for this transfer have been published.

2. **Stamp Fabrication**: Stamps are fabricated with standard PDMS diluted in hexane solvent to reduce the viscosity and improve the penetration of PDMS in nanoholes.

3. **Imprint in AMONIL**: The PDMS stamp is pressed against PMMA/AMONIL resists, which are deposited on a glass substrate, using an EVG 620 mask aligner. The PDMS stamp also reduces the interface adhesion and allows easy separation between the stamp and the AMONIL resist.

4. **Residual Thickness Etching**: Residual thickness etching by RIE is performed.

5. **Cr/Au Evaporation & Lift-Off**: Cr/Au evaporation & lift-off process is carried out to achieve a very regular grating of GNCs.

**Avidin Detection**

Cysteamine, biotin-NHS, dodecanethiol (DDT), avidin, and bovine serum albumin (BSA) were purchased from Sigma Aldrich (St. Louis, MO, USA). The same functionalization procedure was used for GNC (SERS detection) and flat gold film (SPR detection). Functionalization involves steps such as overnight incubation in a 100 mM solution of cysteamine in water, thorough rinsing with water, dip in 10 mM solution of biotin-NHS in dimethylformamide (DMF), rinse with DMF and water, blocking step with DDT, and finally, soaking in 1 mM avidin solution.

**Raman Spectrum Acquisition**

Raman spectra were recorded using a Labram spectrophotometer from Horiba Scientific (Kyoto, Japan) for all experiments. The acquisition parameter was fixed to 500 s for avidin/avidin system. A 633-nm laser was used for all experiments with a power of 100 μW.
excitation was focused on the substrate using a microscope objective \((\times 80, \text{N.A.} = 0.75)\). The same objective was used to collect the Raman signal from SERS substrates in a backscattering configuration. The Raman spectra were recorded with a spectral resolution of \(1 \text{ cm}^{-1}\) and a spatial resolution about 1 \(\mu\text{m}\). For classical Raman measurements in solution, a macro-objective with a focal length of 40 mm (N.A. = 0.18) and a 633-nm laser were used. All obtained spectra have been corrected for acquisition time and laser power so they can be compared.

SPR measurements

The SPR measurements were recorded using a BIAcore 1990 GE Healthcare system (Pewaukee, WI, USA) with bare gold chips. It was mainly used to test the biotin functionalization procedure and the specificity of this biotin-DDT layer. For this experiment, increasing concentrations of avidin (0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, and 100 nM) were used to test the surface sensitivity. Furthermore, the functionalized surface was also exposed to a flow of concentrated BSA (1.5 \(\mu\text{M}\)) in order to determine the surface specificity for avidin detection.

Results and discussion

Gold nanocylinder fabrication

Firstly, the imprint process is realized in the AMONIL, which is deposited on a PMMA resist. The polymerization is performed with UV exposure \((\lambda = 365 \text{ nm})\) with 10 mW/cm² power during time exposure of 20 min. The imprint pressure is 200 mbar. Figure 3a shows a cross section SEM view with nanoholes in the AMONIL resist. The average diameter of holes is around 220 nm, and the residual thickness of AMONIL is around 20 nm. Thus, the AMONIL presence in the hole ground needs a specific RIE process. The removal of the residual AMONIL and the PMMA etch conditions have been reported in reference [17]. During this PMMA etching, we have a good selectivity between the PMMA and AMONIL \((v_{\text{PMMA}}/v_{\text{AMONIL}} = 2.7)\). After this etch, a gold thin layer (50 nm) is deposited by electron beam evaporation in order to realize the plasmonic nanocylinders. Previously, an adhesion layer (Cr) for gold is evaporated (3 nm). Figure 3b shows SEM image of gold nanocylinder arrays on 1 mm². The obtained dimensions are approximately 220 nm for the diameter, 50 nm for the height, and a periodicity of 400 nm. The inset of the Figure 3b is a zoom of a single nanodisk, and we observed a surface roughness relatively important with small tips on the nanodisk border.

Avidin detection

Avidin detection was performed, thanks to the GNC functionalization with biotin (to capture avidin) and DDT (to block the remaining clean gold surface). In order to validate the functionalization protocol, we have performed SPR measurements on bare gold surface prior to the
SERS results obtained on biotin and avidin with UV-NIL GNCs are shown in Figure 4b. Since SERS is highly sensitive to the first layer deposited on the gold surface, the red Raman spectrum should then be relevant of the whole functionalization layer (i.e., cysteamine, DDT, and biotin). Yet, cysteamine and DDT have a low Raman cross section; thus, we assume that they would be barely visible and that the red spectrum is mainly due to the biotin molecule in interaction with cysteamine. It is difficult to compare this spectrum with the biotin-NHS powder spectrum. The main reason for this is that the NHS group has been removed during the reaction between biotin and the cysteamine. The main visible peaks in the red spectrum are located at 1145, 1202, 1276, 1493, 1537, 1572, and 1591 cm\(^{-1}\). In a second step, avidin was added onto the sample, giving rise to the green spectrum. A new set of peaks is seen revealing the interaction between avidin and biotin. The spectrum of avidin on biotin is quite different from the one of the avidin solution. Two explanations are given: the interaction between avidin and biotin (this behavior has already been observed in references [22,23]) and the fact that the avidin-avidin SERS spectrum has been acquired in dry conditions giving rise to probable conformational changes.

