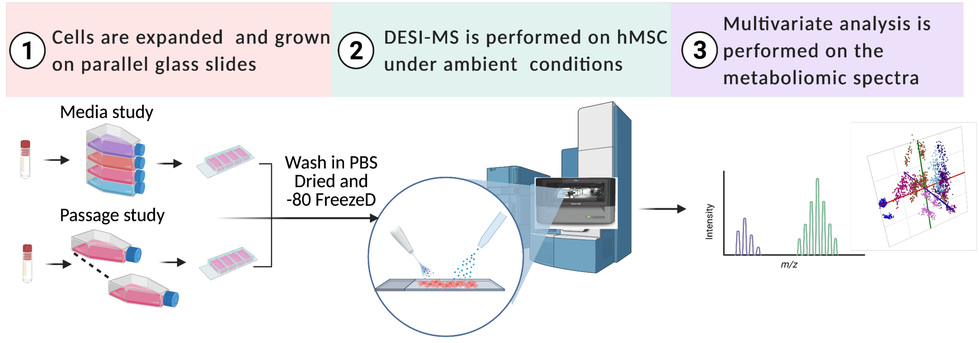
DESI-MSI as novel analytical tool for metabolic profiling of Mesenchymal Stem Cells in different media formulations

Marilina Piemontese[1], Julia Balog[2], Stephanie J Davies[1], Paul Dobson[1], Frank Barry[3]
1.ValitaCell LTD, Dublin, Ireland. 2. Waters Research Center, Budapest, Hungary.
3.Regenerative Medicine Institute, National University of Ireland, Galway, Ireland

Background & Experimental Design

In this study we report on the application of ambient Desorption Electrospray Ionization Mass Spectrometry Imaging (DESI-MSI) for the characterization of hMSCs cultured in different expansion media and across passage for a high resolution and unprecedented view on stem cell states.

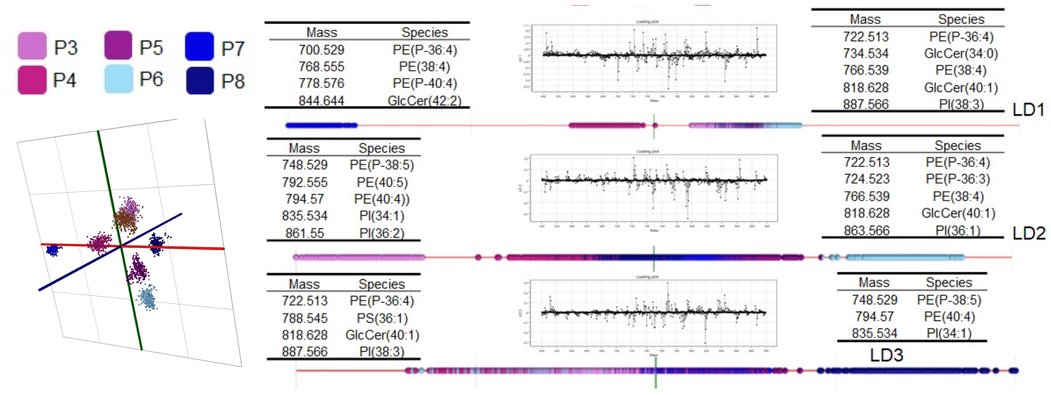
hMSCs where expanded in the conditions of interests and cultured on parallel glass slides for 5 days, washed twice with sterile PBS, dried briefly in an oven at 37°C, and immediately stored at -80 °C until analysis.



The slides were placed on an AutoDESI Imaging system consisting of a DESI-XS sprayer, a heated transfer line (500 °C) and an automated imaging setup fitted with a Xevo G2-XS ToF mass spectrometer (Waters Corporation) for analysis. The acquired spectra was submitted to multivariate analysis.

Passage profiling study

Cells across passages could be differentiated from each other with 94.75% accuracy through PCA followed by an LDA on 25 components. There is no visible pattern recognized in the component distribution of the different membrane phospholipids across passages, more the overall ratio and resulting fingerprint is unique for each passage.

