Microfluidics and surface-enhanced Raman spectroscopy : a perfect match for new analytical tools

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Microfluidics and Surface-Enhanced Raman Spectroscopy: a perfect match for new analytical tools

Gustavo Ochoa-Vazquez[1,4], Boris Kharisov[2], Ana Arizmendi-Morquecho[3], Anaïs Cario[4], Cyril Aymonier[4], Samuel Marre*[4], and Israel López*[1]

***Abstract* — In this perspective article, we emphasize the combination of Surface-Enhanced Raman Spectroscopy (SERS) and Microfluidic devices. SERS approaches have been widely studied and used for multiple applications including trace molecules detection, *in situ* analysis of biological samples and monitoring or, all of them with good results, however still with limitations of the technique, for example regarding with improved precision and reproducibility. These implications can be overcome by microfluidic approaches. The resulting coupling Microfluidics – SERS (MF-SERS) has recently gained increasing attention by creating thundering opportunities for the analytical field. For this purpose, we introduce some of the strategies developed to implement SERS within microfluidic reactor along with a brief overview of the most recent MF-SERS applications for biology, health and environmental concerns. Eventually, we will discuss future research opportunities of such systems.**

***Index Terms* — Microfluidics, Surface-Enhanced Raman Spectroscopy, Lab on a Chip, *in situ* characterization.**

I. Introduction

In the last decades, Raman spectroscopy has gained increasing attention as an analytical tool, being conventionally used to provide researchers with precious data for solving problematics in various topics such as environment, safety, biology and healthcare. This technique collects the inelastic dispersion with respect to an incident wavelength, allowing monitoring the vibrational modes of the molecules or material investigated. Due to the coupling to microscope, Raman spectroscopy can be used to perform (bio)chemical imaging at higher spatial resolution (less than 200 nm) that what can be typically obtained with Infrared spectroscopy [1]. However, this characterization tool is known to have a low sensitivity due to weak Raman scattering, which is one of the major problems associated with this technique. Therefore, for some specific applications, this has pushed researchers to consider one technique now widely used in Raman spectroscopy: The Surface-Enhanced Raman Scattering (SERS). This is a surface approach that enhances Raman scattering (the gain can reach a factor up to 10^{10}, thus allowing the detection of trace molecules) due to the interactions between the investigated molecules and different nanostructured substrates constituted of noble metals generating localized surface plasmons and hot spots in between metals nanoparticles [2]. The fabrication of carefully-designed SERS substrates has been widely investigated; however, various challenges still exist in SERS, limiting its adoption by a wider scientific community, namely: the substrates fabrication reproducibility [3], the difficulty of realizing field measures and the high cost of analysis represent the most current problems [4]. With the purpose of addressing these limitations, the scientific community has look towards microscale processes through the use of microfluidics fundamentals.

Microfluidics is the scientific field that studies the behavior of the fluids and their control at microscale using microchannels displaying dimensions in the order of tens to hundreds of μm [5]. The design, fabrication and use of microfluidic devices have permitted to solve some problems related to the SERS technique, thanks to high precision and reproducibility in the synthetic and/or analytical parameters [6], leading to the so-called MF-SERS approaches.

Over the past 10 years, an increasing number of studies have reported and reviewed the use of MF-SERS to miniaturize the analytical systems and to enhance their reproducibility [7]–[11] (Figure 1). The applications of such systems are wide, from chemistry to biology, including several medical (point-of-care – POC analysis) and analytical applications (trace molecules and drugs detection, etc.). All these researches have grown up over time, developing methods for general and specific applications based on MF-SERS devices.

In this perspective paper, we first introduce the general strategies for designing and fabricating MF-SERS devices and further introduce some of the current reported applications of MF-SERS. Eventually, a general perspective of this new analytical tool possibilities to other scientific fields will be presented.

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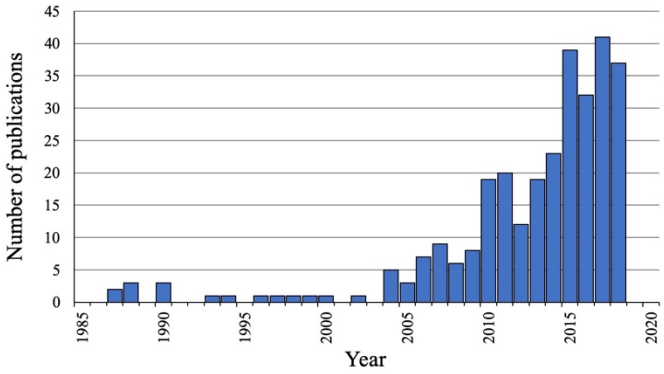

Fig. 1. Advances of MF-SERS over time.

