Attila BONYÁR – Tomáš LEDNICKÝ – Shereen ZANGANA – László JAKAB

GOLD–EPOXY NANOMUSHROOM ARRAYS FOR LABEL–FREE NUCLEOTIDE SENSING

The fabrication technology and two possible application areas of a gold-epoxy surface nanocomposite are presented. The nanocomposite is made of ordered nanoparticles on top of etched epoxy pillars, forming an array of 'nano-mushrooms'. The presented technology enables the large-scale fabrication of such composites (e.g., with a cm^2 surface area) with control over the size and interparticle distance of the nanoparticles. The possibility to utilize such nanocomposites as label-free nucleotide biosensors in LSPR (localized surface plasmon resonance) configurations and also as SERS (surface-enhanced Raman spectroscopy) substrates is presented.

Introduction

Recently, the development of plasmonic nanomaterials became one of the focal points of materials science research, due to their unique optical properties, such as their extremely high absorption and scattering efficiency or excellent sensitivity to the dielectric properties of the surrounding medium. Relying on these properties, plasmonic nanostructures offer a large potential for bioanalytical applications, such as LSPRi and SERS as well. Localized surface plasmon resonance imaging (LSPRi) – the utilization of collective electron oscillations, which are incited on the nanoparticles, as sensors – promises the possibility of high throughput biomolecule sensing integrated into miniaturized point-of-care (PoC) devices (1). Surface-enhanced Raman spectroscopy (SERS) is a promising ultrasensitive method in which the Raman scattering signal strength of molecules, absorbed on the surface of metallic nanoparticles with intensive near-fields, is enhanced with several orders of magnitude (2). Both techniques require nanoparticles or nanostructures, preferably bound to a surface. Besides, the sensitivity of both methods is depending on the intensity of the plasmon field around the particles, which can be enhanced by utilizing plasmonic coupling between closely packed nanoparticles (3-5).

In this work, we present a fabrication technology to prepare gold-epoxy surface nanocomposites (nanomushrooms, as illustrated in Fig.1), where closely packed gold nanoparticles form an array on a large surface area, with controllable particle size and interparticle distance, which is essential for the optimization of these substrates for LSPR and SERS purposes. The application of these nanocomposites for the label-free detection of DNA fragments with these methods is also demonstrated.

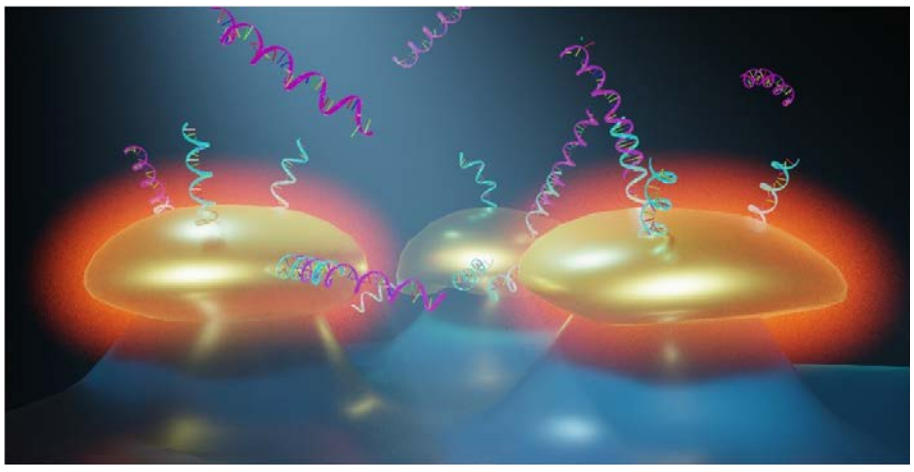

Fig.1: 3D illustration of the fabricated gold-epoxy nanomushroom arrays and their possible use as nucleotide sensors (4).

