The Volatilome of Human Embryonic Stem Cell Cultures using Selected Ion Flow Tube Mass Spectrometry

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Introduction

- Human embryonic stem cells (hESCs) have gathered tremendous attention due to their abilities to multiply indefinitely and generate all cells of the human body, which have multiple applications in regenerative medicine. Unfortunately, the use of hESCs is associated with a risk of tumorigenicity [1] and therefore, it is imperative that any residual hESCs are eliminated before translating stem cell therapies into clinics [2]. A potential method to detect the cells is through measuring their metabolism. A way to study metabolism is using selected ion flow tube-mass spectrometry (SIFT-MS), which is a non-invasive quantitative technique that detects volatile organic compounds (VOCs) in real-time [3,4]. In addition to being a cost-effective and timely approach, VOC analysis using SIFT-MS could provide invaluable information regarding the metabolic processes occurring in hESCs.
- Hypoxia (~2-5% O_2) is known to be a pathophysiological condition as it leads to cellular oxidative stress and, ultimately, premature cell death [5]. However, hESCs develop in a hypoxic environment during the early stages of embryogenesis, and hypoxia has been shown to improve hESC maintenance *in vitro* [5]. Although low O_2 conditions seem beneficial for hESCs, most hESC *in vitro* culture is performed under ambient air (~21% O_2) since hESCs display similar morphology and gene expression cultured in either O_2 condition [5]. Unfortunately, these characterisation assays can only assess changes in hESC functions, which does not reflect changes in their physiology (e.g., metabolism) [6]. Analysing the metabolic state of hESCs under different O_2 conditions could provide more insights into the impact of the surrounding environment on hESC behaviour.
- We also wanted to understand if freezing/thawing of cell samples affects their VOC profiles, as freezing provides a fast, time-effective and convenient method to analyse samples at a later date.
- Therefore, this study aimed to use SIFT-MS to **(1) investigate the VOC profile of hESCs**, and **(2) assess the effect of freeze/thaw on VOC production** across two oxygen conditions (21% and 2% O_2).

Methods

- The hESC line SHEF-1 (UK Stem Cell Bank) [7] was used in this study. SHEF-1 cells were cultured in xeno-free conditions (Essential 8 medium and vitronectin) at 21% and 2% O_2. Medium was changed daily, and passaging occurred every 3 days at the split ratio of 1:6.

