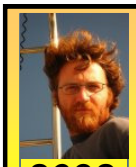

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Characterisation of healthy gingival crevicular fluid proteome by FT-ICR MS/MS

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Overview

- Gingivial crevicular fluid (GCF) is a complex mixture of serum and tissue transudate, saliva and bacterial proteins. It is a potentially rich source of protein biomarkers.
- Using bottom-up proteomics, it is possible to identify thousands of peptides from hundreds of proteins in short periods of time.
- Here we ask: How many proteins can be confidently identify using 2D bottom-up proteomics?

Introduction

- GCF is a complex media with a large dynamic range. It is a rich source of serum proteins and collection is non-invasive. However the proteome is currently poorly defined.
- Tandem mass spectrometry is one of the most powerful tools for analysing complex protein mixtures. High mass accuracy, fast acquisition speed and a large dynamic range allow hundreds of peptides to be identified in less than an hour.
- We show online data-dependent liquid chromatography (LC) tandem mass spectrometry (MS/MS) analysis of GCF performed on a Thermo Fisher Scientific LTQ-FT mass spectrometer.
- The results demonstrate that prefractionating GCF samples using strong cation exchange chromatography prior to LC-MS/MS analysis can increase protein identification 6 fold.

Method

- GCF was collect from 12 healthy sites onto strips of Periopaper™. The twelve strips were split into two groups, placed in screw top tubes and stored in 100 mM ammonium bicarbonate (200 µL).
- Both samples were vortex and the solution extracted.
- The proteins in both samples were reduced and alkylated prior to overnight digestion with trypsin.
- The peptides from one sample were fractionated by strong cation exchange (SCX) liquid chromatography.
- SCX was performed with a Polysulfoethyl A column (PolyLC). Samples were eluted over a 60 minute salt gradient from 0-50% of 500 mM KCl (pH 3). 16 fractions were collected.
- The SCX fractions and the unfractionated sample were each loaded onto a 75 µm C18 reversed phase analytical column (LC Packings). Peptides were separated over a 30 minute gradient from 0 to 40% acetonitrile (0.1% formic acid).
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-FT hybrid mass spectrometer.
- The mass spectrometer performed an initial survey scan and the three most intense multiply charged ions were selected for collision induced dissociation (CID) tandem mass spectrometry.
- Analysed peptides were placed on an exclusion list for 180 seconds to avoid repeat analysis.
- Data were collected using Xcalibur 2.1 (Thermo Fisher Scientific) and analysed with the MASCOT search algorithm against the NCBI protein database.

Conclusion and Future Work

- We have demonstrated that fractionation by strong cation exchange chromatography prior to tandem mass spectrometry improves the coverage of the GCF proteome by 6 times.
- The number of proteins identified is greater than any previously reported in the literature.
- SCX fractionation improved sample "clean up" prior to MS/MS analysis, increasing the ratio of CID events to identifed peptides.
- Further work in this area will include looking deeper into the GCF proteome. By pooling more GCF samples and analysing by 2D-LC-MS/MS the number of proteins identified should increase.

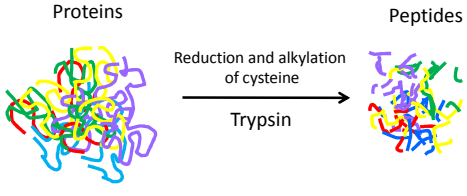


Figure 1: Proteins are digested with the endoprotease trypsin to produce short peptides (≈5-15 amino acids long).

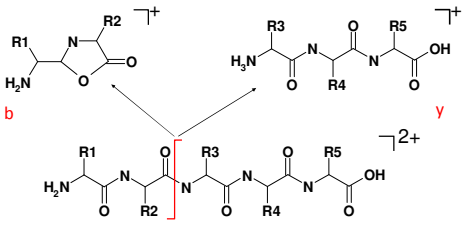
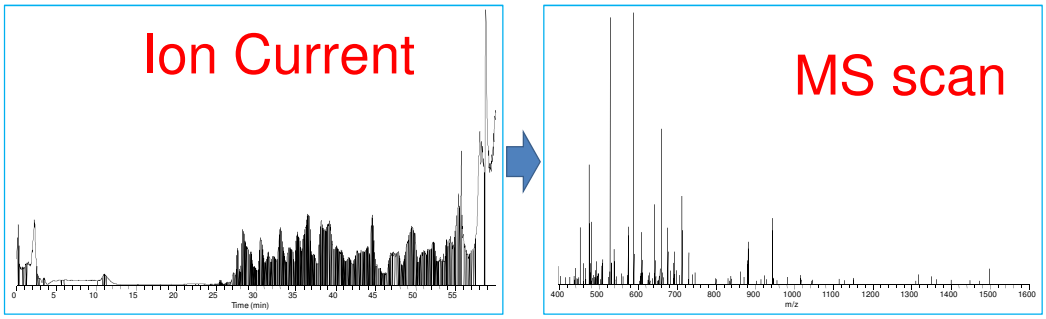
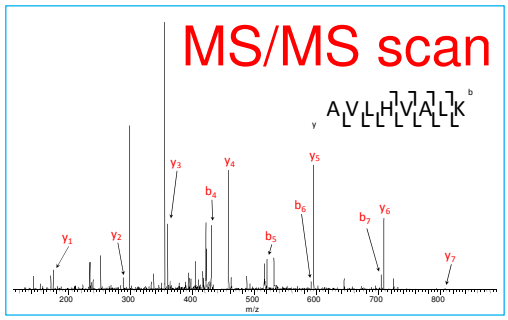


Figure 2: The fragments produced by collision induced dissociation (CID) of a peptide. The N-C_o bond is cleaved resulting in *b* and *y* ions.

