Predicting Pathology: Towards Using SIFT-MS to Facilitate Early Intervention for Age Related Hearing Loss

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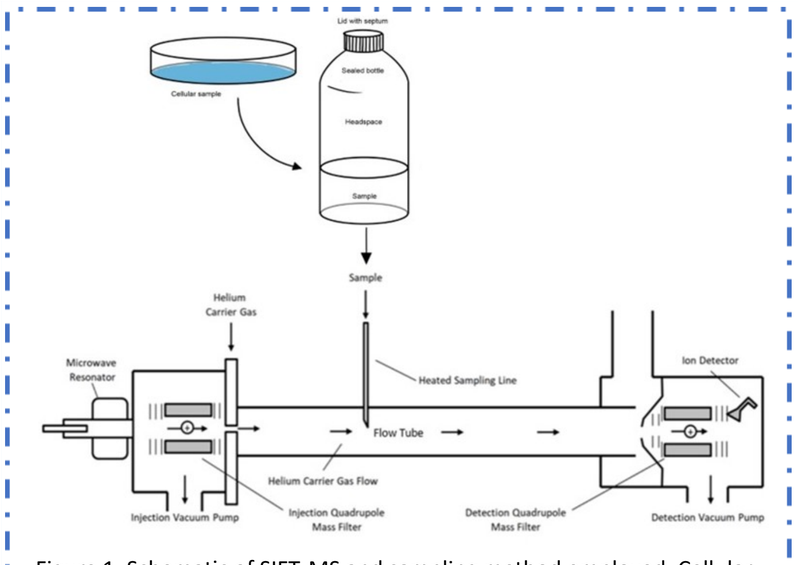


Figure 1. Schematic of SIFT-MS and sampling method employed. Cellular samples are sealed in bottles and headspace measured via needle through the rubber septum of the bottle lid.

INTRODUCTION

Age related hearing loss (ARHL) affects most people over 65, with current treatments unable to offer continued improvement as the condition progresses. Within ARHL, it is widely acknowledged that the damage to spiral ligament fibrocytes seen in metabolic ARHL precedes further inner ear damage [1][2]. Based upon this, it may be stated that fibrocyte damage exists prior to possible detection of ARHL using current testing methods (typically pure tone audiometry[3]) which are reliant upon the poor function of sensory inner ear components. Herein lies the issue where regenerative therapies for spiral ligament fibrocytes are concerned. Though these methods have shown promise in animal trials[4][5], their efficacy in restoration of hearing function is limited by the health of remaining sensory cells, many of which have suffered irreversible damage at the point of diagnosis (often via inflammation). The present research aims to counteract delays in detection and to facilitate intervention in metabolic ARHL before excessive downstream damage occurs. Using selected ion flow tube mass spectrometry (SIFT-MS), we hope to lay the ground work for early detection of spiral ligament fibrocyte death, primarily via inflammation. To date, we have made significant progress towards the development of a non-invasive, real time detection method for metabolic ARHL and facilitating the use of regenerative strategies that, if implemented in a timely manner, may significantly delay or even prevent the further course of metabolic ARHL.

METHODS

Murine cochlear fibrocytes were cultured from explanted spiral ligament tissue sections according to existing methods[6] and characterised via morphological and immunocytochemical (ICC) analysis. Inflammation was induced via addition of IL-1β (concentrations: 1ng/ml, 5ng/ml, 10ng/ml, 25ng/ml, 50ng/ml), with inflammatory state confirmed by examination of IL-6 and IL-8 expression. Samples to be analysed via SIFT-MS were sealed in sterile bottles, air purged and incubated for 16 hours at 37°C and 5% CO_2. Cell samples were seeded at 15,000 per bottle (low cell number) and 100,000 per bottle (high cell number). Measures of volatile organic compounds in the headspace of samples were gathered via Transpectra Profile 3 SIFT-MS (Fig.1), with observations across H_3O^+ and NO^+ precursors for compounds of m/z 1-180. Multi-ion monitor (MUI) mode samples were taken using an uploaded kinetic library for 40 seconds in each bottle for both reagent ions. MUI samples were normalized via water value correction to 4% to account for sample humidity variation. Statistical testing of MUI data was conducted via Mann Whitney U test, performed in SPSS data analysis software to a $p \leq 0.05$ significance level. Full scan data are presented with precursor isotopologues removed.