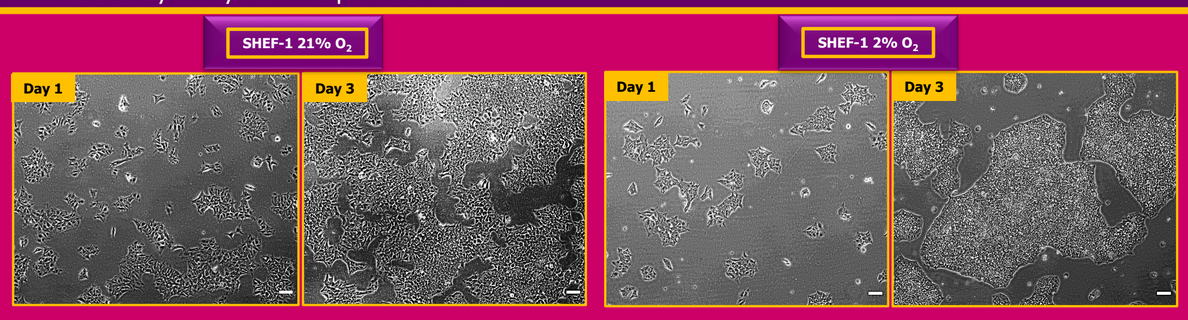
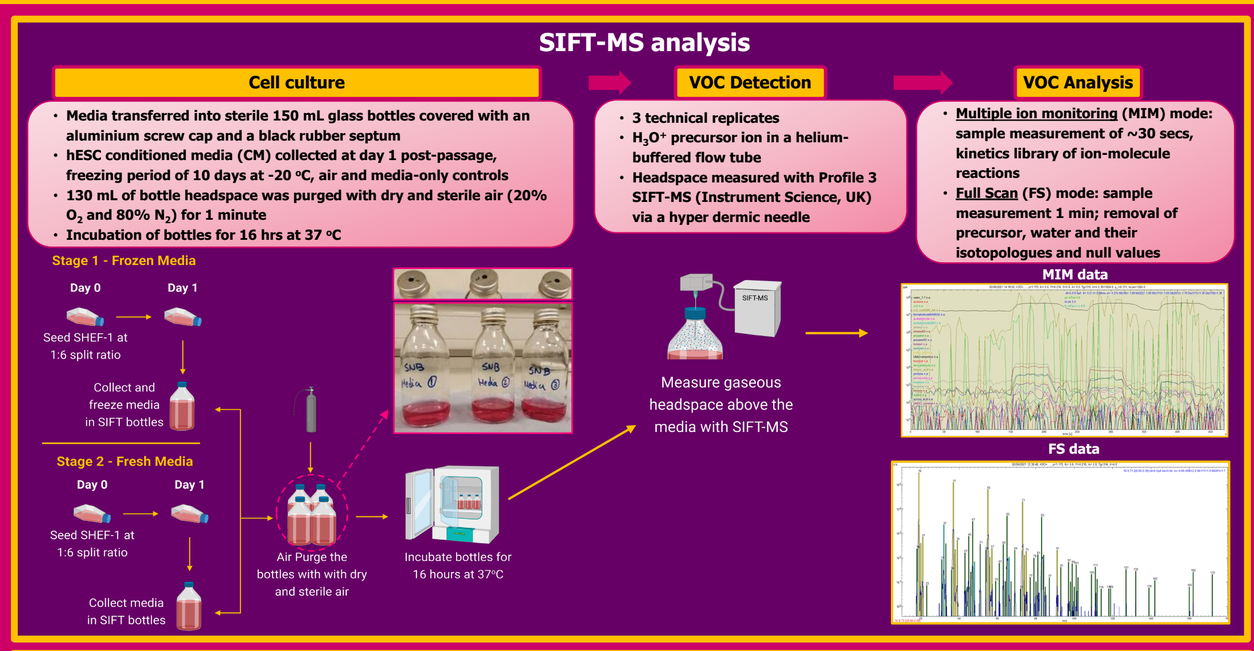
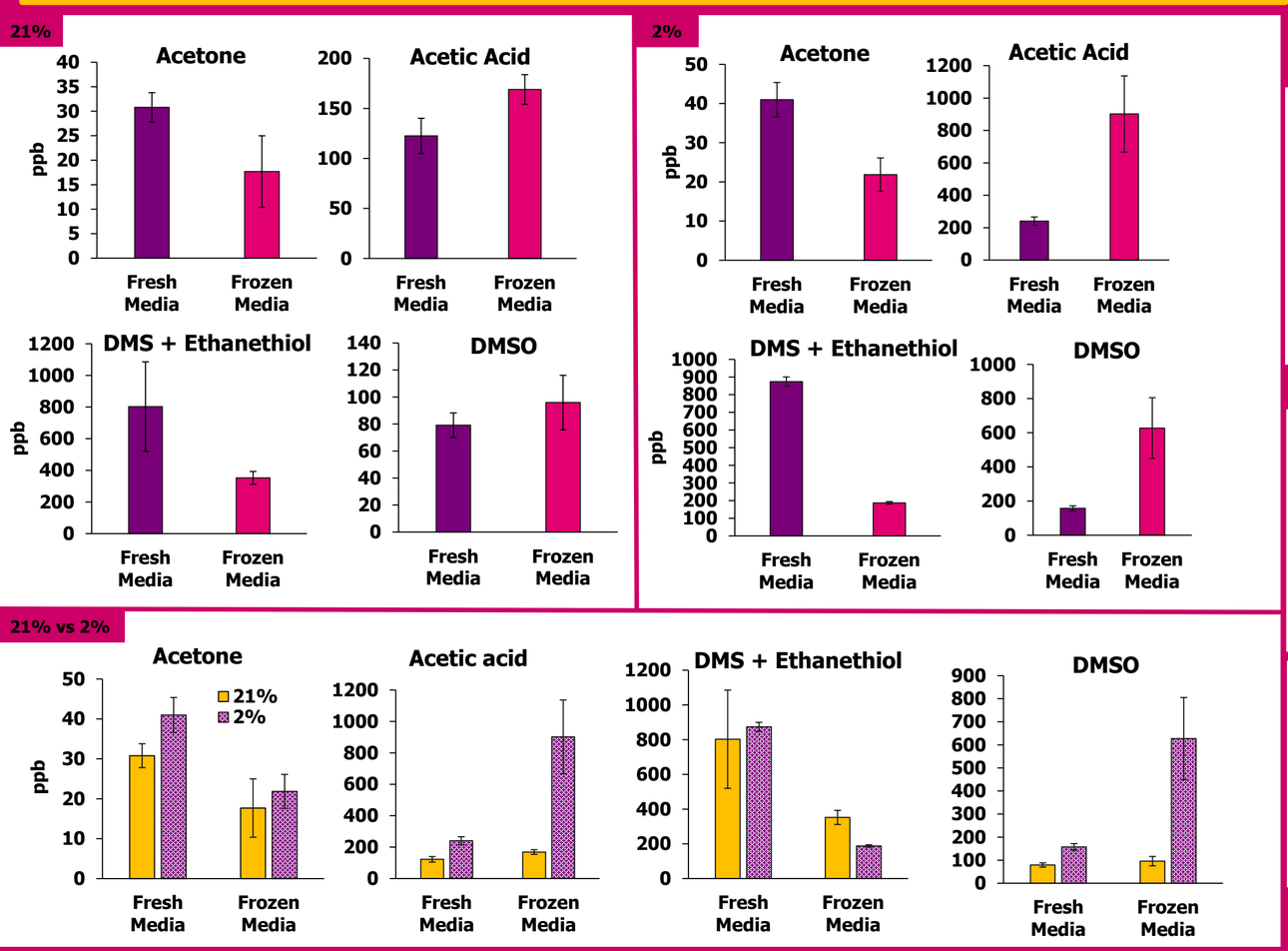