In addition, it is more difficult to make an assumption on the number of detected molecules. This is due to the fact that a functionalization layer was used and hypothesis should be made concerning the quality of the functionalization and the association between the molecules. The comparison between the SERS spectra of avidin-biotin and the Raman spectra of an avidin solution (1 mM) and biotin-NHS powder shows that all peaks from the avidin powder are not found on the avidin-biotin SERS spectrum. An explanation is that the SERS experiment has been performed in dry conditions. Conformation changes are probable consequences of drying a protein, and this is reflected in the molecule Raman fingerprint. The second noticeable point is that the avidin and biotin-NHS signals were multiplied, respectively, by 20 and 5 in order to be compared to the SERS spectrum of avidin-biotin (see Figure 4b). In order to check the substrate sensitivity, we have calculated the enhancement factor of the UV-NIL SERS substrate. We compared the SERS signal of avidin-biotin to the Raman signal of 1 mM avidin solution. The number of excited molecules in the SERS experiment is \(3.8 \times 10^6\) (assuming that the GNC is entirely covered by avidin, that avidin is a sphere with a diameter of 5 nm, and that the collection area is 74.3 \(\mu\)m\(^2\) for a pattern that contains 1024 nanocylinders). In the Raman experiment, the number of excited molecules is \(4.3 \times 10^{12}\). This number is obtained by: \(N_{\text{Raman}} = N_A CV\), where \(N_A = 6.023 \times 10^{23}\) is the Avogadro’s number (mol\(^{-1}\)), \(C\) is the used concentration of Avidin, and \(V\) is the scattering volume (in L). This scattering volume is defined as follows [24]: \(V = A \times H\),
where $A = 7.07 \times 10^4 \, \mu m^2$ (scattering area = disk area of which the diameter is 300 $\mu m$) and $H$ is approximately 100 $\mu m$ (scattering height). If we assume that the peak at 1664 cm$^{-1}$ is shifted to 1630 cm$^{-1}$ due to avidin interactions with biotin and GNC, an intensity ratio ($I_{SERS}/I_{Raman}$) of 32.5 is measured. Then, the enhancement factor (EF) is given by:

$$EF = \frac{I_{SERS}}{I_{Raman}} \times \frac{N_{Raman}}{N_{SERS}}$$

where $I_{SERS}$ and $I_{Raman}$ are the SERS and Raman intensities, respectively. $N_{SERS}$ and $N_{Raman}$ are the number of excited molecules in SERS and Raman experiments, respectively. Thus, we found an EF value of approximately $3.7 \times 10^7$ for avidin/biotin system. This EF value is comparable or slightly higher than that obtained by other fabrication techniques such as EBL. Moreover, the advantages of the UV-NIL technique are the fabrication speed, the low cost, reproducibility, and homogeneity of nanostructures on large pattern areas. These advantages are very important parameters for the SERS application. In addition, giving this EF value and considering the signal/noise ratio of the SERS spectrum, it would be possible to dilute the concentration of avidin at least 100 times and still be able to detect it. Thus, we assume that we could detect avidin at concentrations in the micromolar range, which is in
agreement with the detection limit already calculated for other proteins but using nanocylinders produced by EBL [11].

**Conclusions**

In summary, we have fabricated by the UV-NIL technique a large area of ordered GNCs, which was used as SERS substrate. The use of UV-NIL enabled to reach comparable results with EBL in terms of enhancement factor. Moreover, this UV-NIL technique is faster and less expensive than EBL. The size and shape of nanostructures are homogenous and reproducible on large pattern areas, which are important points for industrial applications of SERS. We detected a weak number of avidin molecules (3.8 × 10^8) by SERS measurements. A great advantage of SERS is the fingerprint provided by each molecule in order to identify the detected molecules. Finally, the SERS substrates fabricated by UV-NIL have thus showed their ability in biosensing with a good sensitivity.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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**References**

1. Blackie EJ, Le Ru EC, Etchegoin PG. Single-molecule surface-enhanced raman spectroscopy of nonresonant molecules. J Am Chem Soc 2009, 131:14466–14472.

2. Le Ru EC, Grand J, Sow I, Somerville WRC, Etchegoin PG, Trégouët-Delapiere M, Charon G, Félix J, Lévy G, Aubard J. A scheme for detecting every single target molecule with surface-enhanced raman spectroscopy. Nano Lett 2011, 11:5013–5019.

