Human Saliva for Periodontitis

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This pilot study aims to investigate whether salivary small extracellular vesicle (sEV)-associated microRNAs could act as potential biomarkers for periodontal disease status. Twenty-nine participants (10 who were healthy, nine with gingivitis, 10 with stage III/IV periodontitis) were recruited and unstimulated whole saliva samples were collected. Salivary sEVs were isolated using the size-exclusion chromatography (SEC) method and characterised by morphology, EV-protein and size distribution using transmission electron microscopy (TEM), Western Blot and Nanoparticle Tracking Analysis (NTA), respectively. Ten mature microRNAs (miRNAs) in salivary sEVs and saliva were evaluated using RT-qPCR. The discriminatory power of miRNAs as biomarkers in gingivitis and periodontitis versus healthy controls was evaluated by Receiver Operating Characteristics (ROC) curves. Salivary sEVs were comparable to sEVs morphology, mode, size distribution and particle concentration in healthy, gingivitis and periodontitis patients. Compared to miRNAs in whole saliva, three significantly increased miRNAs (hsa-miR-140-5p, hsa-miR-146a-5p and hsa-miR-628-5p) were only detected in sEVs in periodontitis when compared to that of healthy controls, with a good discriminatory power (area under the curve (AUC) = 0.96) for periodontitis diagnosis. Our study demonstrated that salivary sEVs are a non-invasive source of miRNAs for periodontitis diagnosis. Three miRNAs that are selectively enriched in sEVs, but not whole saliva, could be potential biomarkers for periodontal disease status.

salivary small extracellular vesicles | size exclusion chromatography | miRNAs | periodontal disease | biomarker discovery

1. Periodontitis

Periodontitis is a complex inflammatory disease, associated with a dysbiotic plaque biofilm and characterised by the destruction of periodontal tissues. Currently, it is clinically diagnosed by clinical attachment loss (CAL), periodontal pocket depth (PPD), bleeding on probing (BOP) and radiographic bone loss. Aside from the fact that these parameters are mostly measures of past disease activity and, in the case of BOP, have poor predictive properties for disease progression, they also require professional dental assessment. Given the widespread incidence of periodontitis in approximately 50% of the global adult population [1], there is a plausible rationale for the development of a biologically based non-invasive diagnostic system for periodontal disease status.

2. Human Saliva

Human saliva is an attractive source of biomarkers for periodontitis; it is easy to access through non-invasive means, is low-cost, and potentially provides a “mirror” of the periodontal status of a patient [2][3]. Salivary levels of pro-inflammatory cytokines [4][5], chemokines [6], matrix metalloproteinases [7][8] and bone remodelling proteins [4][9][10][11] have been investigated for their ability to distinguish between individuals with periodontal disease and those who are healthy. However, most proteins are present in low concentrations in saliva and have a limited diagnostic value due to their poor sensitivity and specificity [11].

3. History

In the past 10 years, the role of extracellular vesicles (EVs) in cell-to-cell communication has been widely explored [12]. EVs are lipid-encapsulated vesicles with the capacity to transport bioactive molecules (i.e., microRNAs (miRNAs)) which can be delivered to other cells to regulate their biological function. There are several types of EVs, which can be classified by their sizes, into small EVs (i.e., sEVs, exosomes), medium (i.e., microvesicles) and large EVs (i.e., macrovesicles and apoptotic bodies). Currently, there is an ongoing discussion in the literature about the heterogeneity of EVs and the correct nomenclature; however, the majority of studies have focused on small EVs such as exosomes. sEVs (< 200 nm), which are abundant in saliva and are emerging as a potential source for the development of diagnostic tools for a variety of diseases [13][14][15][16], owing to their components—nucleic acids (microRNAs, DNAs and other RNAs), lipids and proteins. Salivary sEVs have not been widely explored as a diagnostic tool in periodontology, probably because the methodology for their isolation is still developing. Very recent research has demonstrated that salivary CD9- and CD81-positive (two tetraspanin proteins enriched in EVs) sEVs are decreased in periodontitis compared to healthy controls [17]. Other recent research has demonstrated that the salivary sEV (exosomal) programmed death-ligand 1 (PD-L1) mRNA is significantly increased ($p < 0.01$) in periodontitis patients versus non-periodontitis subjects [18].

4. Development

sEVs can carry a wide range of bioactive molecules and are enriched in small non-coding RNAs [19]. MicroRNAs (miRNAs) are non-coding RNAs, 19–25 nucleotides in length. Very recent research has demonstrated that miR-512-3p and miR-412-3p were upregulated in salivary sEVs from oral squamous cell carcinoma patients compared to the controls [20]. However, no studies have explored miRNAs’ expression patterns in salivary sEVs as potential biomarkers for periodontal status. According to published reviews [21][22][23][24][25][26], ten periodontitis-associated miRNAs (hsa-miR-15a-5p, hsa-miR-29b-3p, hsa-miR-124-3p, hsa-miR-140-5p, hsa-miR-146a-5p, hsa-miR-148a-3p, hsa-miR-155-5p, hsa-miR-223-3p, hsa-miR-301b, hsa-miR-628-5p) have been explored as potential periodontitis biomarkers from one or more sample sources, such as saliva, gingival tissues, or periodontium-derived cells. However, since miRNAs are expressed in a tissue and biofluid-specific manner, it is important to investigate whether these proposed miRNA periodontitis biomarker candidates are also expressed in salivary sEVs. Additionally, whether salivary sEV-associated miRNAs have the same profile as whole saliva-associated miRNAs in periodontitis remains unknown.

This pilot study aimed to evaluate the miRNA expression profile of whole saliva and salivary sEVs obtained from gingivitis, periodontitis and periodontally healthy patients, and to determine the diagnostic potential of miRNAs associated with sEVs as biomarkers of periodontal status.

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