Figure 1. Microscopic pictures of SHEF-1 cells cultured at 21% and 2% O_2.

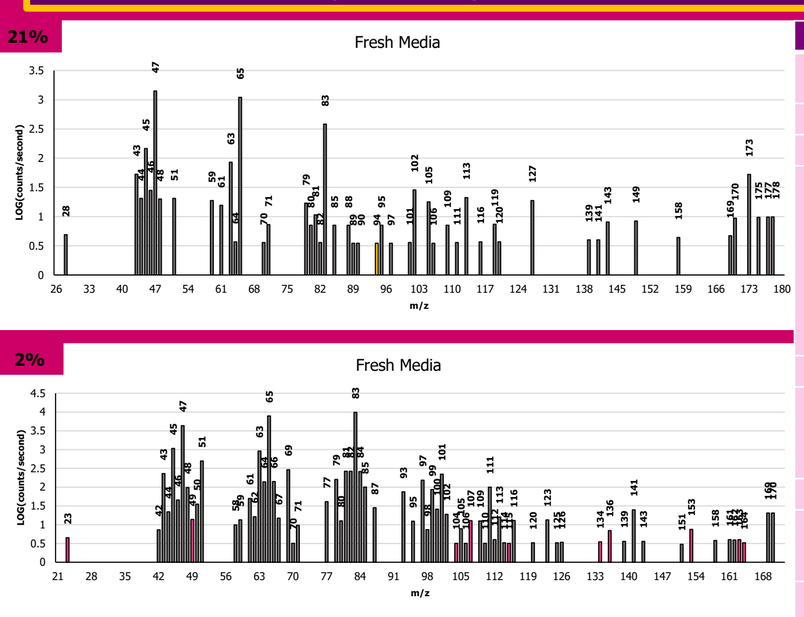


Results

- Preliminary results show that it is possible to detect VOCs in the headspace of hESC CM at trace levels, parts-per-billion (ppb) in both frozen and fresh media. VOCs were detected at different concentrations in frozen and fresh media cultured at either 21% or 2% O_2. For both O_2 conditions, freezing the media led to a decrease in concentration for acetone and dimethyl sulphide (DMS) + ethanethiol, whereas the opposite was observed for acetic acid and dimethyl sulphoxide (DMSO). When comparing the two O_2 conditions, the concentration of most VOCs was higher in 2% for both fresh and frozen media, except for DMS + ethanethiol, where there was a decrease in concentration. Acetic acid and DMSO were also particularly high in frozen media at 2%.



