UNIVERSAL PLATFORM FOR SERS DETECTION OF "NORMAL" AND "TUMOR" CELLS CULTIVATION MEDIA

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Abstract

Surface-enhanced Raman scattering (SERS) has become an attractive analytical tool for recognition of various biomolecules and bio-objects. In this work, we created a universal platform, based on surface modified gold multibranched nanoparticles (AuMs), plasmon coupling between surface plasmon polariton waves (SPP) and localized surface plasmon (LSP), and utilization of advanced statistical methods for analysis and estimation of SERS spectra, measured on the „tumor" cells cultivation media. At the first stage, we used the AuMs which have an extremely high number of plasmonic hot spots per single nanoparticle and provide excellent SERS signal intensification. The surface of AuMs was decorated with charged organic functional moieties to entrap various (bio) molecules from targeted solution. Chemical and morphological structures of the prepared nanoparticles were examined with different techniques such as SEM-EDX and Raman spectroscopy. The decorated AuMs were placed in the cultivation media of melanoma and fibroblast lines in vitro. After interaction with cultivation media the AuMs were drop-deposited on the periodical gold grating, to achieve SPP-LSP coupling and even more enhance the SERS signal from entrapped (bio)molecules. Raman spectra were collected from several kinds of melanoma cells lines and evaluated using the developed mathematical route.

Keywords: Gold nanoparticles, SERS detection, cultivation media, arenediazonium tosylates

1. INTRODUCTION

SERS spectroscopy is a well-known analytical tool [1-4], that offers a number of advantages with other methods of analysis such as Fourier transform infrared (IR) spectroscopy, UV-vis absorption, fluorescence, nuclear magnetic resonance (NMR) and others [5-7]. Also SERS has become an attractive analytical tool for recognition of various biomolecules especially in tandem with plasmonic gold multibranched nanoparticles (AuMs) [8].

AuMs comparing to other nanoparticles (NPs) are famous with their biocompatibility, chemical stability, and plasmon tunability [8,9]. Moreover, they have controllable synthesis, easy surface modification, high molar absorption coefficient and plasmon peaks that are tunable into the near-infrared (NIR) region. It means that AuMs are ideal analysis tools for biochemical assays [9].

However present of the art state of SERS does not allow performing the analyses of the "big" biomolecules, which represent one of central, but up-to-now unsolved challenge in this field. Many attempts to solve this question were performed through the creation of sophisticated SERS substrates (where the targeted molecule is sandwiched between the metal surface and metal nanoparticles) to enhance the SERS and prevent the Raman peak interference; specific functionalization of SERS-active surfaces (with the aim to reach the very definite "specific' entrapping of biomolecules and minimize unspecific sorption); or introduction of advanced algorithm of SERS data analysis. In this study, we aimed to decorate AuMs with charged organic functional

moieties to entrap (bio) molecules from the cultivation media of melanoma and fibroblast lines and collect Raman spectra from several kinds of melanoma cells lines and evaluated using the developed non-linear mathematical route (DMR). To our knowledge this is the first report of detection melanoma and fibroblast lines with DMR. Our results indicate that discrimination of a complex biological systems is possible with the implementation of advanced data evaluation.

2. EXPERIMENTAL

2.1. Materials

Diethyl ether, deionized water, methanol (purist, p.a., absolute, ≥99.8 % (GC)), Chloroauric acid tetrahydrate (HAuCl4·4H2O, 99.9 %), silver nitrate (AgNO3,99.0 %), ascorbic acid (AA, 99.0 %), p-toluenesulfonic acid, acetic acid, terc-butyl nitrite, 4-nitroaniline, 4-aminobenzoic acid (+ 99 %), 4-aminophthalic acid (97.0 %), all were purchased from Sigma-Aldrich and used without further purification.

2.2. Gold grating preparation

Su-8 films were spin-coated onto freshly cleaned glass substrates and patterned using the linearly polarized excimer laser irradiation according to the procedure described in [10]. Gold thin films were deposited onto the patterned surface by vacuum sputtering.

