Microwave-assisted synthesis of surface-enhanced Raman scattering nanoprobes for cellular sensing

By: Kristen Bowey, Jean-François Tanguay, Marinella G. Sandros, Maryam Tabrizian

Abstract:

The fabrication of 4-mercaptobenzoic acid (4-MBA) antibody-functionalized gold nanoparticles *via* microwave technology for surface-enhanced Raman scattering (SERS)-based cellular nanosensing is reported. Nanoprobes were characterized by UV‑vis absorbance, Raman scattering properties, and observed by TEM imaging. Results showed that microwave irradiation rapidly yielded nanoprobes with significant Raman scattering intensity and suitable stability to support antibody conjugation in under 10 min. Functionalized nanoprobes demonstrated the ability to map the expression of vascular adhesion molecule-1 (VCAM-1) in human coronary artery endothelial (HCAE) cells, indicating that microwave fabrication presents a viable and rapid approach to SERS nanoprobe construction. The successful application of SERS nanoprobes to localize biomarker expression *in vitro* may ultimately be used for early diagnostic and preventative functions in medicine.

Graphical abstract
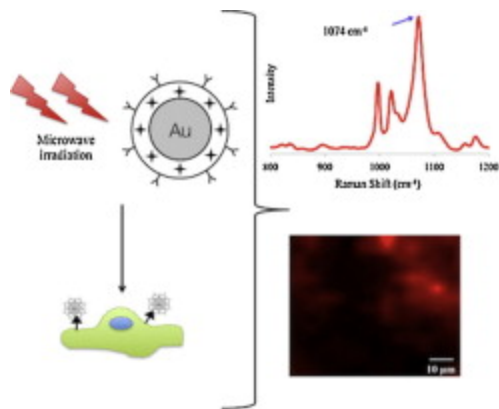
Article:

1. Introduction

Surface-enhanced Raman scattering (SERS) detection using functionalized tags is emerging as a valuable analytical tool in the field of cellular biosensing owing to enhanced signal intensities, up to 10^{13}-10^{14} fold[1]. This dramatic signal enhancement is dominated by localized surface plasmon resonance (LSPR)[2] and [3]. SERS has been applied to label cells[4], detect various analytes, including dyes [5], proteins[6] and biomarkers [7], as well as monitor subtle cellular fluctuations, such as the effect of pH [8] and distinguishing between cell type [9] and [10]. When combined with confocal microscopy, SERS-based detection yields rich spectral characterization data and spatial resolution of biomolecules or targets of interest down to the single cell level [11].

Advantages of SERS compared to conventional detection and optical imaging techniques, such as fluorescence or enzyme-linked immunosorbant assays, include lack of photobleaching, narrow spectral bandwidths, the capability to simultaneously provide structural information and spatial resolution, as well as perform multiplex analysis [12]. SERS signal enhancement occurs when molecules are adsorbed or in close proximity to surface-roughened noble metallic nanostructures. For indirect sensing, this phenomenon can be exploited by fabricating SERS nanoprobes on the order of 20-300 nm that support LSPR. Typically, these probes are composed of gold, silver, copper or a combination thereof with common modifications such as: studding with Raman reporters and attaching antibodies or aptamers to enhance target selectivity [13] and [14]. For target biorecognition, the chemical and physical stability of SERS probes is heavily affected by the percent surface coverage of reporter molecules [15] and coating with a protective shell (*e.g.* polymer) [16]. Typical Raman reporters include nitrogen- and sulfur-containing cationic dyes, or thiol small molecules. For biological applications, thiol modified-probes are best for chemisorption, since they are active in the NIR region and produce significant scattering signals due to the formation of self-assembled monolayers (SAMs) on the surface of nanosubstrates [13].

However, inherent instabilities and aggregation associated with the formation of SAMs can require rigorous optimization and deter successful application *in vitro* [17]. Herein, these challenges have been addressed by investigating the feasibility of microwave technology as a means to facilitate chemisorption of the thiol small molecule to metallic nanostructures. Microwave heating can serve to increase molecular rotations and speed up transfer between molecules resulting in a significant reduction in reaction time for diverse applications [18]. Recently, Grell et al. (2013) demonstrated that the formation of SAMs on thin gold films using selective microwave heating was comparable to the formation of SAMs at room temperature, albeit at a significantly reduced time frame [19]. Therefore, we hypothesized that microwave technology could be used to rapidly fabricate SERS nanoprobes suitable for biomolecule functionalization, with applications as biological nanosensors. Although other groups have employed microwave technology to fabricate SERS substrates[20] and [21], this work establishes for the first time, application toward Raman reporter SAM construction and demonstrated application for biomarker detection.

Recent evidence has highlighted the role of the immune system in the initiation of atherosclerosis[22] and [23], a cardiovascular disease (CVD) characterized by a build-up of plaque in arteries. Consequently, various inflammatory adhesion molecules and cytokines have been investigated as a means to detect and localize plaque during early stages of evolution [24], [25] and [26]. Of particular interest is vascular cell adhesion molecule-1 (VCAM-1), which is upregulated upon activation of endothelial cells [27]. Accordingly, a robust technique to detect and localize VCAM-1 on endothelial cells could provide a practical means for early diagnosis and treatment of atherosclerosis, prior to the onset of clinical symptoms.