II. Coupling SERS with Microfluidics

One of the most important parts to consider when designing a MF-SERS experiment is the correct selection and fabrication/use of SERS substrates. These substrates are generally fabricated from the assemblies of nanoparticles or nanostructures of noble metals allowing generating the desired Plasmon effects, leading to the enhancement of the Raman scattering (nanoM with M = gold or silver). Several types of arrangements have been reported so far including metal NPs self-assemblies or core-shell (nanoM@ Al_2O_3 or nanoM@SiO_2) [12], [13] pickering emulsions [14] or metals NPs-decorated oxides solids substrates [15]. Nevertheless, the complex fabrication procedures mostly exhibit low reproducibility, leading to a dispersion in the results.

When considering the integration of SERS substrates into microfluidics devices, additional difficulties arise, as several other considerations need to be taken into account. First, complicated microfabrication techniques generally have to be considered to integrate the substrates into a microfluidic chip. At the nanometer scale - required for such applications - it is not easy to control either the arrangement of NPs for a bottom-up SERS substrate approach [16] or the top-down manufacturing of substrates [17]. Second, the physicochemical stability of the substrates exposed to additional stresses (fluid flow, chemicals, temperature, etc.) has to be carefully investigated, as it can result in a modification or even a failure of the substrate during experimentation, leading to poor reproducibility.

Nevertheless, there are several strategies that can be employed to generate SERS effect within microfluidic systems depending on whether the SERS substrate is in solution (colloids, nanoparticles aggregates) or fixed on a surface (solid substrates fabricated from top down microfabrication or bottom up *in situ* synthesis), see Figure 2, which corresponds respectively to: (i) SERS substrates dispersed in flow, (ii) integrated SERS substrates (top-down approaches) and (iii) immobilized SERS substrates (bottom-up approaches), which are discussed hereafter.

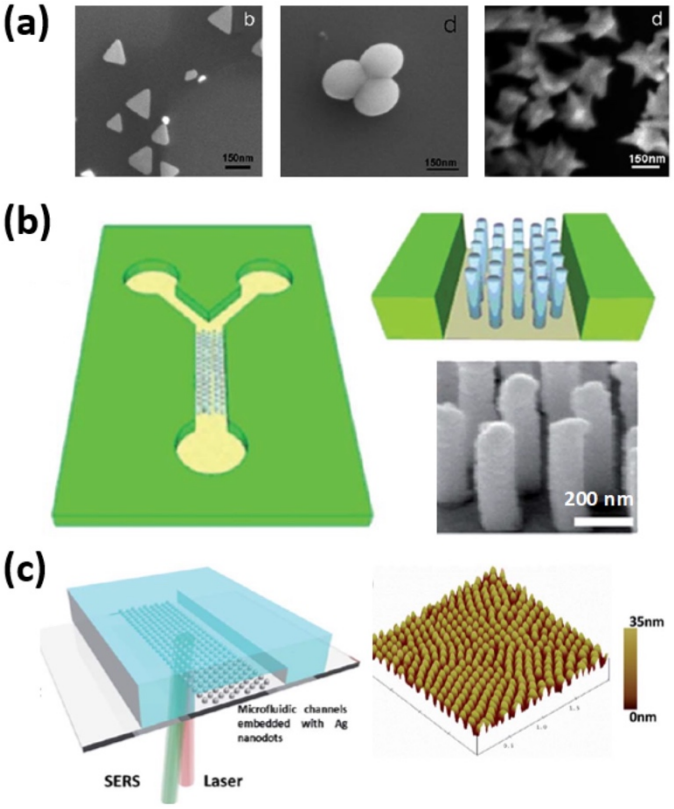

Fig. 2. Strategies for the integration of SERS substrates within microfluidic reactors. (a) examples of SERS colloids: Gold nanostructures (nanotriangles, nanospheres and nanostars) used in flow (adapted from [18] with permission from The Royal Society of Chemistry), (b) microfabricated substrates based on plasmonic nanopillar geometry inside microchannel (adapted from [19] with permission from The Royal Society of Chemistry), (c) Silver NPs deposited inside of a microchannel as a fixed MF-SERS substrate (adapted from [20] with permission from The Royal Society of Chemistry).

