Quantitative proteomic analysis of experimental gingivitis

Andrew J. Creese[1], Melissa M. Grant[1], Gordon Barr[2] Iain L C. Chapple[1], Helen J. Cooper[3]

1. Periodontal Research Group, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN.
2. Department of Chemistry, University of Glasgow, Glasgow, G12 8QQ.
3. School of Biosciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT.

Overview

- Gingivitis is the inflammation of the gum tissue often caused by plaque. It is one of the most prolific diseases in the world and it is estimated that between 70 and 90% of adolescents will develop gingivitis.
- Currently the methods for detecting and assessing the degree of imflammation are subjective, such as bleeding on probing.
- Here we ask: Is it possible to produce a set of proteins biomarkers for early stage gingivitis?

Introduction

- Gingivitis is a reversible condition which does not result in any permanent damage.
- If untreated, gingivitis will develop into periodontitis which can result in tooth loss and has been linked with increased frequency of coronary artery disease.
- Gingival crevicular fluid (GCF) is a complex fluid containing serum transudate, saliva proteins and bacterial proteins with a large dynamic range. It is a rich source of biomarker but currently few proteins have been identified.
- We show online data-dependent liquid chromatography (LC) PQD MS/MS analysis of iTRAQ labelled SCX fractionated GCF samples performed on a Thermo Fisher Scientific LTQ-FT mass spectrometer.
- 186 proteins were identified from the IPI human database. A group of 18 proteins showed an increase in abundance over days 14 and 21 before returning to baseline at day 35.

Method

- Volunteers were provided with a vinyl splint which covered 3 teeth when brushing (referred to as test).
- GCF was collect by inserting strips of Periopaper™ between the tooth and gum from three sites on both control and test sites. Samples were stored in 100 mM ammonium barcarbonate (200 µL).
- Samples were collected on days 0, 7, 14, 21 and 35.
- The GCF samples from ten volunteers were pooled (for each time point) and digested with trypsin.
- The samples were labelled with an iTRAQ 8-plex and combined prior to strong cation exchange chromatography.
- SCX was performed with a Polysulfoethyl A column. Samples were eluted over a 60 minute salt gradient from 0-50% 500 mM KCl (pH 3)
- The fractioned were pooled into 8 samples and desalted prior to loading onto a 75 µm C18 reversed phase analytical column (LC Packings). Peptides were separated over a 30 minute gradient from 3.2 to 44% acetonitrile. All samples were run in triplicate.
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-FT hybrid mass spectrometer.
- A survey scan of eluting peptides was recorded and the top 3 most intense multiply charged ions were fragmented with PQD (normalised collision energy 45) and measured in the ion trap. Dynamic exclusion was used (180 s exclusion).
- Data were analysed with Xcalibur 2.1 and Proteome discoverer sp 1.0 (Thermo Fisher Scientific).
- For quantitation a protein had to be identified 3 times and with two peptides .
- Clustering analysis was performed using PolySnap 3. (Glasgow university)

Conclusion and Future Work

- We have identified and quantitified 186 human proteins from gingival crevicular fluid and an additional 16 proteins from known bacterial families.
- Clustering analysis of the normalised quantification data identified a group of 18 proteins which showed increased abundance over days 14 and 21 before returning to baseline levels at day 35.
- The data for these 18 proteins matches with that observed in the bleeding index, gingival index and plaque index.
- Further work in this area will include analysis of individual patient samples to access the specificity of the up-regulated proteins.

Results

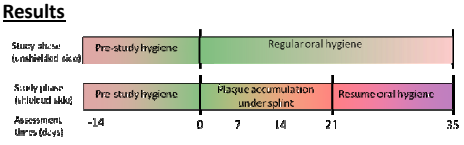

Figure 1: Flow chart of study design (control site on top)

	Volumes of GCF (µL)		iTRAQ labels	
	Control	Covered	Control	Covered
Day 0	2.148	2.148	113	-
Day 7	3.078	5.242	-	114
Day 14	2.137	6.019	115	116
Day 21	3.362	6.427	117	118
Day 35	1.749	2.986	119	121

Table 1: The volumes of GCF collected and the labels used.

Results

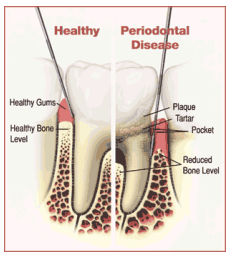

Figure 2: A cut through of a tooth. Currently probing is one of the main methods used for assessing gingivitis.

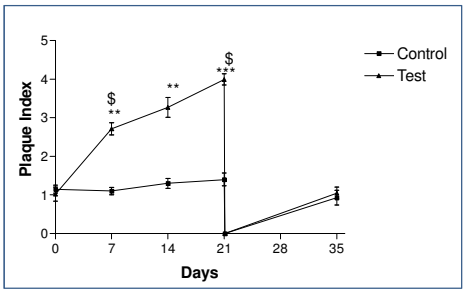

Figure 3: Plaque accumulation index assessed using a modified Quigley–Hein index.

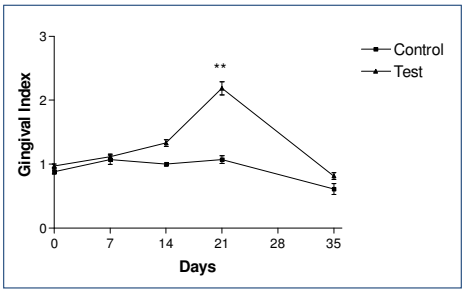

Figure 4: The gingival index, used to measure gingival inflammation.

