SERS Detection of Pathogens using a LAMP–in–Microdroplets platform

Alexandra Teixeira*, Juan L. Paris, Foteini Roumani, Lorena Diéguez, Marta Prado, Begoña Espiña, Sara Abalde-Cela, Alejandro Garrido-Maestu and Laura Rodriguez-Lorenzo

International Iberian Nanotechnology Laboratory, Avenida Mestre José Veiga s/n, 4715-330 Braga, Portugal

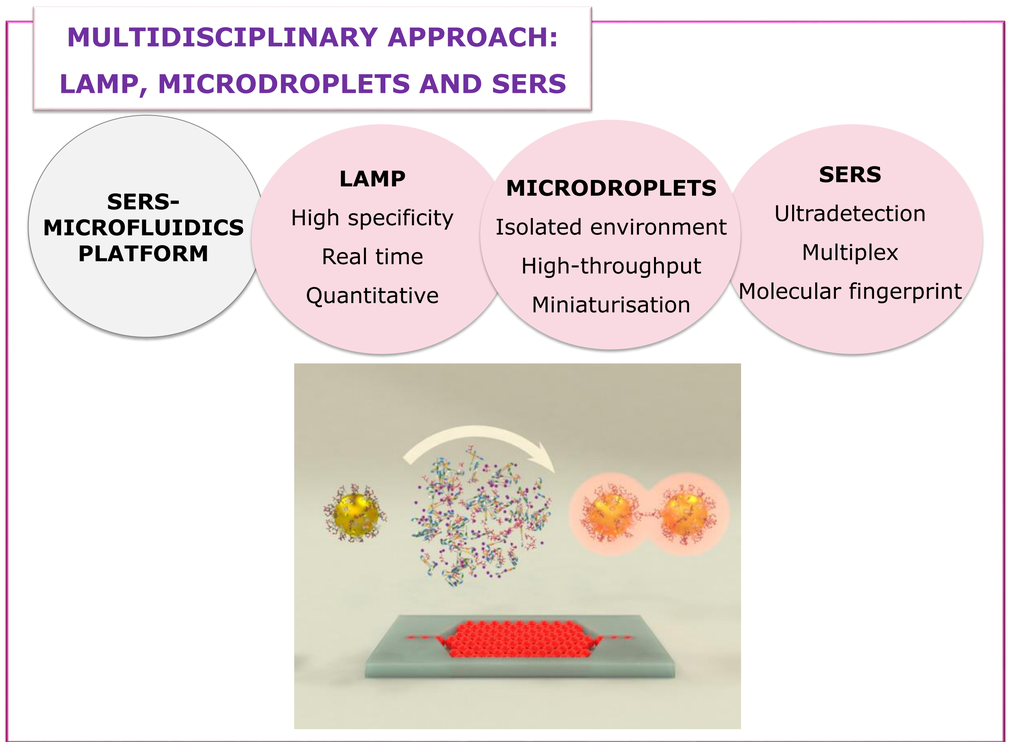
*alexandra.teixeira@inl.int

Introduction

In 2018 more than 2500 cases of Listeriosis were reported in Europe with **a fatality rate of 15.6 %**, which makes it one of **the most aggressive foodborne diseases** [1]. As such, it is extremely necessary to develop novel methodologies for the early detection of this pathogen.

Herein, we present **a bioinspired solution for the on-chip detection** of *L. monocytogenes* by combining the advantages of **molecular (LAMP), engineering (microfluidics) and spectroscopic (SERS) tools**. More precisely, we developed a droplet-based optofluidic system for the detection of foodborne pathogens. In this work, we have shown that the integration **of LAMP with SERS offers an excellent method for DNA ultradetection** [2].