RESULTS AND CONCLUSIONS

- Cochlear fibrocytes were successfully explanted to culture. ICC demonstrates expression of NaKATPase and S100B with little evidence of Aqp1 expression. This verifies the cultured cells as cochlear fibrocytes and suggests a sub-type of II, IV or V (Fig. 2). Inflammation was confirmed via IL-6 and IL-8 expression (Fig.3).
- Multi ion monitor data from fibrocyte SIFT-MS analysis shows significant differences in acetaldehyde (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), butyric acid (U(Nmedia=N15000=3)=2, z=-2.193, p<0.05), benzaldehyde (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) and pyruvic acid (U(Nmedia=N15000=3)=0, z=-2.611, p<0.05) between media and low cell number fibrocyte samples for H3O+ precursor multi ion monitor data (Fig. 3). These compounds are commonly noted in various mammalian biofluids and predominantly vary as a result of processes such as ethanol and glucose metabolism. Such detection capacity is vital when dealing with the similarly low cell numbers seen in vivo.
- Variations seen in multi ion monitor data are supported by full scan mode data (Fig. 5) with several m/z arising that appear to be unique to the cellular profile. Though the associated compounds of these m/z are subject to ongoing analysis, possible compounds identified have been noted within cells (pyrrole- cytoplasm, hexyl acetate and menthone- membranes)[7]. These results highlight SIFT-MS detectable profiles for cochlear fibrocytes.
- Preliminary comparison of IL dosage effects on compounds of interest (H_3O^+ MUI-identified) shows notable compound level variation between undosed and inflamed conditions, particularly at higher dosages (Fig.6). Benzaldehyde in particular appears to demonstrate a visible difference in level with IL-1β applied at its lowest dose (1ng/ml). However, a large margin for error is seen at higher doses, possibly as a result of variations in cell response.
- Nonetheless, even from this preliminary IL SIFT-MS data, it may be asserted that inflammation is detectable at a low level in small, physiologically relevant, samples. Additional sampling, further compound analysis and investigation of full scan profiles is currently underway to determine more representative results.
- To conclude, the suggested novel method for SIFT-MS based measurement of cochlear fibrocyte culture health appears successful thus far, with research demonstrating progress towards the establishment of cochlear fibrocyte health profiles. The next stage of research, following the additional sampling, further compound analysis and investigation of full scan profiles noted above, will focus on the translation of detection of poor health in cochlear fibrocytes via relevant biofluids. Overall, though this research represents only the initial development of the technique, it may be reasonably stated that, if successful, the capacity to non-invasively detect early auditory issues in real time may well be a breakthrough for the practice of Otolaryngology.

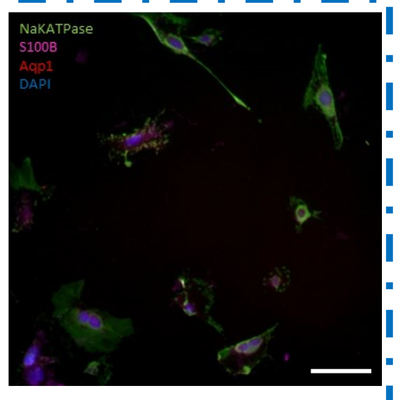


Figure 2. Fluorescence microscopy of cochlear fibrocytes. Scale bar= 100µm .