In the present work, the preparation SERS nanoprobes consisting of 50 nm citrate-capped gold nanoparticles (Au-NPs) coated with the Raman reporter, 4-mercaptobenzoic acid (4-MBA), *via* microwave irradiation is described. For biorecognition and mapping of VCAM-1 in human coronary artery endothelial (HCAE) cells, probes were functionalized with anti-VCAM-1, as depicted schematically in Fig. 1A. To provide a surface for bioconjugation, a protective layer of the cationic polymer, poly(allylamine hydrochloride) (PAH), was coated on the surface of the nanoprobes. Resultant amine modified nanoprobes were activated with glutaraldehyde to bind anti-VCAM-1. The optical absorbance, SERS activity, and structural properties were examined to characterize the nanoprobes. Control data from nanoprobes prepared *via* a conventional SAM formation technique is included to validate the microwave radiation technique. Finally, engineered SERS nanoprobes were tested for their ability to detect and map the VCAM-1 expression in HCAE cells using confocal Raman spectroscopy, represented in Fig. 1B.
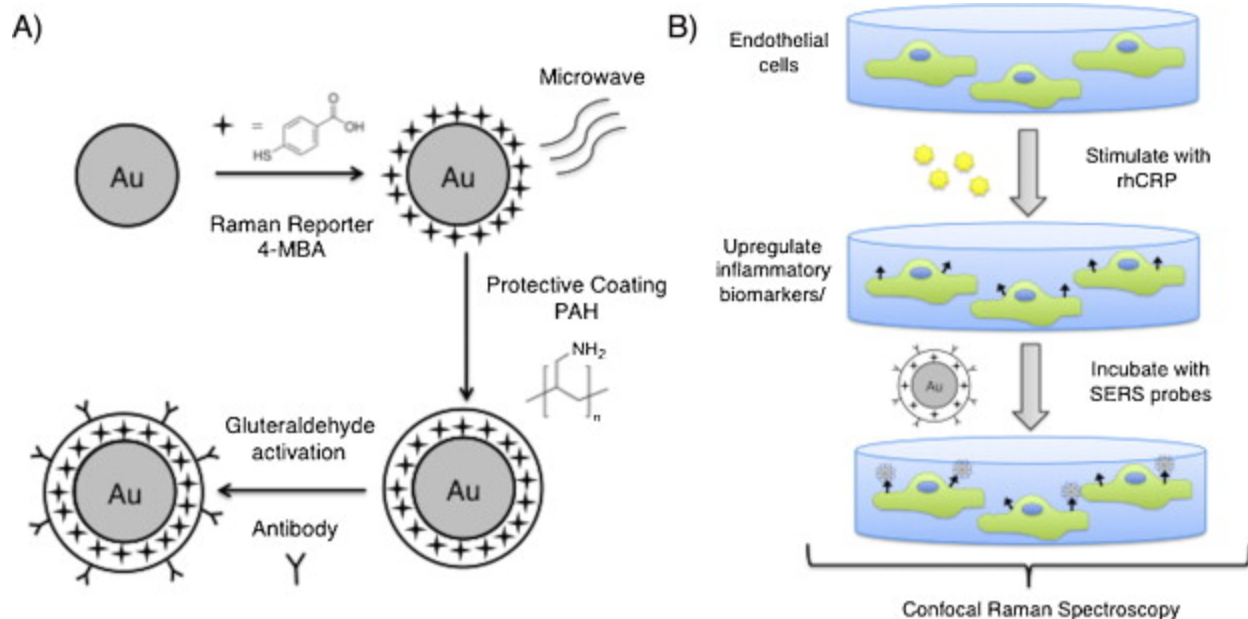
Fig. 1. (A) Fabrication of antibody-conjugated SERS nanoprobes *via* microwave irradiation. (B) Experimental approach to detect inflammatory biomarkers in HCAE cells using SERS nanoprobe technology.

2. Materials and methods

2.1. Materials

Citrate-stabilized gold nanoparticles (3.51×10^{10} particles/mL), 4-mercaptobenzoic acid (4-MBA), 25% gluteraldehyde solution, and poly(allylamine hydrochloride) (PAH, MW 15 kDa) were purchased from Sigma‑Aldrich (St. Louis, USA). Recombinant human C-reactive protein (CRP), anti-VCAM-1 and mouse IgG-1 antibodies were purchased from Fisher Scientific (Rockford, USA). Ultra pure water (UPW) from a Millipore filtration system was used for all experiments. All other chemicals were analytical grade.

