A comprehensive method for quantification and in-depth profiling of secreted metabolites from human adipose stem cells

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ABSTRACT

Human adipose-derived stem cells (hASCs) are multipotent cells capable of differentiating into multiple cell lineages which has increased their functional importance in regenerative medicine. Recently, secretome analysis of hASCs has become a key procedure in the development of cell/tissue-based therapeutics for regenerative medicine.[1]

Common approaches for the secretome analysis are unable to provide a comprehensive analysis of the secreted metabolites, such as NMR and IR. Considering the multitude of possible secreted metabolites, LC-MS/MS provides a strong solution with high sensitivity, selectivity, speed, and robustness enabling unambiguous identification and quantification of multiple analytes over a single analysis.[2]

Herein, a comprehensive profiling of cell culture media components was performed using a highly sensitive LC-MS/MS workflow under a 20 min run time.

INTRODUCTION

The secretome analysis of hASCs is needed to ensure proper development, safety and efficacy of cell/tissue-based biotherapeutics for regenerative medicine. In this study, the rate of exchange of metabolites between hASCs and external medium and the appearance or clearance of existing and/or new metabolites was monitored.

The hASCs were grown in cell culture medium and collected as following: day 1, day 3, day 7, day 14, day 21 and day 28. Cell culture media (CCM) serves as a critical nutrient source for cell growth and aids cell cycle regulation. CCM nutrients are diverse and numerous, as it is a combination of various components, including vitamins, amino acids, nucleic acids, carbohydrates and fatty acids.

Quantification and targeted identification of secreted metabolites from human adipose stem cells was carried out by developing a highly sensitive analytical method. Over a 110 media analytes were monitored by using the method consisting of more than 230 MRM transitions (positive and negative ionization modes). Determination of the CCM components as well as their variation have been studied to define impact cell metabolism, growth and differentiation.[3]

The data generated for cell culture medium and spent media samples were processed for principal component analysis (PCA) and analyte abundance visualization among multiple samples.

MATERIALS AND METHODS

Sample Preparation:
The hASCs were cultured in cell culture medium up to 95% confluence. After 3 expansion cycles, the cells were trypsinized, centrifuged and the cell culture media was collected.

Media samples were diluted in 100 µL of 50:50 (v:v) 0.1% formic acid (FA) in water and 0.1% FA in acetonitrile and centrifuged. The supernatant was diluted 60-fold with 0.1% FA.

HPLC Conditions:
Analytes were separated using a Phenomenex Kinetex F5 column (150 mm × 2.1 mm, 2.6 µm, 100 Å). Total method time was 20 min at a flowrate of 200 µL/min. Mobile phase A was composed of 0.1% FA in water while mobile phase B was composed of 0.1% FA in acetonitrile. Operating column temperature was 40ºC. Injection volume was 5 µL.

MS/MS Conditions:
Samples were analyzed on a SCIEX 7500 system. Scheduled MRM algorithm was used for best accuracy and reproducibility (Figure 1).
Over 110 components were detected using 2 MRM transitions per compound to allow quantitation and identification. Every sample was injected 3 times. Qualitative and quantitative workflows were processed with SCIEX OS software. Statistical analysis (principal component analysis, PCA) was performed using MarkerView software

RESULTS

The developed method was successfully applied for the analysis of secreted metabolites in the hASCs cell line. Figure 1 and 2 report profiles for monitored components in culture medium at day 1, day 3, day 7, day 14, day 21 and day 28. The fast polarity switching and the high sensitivity enabled monitoring and quantification in a single run 196 and 76 transitions in positive (Figure 1) and negative (Figure 2) ion mode, respectively.

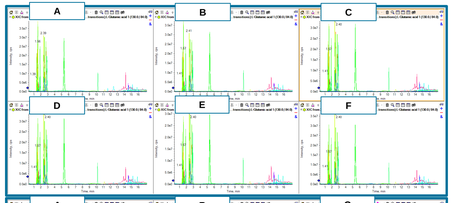

Figure 1. MRM transitions in positive ion mode obtained at day 1 (A), day 3 (B), day 7 (C), day 14 (D), day 21 (E) and day 28 (F).

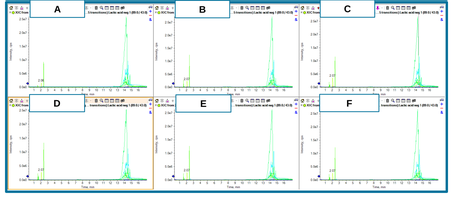

Figure 2. MRM transitions in negative ion mode obtained at day 1 (A), day 3 (B), day 7 (C), day 14 (D), day 21 (E) and day 28 (F).