Dispersed SERS substrates. The first strategy consists in using nanoM assemblies dispersed within the solution to analyze. This simple procedure requires the pre-synthesis of the nanoM assemblies (which size generally ranges from 20 to 200 nm) and their dispersion in the media. Several types of morphologies for the nanoM arrangements have been reported such as metal nanoflowers or nanostars [21]. Indeed, the SERS effect is affected not only by the particle size, but also by their shape, and the distances between the metallic nanoparticles, generating controllable hot spots, driven by the localized electric field (Figure 2-a). The main interest concerns the easy manipulation and preparation of the samples, while the substrates can be recovered between analyses and cleaned to minimize cross contaminations. Nevertheless, solid handling inside microfluidics channels still remains challenging and some deposition of the nanostructured SERS substrates on the microchannel walls can occur over time, leading potentially to clogging of the device. Additionally, random aggregation of such nanoM colloids inside the solution may induce uncontrollable variation in the Raman signals. It is therefore highly desirable to pay attention to the fluidic design of the chips. The design and fabrication of microfluidic devices are not limited to their fabrication with only one microchannel to transport the fluid. Indeed, there are many possibilities to design microfluidic devices with different number of microchannels that expand their applications. For instance, Cui Y. *et al* show the use of a microfluidic system with

different microchannels for the *in situ* synthesis and generation of gold SERS substrates and their performance evaluation [22], [23].

The advantage of this strategy is its easy operation and the use of nanoM previously synthesized as well as commercial nanoM. Also, in this strategy, is possible to recover and to clean the nanoM for reuse. This way, the chip can be used several times. However, undesirable clogging into the microchannels and nanoM agglomerations are hard to avoid, causing variation in Raman signals.

Integrated microfabricated SERS substrates on chip. The second strategy concerns the generation of SERS substrate directly inside the channels, which is now been made possible thanks to the recent development of microfabrication techniques at nanoscale (Figure 2-b). Such approaches allow to design 3D SERS substrates for example through the fabrication of nanopillar arrays from silicon or glass [24], on the top of which thin metals layers can be sputtered or nanoM assemblies can be deposited for generating the desired SERS effect. Similarly, oxide nanoporous layers, nanotubes or nanowires (Al_2O_3, ZnO or TiO_2) can be first grown on a planar surface and later decorated with nanoM assemblies [25]–[27]. These substrates are slightly more complicated to realize but lead so far to the best stability and reproducibility of the results thanks to a more homogeneous adsorption of molecules on the SERS substrates.

Among the various available fabrication techniques, it is worth mentioning the laser irradiation techniques [28], which allows accessing complex multi-materials nanostructures directly on chip. The principle consists in irradiating a metallic film with a laser, which will heat a precursor solution in contact with the film, leading to their decomposition and the heterogeneous growth of nanostructures. This approach was use in particular to grow 3D Ag@ZnO nanostructures for MF-SERS applications [29]. The laser direct writing approaches [30], is yet another interesting methodology, which allows accessing nanoscale structuration of MF-SERS substrates thanks to the recent development of femtosecond laser writing for the precise design of nanoscale structures.

Eventually, nanoprinting methodologies [31], aiming at transferring through a mold sub-micrometer designs to a process able active layer, have quickly evolved over the last decades and can now easily be implemented to design metal SERS substrates at the nanoscale [32].

Despite this strategy is expensive for the distinct microfabrication techniques at nanoscale and their hard training for operations, it presents great advantages. With integrated microfabricated SERS substrates on chip, it is indeed possible to design highly ordered and controlled nanoM SERS substrates for increasing the number of hotspots and the homogeneous adsorption of molecules, and thus to realize reproducible SERS analysis.