2.3. Gold nanoparticles preparation

Gold nanoparticles (AuMs) were synthesized by the seed-mediated growth method with some modifications [11]. Briefly, seed solution was prepared by mixing boiling HAuCl4·4H2O solution (1 mM, 100 ml) with 1 % citrate solution (15 ml) under vigorous stirring. The solution was boiled 15 min, cooled, filtered by a 0.22 μm nitrocellulose membrane and kept in a dark, cold place. Nanostars were prepared by adding the seed solution (100 μl) to HAuCl4 solution (0.25 mM, 10 ml) with HCl (1 M, 10 μl). After that AgNO3 (20 mM, 100 μl) and AA (100 mM, 50 μl) were added simultaneously. After color change AuMs were immediately centrifugal washed [12].

2.4. Diazonium modification

4-Aminobenzenediazonium tosylate (ADT-NH2), 4-carboxybenzenediazonium tosylate (ADT-COOH) and 3,4-dicarboxybenzenediazonium tosylate ADT-(COOH)2 were prepared according to the published procedure [13,14]. AuMs were spontaneously modified due to the published procedure [12].

2.5. Entrapping of biomolecules expressed by the normal and tumor-associated cell lines

Decorated AuMs were placed in the 0.35 mkl of analyte (cultivation medium, cultivation medium from health cells, cultivation medium from melanoma associated cells) and put in a cold, dark place for 3 hours. After that there were taken drops of each analyte and placed on the gold grating. When drops were dried SERS spectra were measured immediately.

3. RESULTS AND DISCUSSION

A schematic representation of the multibranched gold nanoparticles (AuMs) synthesis and surface modification is showing in **Figure 1**.

Prepared AuMs were decorated by several arenediazonium tosylate salts (ADT-NH_2, ADT-COOH, ADT-$(COOH)_2$) with the aim of changing the surface charge of the NPs and introducing functional groups. The AuMs shape and size distribution were examined by TEM after preparation, modification and purification procedures.

The results are presented in **Figure 2**. It is apparent that modification of AuMs did not change the shape of nanoparticles, which contain a great number of plasmonic hot-spots - sharp edges.

