# The use of salivary proteomics to monitor disease activity in periodontitis

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Kevin Stewart[1], Brendan Haigh[2], John Whelan[3], Matthew Barnett[2], Grant Smolenski[2] and Thomas Wheeler[2]
Waikato Institute of Technology[1], AgResearch, Ruakura Research Centre, Hamilton[2], John R. K. Whelan Periodontics and Dental Implants[3]
Kevin.Stewart@Wintec.ac.nz

agresearch

## What is periodontal disease?

• Periodontitis is a common disease characterised by the bacteria-induced inflammatory destruction of the support tissues of the teeth. Symptoms range from mild gum bleeding to severe inflammation and chronic destruction of tissue, ultimately requiring surgery and removal of teeth.

• Periodontitis is episodic in nature; periods of inflammation and tissue destruction can alternate with periods of minor activity.



normal          mild periodontitis          severe periodontitis

## Periodontal disease and saliva

• Saliva is an important component of the host defence system of the oral cavity. It contains a variety of antimicrobial and immunomodulatory components.

• Changes in salivary composition may reflect the presence of disease, as well as modulate the host defence activity of the oral cavity.

• By investigating the effect of periodontal disease on saliva composition, we may identify new biomarkers for periodontal disease and learn how localised inflammation may influence host defence activity throughout the oral cavity.

## Objective of study

To identify changes in the salivary proteome associated with periodontal disease activity

## Experimental design

• 9 participants were recruited from individuals seeking periodontal therapy. Selection criteria included severe periodontitis, good general health, non-smoker and no antibiotics in the past 3 months.

• A saliva sample was collected from each individual prior to beginning periodontal therapy (saliva was stimulated by chewing on a bolus). A 2nd sample was collected at the completion of the periodontal treatment programme, typically 3-4 months later, when participants met clinical criteria for disease remission.

• Saliva samples were separated using 2D SDS PAGE gel electrophoresis and individual spot intensities quantified using PDQuest software.

• Paired t-tests were performed on log-normalised spot intensities from duplicate samples obtained before and after treatment. Spots which showed a significant difference ($p < 0.05$, >1.5 fold change in abundance) were picked from the gels and analysed by mass spectroscopy using MALDI-TOF and LC-MS/MS instruments.

## Results

### Fig 1  Protein spots with altered abundance in saliva during periodontal disease
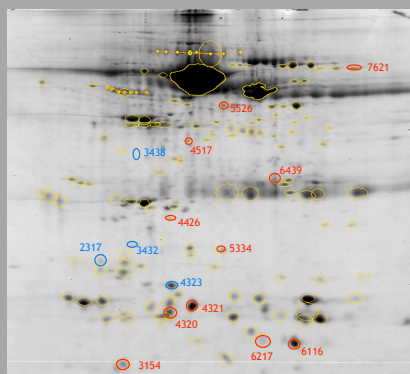


| Spot # | Protein | Fold change in disease |
|---|---|---|
| 7621 | Transketolase | 5.4 |
| 5334 | Haptoglobin | 3.6 |
| 4426 | unidentified | 2.6 |
| 6217 | S100A8 | 2.5 |
| 6116 | S100A8 | 2.3 |
| 6439 | unidentified | 2.3 |
| 5526 | Rab GDP dissociation inhibitor beta | 2.2 |
| 4321 | S100A9 | 2.0 |
| 3154 | S100A6 | 1.6 |
| 4517 | Transaldolase 1 | 1.6 |
| 4320 | S100A9 | 1.5 |
| 2317 | Prolactin inducible protein | 0.7 |
| 3438 | Parotid secretory protein | 0.7 |
| 4323 | Transthyretin | 0.7 |
| 3432 | unidentified | 0.6 |

400 ug of total saliva was focused on a pI 3-10 IPG strip and separated on a 13 % SDS-PAGE gel. Yellow circles indicate protein spots quantified. Red and blue circles identify spots significantly increased or decreased respectively in the disease versus post treatment samples (paired t-test, P<0.05). The table indicates protein identification and average fold change in disease versus post treatment sample.

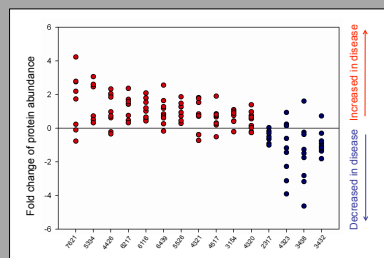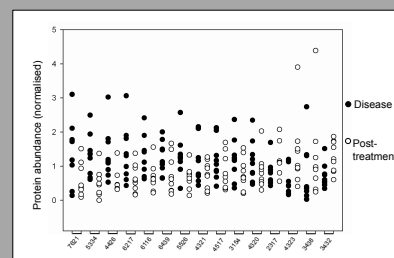### Fig 2  Fold change of protein levels in each individual



### Fig 3  Groupwise comparison of protein levels



Spot abundance normalised to combined average of disease and post treatment samples

• 126 protein spots were compared across all saliva samples and 11 were found to be more abundant in the samples taken from individuals with severe periodontal disease when compared to their respective post-treatment sample (P<0.05), while 4 protein spots were decreased (Figs 1 and 2).

• The predominant alteration observed was an increase in the abundance of the S100 proteins S100A8/A9 (calprotectin) and S100A6. The S100A8/A9 heterodimer is expressed by neutrophils, activated macrophages and squamous epithelia. The increased levels observed in whole saliva likely result from active secretion by infiltrating neutrophils and inflamed keratinocytes, both of which secrete S100A8/A9 in the presence of bacterial lipopolysaccharides.

• Two acute phase response proteins were also identified, haptoglobin and transthyretin, which were increased and decreased in disease respectively. Of the remaining proteins with altered abundance, prolactin inducible protein and parotid secretory protein have previously been associated with host defence, while the function of the ubiquitous intracellular proteins transketolase, transaldolase and GDP-dissociation inhibitor B is less clear, but may reflect cellular damage in the inflamed gingiva.

• The high variation in basal levels of these proteins between individuals (Fig 3) suggests these protein changes would not be suitable for a screening tool to identify affected individuals, but may be useful in monitoring changes in the inflammatory response within an individual during periodontal treatment (Fig 2).

## Conclusions

• Alterations in the salivary proteome were detected in samples from individuals with severe periodontal disease, suggesting local inflammation of gingival tissue may influence host defence activity throughout the oral cavity.

• The predominant alteration was an increase in the abundance of the S100 proteins S100A8/A9 (calprotectin) and S100A6, while changes in the levels of acute phase and other host defence proteins were also observed.

• These proteins may provide useful markers for monitoring disease activity during periodontal treatment.