MULTIDISCIPLINARY APPROACH: LAMP, MICRODROPLETS AND SERS



Methods

1. Nanoparticle Synthesis

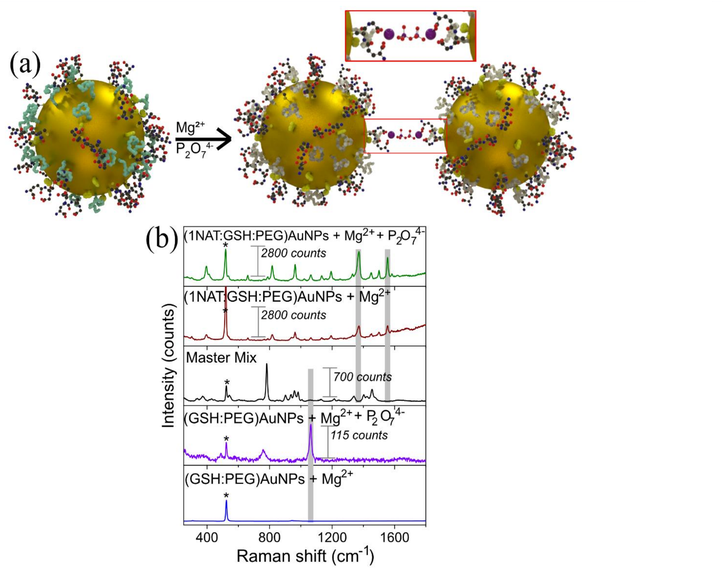


Figure 1. Illustration of the synthesis of AuNPs. (a) Schematic illustration of the designed multifunctional AuNPs. AuNPs were coated with three components with different important roles: *(i)* Glutathione (GSH) molecules (*(ii)* 1-naphtalenethiol (1NAT) as SERS tag; and *(iii)* Poly(ethylene glycol) (PEG); (b) shows the SERS spectra acquired in absence and presence of potassium pyrophosphate ($P_2O_7^{4-}$) at concentration of 6 µM.

2. UV-VIS and Transmission Electron Microscopy (TEM)

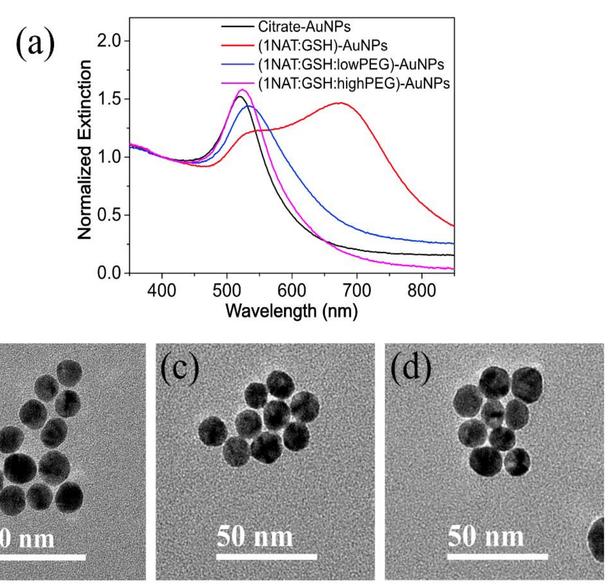


Figure 2. Characterization of the AuNPS. (a) UV-vis spectra of the multifunctional AuNPs set in comparison with initial citrate-AuNPs (black spectrum). Clearly, (1NAT:GSH:highPEG)-AuNPs show the highest colloidal stability, while (1NAT:GSH:lowPEG)-AuNPs are slightly aggregated. (b) TEM image of AuNPs used as core in the preparation of multifunctional AuNPs. Specifically, (c) and (d) show TEM images of (1NAT:GSH:lowPEG)-AuNPs and (1NAT:GSH:highPEG)-AuNPs.

3. Schematic representation of the experimental process of the microdroplet-based SERS LAMP analysis

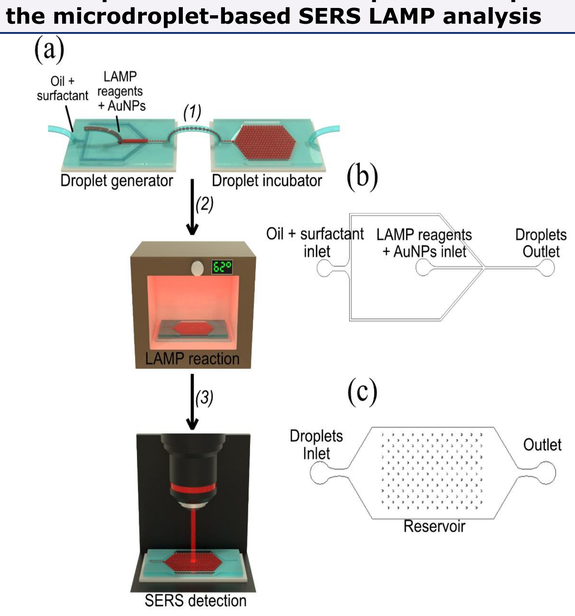


Figure 3. Illustration of the procedure, from the droplet generation to SERS detection. (a) Schematic representation of the experimental setup for microdroplet array-based SERS LAMP analysis: (1) Microdroplet generator device; (2) Stored microdroplets into trapping array were incubated at 62 °C for 1 h to produce the DNA amplification by LAMP and (3) SERS signal emerging from positive droplets was detected on the device using a confocal Raman microscope; (b) this figure shows AutoCAD design for the microdroplets generator and **(c)** AutoCAD design of the microdroplet incubator with an integrated trapping array. The size of microdroplets generated here was around 100 µm.

