The Raman fingerprint of Salivary Extracellular Vesicles for the differential diagnosis of neurodegenerative diseases

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Early diagnosis of neurodegenerative diseases is a critical challenge in contemporary medicine, as it would enable the implementation of timely and personalized therapeutic strategies with a significant impact on disease progression and patients' quality of life. While the importance of biologically based classification of Parkinson's disease (PD) and other neurodegenerative disorders is now widely recognized, the identification of reliable, measurable biomarkers remains an unmet need.
Within this context, the ongoing MINERVA project explores the potential of Raman spectroscopy as a valuable approach for the molecular characterization of saliva-derived extracellular vesicles (EVs) in neurodegenerative diseases, including PD, atypical parkinsonisms (APs), and Alzheimer's disease (AD). A key objective of the project is the validation of a spectroscopy-based method for the differential diagnosis of neurodegenerative disorders.
Patients diagnosed with PD, APs, and AD were recruited after protocol approval by the local Ethics Committee, and saliva samples were collected. EVs were isolated from saliva using a combined procedure of size exclusion chromatography and ultracentrifugation [1]. The repeatability and robustness of the isolation protocol were assessed following the MISEV2023 guidelines [2]. Raman spectroscopic analysis was then performed on air-dried EV samples deposited on calcium fluoride substrates, following a previously optimized measurement protocol [1]. The acquired Raman spectra provided detailed biochemical fingerprints of the EV cargo, with contributions from proteins, lipids, and other biomolecular components.
Preliminary results demonstrate that Raman spectroscopy is able to detect subtle yet reproducible biochemical differences between salivary EVs derived from PD, APs and AD subjects. In particular, significant variations were observed in the Amide I spectral region, consistent with differences in EV-associated protein composition. Multivariate statistical analysis indicated that these spectroscopic differences are independent of age and gender, while enabling discrimination between different movement disorders and showing correlation with clinical assessments.
Upon validation on the full MINERVA cohort of more than 240 subjects, this Raman-based approach has the potential to represent a significant advance in spectroscopic biomarker development for neurodegenerative diseases, offering a non-invasive, rapid, and objective tool for differential diagnosis and supporting the future personalization of therapeutic strategies.
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References

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[2] J.A. Welsh et al, *J Extracell Vesicles* **2024**, *e12404.*