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My name is Sara Cavallaro and I am a PhD student in the Dept. of Applied Physics at KTH Royal Institute of Technology (Stockholm, Sweden). I am currently working on detection and characterization of extracellular vesicles (EVs), nanosized cell derived vesicles involved in intercellular communication, using both ensemble methods and single EV techniques. In particular, I have been using SEM and AFM for the vesicle size estimation, while using electrical- and fluorescence-based sensors for their molecular content characterization. Other than in biosensors, I am interested in biomechanics, tissue engineering and regenerative medicine.

"High-resolution size-based profiling and morphological analysis of extracellular vesicles by scanning electron and atomic force microscopy"

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Introduction: Extracellular vesicles (EVs), nanosized cell derived vesicles, have been found to mediate intercellular communication in physiological and pathological processes[1]. Nevertheless, the understanding of EVs bio-functionality remains elusive, mainly because of their high heterogeneity in molecular content, but also in size (30-2000 nm). Therefore, accurate size measurements of EVs are highly desired, particularly for exploiting their full diagnostic/therapeutic potential[2].
Currently available techniques, such as Nanoparticle Tracking Analysis (NTA), cannot accurately measure EVs smaller than 60-70 nm and are not capable to distinguish them from other particles, e.g. lipids or protein aggregates[3]. On the contrary, electron microscopy (EM) and atomic force microscopy (AFM) techniques allow high-resolution size-profiling and

morphological analysis of EVs over their whole size range. However, their low throughput combined with several long preparatory steps have prevented these methods from being routinely used for EV size profiling.

Methods: We present a method improvement in throughput and reproducibility of EV size-analysis by scanning EM (SEM) and AFM. The SEM technique is based on covalent EV capture onto a silicon wafer, using the protocol reported by Cavallaro et al.[4] up to the Glutaraldehyde step. After immobilization, Critical Point Drying (CPD) is performed to dehydrate EVs before SEM, while preserving their shapes. For the AFM, Quantitative Imaging (QI) based mode is used in liquid for high resolution size analysis of EVs with low vesicle damage.

Results: SEM images, showing the comparison in densities of EVs prepared by covalent and non-covalent coupling to substrate, indicated a good capture efficiency of our covalent protocol. The size distribution analysis showed good agreement between NTA and SEM for EVs >80 nm. For smaller EVs, SEM is more sensitive than NTA, thus more suitable to check the purity of EV-isolation techniques. Last, atomic-force microscopy (AFM) was also used to validate our measurements and as an alternative technique for high resolution EV size analysis.

Summary/Conclusion: To conclude, SEM and AFM can be used to accurately estimate EV size distributions in a wide range, overcoming the well-known limitations of NTA.

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