2.2. Preparation of antibody-conjugated SERS nanoprobes

Au-NPs at room temperature were mixed with 50 mM of 4-MBA under vigorous stirring and reacted in an open vessel under microwave irradiation with a power of 50 W (2.45 GHz) and temperature of 50 °C for 10 min (Discover, CEM Corporation). Excess 4-MBA was removed by washing for 3 rounds of centrifugation at 3000 rpm for 20 min. Next, the SERS-active probes were added drop-wise to a 1 mg/mL solution of PAH and reacted for 3 h. Excess PAH was removed by repeated rounds of centrifugation (3000 rpm, 15 min). To functionalize antibodies onto the surface of the probe, the particles were activated using the glutaraldehyde spacer method. After washing, SERS nanoprobes were rehydrated with a 5% glutaraldehyde solution in borate buffer (pH 9.2) and incubated under gentle rotation for 1 h. Afterwards, anti-VCAM-1 monoclonal antibodies were added to the activated SERS nanoprobe suspension and reacted for

2 h at room temperature. For negative controls, the same procedure was used to prepare SERS NPs conjugated to non-specific murine IgG-1 antibodies. Conventional 4-MBA adsorption was carried out by dropping a solution of 4-MBA into a suspension of Au-NPs, under vigorous stirring. The reaction was carried out overnight at room temperature, since shorter time periods were not sufficient to produce significant Raman scattering signals. Thereafter particles were washed, as described above for the microwave technique. Ultrapure water (UPW) from a Millipore filtration system (resistivity above 18 m Ω) was used for all reactions and washing steps, expect for the glutaraldehyde activation, which was carried out in borate buffer (pH 9.2).

2.3. SERS nanoprobe characterization

Ultraviolet‑visible (UV‑vis) absorption spectra of aqueous nanoparticle suspensions were measured by a Cary Eclipse Fluorescence spectrophotometer (Varian, Palo Alto, USA) with quartz cuvettes of 1 cm path length. Visualization of the shape and surface structure of nanoprobes was carried out by transmission electron microscopy (TEM). Briefly, 10 μL of nanoparticle suspension was dropped onto a carbon-coated copper grid. The sample was then air-dried overnight. Imaging was performed with a Carl Zeiss Libra 120 Plus TEM Microscope operating at an accelerating voltage of 120 kV. Mean particle size was determined by Nanosight measurements, which utilizes nanoparticle tracking analysis technology (NanoSight, North Carolina, USA). The zeta potential (ζ-potential) of colloidal solutions was measured using a ZEN3600 Zetasizer Nano-ZX (Malvern Instruments, Worcestershire, United Kingdom).

2.4. Cell culture and labeling with SERS nanoprobes

HCAE cells used in cell culture experiments were grown in tissue culture flasks, maintained in 5% CO_2 at 37 $^\circ$C. The culture medium was prepared using an EGM-2 BulletKit containing Endothelial Basal Medium-2, 10% FBS, and EGM-2 SingleQuots comprised of appropriate growth factors, cytokines and supplements to support cell growth (Lonza, Basel, Switzerland). Cells to be used for experimentation were released with 0.25% trypsin/0.1% EDTA (Gibco, Invitrogen) and seeded at a concentration of 1 $\times$ 10^4 cells/cm^2 onto glass coverslips, which were placed in culture dishes. Cells were allowed to reach ~80% confluence prior to treatment.

2.5. Analysis of VCAM-1 expression by SERS

Prior to experimentation, sodium azide was removed from CRP preparations by dialysis using a Float-A-Lyzer® G2 device (Spectra/Por, molecular weight cutoff of 3.5‑5 kDa, Spectrum Laboratories Inc. USA). As a secondary purification step, contaminating lipopolysacharrides were removed using a Detoxigel column according to the manufacturer's instructions (Fisher Scientific). To upregulate the expression of the inflammatory biomarker, VCAM-1, by SERS, HCAE cells were treated with 25 μg/mL CRP. After 24 h of incubation, media was removed and cells were fixed with 3.7% paraformaldehyde. After washing, cells were blocked using 1% BSA for 20 min, then incubated with freshly prepared SERS nanoprobes (0.003 pM) for 4 h at

room temperature. Finally, cells were washed and examined under the Raman microscope. The following controls were used: non-specific IgG-1 SERS probes to assess non-specific binding and cells incubated without SERS tags to assess background cell scattering.

2.6. SERS microspectroscopy

An XploRA Raman confocal microscope system (JY Horiba, Edison, NJ) was used for all surface enhanced Raman scattering measurements. Spectra were collected on an inverted microscope with an ULWD 50× (0.55 NA) objective to focus the 785 nm laser with a power of 3 mW onto the sample. Rayleigh scattering was removed using a holographic notch filter. Spatial resolution was obtained using 100- and 200 μ m confocal pinholes. A Peltier-cooled CCD camera was used as detector. Peak frequencies were calibrated with silicon at 520 cm^{-1} prior to each use. Data was analyzed using LabSpec NGS. The integration time was 10 s for all SERS measurements.

3. Results and discussion

Microwave technology is of interest to the field of synthetic and colloidal chemistry since dielectric heating can significantly decrease reaction time and improve system reproducibility [28]. In fact, reactions typically requiring several hours can be reduced to a few minutes with the application of microwave heating [29]. Accordingly, since microwave technology increases molecular rotation and speeds up material transfer, we therefore sought to investigate whether microwave irradiation could be used in the synthesis of stable SERS nanoprobes intended for biosensing applications.

