Label-free nanosensing platform for breast cancer exosome profiling

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Cancer constitutes the second leading cause of mortality worldwide, only surpassed by cardiovascular disease. Based on GLOBOCAN estimates, about 18 million new cancer cases and 9.6 million deaths occurred in 2018 worldwide. Breast cancer is the most frequently diagnosed form of cancer and the leading cause of cancer death among females worldwide. It totalizes 11.6% of the global registered cases in both genders. The diagnosis of breast cancer in the current scenario is made by mammography and its spectroscopy, and breast biopsy test. All these detection techniques are usually expensive, invasive and time-consuming. Thus, the development of simple, reliable, cost effective and non-invasive detection methods for early cancer diagnosis and posterior follow-up is particularly important, due to the disease's prevalence and potential lethality.

Exosomes are small (50–100 nm in diameter) extracellular vesicles secreted by all cells into body fluids such as blood, saliva and urine. These extracellular vesicles show potential for cancer diagnostics, as they transport several molecular contents of the cells from which they originate. Additionally, exosome' analyses are minimally invasive and afford relatively unbiased readouts of the entire tumour burden, less affected by the scarcity of the samples or intra-tumoral heterogeneity. The essential first step of current exosome analysis is purification by ultracentrifugation. Afterwards, exosomes may be analysed using western blot, enzyme-linked immunosorbent assay, flow cytometry and other analytical methods. Though robust and effective, these strategies are expensive, time-consuming and rely heavily on the sample handling skills. Thus, the development of sensor technologies for rapid, inexpensive, simple and on-site exosome screening is necessary. Some exosomes-based sensors have already been reported, such as label-free exosome assay utilizing transmission surface plasmon resonance (SPR), mass-sensitive sensors aptamer-based sensors magneto-electrochemical sensors, among others. [1,2,3]

Surface Enhanced Raman Spectroscopy (SERS) has emerged as a highly sensitive and rapid analytical technique with wide application regimes, from biological analysis to environmental monitoring. At the most basic level, SERS is a way to significantly increase the signal from the weak yet structurally rich technique of Raman scattering. A SERS-active substrate is generally based on a platform coated with a roughened or nanostructured metallic surface (silver, gold, etc.) that enhances the Raman signal due to the Localized Surface Plasmon Resonance (LSPR). However, the commercially available SERS-active substrates come with a high cost and low shelf-life. Cellulose is the most abundant polymer on earth and meets several interesting properties. Being a biocompatible, biodegradable, flexible, recyclable and low-cost material draws the scientific community attention in several applications, from biosensors to electronics. In this work [4], low-cost SERS-active substrates were produced based on silver nanoparticles (AgNPs) grown *in situ* into bacterial cellulose (BC) coming from commercial *nata de coco,* providing a low-cost and simple alternative to the conventional production methods (Figure 1). The AgNPs were grown by hydrothermal synthesis assisted by microwave radiation, that allows well-controlled and fast synthesis. Two routes were followed: one using silver citrate as precursor and another using silver citrate and ammonium citrate dibasic, to induce alkaline environment. A systematic study on the precursor concentration, synthesis time and temperature was performed in order to obtain a good SERS enhancement factor (EF). Ultimately, it was possible to achieve EF from 10^4 to 10^5, detecting rhodamine 6G (R6G) with concentrations as low as 10^{-11} M, which was used as model-molecule. Finally, biological tests were performed on the optimized SERS substrate, with exosomes samples coming from MCF-10A (non-tumorigenic breast epithelium) and MDA-MB-231 (breast cancer) cell cultures lineages and the obtained SERS spectra were subjected to statistical Principal Component Analysis (PCA), to analyse variances in each collected spectrum and group the similar ones. This analysis tool was chosen since exosomes segregated by the same cell may have different Raman fingerprints: this is probabilistic and is related to the way the exosomes may be positioned in the SERS substrate. Since exosomes have several surface's proteins, different positions of the exosomes in the SERS platform will show different Raman spectra, thus inducing some intra sample variation. So, by combining PCA with Raman intra and inter variability in exosomal samples, it was possible to differentiate tumoral and non-tumoral exosomes, with a data grouping of 95% confidence. The obtained 95% confidence ellipses (Figure 2) are especially useful in this work final objective, since they can be used as a form of diagnostic: if an analysed sample Raman spectrum is located inside any of the presented ellipses, it's safe to assure with 95% confidence that the sample belongs to the

respective group. As a proof-of-concept, a close-to real time diagnosis was performed. For this, tumoral and non-tumoral sample SERS spectra were collected and analysed in the previously obtained score plots. It was registered that both samples fell within the respective ellipses, proving PCA as a valuable diagnosis tool, being able to distinguish between exosomes segregated by tumoral and non-tumoral cell lineages trough SERS while using innovative substrates, with an overall cost of 0.39 € per membrane, which can provide several dozens of different measurements. This approach rendered a simple, label-free and easy-to-perform method for exosomes profiling and shows promising results as far as breast cancer diagnostic by SERS is concerned. Moreover, we believe that this novel exosome's profiling method can provide precious information on both the prognosis of the disease and the predictive outcome of a given therapy.