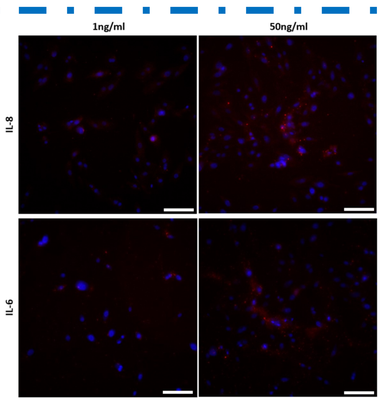
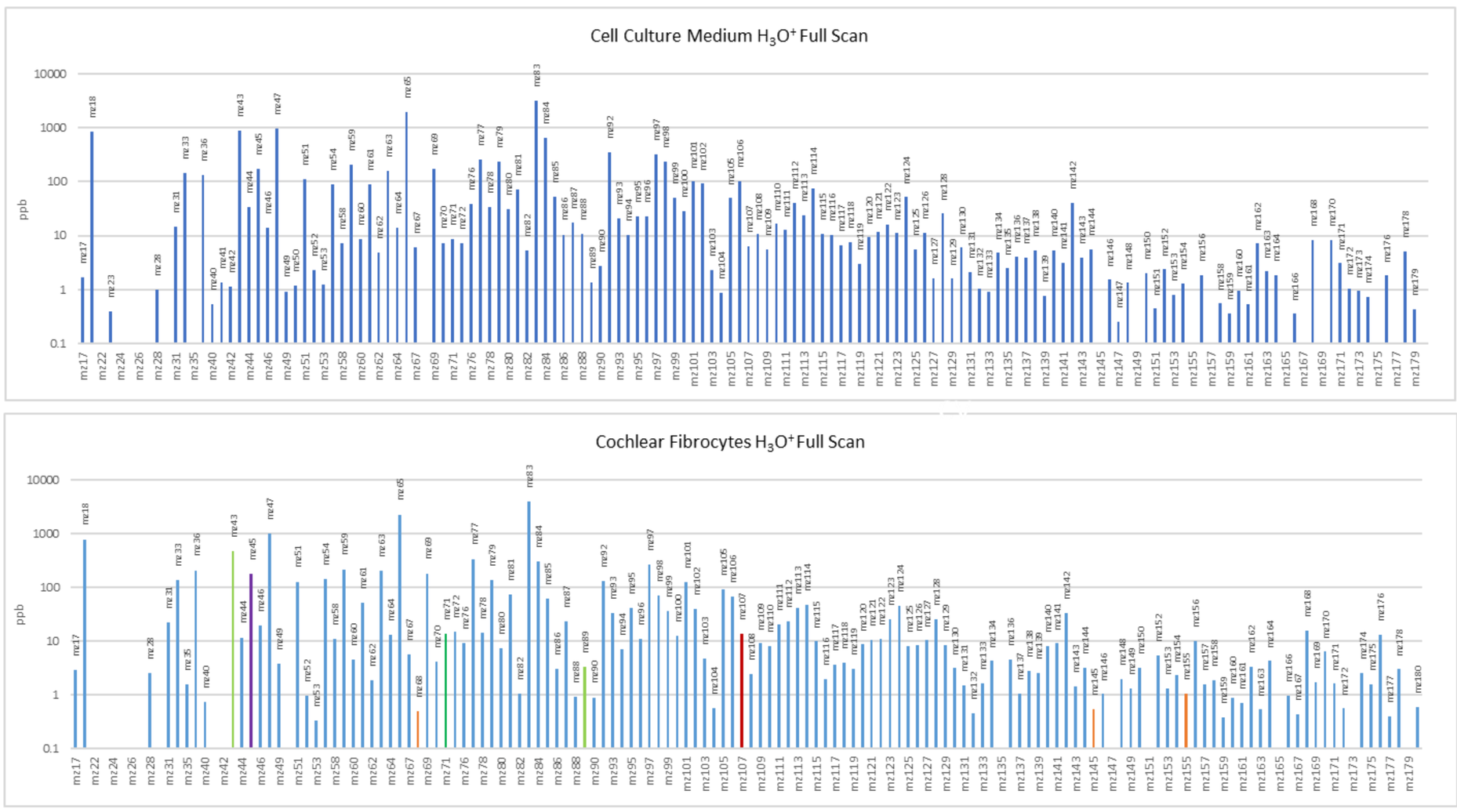