Studies on the optical properties of the nanoprobes demonstrated that the plasmon resonance absorption of 50 nm citrate-capped gold nanoparticles (Fig. 2A, curve a) shifted slightly from 535 to 536 nm after formation of 4-MBA SAMs by microwave irradiation (Fig. 2A, curve b). This small shift likely corresponds to changes in the dielectric medium surrounding the gold nanoparticle surface. Analysis of the SERS spectra of 4-MBA coated nanoparticles (Fig. 2B, curve b) shows that the dominant Raman band of 4-MBA, at around 1074 cm^{-1}, is also present after only 10 min of microwave irradiation compared to uncoated citrate-capped gold nanoparticles, which do not show any SERS activity (Fig. 2B, curve a) [30]. Typically, the reaction between gold nanoparticles and 4-MBA can take anywhere from 3 to 24 h [30], [31] and [32], however a microwave irradiation time of 10 min was shown to be sufficient to yield probes with suitable Raman scattering intensities for detection. Control probes fabricated conventionally by overnight incubation showed no significant changes in optical absorbance (Fig. 2A, curve c), or in Raman scattering data (Fig. 2B, curve c). Furthermore, no morphological changes were observed by transmission electron microscopy (TEM) between nanoprobes fabricated by microwave (Fig. 3A) and conventional adsorption (Fig. 3B), demonstrating that the probes do not undergo any damaging effects after microwave irradiation.
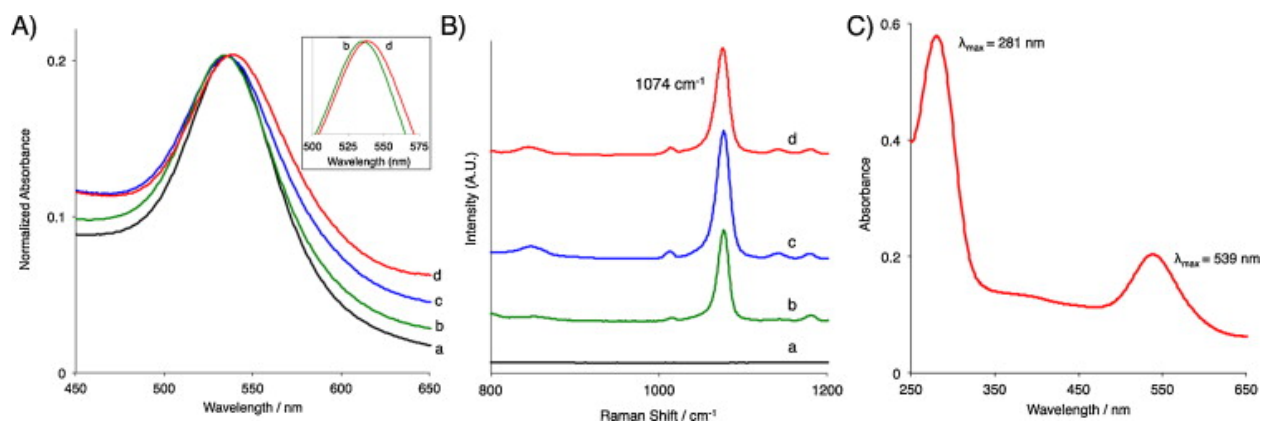
Fig. 2. (A) Visible absorbance of (a) citrate-capped Au-NPs, Au-NPs encoded with 4-MBA by (b) microwave and (c) conventional techniques, (d) anti-VCAM-1 studded SERS probes. (B) SERS spectra of (a) citrate-capped Au-NPs, nanoparticles coated with 4-MBA by (b) microwave and (c) conventional methods, (d) anti-VCAM-1 conjugated nanoprobes. (C) Expanded visible absorbance spectrum of anti-VCAM-1-conjugated SERS nanoprobes (λ_{max} = 281 nm, 539 nm).
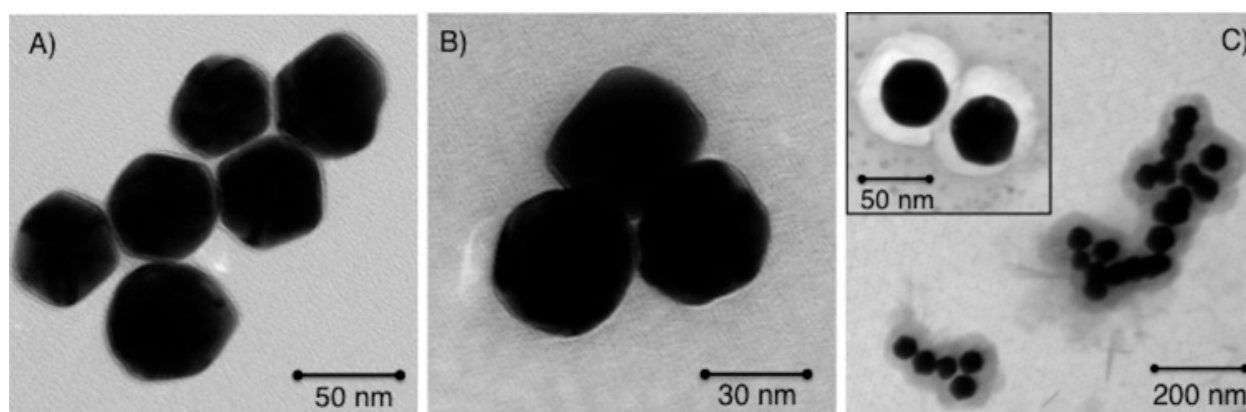

Fig. 3. TEM of 4-MBA coated nanotags prepared by (A) microwave irradiation, (B) conventional adsorption, (C) anti-VCAM-1-conjugated SERS nanoprobes prepared by microwave.