Fabrication Technology

The main steps of the fabrication technology are presented in Figure 2. A detailed description of the different steps can be found in our previous works (4,5). In short, the nanoparticles are synthesized by solid-state dewetting on nanobowled aluminum templates, which are prepared by the selective chemical etching of porous anodic alumina (PAA) grown on an aluminum sheet with controlled anodic oxidation. The nanoparticles are transferred to the surface of epoxy substrates, which are subsequently selectively etched (5).

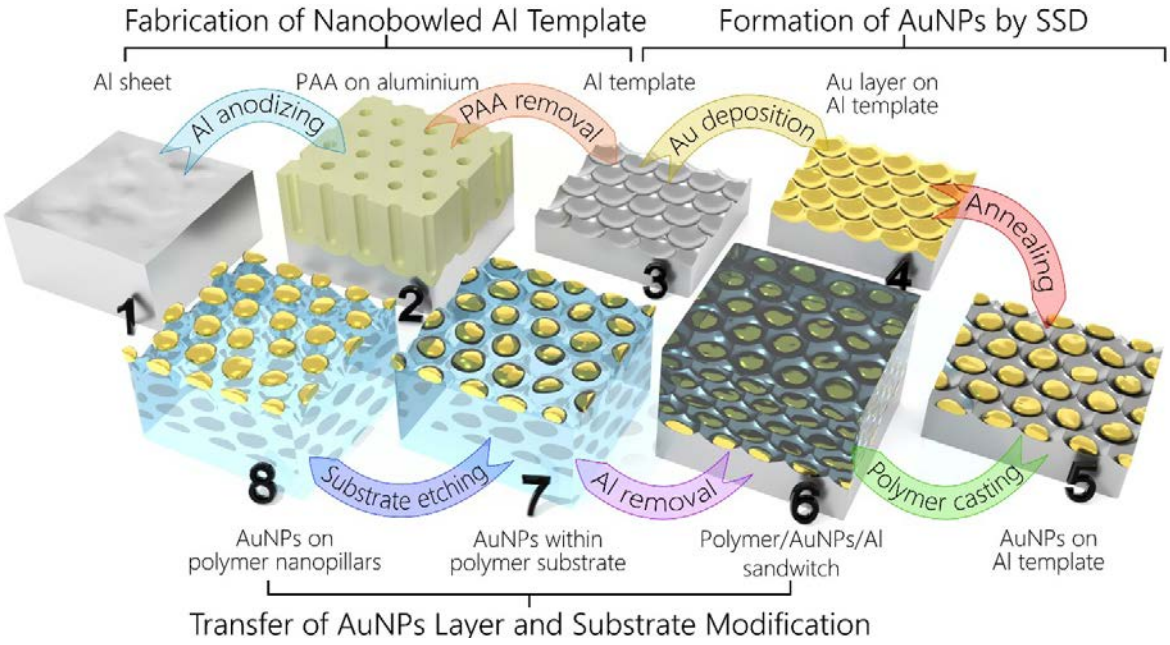

Fig.2: The main fabrication steps of gold-epoxy nanomushroom array preparation. (4)

This flexible fabrication technology provides proper control over the nanoparticle size, shape, and interparticle distance over a large surface area (several cm^2), which enables the fine-tuning and optimization of their plasmonic absorption spectra for LSPR and SERS applications between 535–625 nm. As illustrated in Fig. 3, by controlling the pore size and the deposited layer thickness, the size and interparticle distance of the spheroidal particles can be influenced. For type A substrates, the average size of the pores is 68 ± 3 nm; for type B it is 109 ± 4 nm. With subsequent gold thin film deposition and dewetting (thermal annealing) the size of the resulting particles can be set [4,5]. In the examples presented in this paper B2-type particle arrangements were used (corresponding to an average particle diameter of 92 ± 6 nm).