Figure 3. Fluorescence microscopy of cochlear fibrocytes dosed with IL-1β for 24 hrs. Scale bar= 100µm .



m/z	Suggested Source
43	Pyruvic/Butyric Acid
45	Acetaldehyde
68	Pyrrole
71	Butyric Acid
89	Pyruvic/Butyric Acid
107	Benzaldehyde
145	Hexyl Acetate
155	Menthone

Figure 4. Full scan mode H_3O^+ data for cell culture media and low number (15k) cochlear fibrocyte samples highlighting compounds of interest and unique to cells compounds (with associated table).

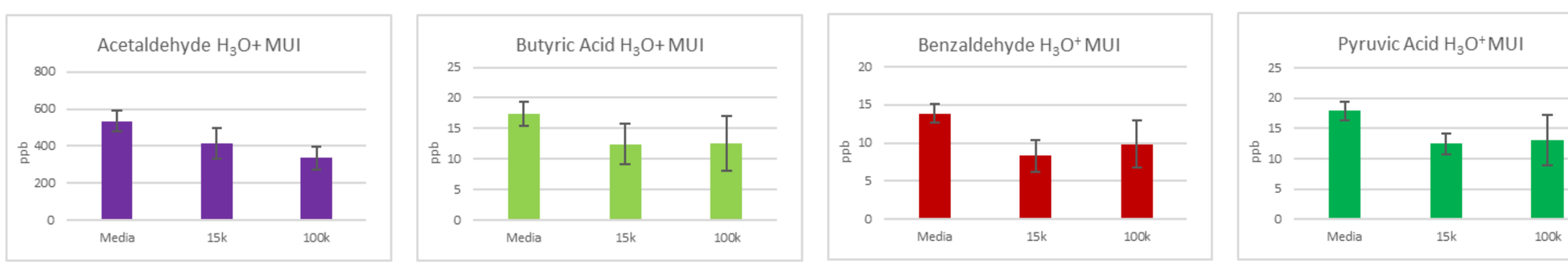


Figure 5. Compounds displaying significant differences between Media and 15k fibrocyte conditions from multi ion monitor H_3O^+ SIFT-MS analysis.

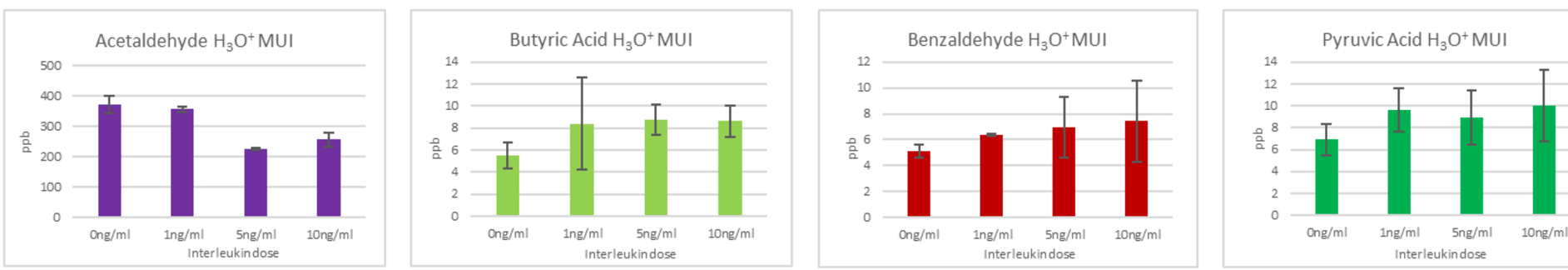


Figure 6. Variations in compounds of interest (identified above) in 15k fibrocytes with IL-1β dosage from multi ion monitor H_3O^+ SIFT-MS analysis.

1) Hequembourg & Liberman, 2001 2) Mahendrasingam et al., 2011 3) Uy & Forciea, 2013 4) Sun et al., 2012 5) Kamiya et al., 2017 6) Gratton et al., 1996 7) Human Metabolome Database

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