Acoustic seed-trapping enables rapid enrichment and purification of nanovesicles involved extracellular signalling.

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Abstract

Extracellular vesicles (EV) encompass several different cell-derived nanometer scale vesicles, which all play important roles in intercellular communication, e.g. through membrane integrated proteins that target cells and trigger intracellular signalling pathways or fuses with the target cell delivering gene-regulating components such as mRNA or microRNA (miRNA). Exosomes are small intraluminal vesicles (50-100 nm) secreted via so called multivesicular endosomes and are recognized as an important mode of cell-independent communication and immune system regulation. Exosomes are present in all biofluids and contain a wide range of proteins and RNAs that reflect their tissue of origin. Microvesicles (microparticles) are larger in size, 100-1000 nm, and are disseminated from cells by budding from the plasma membrane into the extracellular space, having similar function in extracellular communication.
The study of extra cellular vesicles involves extensive ultracentrifugation protocols to isolate exosomes and microvesicles. In order for ultracentrifugation to be functional, sufficient material must be available to allow the formation of a visible pellet after the centrifugation. This usually requires several 2-5 mL of biofluid and is a major bottle neck in advancing research in this area due to the limited access to such large sample volumes.
Our group has recently reported that bacteria as well as nanoparticles (110 nm) can be enriched by means of capillary based acoustic trapping configured in the so called seed-trapping mode. Acoustic seed-trapping utilises inter particle forces, occurring as ultrasound waves are scattered between two particles. By seeding the acoustic trap with larger particles ($\approx$ 10 um) that can easily be retained against flow by the primary acoustic radiation force, when exciting a capillary with a local ultrasonic vibration, nanometer sized particles in a sample that is exposed to the larger seed particles in the acoustic trap will be attracted to the seed particles, aggregate and be retained against flow.
This mechanism enables rapid enrichment of nanometersized solid particles as well as biological nanoparticles, i.e. bacteria, exosomes and microvesicles. The basics of acoustic trapping will be discussed and the application of acoustic seed-trapping to realise a rapid microfluidic system for detection of bacteria in blood will be described and the first tests of this in a clinical setting on 57 patient samples will be discussed. The seed-trapping platform has also been investigated for the enrichment and enumeration of platelet derived microvesicles in blood plasma from patients with myocardial infarction, demonstrating analogous data to what was obtained by ultracentrifugation based sample preparation. Initial data on exosome and micro vesicle enrichment from cell cultures, cerebrospinal fluid and blood plasma will also be presented, showing our first data on protein content in these vesicles using LC MS/MS analysis and detection of short RNA and microRNA by qRT-PCR. The development of acoustic seed-trapping for nanoparticle preparation now opens up a Holy Grail for biomarker research and diagnostics in small sample volumes (50-200 uL) which are not accessible for ultra centrifugation and hence extensive studies of extracellular vesicles in cryopreserved biobank samples based on large population-based cohorts may now be possible.