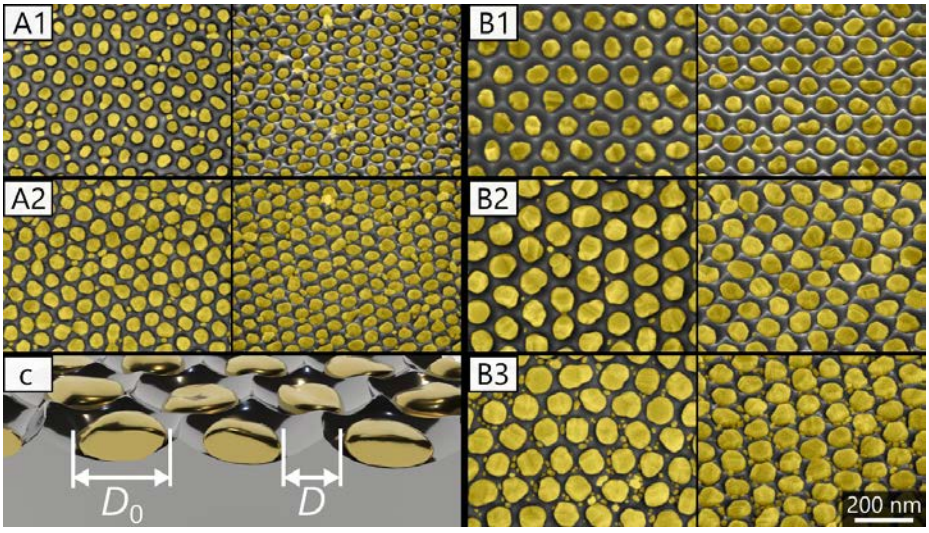

Fig.3: SEM images (false colours) of top and tilt view (45°) presenting the size distribution of the five different nanoparticle array types. Image c) illustrates the shape and position of the nanoparticles inside the nanobowls. The particle diameter will be referred to as D0, while the interparticle distance is denoted as D. (4)

LSPR

In order to demonstrate the applicability of the fabricated sensor elements for LSPR sensing, optical spectroscopy measurements were performed in a transmission-based optical setup with an Avantes Avaspec 2048-4DT spectrometer and an Avantes Avalight DHS halogen light source. The bulk refractive index sensitivity of the plasmonic sensors was tested by changing the medium above the samples between air, de-ionized water, and a sucrose dilution series (25 %, 50 %, 75 % and 100% in di water, as shown in Fig. 4.A). The sensor surface was illuminated in a circular area with a diameter of 8 mm and a glass microscope sheet was used to cover the dispersed media on the samples. The obtained RI sensitivity of our sensors was between 85–120 nm/RIU, depending on the fabrication technology (5).

