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# Raman spectroscopy for the biochemical characterization of human salivary extracellular vesicles as a valuable source of brain biomarkers

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Extracellular vesicles (EVs) are natural nanoparticles secreted under physiological and pathological conditions. Thanks to their diagnostic potential, EVs are increasingly being studied as biomarkers of a variety of diseases, ranging from neurodegenerative to cancer disorders (1). To date, most studies on EV biomarkers use blood as the source, despite different disadvantages that may cause an impure EV isolation (2). However, saliva could be a valuable source of EVs that could be studied as brain biomarkers in an easily accessible biofluid, as it is also reported that brain-derived EVs have been found in the saliva of patients with Parkinson's disease (3).
Herein, we propose the isolation of human salivary EVs and their subsequent characterization by Raman spectroscopy to obtain a comprehensive biochemical characterization that can be easily translated to diagnostics.
Using a comparable protocol for the isolation of EVs from both serum and saliva in parallel for the same subject, salivary EVs showed greater purity in terms of co-isolates, including both lipoproteins and protein corona, evaluated by nanoparticle tracking analysis and Conan test. After having demonstrated the presence of brain-derived EVs in both saliva and serum samples by using a previously optimized Surface Plasmon Resonance (SPR) - based biosensor (4), Raman spectroscopy allowed the identification of the overall biochemical composition of EVs coming from the two different biofluids. Even considering the limited amount of EVs that can be isolated from saliva, the use of Raman spectroscopy was not hampered, and it was able to provide with a good sensitivity a comprehensive characterization of EVs in a high throughput and repeatable manner (5).
Raman spectroscopy can thus represent a turning point in the application of salivary EVs in clinics, taking advantage of the simple method of collection of the liquid biopsy and of the quick, sensitive and label-free biophotonics-based approach.

(1)Hill, A.F. Extracellular Vesicles and Neurodegenerative Diseases. J. Neurosci. 2019, 39, 9269–9273

(2)Martins, T.S.; Vaz, M.; Henriques, A.G. A Review on Comparative Studies Addressing Exosome Isolation Methods from Body Fluids. Anal. Bioanal. Chem. 2022, 1–25

(3)Rani, K.; Mukherjee, R.; Singh, E.; Kumar, S.; Sharma, V.; Vishwakarma, P.; Bharti, P.S.; Nikolajeff, F.; Dinda, A.K.; Goyal, V.; et al. Neuronal Exosomes in Saliva of Parkinson's Disease Patients: A Pilot Study. Park. Relat. Disord. 2019, 67, 21–23.

(4)Picciolini, S; Gualerzi, A; Carlomagno, C; Cabinio, M; Sorrentino, S; Baglio, F; Bedoni, M. An SPRi-based biosensor pilot study: Analysis of multiple circulating extracellular vesicles and hippocampal volume in Alzheimer's disease. Journal of Pharmaceutical and Biomedical Analysis, 2021; 192: 113649

(5)Mangolini, V; Gualerzi, A; Picciolini, S; Rodà, F; Del Prete, A; Forleo, L; Rossetto, RA; Bedoni M. Biochemical Characterization of Human Salivary Extracellular Vesicles as a Valuable Source of Biomarkers. Biology. 2023, 12, 227

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