Immobilized SERS substrates within microchannels. This last strategy refers to the ordered spatial deposition of single metals nanoparticles or nanostructured materials inside of a microchannel (Figure 2-c). These immobilized SERS substrates constituted of nanoM arrays allow a fine control of the interparticle distance for improving the electrical field and the subsequent SERS effect. Thanks to advances in surface chemistry on glass or polymers, coupled to either nanoimprint lithography [33], electron beam lithography [34], focused ion beam [35], or nanosphere lithography [36] approaches, these types of substrates can be easily modified for generating either homogeneous or heterogeneous patterns. Compared to the first strategy of flowing SERS substrates, these immobilized substrates reduce the Raman signal fluctuations and no specific microfluidic design are needed to handle solids assemblies. However, the main limitation remains: (i) the non-homogeneous molecules adsorption on the surface of the SERS substrate, (ii) the relatively high cost of fabrication, especially for the patterning part and (iii) the possible contamination of the MF-SERS devices over time as the cleaning of such substrates is not straightforward and might lead to particles desorption from the surface. Despite these limitations, such strategy is probably the most promising one to increase reliability of the MF-SERS devices as it provides ways towards the synthesis and the assembly of colloids directly on chip in a control manner. Since nanomaterials synthesis in microfluidics devices have been largely reported [37], their further *in situ* assemblies (self- or orientated) could constitute a real advantage compared to the other reported strategies as the SERS substrate could be synthesized and shaped directly in a single step within microchannels. For example, Sepaniak *et al*. have worked with the microfluidic synthesis of nanostructures since 2004, creating different microfluidic SERS systems and testing their applications in various domains [38]–[40]. Summarizing their work, it was observed that for the correct synthesis and coupling of these materials, is necessary to provide an accurate design and fabrication of the microfluidic devices. Similarly, Xu and Giorgis have reported different techniques and materials of fabrication for the microfluidic devices, (PDMS, PDMS/silicon, PDMS/glass, glass-glass, paper using 3D printing, molding and soft lithography) [41], [42]. They have demonstrated for all these materials the integration of synthesized nanostructures as SERS substrates for the generation of MF-SERS systems capable of performing *in situ* analysis [22].

In spite of their disadvantages as high cost of fabrication, facile contamination and hard cleaning procedures, the principal advantage of this approach is the possibility for synthesizing and assembling the nanoM *in situ* into the microchannel and, with some microfabrication techniques create nanoM arrays with a specific interparticle distance.

We summarize hereafter (Table 1) the major advantages / limitations of each strategy. As described in the previous sessions, the main difference between these strategies lies in the investment / production costs and the enhancement factors (EF) and limits of detection (LOD) achieved.

Table 1: Comparison of the advantages and limitations of each above-mentioned methodology.

Feature	Dispersed MF-SERS substrates	Integrated MF-SERS substrates	Immobilized MF-SERS substrates
Fabrication cost	$	$$$	$$
Viability of reuse	easy	hard	medium
Maximum EF reported	10^9	10^{12}	10^{10}
Minimum LOD reported	10^{-8} M	10^{-6} M	10^{-12} M
Chip platform	PDMS-glass	PDMS-glass, PDMS-Si	PDMS-glass, all-PDMS
Analysis time	60-80 min	5 min	5 min
Manufacturing infrastructure	basic	sophisticated	intermediate
Example applications	biomolecules, cancer diagnosis	biomolecules, cells	biomolecules, drugs, dyes

We will detail in the next section some applications of MF-SERS devices for several domains of research.

III. MF-SERS: A FULL RANGE OF APPLICATIONS

The first mention about flow-injection analysis coupled to SERS (FIA) at small scale was reported in the late 1980s by Laserna, Berthod, and Winefordner. They were able to detect 4-aminobenzoic acid using SERS in submillimetric channels [43]–[45]. In the next decade, Caballin, Ruperez, and Laserna developed a windowless flow

cell for the SERS detection of amiloride, amiphenazole, 2-mercaptopyridine, pemoline and triamterene [46], [47]. Since then, several other applications have been reported, among which are the research domains of environment, food monitoring for analytes detection and cells management and analysis on a chip, which are discussed below.