Results

4. SERS Detection

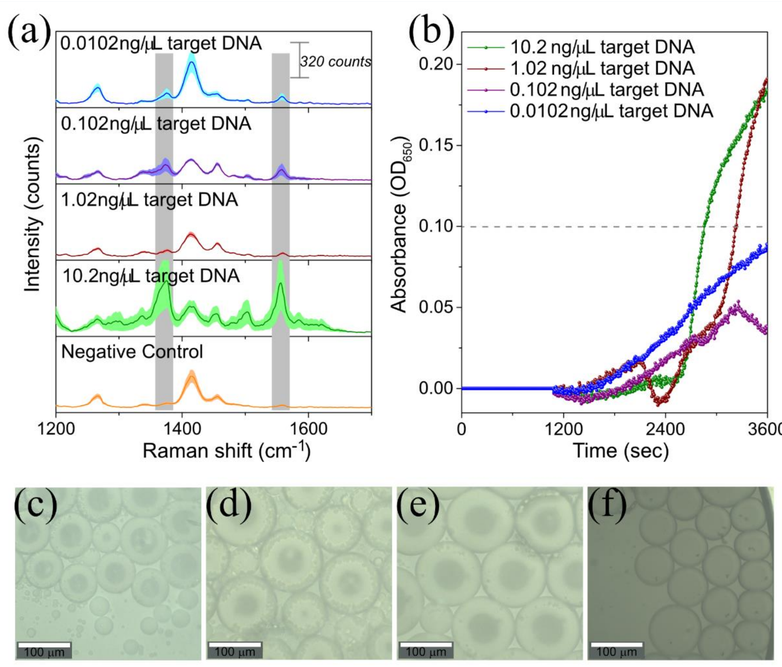


Figure 4. Comparison between LAMP-on-a-chip SERS detection with conventional method. (a) LAMP-on-a-chip SERS detection; (b) LAMP real-time turbidity detection for DNA extracted from *Listeria monocytogenes.* A positive reaction was indicated by (a) and the enhancement of SERS signal of 1NAT due to the co-complexation of (1NAT:GSH:lowPEG) AuNPs, Mg^{2+} and pyrophosphate, byproduct of DNA amplification in the case of (b). Gray shaded areas in (a) indicate the characteristic peaks of 1NAT (1368 and 1553 cm^{-1}, ring stretching). Representative bright field images of stored microdroplets into trapping array and after target DNA amplification by LAMP-on-a-chip at target concentration of (c) 10.2 ng/µL (e) 1.02 ng/µL, (d) 0.102 ng/µL and (f) 0.0102 ng/µL. In the negative control, sterilized water was added instead of extracted DNA.

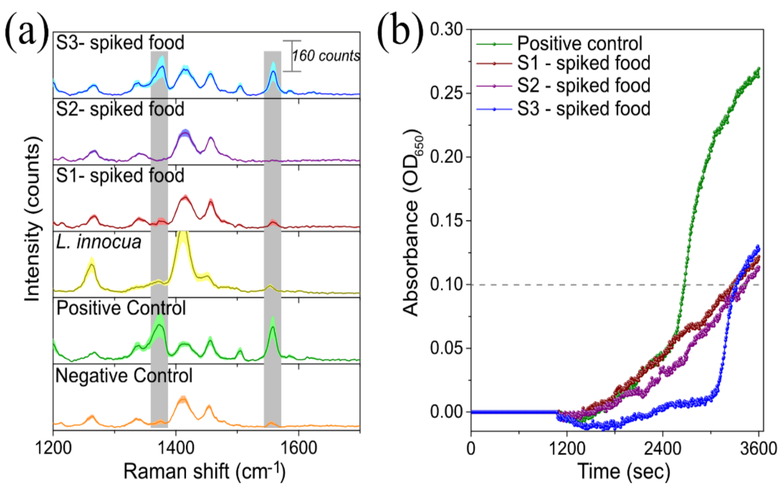


Figure 5. Detection of *Listeria monocytogenes* DNA from spiked food sample. (a) LAMP-on-a-chip SERS detection for target Listeria monocytogenes DNA from spiked food sample; (b) LAMP real-time turbidity detection was performed as reference. Positive control consists on target DNA extracted from Listeria monocytogenes at concentration of 10.2 µg/µL. In the negative control, sterilized water and DNA extracted from *Listeria inocua* were added instead of target DNA.

Conclusion

- LAMP-SERS approach demonstrated a higher sensitivity than LAMP-turbidity detection (0.102 vs 1.02 ng/µL of target DNA), suggesting LAMP-on-a-chip-SERS technology as a potential alternative method for foodborne pathogens detection;
- The fact of using LAMP eases the process of the integration in microfluidic devices as no temperature cycles are needed, widening the possibilities of expansion of the technology such as the use of novel materials in microfluidics for scale-up.

This work allowed the development of a platform ***with isothermal amplification*** *and* ***real-time*** *identification capabilities.*

References

1] E. F. S. A. and E. C. for D. P. and C. (EFSA and ECDC), "The European Union One Health 2018 Zoonoses Report," *EFSA J.*, vol. 17, no. 12, p. e05926, 2019.

[2] A. Teixeira *et al.*, "Multifuntional Gold Nanoparticles for the SERS Detection of Pathogens Combined with a LAMP–in–Microdroplets Approach," *Materials (Basel).*, vol. 13, no. 8, p. 1934, Apr. 2020.

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