Editorial

Raman spectroscopy in biomedicine: new advances in SERRS cancer imaging

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Raman spectroscopic technologies have proven to be valuable biomedical tools providing true biochemical fingerprints. For example, they have been used to identify and differentiate several pathogenic microorganisms found in bloodstream infections in intensive care unit patients (1). Raman spectroscopy can also discriminate healthy tissue from diseased tissue, due to the chemical changes resulting from a disease. For instance Huang *et al*. (2) used near infrared Raman spectroscopy to distinguish healthy bronchial tissue from tumor tissue, characterized by higher percentages of nucleic acids and lower percentages of phospholipids compared with healthy tissue. Haka *et al*. (3) demonstrated the potential of Raman spectroscopy to diagnose breast cancer lesions *ex vivo* with 94% sensitivity and 96% specificity. Although very informative and accurate, the Raman signal is intrinsically weak, which limits its applications for early diagnosis. SERS, or Surface Enhanced Raman Spectroscopy, represents a very exciting possibility to amplify and use this sharp and specific Raman signal. In turn, this enables much better limits of detection, even enabling single-molecule detection levels (4).

SERS is based on the excitation of surface plasmons in a rough metallic surface, which provides an amplification of the Raman signal of any molecule adsorbed on the metallic surface by a factor of 10^6 (5). The spectral fingerprints display intense and sharp bands that are much narrower than fluorescence bands (6,7), and show no photobleaching or background fluorescence, in marked contrast with fluorescence- and bioluminescence-based methods (8). This gives SERS a great sensitivity and outstanding multiplexing abilities (9). SERS has notably been developed for the detection of specific DNA sequences: Driskell *et al*. (10) have for instance developed a SERS platform for the detection and discrimination of microRNAs as biomarkers for cancer. Several *in vitro* molecular diagnostic methods for cancer have emerged from SERS-based technologies [see reference (7) for a review]. Immunoassays where SERS substrates are conjugated with antibodies allow for the identification of targeted biomarkers. For example, Wang *et al*. (11) developed a SERS-assay to detect in serum samples a protein aberrantly expressed in pancreatic cancer cells, MUC4, that was not detected by traditional immunoassays (ELISA). Gold nanoparticles, used as a SERS substrate, were functionalized with a SERS reporter and 8G7 antibodies that would specifically attach to the MUC4 protein, enabling its detection. These types of immunocomplexes are also very useful for cancerous cell mapping, by targeting membrane cell receptors (12). Multiplexed cancer cell detection has been reported using gold nanoparticles conjugated with cyanine and triphenylmethine as SERS reporters (13).

Such SERS-engineered nanoparticles made of a metallic core coated with a Raman-active layer, and further functionalized depending on specific diagnostics needs, have led to a fast development of new *in vivo* molecular imaging methods in preclinical animal models. Keren *et al*. (14) performed for the first time non-invasive imaging of live mice using silica-encapsulated SERS-nanoparticles with a gold core, Raman active molecules and a protective glass coating, whose surface chemistry is well known. These nanoparticles are detected at a picomolar level and a maximum depth of 5.5 mm, and *in vivo* multiplex detection has been demonstrated for up to 10 of those SERS nanoparticles (15). The glass-coated SERS nanoparticles can be functionalized

for tumor targeting, for instance to specifically target the EGFR (epidermal growth factor receptor), a surface biomarker which is expressed to a much higher level in colon cancer cells compared to normal cells (16). Similar SERS-nanoparticles have been designed by Kircher *et al*. (17) in a tri-modal study involving MRI, photoacoustic and SERS imaging of brain tumors in live mice. The nanoparticles were hypothesized to enter cancerous cells specifically due to the EPR effect (enhanced permeability and uptake), which is an increased vascular permeability of tumor tissue (18). It increases the uptake of macromolecules in cancerous tissue, and would allow for the accumulation of nanoparticles in cancer cells without the need for a specific labeling. The tri-modal nanoparticles allowed for the co-localisation of tumors using the three imaging techniques, and provided guidance during the surgical resection of the tumors by precisely delineating the margins of the tumor. SERS imaging identified small cancerous *loci* invisible to the naked eye (17) that would have been left behind in a traditional surgery.

A recent study by Harmsen *et al*. (19) reports a new type of SERS nanoparticles called SERRS nanostars that push the limits of SERS cancer imaging even further. These glass-coated SERRS nanostars allow for the successful detection of both cancerous and pre-malignant cells, with detection limits of 1.5 fM, which is 400 times lower than previously obtained by Kircher *et al*. (17). These limits of detection allow for a more precise detection of the true spread of cancer, with the potential to detect microsatellite lesions and diffuse margins. The SERRS nanostars also enable remarkably versatile detection of several cancer types without the need of *a priori* labeling with targeting molecules. Such a universal probe for cancer could lead to crucial gains in time both in diagnostics and treatment. The SERRS nanostars fulfill all criteria required for accurate and universal cancer imaging: affinity for all cancer types, high sensitivity and specificity for cancer cells compared to healthy tissue, and very low detection limits allowing for high spatial resolution.