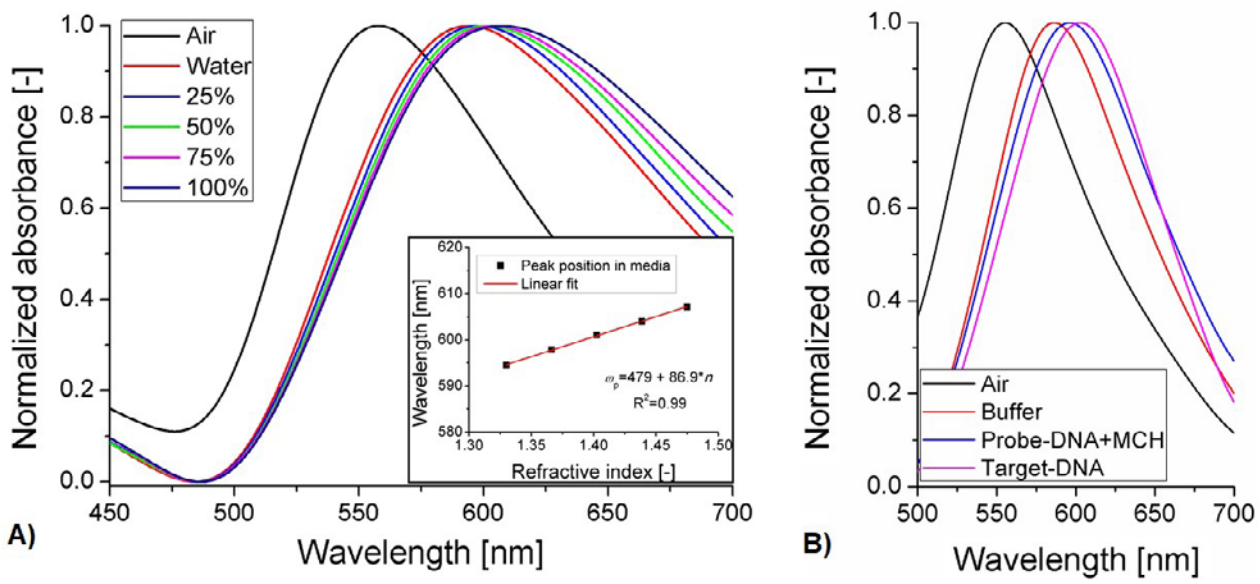

Figure 4. A) LSPR absorbance spectra of a nanocomposite sensor in different calibration media (sucrose solution). Insert: the corresponding peak positions and linear fit. B) The response of the sensor to probe- and target single-stranded DNA, respectively. (5)

Nucleotide sensor tests were performed by using 20 bases long probe- and target-DNA molecules that form a specific sequence from the parasite *Giardia lamblia* (the β-giardin gene) (6). The DNA base sequences are the following (from 5′ to 3′): probe-DNA: CGTACATCTTCTTCCTTTTT-[ThiC6]; target-DNA: AGGAAGAAGATGTACGACCA. We used the same DNA functionalization protocols, which were tested in a previous work (7), including 1) overnight probe immobilization from 1 µM solution; 2) 30 min 6-mercapto-1-hexanol (MCH) treatment after probe immobilization to reduce nonspecific binding of DNA on the gold surface, 3) 2 h binding of target-DNA from a 1 µM solution. As shown in Fig. 4. B), these treatments caused a redshift of 9.4 ± 0.8 nm after probe immobilization and 6.6 ± 0.7 nm after target hybridization. As demonstrated in our paper (5), with these protocols, it was possible to reach a limit of detection around 5 nM for this 20 bp long target DNA.

SERS

Surface-enhanced Raman spectroscopy measurements were performed with a Renishaw InVia Raman spectrometer at 785 nm laser wavelength on a nanocomposite sample with an acquisition time of 10 s. Fig. 5 shows the obtained SERS spectrum of a 20 base pair long ds-DNA bound on the gold NPs. The observed

peaks correspond well with Raman bands previously associated with the bases (adenine, cytosine, guanine, and thymine) or the sugar-phosphate backbone (deoxyribose) (8,9). With the Raman spectroscopy experiments we have found that by optimizing the fabrication parameters and maximizing the interparticle coupling, the characteristic Raman intensities of the DNA molecules could be increased by more than 2.5 orders of magnitude. With the optimized geometrical parameters the plasmonic sensor elements demonstrated an analytical SERS enhancement factor of around 105 (10).

