

Exosomes: Human Saliva-derived nanoBiomarkers for Use in Clinical Dentistry?

Exosomas: ¿nanoBiomarcadores Derivados de Saliva Humana para Uso en Odontología Clínica?

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EDITORIAL

Exosomes are small (30–120 nm) endocytic cell membrane-derived nano-vesicles (from the larger ~1 µm late endosomal multivesicular body-derived circulating vesicles; after fusion with the plasma membrane - released from activated cell surfaces), discovered in the mid-80s, and for long considered to serve as “garbage bags” for cells to discard undesirable cargo. Today, exosomes are attracting exquisite snow-balling attention as they are known to be present in all biological fluids including blood, urine, amniotic fluid, breast milk, ascitic and hydrothoracic fluid. This also includes non-invasively obtained bodily fluids such as saliva, harboring a variety of proteins, nucleic acids, and lipids (predominantly cholesterol, sphingolipids, phospholipids, and bisphosphates). Exosomes have a unique and complex composition. Indeed, the latest exosome content database identifies: 4,563 proteins, 194 lipids, 1,639 mRNAs, and 764 miRNAs, in exosomes from multiple organisms (Exocarta V4; available at: www.exocarta.org). Exosomes, which carry various molecular constituents of their cell of origin, including proteins, lipids, mRNAs, and microRNAs (miRNAs), have characteristic surface markers (such as CD63, CD9, CD81, and TSG101) and participate in cell-to-cell communications and protein and RNA delivery, thereby, influence physiological as well as pathological processes (note: exosomes are shed by cells under both normal and pathological conditions). For example, while several studies report that miRNA contents of circulating exosomes correspond to those of their originating cancer cells (tumor cells release exosomes containing tumor-specific RNAs), other studies report that miRNAs can be detected in exosomes isolated from saliva. Hence, exosomes are suggested to have potential for

cancer diagnostics and offer a great utility promise as novel biomarkers (and perhaps, targeted delivery systems) for clinical diagnosis and management/treatment of pathologies and conditions. As mentioned earlier, while exosomes originate (or are released - via exocytosis) from many cell types (primarily, hematopoietic cells), such as platelets, reticulocytes, lymphocytes (B and T), mast cells, epithelial cells, endothelial cells, dendritic cells, macrophages and neurons; molecular constituents are often associated with certain diseases and treatment responses. Thus, exosomes have also emerged as an exciting potential candidate for immune-therapy, vaccination and as a novel vector for gene therapy, beyond diagnostics.

Given their small size and low density, exosomes are usually isolated from bodily fluids and cell culture media via labor-intensive, technically-sensitive, time-consuming and expensive differential ultracentrifugation, in a laboratory setting, typically. To obtain exosomes from human saliva, for example, stimulated submandibular/sublingual and parotid glandular saliva (bi-lateral, using 2 % citric acid; repeated up to 8-10 times) is collected and immediately placed on ice, and centrifuged at 1500 g for 10 min at 4 °C. The supernatant is then removed, placed in another tube and centrifuged at 17000 g for 15 min at 4 °C to further remove unwanted organelles and cell fragments. The supernatant is again transferred to sterile tubes and ultra-centrifuged at 160000 g for 1 h at 4 °C. The pellet containing the exosomes is washed with phosphate-buffered saline and ultra-centrifuged again at 160000 g for 1 h at 4 °C. Again, the supernatant is removed and the pellet briefly allowed to dry. Samples can then be passed for protein and/or RNA isolation.

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To permit clinical application, micro- and ultra-filtration techniques, have been recently introduced; allowing the rapid isolation of exosomes (urinary). Other methodologies also exist. In our experience at BioMAT'X, exosomes (serum and salivary) are simply isolated following a precipitation protocol using the readily-available and efficient ExoQuick™ kit (System Biosciences, Mountain View, CA, USA). Amongst others, we do recommend this technique for exosomal miRNA profiling and exosomal RNA analysis (no conflict of interest exists). While it allows the use of small bio-fluid samples, with simplicity and rapidity, pellets will contain larger-sized exosomes. It is noteworthy also that, when compared to ultra-centrifugation, resulting samples from precipitation, will contain more non-exosomal-related proteins, microvesicles and membranous debris (biological impurities). Hence, pros and cons of each method should be taken into consideration, and selected per planned aims, objectives and requirements, for the research and/or use.

Quick Comments on Exosomes in Oral Cancer – available studies are enormously limited. It seems thus far, that oral cancer-derived exosomes (salivary), when compared to those from healthy patients, are larger (20-400 nm) in size, irregular in morphology (appear under Atomic Force Microscopy as circular or bulging vesicles) and are presented with elongated extra-vesicular nanofilaments, of un-known origin or function.

While both exosomes share similar protein markers (exosomal surface), variance in concentrations exist; higher expression of CD63 molecules in cancerous exosomes. Conversely, others reported decreases in CD9 and CD89 concentrations (via ELISA; insignificant differences). Recently, exosomes isolated from patients diagnosed with oral squamous cell carcinoma or OSCC, demonstrated an average size of 50-200 nm, using Scanning Electron Microscopy. Further, higher concentration levels of CD63 and CD81 molecules were detected via Western Blotting. Likewise, concentration levels of miR-21 were found to be higher than in healthy donors, interestingly. It is safe to say, again, nonetheless, that OSCC-related exosome literature is still sparse, as more focus or prioritization is given to characterizing and understanding human-derived salivary exosomes as novel nanobiomarkers for application in clinical diagnostics and therapeutics. In our BioMAT'X, ongoing efforts aim to explore potential use of exosomal concentration levels of CD63 and that of miR21 in circulating exosomes, for example, as an early indicator(s) for oral cancer onset, a measure for tumour aggressiveness (via studying oral pre-malignant

lesions) and a biomarker for the diagnosis of oral cancer(s).

KEY WORDS: Exosomes; salivary diagnostics; salivary biomarker; oral fluid diagnostics; oral squamous cell carcinoma; oral cancer; salivaomics; proteomics; vesicles; nanosize.

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