References

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Figures

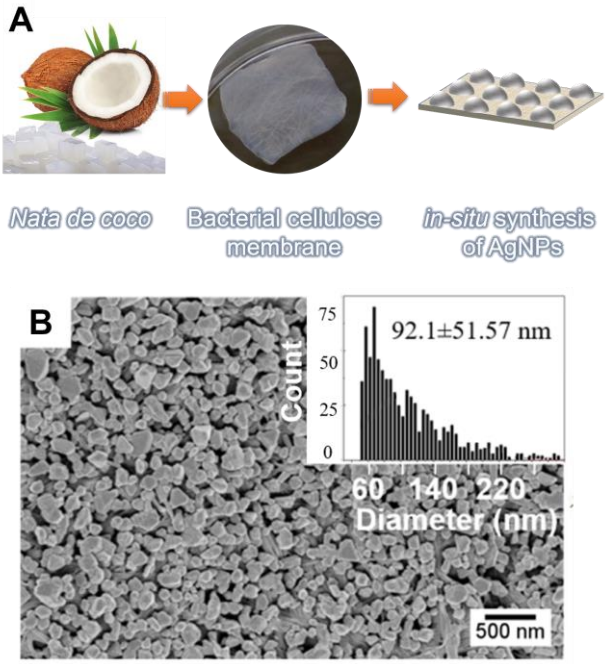

Figure 1. A: Schematic workflow of the active-SERS substrate's production: from the source (*nata de coco*) to the final BC/AgNPs platform; B: SEM imaging and AgNP's diameter histogram of the optimized active-SERS platform using BC/AgNPs composites.

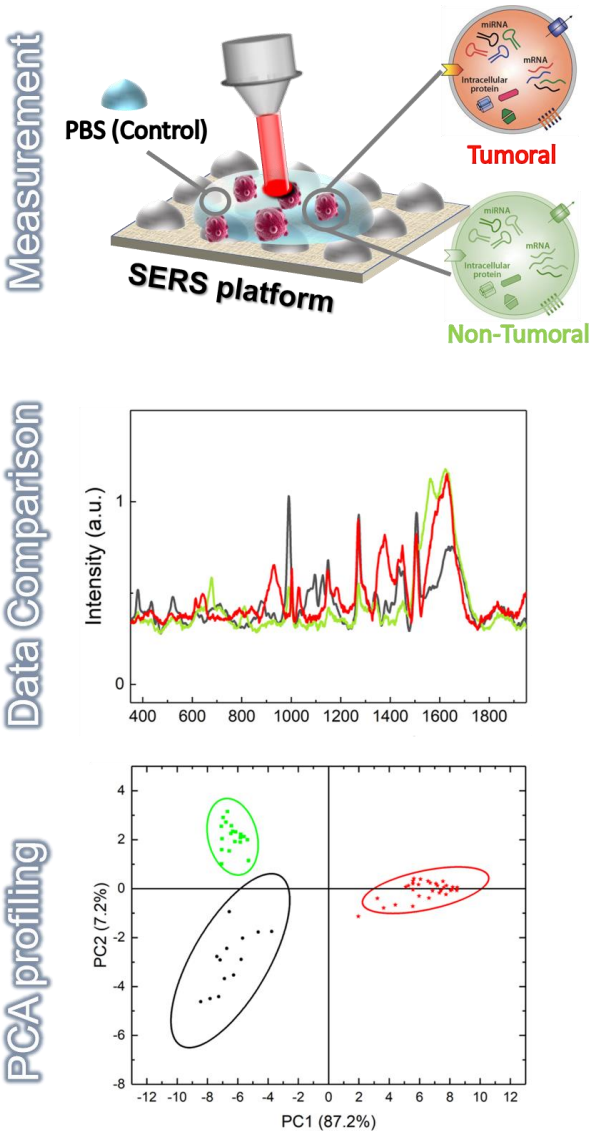

Figure 1. Schematic representation of the detection system used in this work. Measurement of exosome's samples, spectra data comparison and PCA profiling: PCA resultant score plot using PC1 and PC2 as the analysed principal components: PBS (control) in black, MCF-10A (green) and MDA-MB-231 (red) exosomes sample analysed.

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