Analytes detection. The combination between microfluidic devices and SERS technique has allowed the better handling of analytes and the improvement of their assays. Thanks to the high sensitivity of SERS, miniaturized devices have been developed for portable detection of various molecules including pollutants [48], drugs or narcotics [49], as well as biomolecules such as DNA or pathogens [50]. In particular, the most studied analytes are dyes including rhodamine 6G, crystal violet, and other Raman tags such as 4-MBA, due to their signal intensity, photostability, and their functional groups that permit specific bonding between these molecules and the SERS substrates [51]–[53]. Furthermore, this combination also has permitted the analysis of diverse inorganic samples highlighting the detection of metallic ions for applications in food safety and environmental monitoring [54], such as Hg(II) [55], As(III) [56], Cr(III) [57] or Fe(II) [58]. The efficiency of MF-SERS platforms to be very sensitive, rapid, low-cost, non-invasive, highly selective and specific, has found various applications in clinical studies and drugs detection such as morphine and cocaine traces [59]. Additionally, other interesting applications include the detection of explosives molecules as traces, mostly for security checking and military on site applications. Indeed, MF-SERS allow the fabrication of small, portable devices, which can be utilized in real environments. Besides, the MF-SERS analysis of explosive samples provide safer operating conditions with a small amount of sample, as reported for 4-ABT [60], DTN [61], TNT [62]. Other research areas that have been favored for MF-SERS use are biology and medicine for the detection of biomolecules, biomarkers and pathogens. Adenine, DNA, RNA, and different proteins are some biologic probes that are analyzed using MF-SERS combination due to facilitate the handling of samples using microfluidics parameters [63]–[65]. Similarly, diverse pathogens can also be detected with this approach, as reported for such as *Escherichia coli*, *Staphylococcus aureus*, and different mycotoxins in real samples using MF-SERS [66], [67]. Eventually, the detection of different viruses as human immunodeficiency virus, HIV, type 1 [68] and avian influenza virus, AIV, H7N9 [69] has been possible with the development of microfluidic devices and their link-up with SERS due to their ability to discriminate selectively the infected cells. All these proves of concept of SERS in medical applications and the miniaturization of the instrumentation permitted by the microfluidic devices have led to the fabrication of point-of-care (POC) devices. The POC systems present advantages as low-cost, field analysis as well as time saving in the training of personnel [70]. Nowadays, it is possible to realize different analysis in patients without high personal training and in a faster way. There are several reports about POC tests using blood, saliva and urine as samples for the opportune detection of disease [71], [72]. This is currently the main trend for the development of MF-SERS devices.

MF-SERS for cell analysis. The combination of microfluidic systems and SERS allows a high sample control of fluids flow and the generation of reproducible substrates for *in situ* analysis. Cooper *et al*., confirmed that the use of microfluidic systems allows a better sample control for cell characterization. Cells or bacteria can be manipulated one by one using microfluidic chips, due to the dimension of the