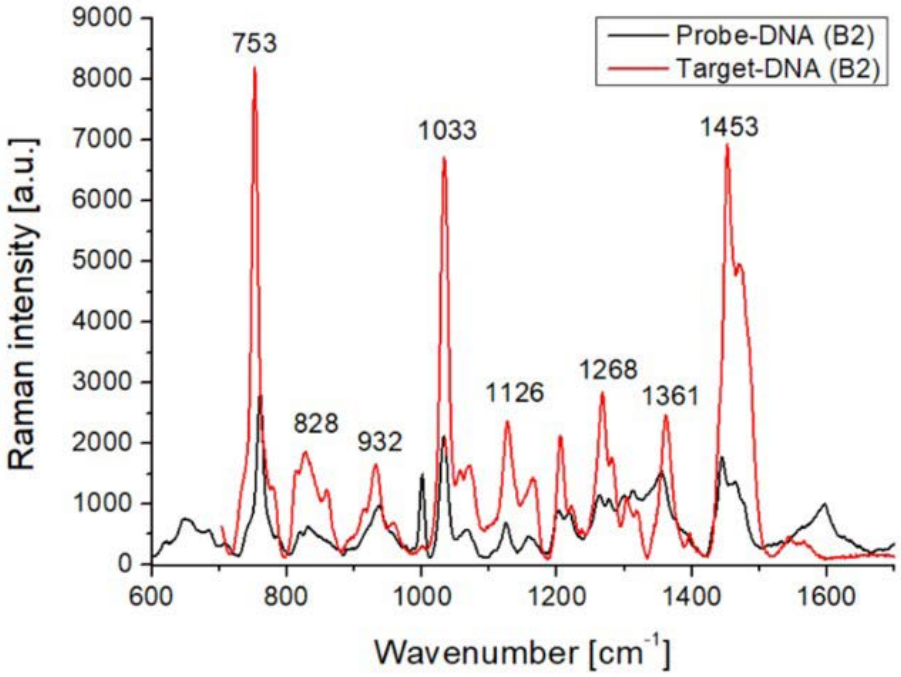

Figure 5. SERS spectrum of DNA molecules after probe-DNA immobilization and subsequent target binding, measured on a B2 type gold-epoxy nanocomposites obtained with 785 nm excitation (10).

Conclusions

Two application areas were demonstrated for the introduced gold/epoxy surface nanocomposites, namely LSPR sensors and SERS substrates. Our proposed fabrication technology has the following distinct advantages compared to other technologies: 1.) Controlled synthesis: the particle size and interparticle distance can be precisely controlled in a fixed hexagonal distribution, and thus the plasmonic absorption peak (and sensitivity) can be fine-tuned for individual applications (which is important to both LSPR and SERS). 2.) Large scale fabrication: the lateral size of the substrate is not limited, sensors with several cm^2 surface area can be easily prepared, and the nanoparticle size/distribution is homogenous on the whole surface. 3) Robustness: the prepared nanocomposite – gold nanoparticle arrangement on fixed on epoxy pillars – is completely stable, there is no particle removal exposed to fluidic environments. More detailed information regarding the fabrication and application of these surface nanocomposites can be found in our previous publications (1,4,5).

Acknowledgments

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Attila BONYÁR, is an associate professor and head of the Nanotechnology laboratory at the Department of Electronics Technology, Faculty of Electronics Engineering and Informatics, Budapest University of Technology and Economics. He has two M.Sc. degrees in electrical engineering (2009) and biomedical engineering (2011), a Ph.D. (2014), and habilitation (2021) in electrical engineering. He is the chair for IEEE Nanotechnolgy Council's Nanopackaging Technical Committee (since 2022, co-chair since 2020) and he is also the chair of the IEEE Hungary&Romania EPS&NTC Joint Chapter since 2019. His current research activities are focused on the development of optical biosensor technologies utilizing plasmonics, low-dimensional nanomaterials, nanocomposites and nanometrology.

Shereen ZANGANA, received her BSc (2008) and MSc (2015) degrees in laser & optoelectronics engineering at Al-Nahrain University, Baghdad, Iraq. Between 2018-2019 she was vice head of medical instrumentation techniques at Al-Farahidy University. Since 2019 she has been a PhD student at the Department of Electronics Technology, Budapest University of Technology and Economics, under the supervision of Attila Bonyár. Her main research area is plasmonic sensors and Raman-spectroscopy.

Tomáš LEDNICKÝ, is a postdoc at Central European Institute of Technology, Brno University of Technology. He has received M.Sc. degree in Physical Engineering and Nanotechnology (2014) and Ph.D. in Advanced Nanotechnologies and Microtechnologies (2021). His research activities are focused on the development of fabrication technologies for low-dimensional nanomaterials for plasmonic applications utilising self-assembly properties of porous anodic alumina.

Prof. Dr. László JAKAB, is a university professor at the Faculty of Electrical Engineering and Informatics at the Technical and Economic University of Budapest (BME). Studies at the BME: 1977–1981 (electrical engineer), 1985: university doctor, 1998: PhD title. 2013: Habilitation Doctor, 2014: University Professor, 2014: Doctor of the Hungarian Academy of Sciences (DSc). Main research areas: Research and development of acoustic-optical elements and systems, surface acoustic wave elements, integrated optical elements and systems, optical signal processing systems, automatic optical test systems and electronics technology, including automatic optical inspection and production.