- After removing unrelated ions (m/z) from the dataset (e.g. precursors, water and their isotopologues, and null values) and comparing to controls, the FS data showed a couple of ions in fresh hESC CM unique to 21% and 2% O_2 (figures below). m/z values were then checked in the human metabolome database and chemical entities of biological interest (ChEBI) to identify potential relevant compounds (tables below). FS data demonstrated that hESC CM cultured under 2% O_2 released more VOCs (71) compared to 21% (68), and most of them were present at higher concentrations.



21%

m/z	Potential Compound	Relevance
94	1. Chloromethy oxirane 2. Phenolate	1. Type of epoxide (involved in the development of cancers; xenobiotic metabolite) 2. Xenobiotic metabolite

2%

m/z	Potential Compound	Relevance
23	Ditritium oxide	Human metabolite
49	Methanethiol	Human metabolite
104	1. Dimethylglycine 2. Aminobutyric acid 3. 3-Aminoisobutanoic acid	1. Involved in betaine, glycine and serine and methionine metabolism 2. Involved in glutamate metabolism/human metabolite 3. Pyrimidine metabolism
107	Glyceric acid	Primary metabolite
115	1. Trifluoroacetic acid 2. Dihydrouracil	1. Xenobiotic metabolite 2. Involved in beta-alanine & pyrimidine metabolism
134	Aspartic acid	Primary metabolite
136	1. Adenine 2. Homocysteine	1. Involved in purine metabolism 2. Primary metabolite
153	1. Hydroxy-phenylacetic acid 2. Oxidized dithiothreitol 3. Phenoxyacetic acid	1. Involved in tyrosine metabolism; xenobiotic metabolite 2. Involved in vitamin K metabolism 3. Involved in rosiglitazone metabolism; primary metabolite
163	1. Molybdic acid 2. 4-Hydroxy-2-oxoglutaric acid	1. Primary metabolite 2. Involved in Arginine & proline metabolism
164	1. 2,5-Dichlorophenol 2. N-propylbenzamide	1. Primary/xenobiotic metabolite 2. Observed in cancer metabolism

Conclusions

- Here, it was shown that freeze/thaw affected the yield of VOCs depending on what they were. Different temperatures might be leading to the release of VOCs at specific concentrations compared to low temperatures, consequently influencing the VOC composition. Thus, even though freezing can be practically advantageous as it allows for the preservation of precious samples from expensive and small cultures for periods of time, results should be treated with caution as inevitably changes in VOCs will be observed, potentially misleading the final biological interpretation.
- Most VOCs were higher in SHEF-1 cultured at 2% O_2 compared to 21% O_2. Different O_2 conditions can change the metabolism of hPSCs [5], which can result in different VOC profiles. Lower O_2 conditions seem to benefit self-renewal, genomic stability and quality of hPSCs [5,9]. Thus, cells are healthier and possibly generate larger quantities of certain VOCs that reflect their state. Additionally, different O_2 levels can alter the pH of the culture and subsequently change the volatilome [10,11]. This shows that more awareness is needed regarding what type of gases the cells are being exposed to during routine culture, which could affect their behaviour.
- This study also demonstrated that SIFT-MS is able to distinguish the difference between cells grown under distinct O_2 conditions, with FS data indicating that these differences are seen across VOCs with metabolic significance. Thus, SIFT-MS could be used to monitor the changes in hESC metabolism acquired when growing the cells under an increasingly physiologically relevant culture method.

Future Plans

- The experiment will need further optimisation, include samples collected at day 3 of culture (just before passaging) and potentially testing different storage temperatures (4 ºC & -80 ºC). Additionally, these results will be further compared to other human pluripotent stem cell (hPSC) lines to establish a detailed VOC blueprint of the cellular state of hPSCs and evaluate if freezing/thawing has similar effects across different cell lines.

Acknowledgements

Dr Abbie V Rutter Lab
Prof. Nicholas R Forsyth Lab
Biorender (USA)

References

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