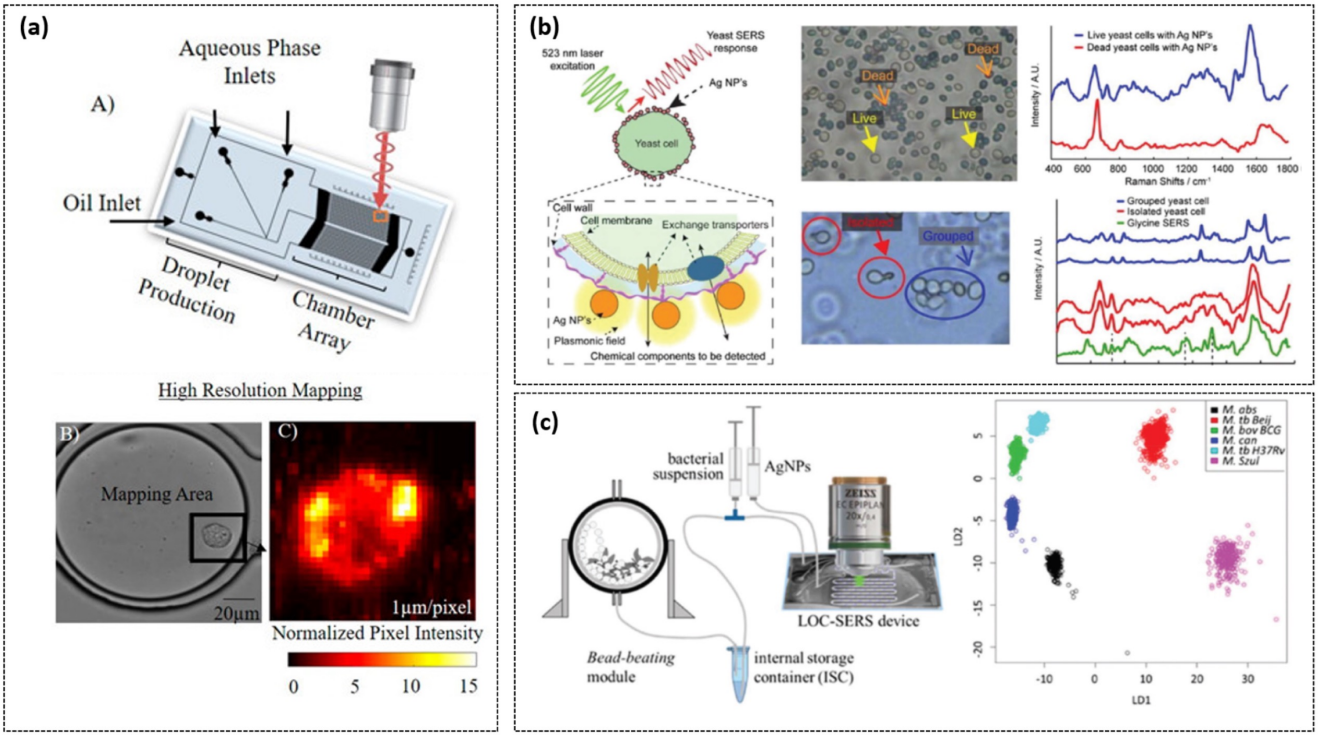

Fig. 3. Examples of MF-SERS applications in the biological field. (a) droplets-based single cell microfluidics strategy coupled to SERS to investigated glycan expression in cancer cells at cellular resolution (adapted from [76] with permission from American Chemical Society), (b) scheme of a MF-SERS set-up and comparison results of both live and dead cell and isolated and grouped yeasts cells (adapted from [78] with permission from Elsevier B.V.). (c) MF-SERS set-up and results for the identification of six different mycobacteria from blood samples (adapted with permission from [79] with permission from American Chemical Society).

microchannels and the low volume to control, allowing to analyze cells in an individually way. MF-SERS systems have been used for diverse biological analysis [73]–[75] including single-cell analysis for improving the understanding of cellular systems. Indeed, cells can be encapsulated within droplets, which can be later stored in a microfluidic device and analyzed by SERS at cellular resolution [76] (Figure 3-a). Originally limited to medical / clinical studies, the use of MF-SERS could be extended to environmental biology for the on-field investigation of microbes [77]. In this research field, MF-SERS can be adapted to target the study of different microorganisms (fungi, viruses, yeasts and bacteria) based on their specific variations in spectral features. As an example, isolated and grouped yeasts cells were monitored *in situ* using MF-SERS coupled to integrated electrophoresis [78]. This approach was also used for the rapid identification of live and dead cells within aqueous environments, demonstrating the capability of MF-SERS to analyze biochemical molecules secreted during the cell growth, metabolism, proliferation and apoptosis (Figure 3-b). Another example concerns the used of MF-SERS for the differentiation of various mycobacteria in blood samples in short time demonstrating the high potential of these approaches for personalized medicine [79] (Figure 3-c). All these types of *in situ* analyses and monitoring approaches are of primary importance for several biological applications using microfluidics tool at lab scale.

MF-SERS in cells cytometry and liquid chromatography. The SERS effect can be used as an efficient *in situ* detection tool coupled to flow processes at microscale. In particular, separation methods have largely benefited from microfluidic principles such as liquid chromatography, by introducing variants of the sheath-flow (hydrodynamic flow focusing) method [80], which are also widely used in cytometry. The sheath-flow is based on the splitting of a liquid sample within droplets or slugs thanks to the squeezing of the sample by another fluid (liquid or gas) within a microfluidic channel. In this context, Schultz *et al*. have been developing and using this method by controlling microfluidic parameters for sample separation and their analysis using SERS, generating different continuous processes [51], [81]. Similarly, SERS technologies have designed for flow cytometry (SERS-activated cells sorting) [82] and cell counting at microscale allowing detecting Raman signals in ultra-short time, as reviewed by Zhang *et al* [83]. *In situ* SERS effect was also demonstrated for the detection and separation of molecules in liquid chromatography. For instance, Wang et al. have coupled HPLC with SERS for continuous separation and further detection of several analytes, providing an online characterization mean for the acquisition of molecular structural information of solutions [84].