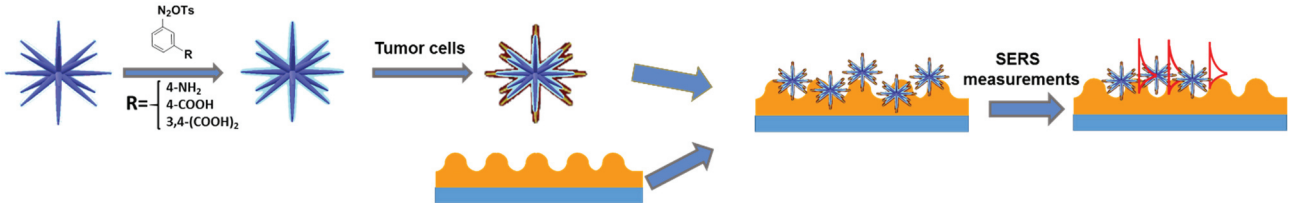


Figure 1 Schematic representation of AuMs synthesis and surface modification

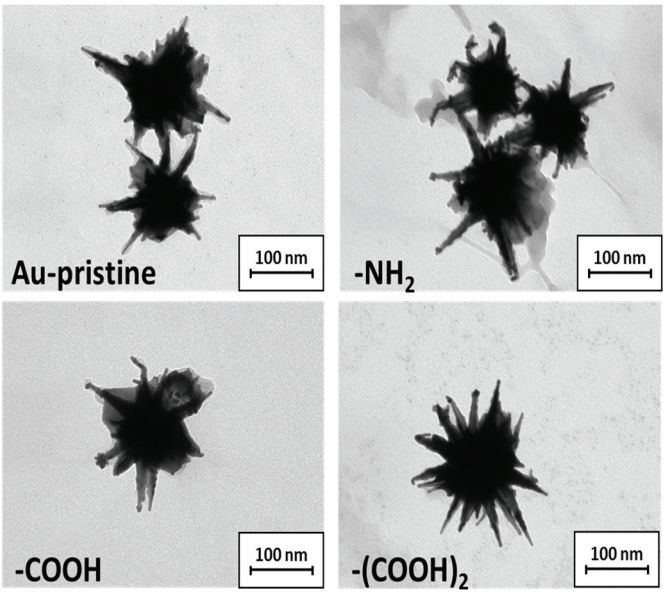
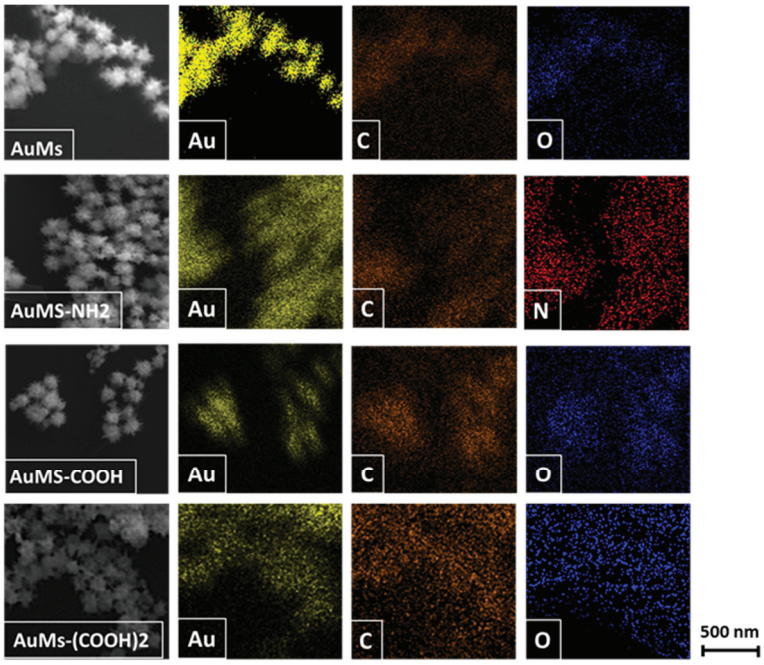


Figure 2 TEM images of modified gold multibranched nanoparticles. The scale bar is 100 nm.

The results of AuMs surface modification and organic functional group (OFG) grafting were also checked by SEM-EDS (**Figure 3**). EDS analysis confirmed the presence of OFGs characteristic elements on the NP surfaces with a spatial distribution correlating to the physical positions of AuMs on the SEM scans (**Figure 3**). The element ratio values (presented in the Table below **Figure 3**) correlate well with the composition of the attached OFGs.

Figure 4 presents the absorption spectra of the gold grating where the apparent plasmon-polariton absorption band at 780 nm is well visible (i.e. the effective excitation of SPP takes place). Additionally, **Figure 4** shows the absorption spectra of pristine and modified AuNPs. As evident, the main plasmon absorption peak of AuNPs is also located near the 780 nm. It must be also noted that used AuMs have an extremely high number of plasmonic hot spots per single nanoparticle that provide excellent SERS signal intensification, which can be further enhanced through the SPP wave, after the deposition of AuNPs on the gold grating surface.

After interaction with cultivation media the AuMs were drop-deposited on the periodical gold grating, to achieve surface plasmon polariton waves - localized surface plasmon (SPP-LSP) coupling and even more enhance the SERS signal from entrapped (bio)molecules. Raman spectra were collected from several kinds of melanoma cells lines and evaluated using the developed mathematical route. The SERS spectra of line A2058 tumor melanocytes are presented in **Figure 5**. As is evident, the as-measured SERS spectra do not contain any apparent Raman band and the evaluation of results seems to be complicated. However, utilization of non-linear mathematical algorithm provide an possibility to effectively process the Raman signal and clearly distinguish the spectral areas, attributed to the different kinds of (bio) molecules.



