Developing new diagnostic tools based on SERS analysis of filtered salivary samples for oral cancer detection

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Supplementary Table S1. Demographic data and cancer-related information of the oral cancer patients enrolled in the study.

Number	Age (years)	Sex	Urban (U)/rural (R)	Smoker	Special Diet	General pathologies
1	66	F	U	NO	NO	YES
2	58	M	U	YES	NO	YES

3	48	M	U	NO	NO	YES
4	71	F	U	NO	NO	YES
5	66	F	R	YES	YES	YES
6	49	M	R	YES	NO	YES
7	75	F	R	NO	NO	YES
8	52	M	U	YES	NO	YES
9	64	M	R	YES	NO	YES
10	60	M	U	YES	NO	YES
11	61	M	M	YES	NO	YES
12	73	M	U	YES	NO	YES
13	65	M	R	YES	NO	YES
14	66	M	R	YES	NO	YES
15	45	F	R	NO	NO	NO
16	75	F	U	YES	YES	YES
17	71	M	U	YES	NO	YES

Supplementary Table S2. Demographic data of controls enrolled in the study.

Number	Age (years)	Sex	Urban (U)/rural (R)	Smoker	Diet	General pathologies	Medication
1	31	F	R	NO	NO	NO	NO
2	33	F	R	NO	NO	NO	NO
3	33	M	R	NO	NO	NO	NO
4	28	M	U	YES	NO	NO	NO
5	22	M	U	NO	NO	NO	NO
6	24	M	U	NO	NO	NO	NO
7	21	M	U	YES	NO	NO	NO
8	24	F	U	NO	NO	NO	NO
9	24	F	U	NO	NO	NO	NO
10	24	M	U	NO	NO	NO	NO
11	24	M	U	NO	NO	NO	NO
12	25	M	U	NO	NO	NO	NO
13	24	F	U	NO	NO	NO	NO
14	26	M	U	NO	NO	NO	NO
15	26	F	U	YES	NO	NO	NO
16	24	F	U	YES	NO	NO	NO
17	24	F	U	NO	NO	NO	NO
18	25	F	U	NO	NO	NO	NO

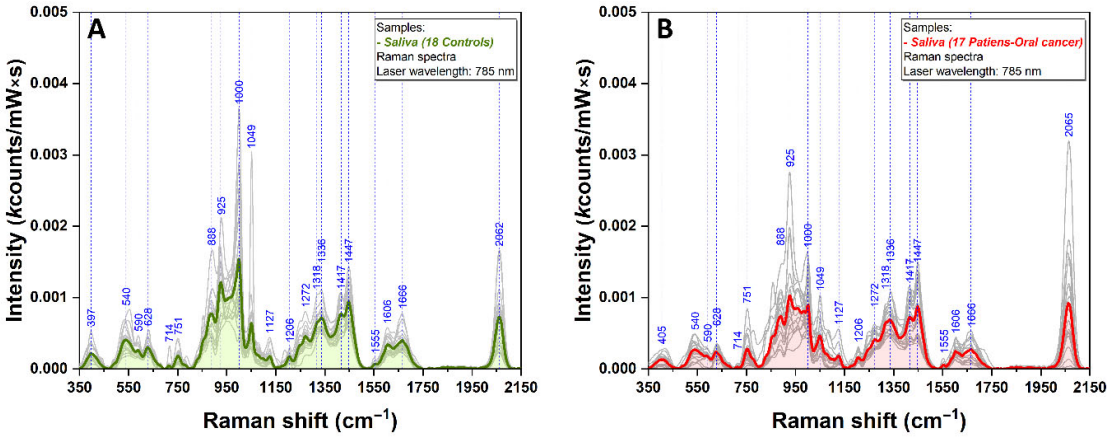

Figure S1. Mean Raman spectra of filtered salivary samples collected from control (A, green) and oral cancer (B, red) group samples using a 785 nm excitation laser.