MF-SERS integrated platforms for micro-assays. Some of the latest developments of MF-SERS concern the fabrication and use of all-in-one devices for fast screening at microscale. In particular, Popp et al. have worked on such microfluidics platforms for the synthesis, the separation, the mixing and the analysis of various types of samples. These platforms have been integrated with SERS for biological probe detection of cells in liquid media or biomolecules in urine samples [85]–[87]. Another application that uses the microfluidic concept is the lateral-flow assay (LFA). These assays are realized in paper strips in which capillary forces actions can carry an analyte in different stages onto the strip from their processing to the analysis stage [88]. Choo et al. employed this technique for realizing immunoassays [89], virus [68] and pollutant detection [90], [91]. The concept of microfluidic platforms for fast screening of the operating parameters has led to the realization of immunoassays using SERS as an analytical tool. These on chip devices have been used in particular for Immunotherapeutic molecules screening. [92] Besides, the possibility to have different stages to realize different processes as LFA technique, plus all the possible applications that already exist in the state of the art, have incited researchers to study, design and fabricate devices as the Lab on a Chip (LOC) equipped with SERS probes [93].

IV. Challenges and Opportunities

The use of microfluidic fundaments for the manufacturing of systems capable to control, synthesize, separate and analyze very small volumes are promoting the growth of a wide panorama of possibilities and applications. Now that the coupling of microfluidics with SERS has been validated and used for several applications, the main challenges associated with MF-SERS could be the development of microsystems able to withstand harsher conditions to opens ways for analyzing more complex samples for applications in industrial processes (including high pressure, high temperature or aggressive chemical conditions). All the current publications on MF-SERS have mostly investigated aqueous media at room conditions, therefore leading to the use of polymers (PDMS mostly) as the microdevices fabrication materials. Being able to couple SERS with microreactors made of glass or silicon-Pyrex would provide more robust microsystems for enlarging the range of applications. This challenge would then also be associated to the need of developing stable SERS substrates integrated in such devices.

Besides enlarging the range of conditions, which could be used by MF-SERS devices, new nano-micro-fabrication processes are also highly desirable. In this view, the recent development of 3D nanoprinting approaches opens avenues towards the precise spatial design of MF-SERS devices down to few hundreds of nanometers [94]. This approach based on two photon polymerization technique and shadow masking for micropatterning was originally developed for tissues engineering [95] before moving to micro electromechanical systems development (MEMS) [96] and could possibly be easily adapted to designed MF-SERS devices.

Among the potential future applications of MF-SERS, POC devices are probably the most promising ones, given the growing demand for personal healthcare solutions. To reduce the fabrication cost of MF-SERS devices for such applications, paper-based SERS microfluidics substrates [97]–[100] should be a great alternative to conventional microfabrication techniques. The low cost and easy fabrication principle consist in impregnating noble metal nanoparticles within a conventional paper microreactor. Besides their cheapness, these MF-SERS substrates also allow overcoming the signal fluctuations sometimes encountered in liquid-based MF-SERS.

Environmental biology is yet another example of future applications for MF-SERS thanks to the portability and miniaturization of the devices, which can be used directly on field. In this context, such tools could be used to investigate the biosphere. Indeed, the vast majority of microbes from natural environments is still mostly unknown, but largely contribute to the Earth global carbon cycle. MF-SERS platforms operating in harsh conditions could therefore offer a unique opportunity to better understand endemic microbes in their environments.

It is also worth mentioning the recent use of 3D living cells mapping with confocal Raman spectroscopy, which could also largely benefits form the combination of microfluidics systems with SERS effect. The major advantage of Raman in such studies relies on the ability to work with label-free sample, preventing from bias, which can be induced by the use of tag molecules, generally needed in fluorescent laser confocal microscopy [101]. Nevertheless, the major limitations mostly reside in the low signal to noise ratio of some particular vibration bands characteristics from specific proteins or biomolecules. In that view, SERS could easily be used to overcome this limitation [102], [103] and combined to microfluidics, this should provide advanced working

environments for the culture, detection and analysis of living cells (Figure 4-a) and the microbiology research field in general (Figure 4-b).