Instead of SERS, the SERRS-nanostars use SERRS (for Surface Enhanced Resonant Raman Spectroscopy), which has already shown great promise for DNA detection for instance (9,20). In addition to the SERS electromagnetic enhancement, SERRS relies on the resonance of the adsorbed Raman reporter when the excitation wavelength is close to its maximum absorption wavelength, leading to amplification of the Raman signal of up to 10^{14} times (21). Here, the Raman-active molecule, IR-780 perchlorate, resonates when excited by a laser with a wavelength of 785 nm. This chosen excitation wavelength in the near infrared also provides an optimum optical penetration in the tissues. The carefully engineered 75 nm star-shaped gold core provides hot spots of signal amplification at its tips and edges (22). Similar "hot spot" strategy has been reported by using as a core an aggregate of two metallic nanoparticles instead of one (12). With a coating of silica and polyethylene glycol (PEG), the 140 nm SERRS nanostars have a high stability under physiological conditions (37 ℃) with only 3.2% decrease of SERRS signal intensity in 72 h. Because they are mostly made of inert material, such nanoparticles present very low cytotoxicity.

The efficiency of the SERRS nanostars was successfully tested on several murine cancer models selected for their high relevance to human health in incidence, morbidity or recurrence: breast, prostate and pancreatic cancer, as well as sarcoma. More specifically, Harmsen *et al*. (19) conducted SERRS-imaging on living mice, both pre-surgery and during tumor surgical resection, 16 h after a tail intravenous injection of a fixed quantity of SERRS nanostars. Raman imaging was performed *in vivo* in near-real time, and a histopathologic study of the tissues was conducted for comparison and confirmation; tissues were stained for tumor biomarkers, as well as for anti-PEG immunohistochemistry, indicating the presence of SERRS nanostars in the tissues. SERRS imaging succeeded in assessing the margins of macroscopic tumors in all the cancer types tested. More excitingly, after primary tumor resection, SERRS-imaging of the tissue directly surrounding the primary tumor in the mouse MMTV-PyMT breast cancer model revealed several sub-millimeter cancerous lesions, with SERRS-nanostars fingerprints confirmed by histopathology. Sub-millimeter infiltrating lesions were observed as well in the Ink4a/Arf$^{-/-}$ fibrosarcoma model and in the implanted human dedifferentiated liposarcoma model, but in the latter, microscopic lesions as small as 100 microns were also found much further from the bulk tumor, up to 10 mm. Histopathology confirmed they were metastatic satellite lesions. All these microscopic lesions were invisible to the naked eye and without SERRS nanostars imaging they would have been left behind.

Harmsen *et al*. (19) also demonstrate the potential of SERRS nanostars to detect pre-cancerous lesions. Using the KPC mouse model as an analog of human pancreatic cancer, they performed *in situ* pancreas SERRS-imaging after exposure of the pancreas, and were able to not only delineate the main tumor but also several sub-millimeter SERRS-nanostar positive *loci*. The histological examination

of these foci revealed the presence of a precursor of invasive pancreatic cancer, PanIN (Pancreatic Intraepithelial Neoplasia). Mice from the Hi-Myc prostatic cancer model also tested positive with a SERRS nanostar signal in a region made of pre-malignant cells (Prostatic Intraepithelial Neoplasia) in addition to the main tumors. One final question addressed by Harmsen *et al*. (19) is the mechanism of nanoparticle uptake by cancerous cells. Going beyond the hypothesis of the EPR effect (Enhanced Permeability and Uptake), they highlight macropinocytosis, a bulk endocytic process recently proven to be up-regulated in cancer cells (23), as one of the uptake mechanisms. Indeed, after incubation with inhibitors of macropinocytosis, a 70% to 90% decrease in the uptake of SERRS nanostars was observed. However, other endocytic and phagocytic process could also be involved.

This very promising study demonstrates that *in vivo* near-real-time SERRS-imaging has the potential to be groundbreaking in cancer imaging, as it could allow for a precise delineating of tumor margins and microsatellite lesions during surgery. This could drastically reduce the number of cancer recurrences, and thereby improve the prognosis for many patients. A few limitations remain, notably the low depth of penetration of Raman spectroscopy, currently limiting SERRS nanostars to imaging during a resection surgery, when the tissues are exposed. Other imaging methods such as magnetic resonance imaging (MRI) will remain essential for whole body scans and initial cancer diagnostics. Rapid evolution of Raman instrumentation and its adaptation to the clinical environment, notably optical fiber Raman probes with high throughput, could help to fast-forward the translation of such molecular imaging agents to a clinical setting. Providing that the uptake mechanism is known in greater detail both in animal models and humans, one can also envision the coupling of SERRS-nanostars to endoscopic or laparoscopic probes for accurate tumor delineation prior to resection surgery.