To test the biomarker targeting properties of the SERS nanoparticles, probes were conjugated with anti-VCAM-1, since VCAM-1 expression is a marker for the early onset of atherosclerotic plaque formation [33]. Following PAH coating and antibody functionalization by glutaraldehyde activation, nanotags prepared by microwave radiation exhibited a further redshift of ~3‑539 nm in optical absorbance (Fig. 2A, curve d). This is likely due to the presence of the antibody on the nanoprobe surface, which causes an increase in the local refractive index of the medium [34]. The extinction spectrum of anti-VCAM-1 biofunctionalized SERS labels also showed an absorption peak at 281 nm, representing the presence of the antibody after activation and conjugation (Fig. 2C). The characteristic peak of 4-MBA at 1074 cm^{-1} was likewise maintained after biofunctionaliztion (Fig. 2B, curve d), while TEM imaging of the final probes demonstrated the

presence of a PAH/anti-VCAM-1 shell (Fig. 3C). The mean particle size and ζ-potential of the particles recorded after the adsorption of 4-MBA *via* microwave irradiation and bioconjugation of the SERS nanoprobes are presented in Table 1. Results showed an increase in mean diameter of the particles after each step of the synthesis. The ζ-potential of the particles was recorded to be >10‑20 mV, the absolute value of which indicates adequate particle disparity and stability of the system [35]. Taken together, these results demonstrate the successful coupling of antibody to SERS active gold nanoparticles synthesized *via* microwave radiation, with reproducible SERS spectra and no loss of the characteristic 4-MBA Raman peak.

Table 1. Results of particle size and ζ-potential analysis of SERS nanoprobes fabricated by microwave irradiation. Data represent average $\pm$ standard deviation ($n = 3$).

Sample	Size (nm)	ζ-Potential (mV)	pH
Citrate-capped Au NPs	47 ± 33	−34 ± 2	7.8
4-MBA Au NPs	64 ± 43	−42 ± 6	7.8
Anti-VCAM-1/PAH/4-MBA Au NPs	119 ± 36	−39 ± 6	9.2

To demonstrate the functionality of developed SERS tags, antibody-functionalized SERS biosensors were employed to carry out detection of VCAM-1 in HCAE cells after CRP challenge. Recently, the authors have shown that CRP increases the inflammatory response in HCAE cells and specifically upregulates the expression of VCAM-1 [36]. Accordingly, cells were treated with CRP for 24 h to upregulate VCAM-1 expression, then fixed, blocked with 1% BSA, and incubated with anti-VCAM-1 SERS nanoprobes, as depicted schematically in Fig. 1B. SERS spectra from HCAE cells incubated with anti-VCAM-1 bioconjugated probes are shown in Fig. 4A. Spectra from three different intracellular locations, represented in the corresponding light micrograph (Fig. 4B), show the characteristic 4-MBA SERS peak at 1074 cm^{-1} indicating cellular binding of the nanoprobes after incubation and washing. Raman spectra were also measured on cells incubated with IgG-1-conjugated SERS nanoprobes as a non-specific control, as well as cells devoid of nanoprobes to assess background scattering. Relative to the anti-VCAM-1 bioconjugated probes, no signal was observed at 1074 cm^{-1} for cells incubated with IgG-1-conjugated SERS probes (Fig. S1A, Supplementary information), nor for cells lacking nanoprobes (Fig. S2A, Supplementary information), which were similarly collected from distinctive points on the cell (Fig. S1B and S2B, Supplementary information).
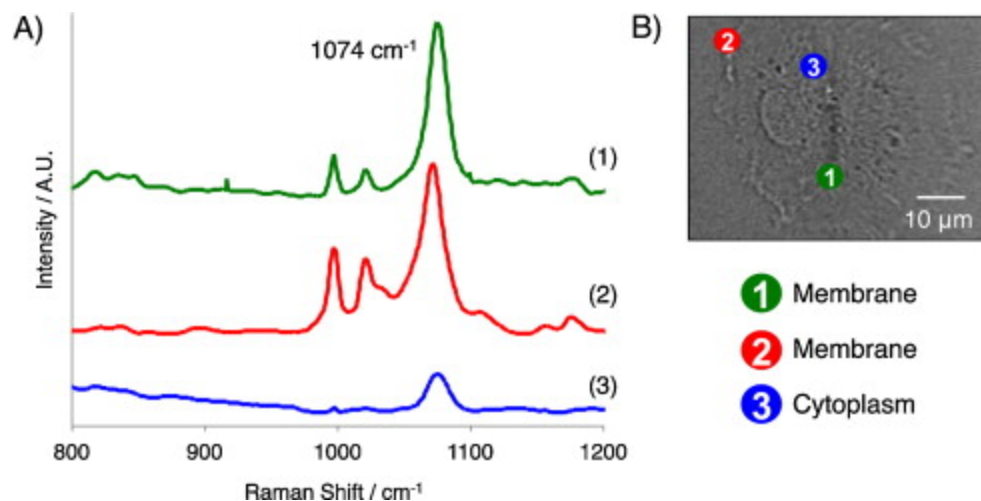
Fig. 4. (A) Representative SERS spectra of intracellular regions of HCAE cells incubated with anti-VCAM-1 SERS nanoprobes. (B) Light micrograph of HCAE cells incubated with anti-VCAM-1 SERS nanoprobes.