Element	Apparent Concentration, wt%			
	Au-Ms	AuMs-NH2	AuMs-COOH	AuMs-(COOH)2
C	8,8	19,1	21,5	23,8
N	-	3,7	-	-
O	0,9	-	3,2	3,5
Au	90,3	77,2	75,3	72,7

Figure 3 SEM-EDS analysis of pristine and modified (-NH2, -COOH, -(COOH)2) AuMS images of modified gold multibranched nanoparticles. The weight concentrations of organic elements

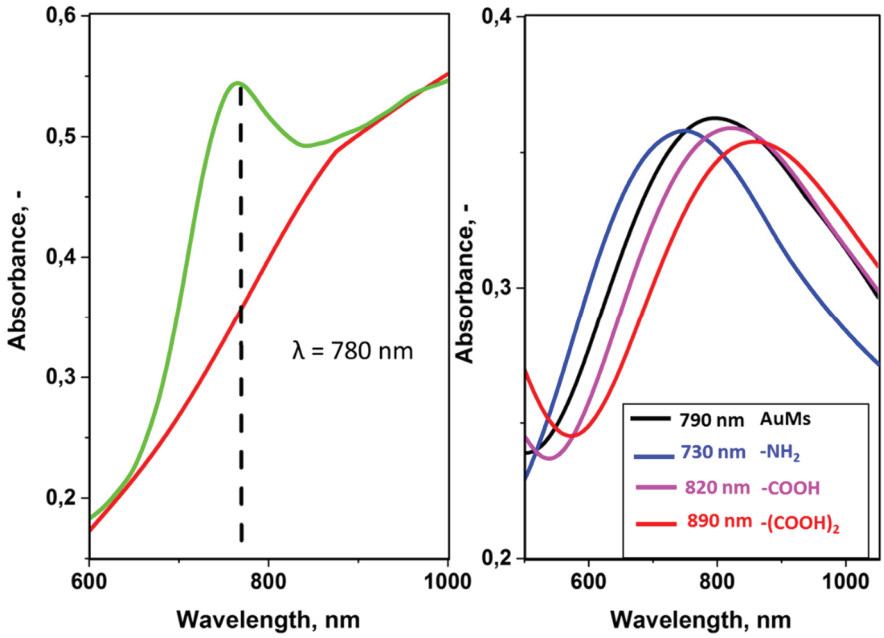


Figure 4 UV-Vis absorption spectra of the gold grating and pristine and modified (AuMs-NH2, AuMs-COOH, AuMs-$COOH)_2$) NPs