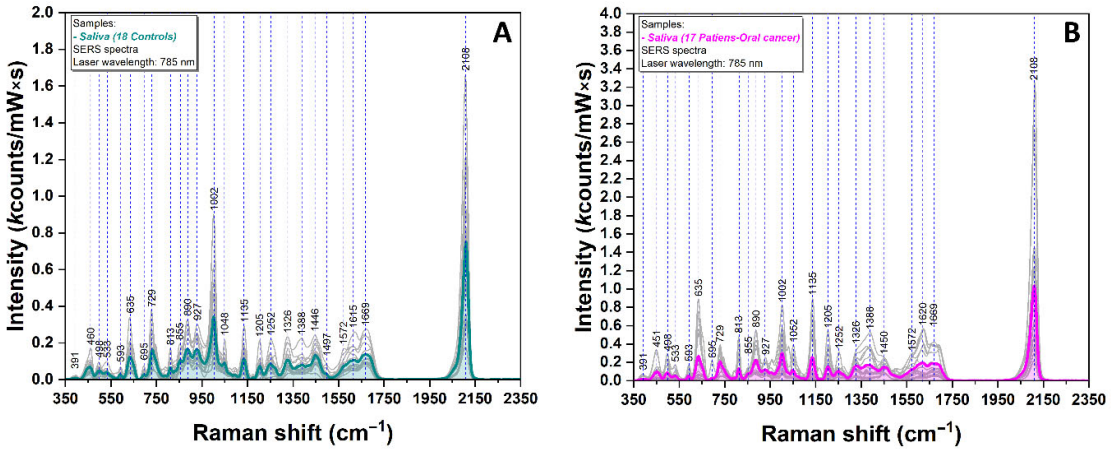

Figure S2. Mean SERS spectra of filtered salivary samples collected from control (A, jade) and oral cancer (B, magenta) group samples using a 785 nm excitation laser.

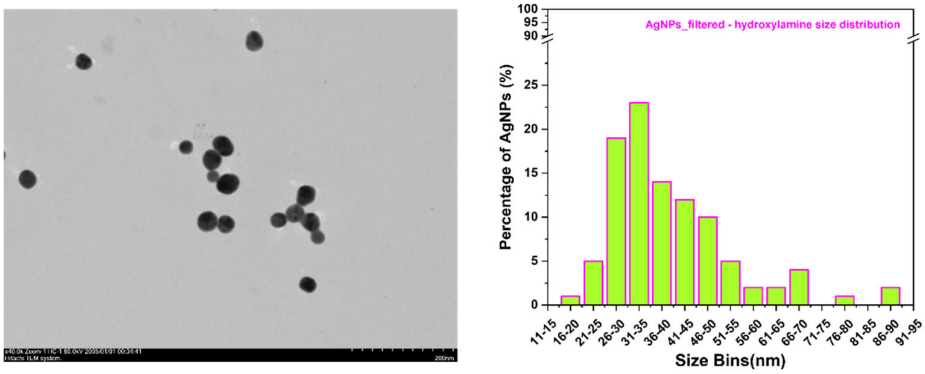

Figure S3. TEM image and size distribution graph of purified silver nanoparticles.

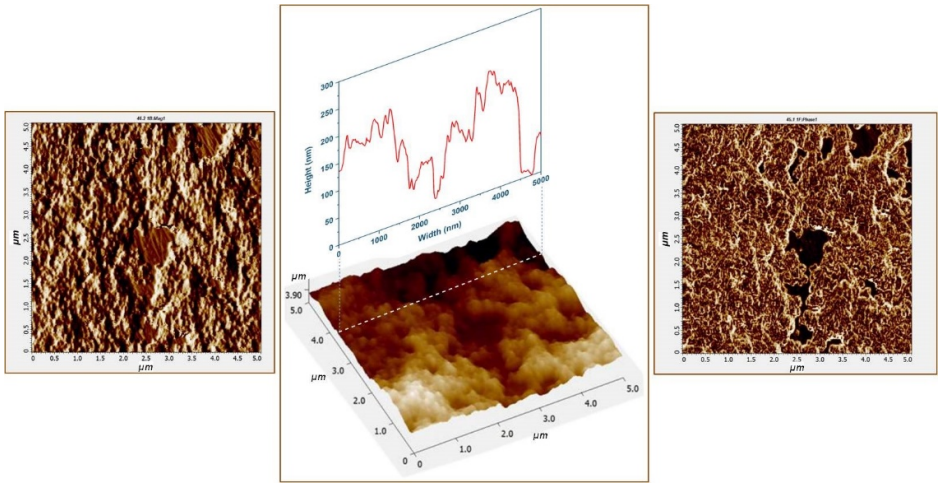

Figure S4. AFM topographic images of silver solid plasmonic substrates.

Calculation of the EF

In our paper we have used rhodamine 6G (R6G) molecules for the calculation of substrates' enhancement factor (EF). According to a procedure proposed by Gupta and Weimar [1] the EF can be calculated by using the following equation:

$$EF = \frac{M_{Raman} \times S_{Surf} \times I_{Surf}}{M_{Surf} \times S_{Raman} \times I_{Raman}} \quad (1)$$

where M_{Surf} and M_{Raman} are the numbers of molecules dropped onto the solid plasmonic substrate (SERS measurements) and CaF_2 (Raman measurements), $S_{Surface}$ and S_{Raman} are the geometrical areas of the molecular films and I_{Surf} and I_{Raman} are the SERS/Raman intensities of the most intense vibrational band that has been used for the calculation of EF (1508/1510 cm^{-1}). Both measurements were performed using a 50× objective and an excitation laser of 785 nm. In the case of Raman measurements, a 100% laser power was used, the acquisition time was 10 s and a number of 4 acquisition was recorded. For SERS measurements the laser power was set to 0.1%, all other conditions being identical. In order to improve the accuracy of EF calculation we measured the laser intensity on the sample surface in the two cases and the nominal values were 113 mW (100 % laser power) respectively 0.22 mW (0.1% laser power). The intensities of the 1508/1510 cm^{-1} vibrational band, in both SERS and Raman spectra have been plotted in *kcounts*/(mW × s) units. For both measurements we have used 10^{-3} M aqueous solutions of R6G. The circular spots had a diameter of ~2 mm in both cases. The values of 1508/1510 cm^{-1} intensities were 16.9 respectively 0.0054 kcounts/(mW × s).