SERS maps at 1074 cm^{-1}, which is the most intense peak of 4-MBA, were then measured to assess the expression and distribution of VCAM-1. Fig. 5A shows a SERS map of anti-VCAM-1 nanoprobes on HCAE cells. In tandem with the light micrograph and overlay (Fig. 5B and C), the outer membrane of the cells appeared to be labeled with a dense population of nanotags as compared to probes functionalized with IgG-1 (Fig. S3, Supplementary information) and control cells devoid of nanoprobes (Fig. S4, Supplementary information), which showed limited non-specific adsorption and low levels of background scattering. These observations suggest that microwave-assisted SERS nanoprobes can successfully be applied to localize and detect VCAM-1 expression *in vitro*.
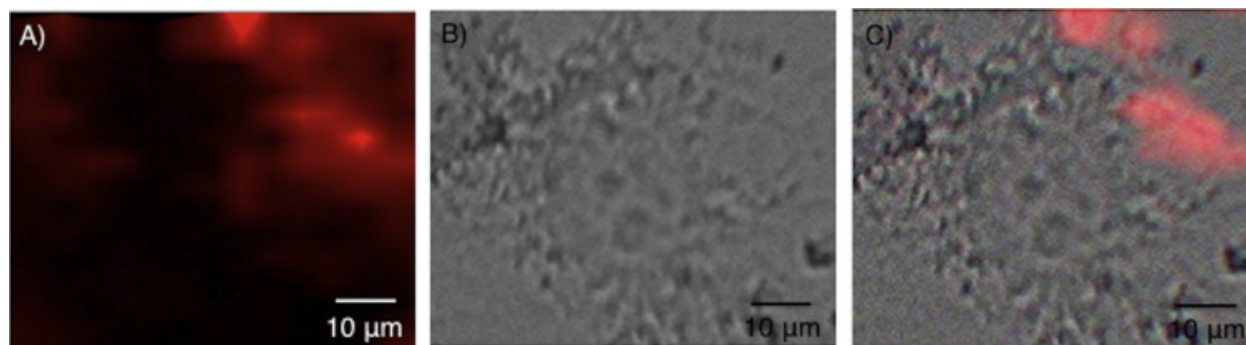

Fig. 5. (A) SERS map measured at 1074 cm^{-1} showing the cellular distribution of anti-VCAM-1 SERS nanoprobes. Corresponding (B) light micrograph and (C) overlay image.

A precise analytical technique for sensing and localizing the distribution of inflammatory cell adhesion markers, such as VCAM-1, is critical to diagnosis atherosclerosis and detect plaque in the early stages of formation [24]. Ideally, this technique would be non-invasive, non-destructive,

and provide both highly specific target identification, as well as localization information. Although traditional methods of biomolecule detection, such as fluorescence and quantum dots, can yield distribution information, surface-enhanced Raman scattering offers this dual advantage of both localization and accurate target identification. Furthermore, the quick and non-destructive nature of Raman spectroscopy makes it ideally suited for work in cells, with great potential for translation to *in vivo* models [37]. Despite these advantages, the complexity, time intensive, and sensitive nature of SERS nanoprobe construction can be a deterrent to more widespread use. Therefore, an alternative method to produce Raman-active nanoprobes using microwave technology is presented. Microwave technology serves to decrease reaction time for SAM construction, while yielding nanoprobes with suitable signal intensity and stability for functionalization. Compared to traditional heating, which relies on energy migration from the outside of the vessel (slow and non-specific transfer), during microwave heating the energy transfer occurs in fractions of a second (with each cycle of electromagnetic energy). This allows for a large amount of energy to be directly applied in a very efficient manner, resulting in rapid molecular rotations and therefore significantly reduces the time required for functionalization [18].

Overall, results presented in this paper indicated that microwave irradiation can be used to successfully fabricate SERS probes with appropriate stability and functional capabilities for antibody conjugation. The approach is versatile since it could be applied to fabricate SERS probes bearing various Raman reporter molecules, which is especially important in multiplex analysis [38]. Furthermore, microwave technology could be applied to prepare targeted SERS nanoprobes for a variety of applications, in addition to CVD biomolecule detection demonstrated in this work. For example, to trace intracellular drug delivery, distinguish cancerous cells, or measure cell viability. Future investigations will explore the microwave-assisted fabrication of probes for simultaneous multiplexing of cardiovascular disease biomarkers, which has the potential to significantly improve the prognosis of atherosclerosis and inflammatory-based diseases.

4. Conclusions

The synthesis, characterization, and *in vitro* application of a SERS-based nanoprobe fabricated *via* microwave technology for the analytical nanosensing of atherosclerotic biomarkers was presented in this paper. Nanoprobes were shown to exhibit strong SERS scattering signals after coating with Raman reporter, 4-MBA, *via* microwave, and were subsequently capable of straightforward functionalization with a protective polymer and target antibody. Using these microwave-assisted nanoprobes with surface-enhanced Raman spectroscopy, the detection and localization of cellular adhesion molecules in HCAE cell was demonstrated. Overall, results indicated that microwave technology can be a viable option to rapidly fabricate SERS nanoprobes for biomarker detection. This approach has potential for use as a simple and rapid fabrication technique in the burgeoning field of SERS-based nanosensing and immunoassays.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2014.07.040.