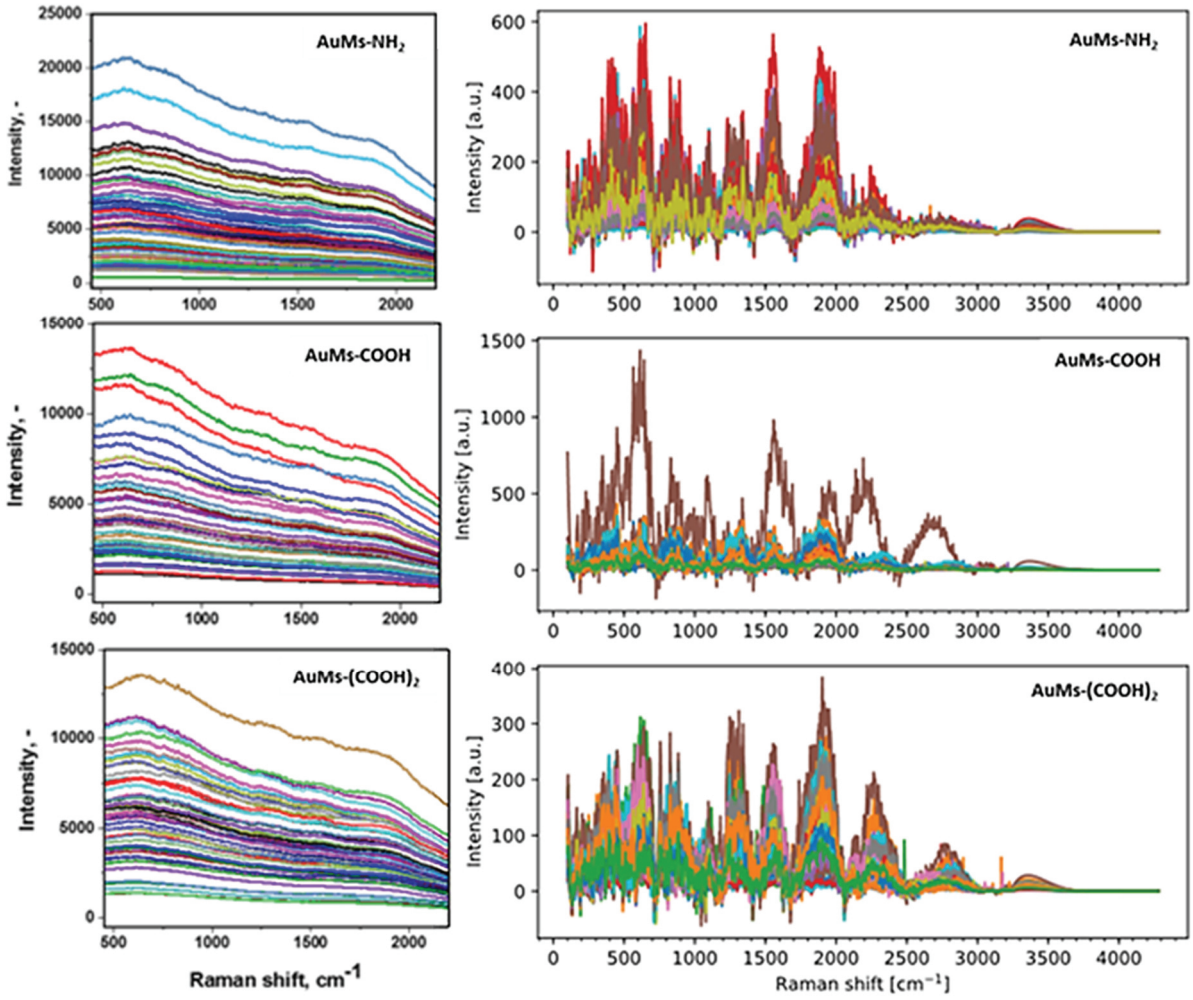


Figure 5 SERS spectra of line A2058 tumor melanocytes deposited on the AuMs decorated with -NH2, -COOH, -(COOH)2 organic groups to entrap (bio)molecules from solution (excitation wavelength - 785 nm)

4. CONCLUSION

We have developed and analytical route to apply the SERS spectroscopy for the analysis of sophisticated (bio)objects. Proposed procedure includes the combination of gold nanoparticles surface functionalization, utilization of plasmonic coupling and advanced Raman spectra evaluation using non-linear mathematical algorithms. In particular, we analysis the melanoma lines with DMR using Raman spectra of decorated AuMs with charged organic functional moieties and perform the transition from the initially complexed spectrum to the clearly distinguishable Raman information.

ACKNOWLEDGEMENTS

This work was supported by the Grant Agency of Health Ministry 15-33459A.

REFERENCES

[1] Martin. Surface-enhanced Raman Spectroscopy: A Brief Retrospective. Journal of raman spectroscopy journal of raman spectroscopy. 2005. vol. 36. pp. 485-496

[2] KNEIPP, Katrin. Surface-enhanced Raman scattering. Physics Today. 2007. vol. 60. pp. 40-46

[3] BRUS, Louis. Noble Metal Nanocrystals: Plasmon Electron Transfer Photochemistry and Single-Molecule Raman Spectroscopy. Accounts of Chemical Research. 2008. vol. 41. pp. 1742-1749