3. Laframboise PD, Le Ru EC, Etchegoin PG. Guiding molecules with electrostatic forces in surface enhanced raman spectroscopy. ACS Nano 2009, 3566–72.

4. Iosin M, Todenas F, Baldeck RL, Astilean S. Study of protein-gold nanoparticle conjugates by fluorescence and surface-enhanced raman scattering. J Mol Struct 2009, 924-926:196–200.

5. Graham D, Goodacre R. Chemical and bioanalytical applications of surface enhanced raman scattering spectroscopy. Chem Soc Rev 2008, 37:883–884.

6. Stevenson R, Ingram A, Leung H, McMillan DC, Graham D. Quantitative SEERS immunoassay for the detection of human PSA. Analyst 2009, 134:842–844.

7. Sim HR, Wark AW, Lee HJ. Attomolar detection of protein biomarkers using biofunctionalized gold nanorods with surface plasmon resonance. Analyst 2010, 135:842–844.

8. David C, Foley S, Mavon C, Enescu M. Reductive unfolding of serum albumins uncovered by raman spectroscopy. Biopolymers 2008, 89:523–634.

9. Guillo N, Shen H, Frémaux B, Péron O, Rinnert E, Toury T, Lamy de la Chapelle M. Surface enhanced raman scattering optimization of gold nanocylinder arrays: influence of the localized surface plasmon resonance and excitation wavelength. Appl Phys Lett 2010, 97:023113.

10. Grand J, Lamy de la Chapelle M, Bjeen JL, Adam PM, Vial A, Royer P. Role of localized surface plasmons in surface-enhanced raman scattering of shape-controlled metallic particles in regular arrays. Phys Rev B 2009, 72:205420.

11. David C, Guillo N, Shen H, Toury T, Lamy de la Chapelle M. SERS detection of biomolecules using lithographed nanoparticles towards a reproducible SERS biosensor. Nanotechnology 2010, 21:75501.

12. Hering K, Cialla D, Ackermann K, Dörfer T, Möller R, Schneidewind H, Matthias R, Fritzschke W, Rösch P, Popp J. SERS detection of biomolecules using lithographed nanoparticles towards a reproducible SERS biosensor. Anal Bioanal Chem 2008, 390:113–124.

13. Camden JP, Dieringer JA, Zhao J, Van Duyne RP. Controlled plasmonic nanostructures for surface-enhanced spectroscopy and sensing. Anal Bioanal Chem 2008, 416:1663–1661.

14. Haynes CL, Van Duyne RP. Nanosphere lithography: a versatile nanofabrication tool for studies of size-dependent nanoparticle optics. J Phys Chem B 2001, 105:5559–5611.

15. Chou SY, Krauss PR, Renstrom PJ: Nanoimprint lithography. J Vac Sci Technol B 1996, 14:4129–4133.

16. Barbilignon F, Hamouda F, Held S, Gogol P, Bartenlian B. Gold nanoparticles by soft UV nanoimprint lithography coupled to a lift-off process for plasmonic sensing of antibodies. Microelectron Eng 2010, 87:1001–1004.

17. Hamouda F, Barbilignon G, Held S, Agnus G, Gogol P, Maroutian T, Scheuring S, Bartenlian B. Nanoholes by soft UV nanoimprint lithography applied to study of membrane proteins. Microelectron Eng 2009, 86:583–585.

18. Kim A, Ou F, S, Ohlberg DAA, Hu M, Williams RS, Li Z. Study of molecular trapping inside gold nanofinger arrays on surface-enhanced raman substrates. J Am Chem Soc 2011, 133:8234–8239.

19. Ou FS, Hu M, Naumov I, Kim A, Wu W, Bratkovsky AM, Li X, Williams RS, Li Z. Hot-spot engineering in polygonal nanofinger assemblies for surface enhanced Raman spectroscopy. Nano Lett 2011, 11:2538–2542.

20. Galarreta BC, Norton PR, Lagugné-Labarthet F. SERS detection of streptavidin/biotin monolayer assemblies. Langmuir 2011, 27:1494–1498.

21. Hamouda F, Sahaf H, Held S, Barbilignon F, Gogol P, Moyen E, Aassime A, Moreau J, Canva M, Lourtcoz JM, Hanbücken M, Bartenlian B. Large area nanopatterning by combined anodic aluminium oxide and soft UV-NIL technologies for applications in biology. Microelectron Eng 2011, 88:2444–2446.

22. Fagnano C, Torreggiani A, Fini G. Raman spectroscopic studies of the anhydrous complexes of avidin and streptavidin with biotin. Biospectroscopy 1996, 2253–232.

23. Torreggiani A, Fini G. Raman spectroscopic studies of ligand-protein interactions: the binding of biotin analogues by avidin. J Raman Spectrosc 1998, 29:229–236.

24. Le Ru EC, Blackie EJ, Meyer M, Etchegoin PG. Surface enhanced Raman scattering enhancement factors: a comprehensive study. J Phys Chem C 2007, 111:13794–13803.