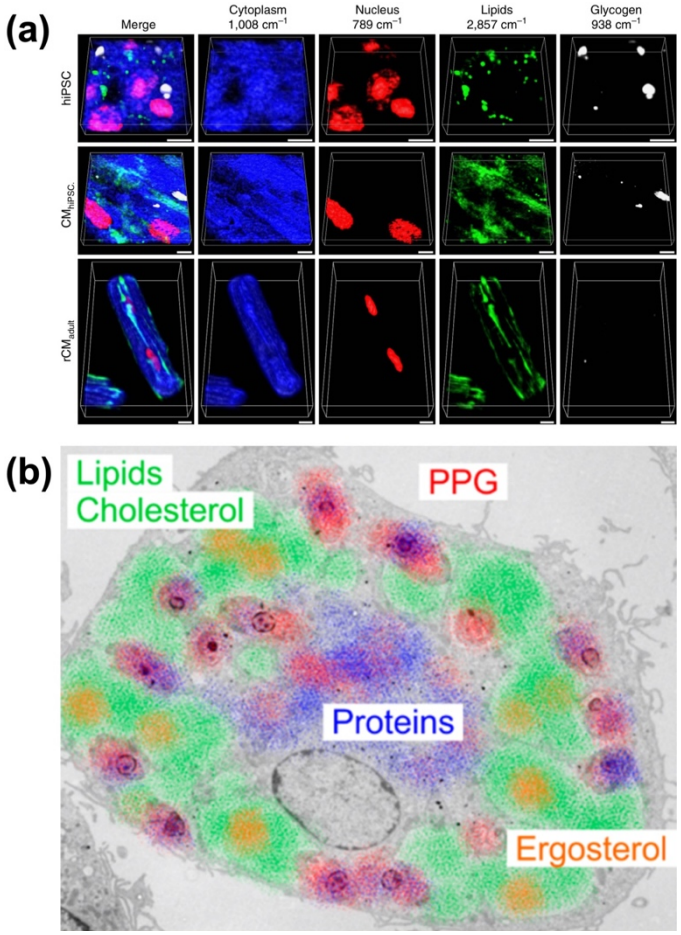

Fig. 4. Examples of living cells SERS mapping. (a) 3D volumes of intensity distributions of human induced pluripotent stem cells, and adult rat ventricular cardiomyocytes for selected bands: 1,008 cm^{-1} for cell cytoplasm (blue), 789 cm^{-1} for cell nucleus (red), 2,857 cm^{-1} for lipids (green), 485 cm^{-1} for glycogen (white) and a merge of all components; scale bar 10 µm. (adapted from [104] with permission from Springer Nature), (b) molecular composition of *Leishmania*-infected macrophage cells *in vitro* by SERS (adapted from [105] with permission from American Chemical Society).

Eventually, Stable Isotope probing strategies could also be implemented in MF-SERS for identifying microbial cells at different levels of substrate incorporation in a complex microbial community, as recently exemplified using conventional SERS [106], [107]. Overall, from the current research general tendency, it is foreseen that MF-SERS will move within the next 10 years from conventional laboratory uses to the palm of our hands.

V. Conclusion

Microfluidic has allowed to improve the separation process and analysis of inorganic, biological and environmental samples. Furthermore, the nature of design and fabrication for microfluidic devices has permitted the coupling of the SERS analytical technique, generating new technologies capable of carrying out more than one process in one place as shown by the multiple applications and potential of the MF-SERS devices.

Recent strategies have been employed to generate reproducible SERS substrates depending on if the substrate is in solution or fixed on the surface. Dispersed SERS substrates in flow, integrated microfabricated SERS substrates on chip and immobilized SERS substrates within microchannels are the most recent strategies for SERS substrates microfabrication. However, some limitations as random aggregation of nanoM colloids, uncontrollable synthesis inside of a microchannel, non-homogeneous molecules adsorption and the relative high cost of fabrication are present in these strategies it is important try to solve them. Even so, with all these limitations, the coupling between microfluidics and SERS technique has permitted a wide range of applications.

Nowadays, MF-SERS had allowed different applications as analytes detections, cells analysis, cytometry and liquid chromatography. Different analytes have been detected as pollutants, drugs, explosives, biomolecules, DNA or pathogens because of the facility of portable microfluidic devices fabrication. Due to the wide use of these devices on medicine and biology, it has been extended the use of POC as a field analysis tool for the opportune detection of disease.

Besides, MF-SERS devices have allowed *in situ* analysis. Different microorganisms as fungi, viruses, yeasts and bacteria, can be handled one by one using these devices, analyzing them in an individually way.

The separation methods have benefited with the control of flow processes using microfluidic devices. The cytometry and the liquid chromatography are techniques that have been improved with the coupling of MF-SERS devices, developing SERS-activated cell sorting and cell counting. Also, HPLC with SERS provides an online characterization.

Another use of MF-SERS is in the design of platforms for microassays. Lateral-flow assays and all-in-one devices have been developed to realize different processes at the same time, becoming them to LOC devices.

Therefore, MF-SERS devices have permitted an improvement in the way to analyze and to control different samples, opening a wide range of possibilities that will allow to enlarge the knowledge on the fields of chemistry, biology and medicine.

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