References

[1] S. Nie, S.R. Emory, Probing single molecules and single nanoparticles by surface-enhanced Raman scattering, Science 275 (1997) 1102–1106.

[2] E.C. Le Ru, P.G. Etchegoin, Principles of Surface-Enhanced Raman Spectroscopyand Related Plasmonic Effects, Elsevier, Amsterdam, 2009.

[3] E.C. Le Ru, P.G. Etchegoin, Basic electromagnetic theory of SERS, in: Surface-Enhanced Raman Spectroscopy: Analytical, Biophysical and Life ScienceApplications, Wiley-VCH, Weinheim, Germany, 2011.

[4] J.B. Song, J.J. Zhou, H.W. Duan, Self-assembled plasmonic vesicles of SERS-encoded amphiphilic gold nanoparticles for cancer cell targeting and traceableintracellular drug delivery, J. Am. Chem. Soc. 134 (2012) 13458–13469.

[5] B.V. Parakhonskiy, Y.I. Svenskaya, C. Yashchenok Acapital Em, H.A. Fattah, O.A.Inozemtseva, F. Tessarolo, R. Antolini, D.A. Gorin, Size controlled hydroxy-apatite and calcium carbonate particles: synthesis and their application astemplates for SERS platform, Colloids Surf. B Biointerfaces 118 (2014) 243–248.

[6] U.S. Dinish, C.Y. Fu, K.S. Soh, B. Ramaswamy, A. Kumar, M. Olivo, Highly sensitiveSERS detection of cancer proteins in low sample volume using hollow corephotonic crystal fiber, Biosens. Bioelectron. 33 (2012) 293–298.

[7] I. Kim, I. Junejo, M. Lee, S. Lee, E.K. Lee, S.I. Chang, J. Choo, SERS-based multi-ple biomarker detection using a gold-patterned microarray chip, J. Mol. Struct.1023 (2012) 197–203.

[8] T. Laurence, C. Talley, T. Huser, M. Colvin, Application of SERS nanoparticles tointracellular pH measurements, Biophys. J. 88 (2005) 553A.

[9] F.C. Pascut, S. Kalra, V. George, N. Welch, C. Denning, I. Notingher, Non-invasivelabel-free monitoring the cardiac differentiation of human embryonic stemcells in-vitro by Raman spectroscopy, Biochim. Biophys. Acta 1830 (2013)3517–3524.

[10] A. Huefner, W.L. Kuan, R.A. Barker, S. Mahajan, Intracellular SERS nanoprobesfor distinction of different neuronal cell types, Nano Lett. 13 (2013)2463–2470.

[11] J.P. Nolan, E. Duggan, E. Liu, D. Condello, I. Dave, S.A. Stoner, Single cell analy-sis using surface enhanced Raman scattering (SERS) tags, Methods 57 (2012)272–279.

[12] S. Lee, H. Chon, J. Lee, J. Ko, B.H. Chung, D.W. Lim, J. Choo, Rapid and sensitivephenotypic marker detection on breast cancer cells using surface-enhancedRaman scattering (SERS) imaging, Biosens. Bioelectron. 51 (2014) 238–243.

[13] S. Schlucker, SERS microscopy: nanoparticle probes and biomedical applica-tions, Chemphyschem: Eur. J. Chem. Phys. Phys. Chem. 10 (2009) 1344–1354.

[14] S. Suarasan, M. Focsan, D. Maniu, S. Astilean, Gelatin–nanogold bioconjugatesas effective plasmonic platforms for SERS detection and tagging, Colloids Surf.B Biointerfaces 103 (2013) 475–481.

[15] B. Kustner, M. Gellner, M. Schutz, F. Schoppler, A. Marx, P. Strobel, P. Adam, C.Schmuck, S. Schlucker, SERS labels for red laser excitation: silica-encapsulatedSAMs on tunable gold/silver nanoshells, Angew. Chem. Int. Ed. 48 (2009)1950–1953.

[16] X.M. Qian, X.H. Peng, D.O. Ansari, Q. Yin-Goen, G.Z. Chen, D.M. Shin, L. Yang,A.N. Young, M.D. Wang, S.M. Nie, In vivo tumor targeting and spectroscopicdetection with surface-enhanced Raman nanoparticle tags, Nat. Biotechnol. 26(2008) 83–90.

[17] V. Joseph, A. Matschulat, J. Polte, S. Rolf, F. Emmerling, J. Kneipp, SERS enhance-ment of gold nanospheres of defined size, J. Raman Spectrosc. 42 (2011)1736–1742.

[18] S.A. Galema, Microwave chemistry, Chem. Soc. Rev. 26 (1997) 233–238.

[19] T.A. Grell, A.M. Alabanza, K. Gaskell, K. Aslan, Microwave-accelerated surfacemodification of plasmonic gold thin films with self-assembled monolayers ofalkanethiols, Langmuir 29 (2013) 13209–13216.