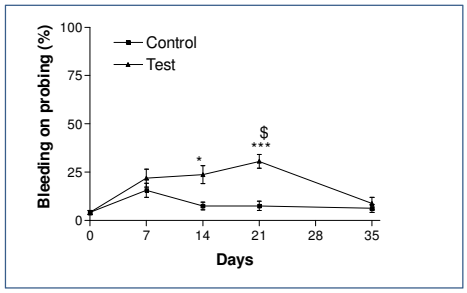

Figure 5: The bleeding index for all sites. Represented as a percentage of sites which bled on probing

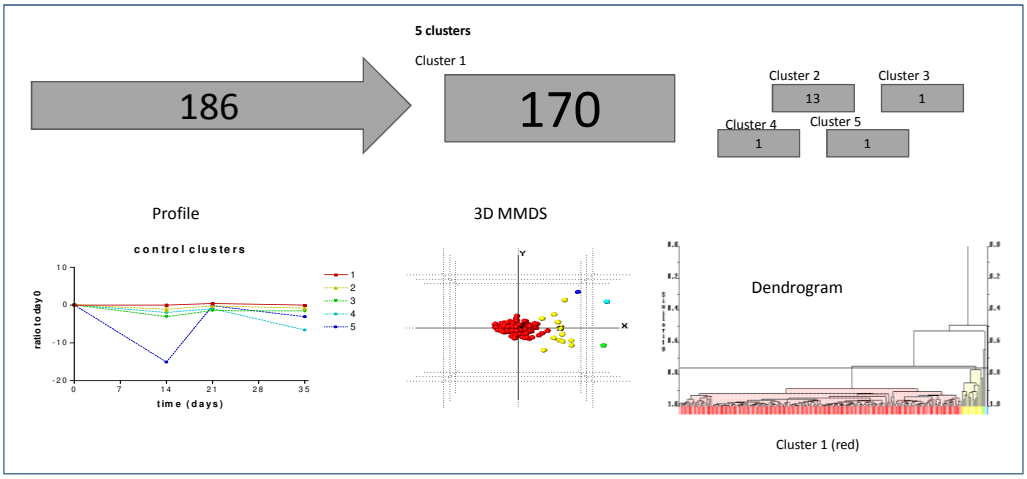

Figure 6: Cluster analysis of the human protein dataset. The control data is clustered. 170 proteins are identified with a normal baseline.

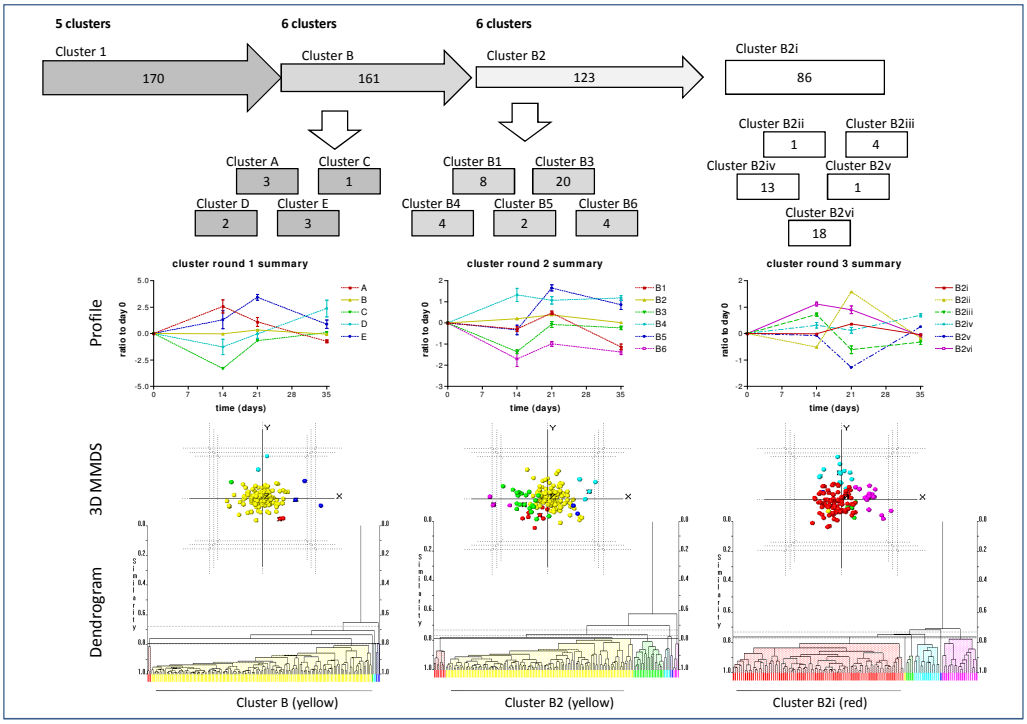

Figure 7: Cluster analysis of dataset showing the workflow and clusters generated. The 170 proteins are normalised to the control data.

Andrew Creese, School of Dentistry, University of Birmingham, St Chads Queensway, Birmingham, B4 6NN, UK. E-mail:a.creese.2@bham.ac.uk