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Footnote

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References

1. Choo-Smith LP, Edwards HG, Endtz HP, et al. Medical applications of Raman spectroscopy: from proof of principle to clinical implementation. Biopolymers 2002;67:1-9.
2. Huang Z, McWilliams A, Lui H, et al. Near-infrared Raman spectroscopy for optical diagnosis of lung cancer. Int J Cancer 2003;107:1047-52.
3. Haka AS, Shafer-Peltier KE, Fitzmaurice M, et al. Diagnosing breast cancer by using Raman spectroscopy. Proc Natl Acad Sci U S A 2005;102:12371-6.
4. Nie S, Emory SR. Probing Single Molecules and Single Nanoparticles by Surface-Enhanced Raman Scattering. Science 1997;275:1102-6.
5. Graham D, Smith WE, Linacre AM, et al. Selective detection of deoxyribonucleic acid at ultralow concentrations by SERRS. Anal Chem 1997;69:4703-7.
6. Faulds K, Barbagallo RP, Keer JT, et al. SERRS as a more sensitive technique for the detection of labelled oligonucleotides compared to fluorescence. Analyst 2004;129:567-8.
7. Vendrell M, Maiti KK, Dhaliwal K, et al. Surface-enhanced Raman scattering in cancer detection and imaging. Trends Biotechnol 2013;31:249-57.
8. Troy T, Jekic-McMullen D, Sambucetti L, et al. Quantitative comparison of the sensitivity of detection of fluorescent and bioluminescent reporters in animal models. Mol Imaging 2004;3:9-23.
9. Graham D, Faulds K. Quantitative SERRS for DNA sequence analysis. Chem Soc Rev 2008;37:1042-51.
10. Driskell JD, Seto AG, Jones LP, et al. Rapid microRNA (miRNA) detection and classification via surface-enhanced Raman spectroscopy (SERS). Biosens Bioelectron 2008;24:923-8.
11. Wang G, Lipert RJ, Jain M, et al. Detection of the potential pancreatic cancer marker MUC4 in serum using surface-enhanced Raman scattering. Anal Chem 2011;83:2554-61.
12. Xia X, Li W, Zhang Y, et al. Silica-coated dimers of silver nanospheres as surface-enhanced Raman scattering tags for imaging cancer cells. Interface Focus 2013;3:20120092.

13. Maiti KK, Samanta A, Vendrell M, et al. Multiplex cancer cell detection by SERS nanotags with cyanine and triphenylmethine Raman reporters. Chem Commun (Camb) 2011;47:3514-6.
14. Keren S, Zavaleta C, Cheng Z, et al. Noninvasive molecular imaging of small living subjects using Raman spectroscopy. Proc Natl Acad Sci U S A 2008;105:5844-9.
15. Zavaleta CL, Smith BR, Walton I, et al. Multiplexed imaging of surface enhanced Raman scattering nanotags in living mice using noninvasive Raman spectroscopy. Proc Natl Acad Sci U S A 2009;106:13511-6.
16. Jokerst JV, Miao Z, Zavaleta C, et al. Affibody-functionalized gold-silica nanoparticles for Raman molecular imaging of the epidermal growth factor receptor. Small 2011;7:625-33.
17. Kircher MF, de la Zerda A, Jokerst JV, et al. A brain tumor molecular imaging strategy using a new triple-modality MRI-photoacoustic-Raman nanoparticle. Nat Med 2012;18:829-34.
18. Maeda H, Nakamura H, Fang J. The EPR effect for macromolecular drug delivery to solid tumors: Improvement of tumor uptake, lowering of systemic toxicity, and distinct tumor imaging in vivo. Adv Drug Deliv Rev 2013;65:71-9.
19. Harmsen S, Huang R, Wall MA, et al. Surface-enhanced resonance Raman scattering nanostars for high-precision cancer imaging. Sci Transl Med 2015;7:271ra7.
20. Feuillie C, Merheb MM, Gillet B, et al. Enzyme-free detection and quantification of double-stranded nucleic acids. Anal Bioanal Chem 2012;404:415-22.
21. Maher RC, Cohen LF, Etchegoin P. Single molecule photo-bleaching observed by surface enhanced resonant Raman scattering (SERRS). Chem Phys Lett 2002;352:378-84.
22. Halvorson RA, Vikesland PJ. Surface-enhanced Raman spectroscopy (SERS) for environmental analyses. Environ Sci Technol 2010;44:7749-55.
23. Commisso C, Davidson SM, Soydaner-Azeloglu RG, et al. Macropinocytosis of protein is an amino acid supply route in Ras-transformed cells. Nature 2013;497:633-7.

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