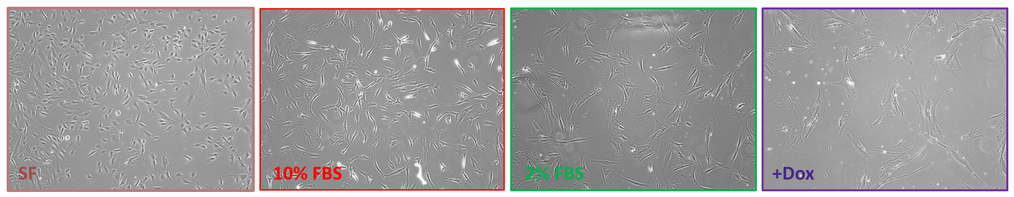


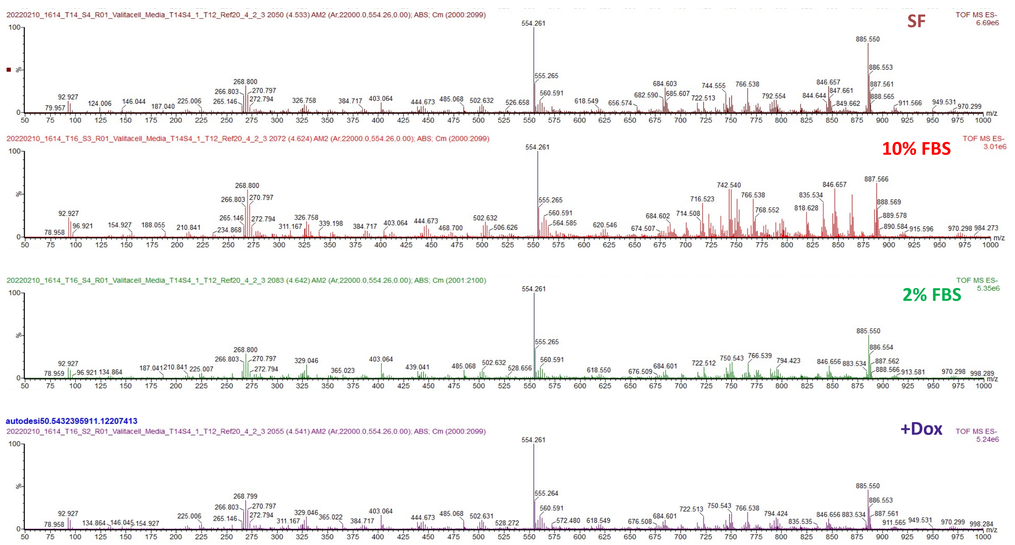
MEDIA profiling study

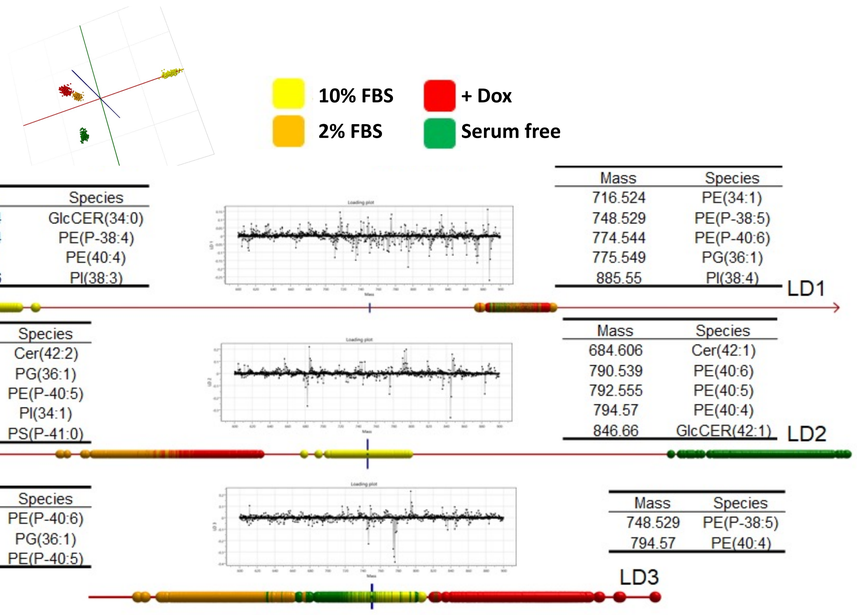
DESI analysis of hMSCs produced a complex metabolic fingerprint containing small molecules (m/z 100-250), fatty acids (m/z 250-350) and metabolites and glycerophospholipids mainly from the cell membrane (m/z 500-1000). Although the molecular compounds are similar, the ratio of the molecules is unique depending on the media composition. Multivariate analysis of hMSC lipidomic profile shows a shift towards more unsaturated glycerophospholipids and a decrease in the amount of Phosphatidyl-ethanolamines (PE) in presence of Doxorubicin. In the serum free condition, there was an even more significant shift towards unsaturated (4-5 double bonds) PEs and Phoshatidyl-inositols (PI) all suggesting differences in the membrane fluidity of each cell state.

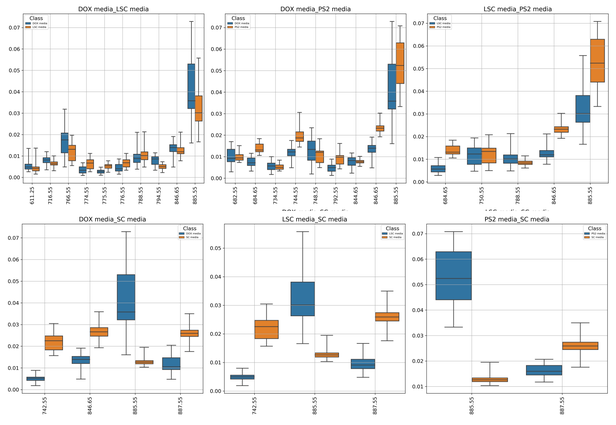
MSC p3	Cell size (µm)	Doubling time (days)
SF	14	1.65
10% FBS	15.8	1.82
2% FBS	16.4	2.98
+Dox	18.4	2.72

- **hMSCs expanded in the four media conditions displayed different proliferation capacity, size and morphology, in line with previously reported findings .**
- **There is an enrichment in highly unsaturated PEs in the serum free media compared to standard conditions, while there are more PGs and PSs identified in the cells expanded on low serum and doxorubicin containing media**









- **LDA loadings and ion species with large coefficient in the LDA loading.**
- **LSVC was used to select the most important peak set, which can be used to separate the classes from each other.**
- **Interestingly, there is a significant shift from PI(38:4) (a membrane molecule active in the PIP signalling pathway) to PI(38:3) in standard serum containing media compared to the others**

Conclusions

While the biological significance of these findings is under investigation, we have demonstrated the application of DESI-MSI as a novel analytical tool to profile hMSC expanded in different media formulations, which can be exploited to track hMSC divergence during expansion, to identify novel markers indicative of hMSC health during manufacturing and to inform advanced media design programmes.

CELLIMAGE
Disruptive Technologies Innovation Fund

marilina.piemontese@valitacell.com