[20] C. Yuen, W. Zheng, Z.W. Huang, Low-level detection of anti-cancer drug in bloodplasma using microwave-treated gold-polystyrene beads as surface-enhancedRaman scattering substrates, Biosens. Bioelectron. 26 (2010) 580–584.

[21] L. Xia, H. Wang, J. Wang, K. Gong, Y. Jia, H. Zhang, M. Sun, Microwave-assistedsynthesis of sensitive silver substrate for surface-enhanced Raman scatteringspectroscopy, J. Chem. Phys. 129 (2008) 134703.

[22] K.J. Moore, I. Tabas, Macrophages in the pathogenesis of atherosclerosis, Cell145 (2011) 341–355.

[23] P. Libby, Y. Okamoto, V.Z. Rocha, E. Folco, Inflammation in atherosclerosis:transition from theory to practice, Circ. J. 74 (2010) 213–220.

[24] B.A. Kaufmann, J.M. Sanders, C. Davis, A. Xie, P. Aldred, I.J. Sarembock, J.R.Lindner, Molecular imaging of inflammation in atherosclerosis with targetedultrasound detection of vascular cell adhesion molecule-1, Circulation 116(2007) 276–284.

[25] M.J. Lipinski, J.C. Frias, V. Amirbekian, K.C. Briley-Saebo, V. Mani, D. Sam-ber, A. Abbate, J.G. Aguinaldo, D. Massey, V. Fuster, G.W. Vetrovec, Z.A.Fayad, Macrophage-specific lipid-based nanoparticles improve cardiac mag-netic resonance detection and characterization of human atherosclerosis, JACCCardiovasc. Imaging 2 (2009) 637–647.

[26] J.H. Revkin, C.L. Shear, H.G. Pouleur, S.W. Ryder, D.G. Orloff, Biomarkers inthe prevention and treatment of atherosclerosis: need, validation, and future,Pharmacol. Rev. 59 (2007) 40–53.

[27] P. Libby, M. DiCarli, R. Weissleder, The vascular biology of atherosclerosis andimaging targets, J. Nucl. Med. 51 (Suppl. 1) (2010) 33S–37S.

[28] I. Bilecka, M. Niederberger, Microwave chemistry for inorganic nanomaterialssynthesis, Nanoscale 2 (2010) 1358–1374.

[29] T. Ahnfeldt, J. Moellmer, V. Guillerm, R. Staudt, C. Serre, N. Stock, High-throughput and time-resolved energy-dispersive X-ray diffraction (EDXRD)study of the formation of CAU-1-(OH)2: microwave and conventional heating,Chemistry 17 (2011) 6462–6468.

[30] J. Yang, Z. Wang, S. Zong, C. Song, R. Zhang, Y. Cui, Distinguishing breast can-cer cells using surface-enhanced Raman scattering, Anal. Bioanal. Chem. 402(2012) 1093–1100.

[31] Z. Wu, Y. Liu, X. Zhou, A. Shen, J. Hu, A turn-off SERS-based detection platformfor ultrasensitive detection of thrombin based on enzymatic assays, Biosens.Bioelectron. 44 (2013) 10–15.

[32] S.P. Xu, X.H. Ji, W.Q. Xu, X.L. Li, L.Y. Wang, Y.B. Bai, B. Zhao, Y. Ozaki,Immunoassay using probe-labelling immunogold nanoparticles with silverstaining enhancement via surface-enhanced Raman scattering, Analyst 129(2004) 63–68.

[33] M. Nahrendorf, F.A. Jaffer, K.A. Kelly, D.E. Sosnovik, E. Aikawa, P. Libby, R.Weissleder, Noninvasive vascular cell adhesion molecule-1 imaging identi-fies inflammatory activation of cells in atherosclerosis, Circulation 114 (2006)1504–1511.

[34] C. Wang, J. Irudayaraj, Gold nanorod probes for the detection of multiplepathogens, Small 4 (2008) 2204–2208.

[35] M.D. Porter, R.J. Lipert, L.M. Siperko, G. Wang, R. Narayanan, SERS as a bioassayplatform: fundamentals, design, and applications, Chem. Soc. Rev. 37 (2008)1001–1011.

[36] E. Cossette, I. Cloutier, K. Tardif, G. DonPierre, J.F. Tanguay, Estradiol inhibitsvascular endothelial cells pro-inflammatory activation induced by C-reactiveprotein, Mol. Cell Biochem. 373 (2013) 137–147.

[37] H. Kang, S. Jeong, Y. Park, J. Yim, B.H. Jun, S. Kyeong, J.K. Yang, G. Kim, S. Hong,L.P. Lee, J.H. Kim, H.Y. Lee, D.H. Jeong, Y.S. Lee, Near-infrared SERS nanoprobeswith plasmonic Au/Ag hollow-shell assemblies for in vivo multiplex detection,Adv. Funct. Mater. 23 (2013) 3719–3727.

[38] K.K. Maiti, U.S. Dinish, A. Samanta, M. Vendrell, K.S. Soh, S.J. Park, M. Olivo,Y.T. Chang, Multiplex targeted in vivo cancer detection using sensitive near-infrared SERS nanotags, Nano Today 7 (2012) 85–93.