Using these data, the EF of the here proposed solid substrates has a value of ~3×10^3.

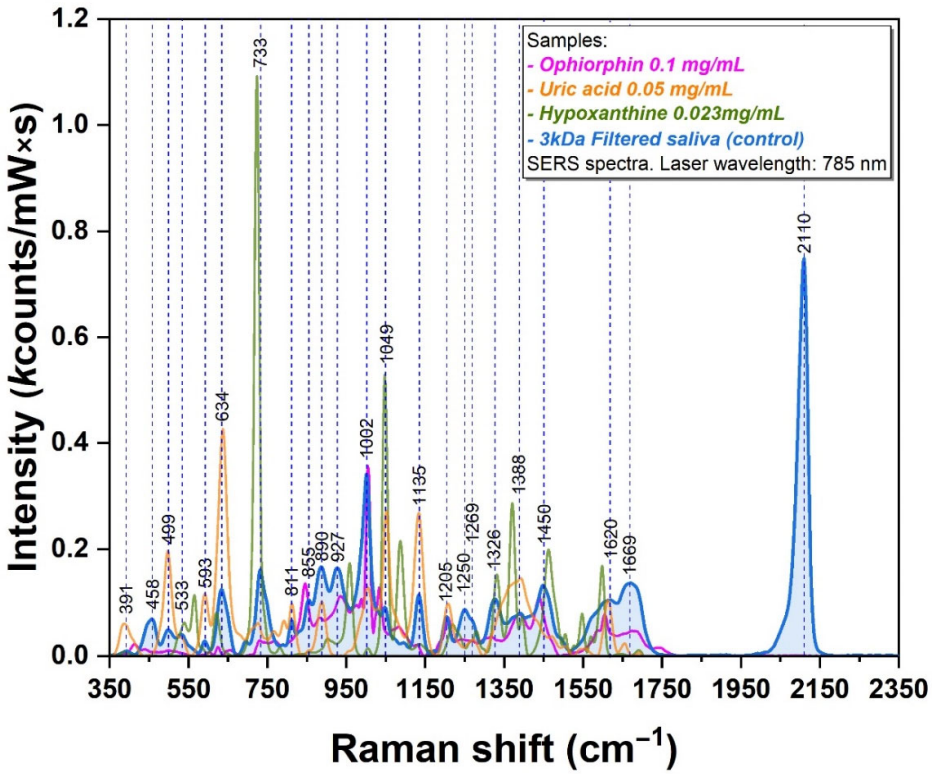

Figure S5. SERS spectrum of opiorphin, uric acid, hypoxanthine and salivary control probes using an excitation wavelength of 785 nm

Supplementary Table S3. Tentative assignment of Raman and SERS molecules

Molecule	SERS vibrational band (cm^{-1})	Contributions
Amide I	1049, 1669	[2–5]
Amide III	1205	[6,7]
Amide VI	593	[8]
Ascorbic acid	593	[8]
Cholesterol ester	533	[8]
Collagen	811	[9]
D-galactosamine	890	[9]
D-mannose	1135	[8,9]
Glycine	890	[10]
Histidine	1135, 1269	[10]
Hypoxanthine	533, 634, 733, 1049, 1205	[8,9,11,12]
L-arginine	1326	[10]
L-glutamate	811, 1049	[10]
Lipids	1326, 1450	[7,13]
L-Phenylalanine	855, 1002	[2–5,7–11,14–18]
L-proline	855, 1049, 1269, 1326	[10]
L-serine	811	[8]
L-serine	1135, 1388	[10]
L-Tryptophan	458, 593, 890, 1049, 1205, 1326, 1388, 1620	[3,8–10]
L-tyrosine	634, 1049, 1135, 1205, 1269, 1326	[4,7–11]
L-valine	890, 1620	[10]

Lysozyme	533	[17]
Nucleic acids	458, 890, 1135, 1269, 1326	[6,10,19–21]
Proteins	890, 927, 1049, 1205, 1450	[3,4,6,7,13–15,17,22]
Saccharides	458	[17]
Thiocyanate	2110	[3,17,23]
Uric acid	391, 499, 593, 634, 811, 890, 1002, 1049, 1135, 1269, 1388	[11,12,22,24]
Xanthine	1250	[25]

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