Results





Proteomic analysis of 6 GCF strips	
Number of CID events	1402
Number of peptides identified	256
Number of proteins identified	34
Number of peptides per protein	7.5

Table 1: The number of CID events, proteins and peptides identified from the analysis of 6 GCF strips using LC-MS/MS.

Figure 3: The total ion chromatogram (top left) for the unfractionated analysis of 6 GCF strips. A high resolution full MS scan (top right) from which the ion m/z 439.78 was selected for fragmentation. The CID mass spectrum of the peptide AVLHVALR (m/z 438.78, bottom right) from glucose-6-phosphate isomerase. All of the N-C_o bonds were cleaved.

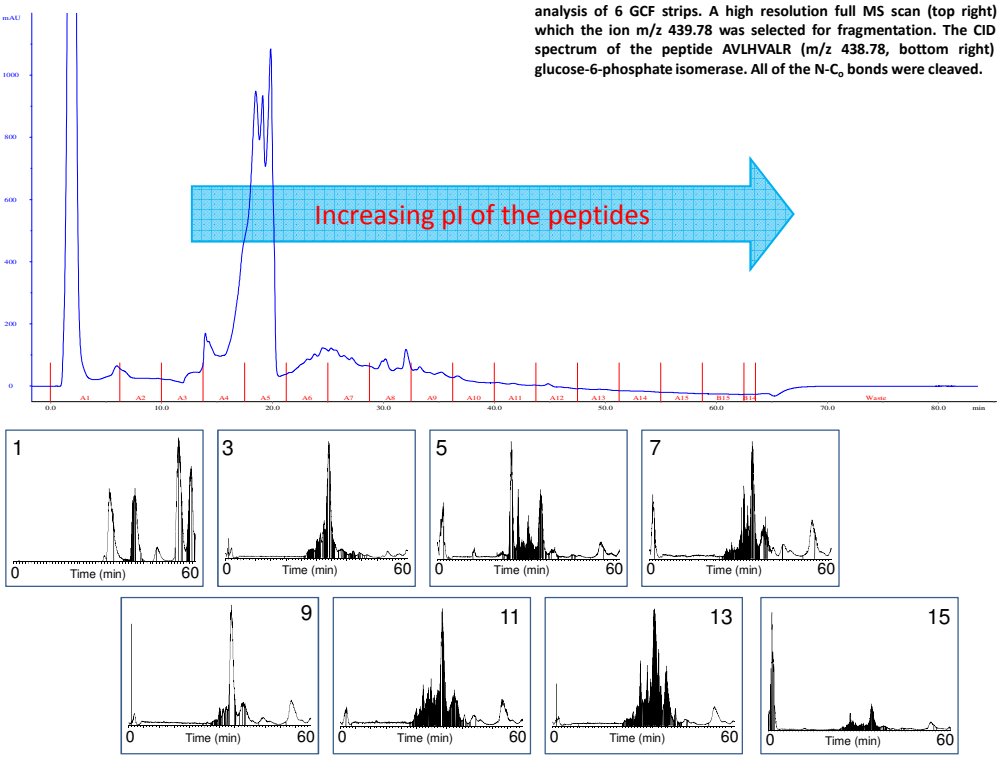


Figure 4: The strong cation exchange chromatogram (top) for the digested GCF sample (UV 214 nm). 16 fractions were collected. The 8 ion chromatograms shown represent fractions 1, 3, 5, 7, 9, 11, 13 and 15 (all chromatograms were collected over 60 minutes).

Proteomic analysis of 6 GCF strips fractionated by SCX	
Number of CID events	3652
Number of peptides identified	2129
Number of proteins identified	201
Number of peptides per protein	10.6
Protein identification fold change	**5.9**

Table 2: The number of CID events, proteins and peptides identified from the combined analysis of 6 GCF strips fractionated by strong cation exchange chromatography prior to mass spectrometry. The fold change is also shown.

A group of proteins only identified in the SCX fractions	
Annexin 1	Defensin α1
Cystatin	Tropomyosin
Heat shock protein 70 kDa	Gelsolin
S100 calcium binding protein A9	small proline-rich protein 1
Kallikrein	Matrix metalloproteinase 9

Table 3: Some of the additional proteins identified using strong cation exchange chromatography to fractionate the GCF sample.

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