Breast cancer and biomineralization: New insights by means of infrared nanospectroscopy

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Introduction – Breast microcalcifications (BMC) are calcium-based mineral deposits within the breast tissue that can be either benign or malign. Their presence on mammograms helps the clinician, as they are considered early signs of breast cancer. However, the correlation between the chemical composition of BMC and breast cancer has been poorly investigated and is still not fully understood. BMC size can range from tens of nanometres to a few hundreds of micrometres. Hence, conventional IR spectroscopy can be used for characterizing the larger BMC but is not suitable for properly describing BMC at the early stage of biomineralization. However, this issue can be addressed by using photothermal infrared spectroscopy such as atomic force microscopy-infrared (AFM-IR) and optical photothermal infrared (OPT-IR) micro-spectroscopy systems.

AFM-IR and OPT-IR are infrared (IR) characterization techniques, based on photothermal phenomena, meaning they probe the thermal expansion of the sample when it absorbs the IR light. OPT-IR uses a visible laser as a probe and has a spatial resolution between 500 nm and 1 µm [1], while AFM-IR senses the thermal expansion of the sample through the tip of an atomic force microscope and has a spatial resolution up to 20 nm [2]. These two technics are non-destructive and do not require specific sample preparation. Therefore, they open new perspectives for the description of complex biological samples at the sub-micrometric scale. For example, AFM-IR was already used to describe, directly in kidney biopsies, vancomycin casts, and more recently crystals involved in cystinosis [3,4]. In that regard, we aim to emphasize that OPT-IR and AFM-IR can contribute to a better understanding of biomineralization in the breast and BMC etiology. Indeed, to this day, two chemical phases - calcium oxalate dihydrate and calcium phosphate apatite - are considered in the breast microcalcification classification. Yet, using IR nanospectroscopy, we will demonstrate the presence of other chemical phases, as well as BMC chemical heterogeneity at the nanoscale.

Materials and Methods – BMC were investigated in patients diagnosed with either in-situ or invasive carcinoma, as well as mastopathies (benign microcalcifications used as control). 4 to 8 µm thick slices of the paraffin- embedded biopsies were placed on low-e microscope slides (substrate compatible with both SEM analysis and IR measurements; MirrIR, Kevley Technologies, Tienta Sciences, Indianapolis). Paraffin was removed using xylene, and microcalcifications chemical analyses were conducted by OPT-IR and AFMIR. OPT-IR measurements were performed using mIRage™ IR microscope from Photothermal Spectroscopy Corp., while AFM-IR measurements were done, in both contact and tapping mode, using NanoIR2 from Bruker nano.

Results – First, we show that AFM-IR and OPT-IR systems are suitable for the description of heterogenous samples and enable the characterization of microcalcifications within their native environment. Thereafter, we demonstrate that a sub-micrometric description of BMC is critical to understand the relationship between biomineralization and breast cancer. We have detected the presence of chemical phases (Figure 1B - dark blue and purple spectra) that aren't considered in the BMC chemical classification (Figure 1B - black and grey spectra) and that have not been described yet by conventional IR spectroscopy. In addition, we highlight BMC local chemical heterogeneity showing that both techniques have a great potential for characterizing BMC and investigating the pathological mineralization processes in breast tissue.

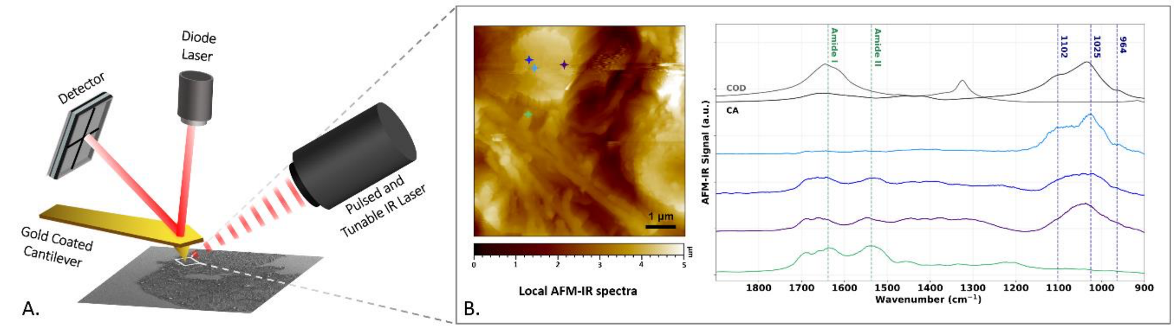

Figure 1 – (A) Representation of the top-down illumination AFM-IR system used to investigated BMC in breast biopsies. **(B)** AFM topography and AFM-IR local spectra in a breast biopsy diagnosed with invasive ductal carcinoma. The coloured stars on the topography indicate the location of the AFM-IR spectra. Grey and black spectra are FTIR reference spectra and correspond, respectively, to Calcium Oxalate Dihydrate (COD) and Calcium Phosphate Apatite (CA): the two chemical phases on which is based the current BMC classification.

Conclusion – Photothermal IR spectroscopy techniques are essential to characterize complex biological samples at the sub-micrometric scale and provide information that isn't accessible through conventional IR micro-spectroscopy. Our results emphasize that BMC implications are still not well understood nor described and prove that AFM-IR and OPT-IR are valuable tools to investigate organic crystals in the breast and evaluate their part in the pathology.

References

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