[4] STILES, Paul L., DIERINGER, Jon A., SHAH, Nilam C., and VAN DUYNE, Richard P. Surface-Enhanced Raman Spectroscopy. Annual Review of Analytical Chemistry. 2008. vol. 1. pp. 601-626

[5] PETRY, Renate, SCHMITT, Michael and POPP, Jürgen. Raman Spectroscopy--a Prospective Tool in the Life Sciences. *Chemphyschem: A European Journal of Chemical Physics and Physical Chemistry*. 2003. vol. 4. pp. 14-30

[6] BAENA, Josefa R. and LENDL, Bernhard. Raman Spectroscopy in Chemical Bioanalysis. Current Opinion in Chemical Biology. 2004. vol.8. pp. 534-539

[7] ABALDE-CELA, Sara, ALDEANUEVA-POTEL, Paula, MATEO-MATEO, Cintia, RODRÍGUEZ-LORENZO, Laura, ALVAREZ-PUEBLA, Ramón A. and LIZ-MARZÁN, Luis M. Surface-Enhanced Raman Scattering Biomedical Applications of Plasmonic Colloidal Particles. Journal of the Royal Society Interface. 2010. vol. 7, pp. 435-450

[8] YUAN, Hsiangkuo, KHOURY, Christopher G., HWANG, Hanjun, WILSON, Christy M., GRANT, Gerald A.and VO-DINH, Tuan. Gold Nanostars: Surfactant-Free Synthesis, 3D Modelling, and Two-Photon Photoluminescence Imaging. Nanotechnology. 2012. vol. 23. pp. 075102

[9] CHEN, Yiping, XIANYU, Yunlei and JIANG, Xingyu. Surface modification of gold nanoparticles with small molecules for biochemical analysis. Accounts of chemical research. 2017: vol. 50.2. pp. 310-319

[10] SVANDA, J., KALACHYOVA, Y., SLEPICKA, P., SVORCIK, V., & LYUTAKOV, O. Smart component for switching of plasmon resonance by external electric field. ACS applied materials & interfaces. 2015. vol. 8(1). pp. 225-231

[11] KALACHYOVA, Yevgeniya, MARES, David, JERABEK, Vitezslav, ULBRICH, Pavel, LAPCAK, Ladislav, SVORCIK, Vaclav and LYUTAKOV, Oleksiy. Ultrasensitive and reproducible SERS platform of coupled Ag grating with multibranched Au nanoparticles. Physical Chemistry Chemical Physics. 2017. vol. 19(22). pp. 14761-14769.

[12] KALACHYOVA, Yevgeniya, OLSHTREM, Anasiya, GUSELNIKOVA, Olga, POSTNIKOV, Pavel, ELASHNIKOV, Roman, ULBRICH, Pavel, RIMPELOVA, Silvie, ŠVORČÍK, Václav and Lyutakov, Oleksiy. Synthesis, Characterization, and Antimicrobial Activity of Near-IR Photoactive Functionalized Gold Multibranched Nanoparticles. 2017. ChemistryOpen. vol.6(2). pp. 254-260

[13] GUSELNIKOVA, Olga, POSTNIKOV, Pavel, ELASHNIKOV, Roman, TRUSOVA, Marina, KALACHYOVA, Yevgeniya, LIBANSKY, Milan, BAREK, Jiri, KOLSKA, Zdenka, ŠVORČÍK, Vaclav and LYUTAKOV, Oleksiy. Surface modification of Au and Ag plasmonic thin films via diazonium chemistry: Evaluation of structure and properties. 2017. Colloids and Surfaces A: Physicochemical and Engineering Aspects. vol. 516. pp.274-285

[14] GUSELNIKOVA, O., POSTNIKOV, P., ERZINA, M., KALACHYOVA, Y., ŠVORČÍK, V., and LYUTAKOV, O. Pretreatment-free selective and reproducible SERS-based detection of heavy metal ions on DTPA functionalized plasmonic platform. Sensors and Actuators B: Chemical. 2017. vol. 253. pp. 830-838