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 (INL), Avda Mestre José Veiga, 4715-310 Braga, Portugal
*alexandra.teixeira@inl.int

In recent years, cases of listeriosis have been increasing and are considered one of the most serious foodborne diseases under EU surveillance [1], emphasising the need for seeking methodologies for the early detection of this pathogen. Traditional molecular techniques are normally based on the detection of specific DNA or RNA target sequences using amplification processes, like the polymerase chain reaction (PCR) [2]. Nevertheless, isothermal nucleic acid amplification technology, specifically loop-mediated isothermal amplification (LAMP), has been widely proposed as an alternative methodology because it exhibits good performance in ultrasensitive detection and biosensing, as compared to PCR [3]. LAMP has been successfully implemented in lab-on-chip systems, however the low volumes inherent to a microfluidic system force these strategies to be coupled to highly sensitive detection techniques. Microdroplets have been used as laboratory flasks for the detection of pollutants, bacteria and even single cells [4]–[6]. Surface-enhanced Raman scattering (SERS) spectroscopy is an ultrasensitive sensing tool, that when combined with microfluidics/microdroplets offers a great potential for the development of automated and sensitive diagnostic platforms.
Herein, we developed a droplet-based optofluidic system for the detection of foodborne pathogens. Specifically, LAMP was combined with SERS, which offers an excellent method for DNA ultradetection. For this, were prepared multifunctional AuNPs involving three components with key roles: (1) thiolated poly(ethylene glycol) as stabilizing agent, (2) 1-naphthalenethiol (1-NAT) as Raman reporter, and (3) glutathione as a bioinspired chelating agent of magnesium (II) ions. The variation of the SERS signal of 1-NAT was controlled by the aggregation of AuNPs triggered by the complexation of pyrophosphate and glutathione with free magnesium ions. Using this strategy, we detected *Listeria monocytogenes*, not only in buffer, but also in a food matrix (i.e., ultra-high temperature milk) enabled by the massive production of hotspots as a result of the self-assemblies that enhanced the SERS signal. This allowed the development of a microdroplet-LAMP-SERS platform with isothermal amplification and real-time identification capabilities.[7]

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FIGURES

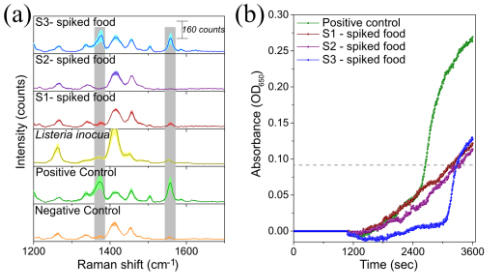

Figure 1: Ability of LAMP-on-a-chip SERS assay to detect L. monocytogenes in UHT milk. (a) SERS (i.e., using LAMP-on-a-chip SERS assay) and (b) turbidity (e.i. LAMP real-time turbidity detection) as reference.