Both qualitative and quantitative variations were observed during different stages of cell grown across various secreted metabolites groups. In general, all samples showed highest content in terms of amino acids (lysine, phenylalanine, tyrosine, arginine (Figure 3)), organic acids (Figure 4) and carbohydrates.

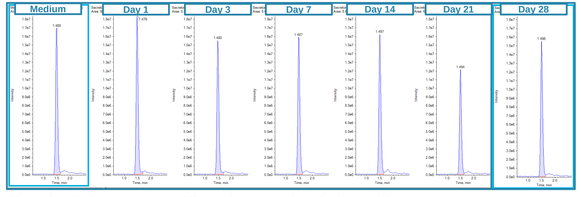

Figure 3. XICs for L- arginine at different time series. (positive ion mode).

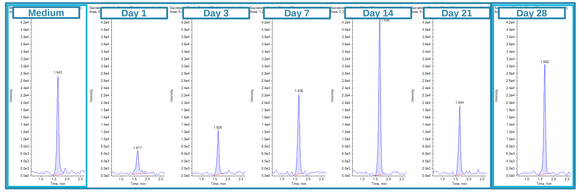

Figure 4. Gluconic acid at different time series (negative ion mode).

Supervised PLSDA (Figure 5) discriminates among medium group (on the top) and samples (bottom). Among the sample group, it's possible to distinguish groups by each growth day (1, 7, 14, 21 and 28 days) and also group by initial growth days (1 and 3) which are located at highest positive values of D1 score (red and purple circles), middle growth days (7 and 14), at positive values of D2 score (green and blue circles) and end days (21 and 28) at highest negative values of both D1 and D2 (yellow and orange circles).

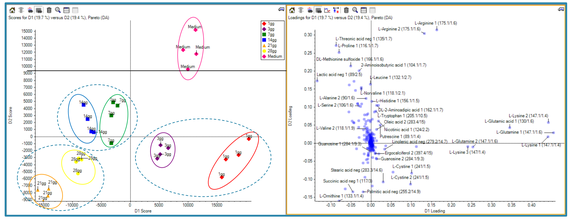

Figure 5. PLSDA score and loading plots of medium and spent media at each growth day (1, 7, 14, 21 and 28 days).

In addition, t-test highlighted variation in secreted metabolites during different stages of cell growth.

For example, high contents of lactic acid (Figure 6) were easily detected across all samples with the highest content on day 7.
Instead, glutamine (Figure 7) and glutamic acid showed a decreasing trend during the growth with the lowest level on day 21 and 28, respectively. An important trend was also revealed by succinic acid which showed same levels in medium and on day 1, 3, 7 and 14 and then a huge increase on day 21 and 28.

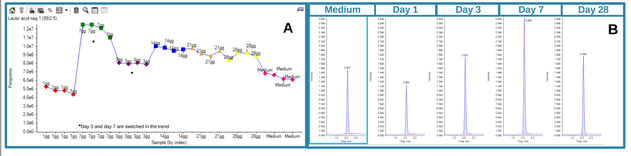

Figure 6. Quantitative trend for lactic acid in all samples (A) and representative XICs (B).

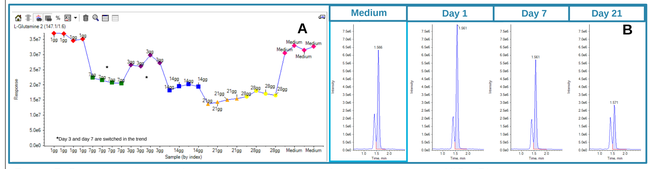

Figure 7. Quantitative trend for L-glutamine in all samples (A) and representative XICs (B).

Moreover, components such as pyruvic acid which are typically present in low abundance were also detected on day 14 with excellent sensitivity.

CONCLUSIONS

Highly sensitive and comprehensive LC-MS/MS method was developed and successfully applied for profiling secreted metabolites from human adipose stem cells at different time point of growth.
Statistical analysis approach (PCA and PLSDA) illustrated variations in composition of CCM components during different stages of cell growth involved in important pathway. Lactic acid, pyruvic acid and gluconic acid are involved in glycolysis and fermentation which are the main energy production pathways in 3D culture systems. In particular, lactic acid increases all along the *in vitro* culture as the results of pyruvic acid reduction.[4]
Lysine, glutamine and arginine decrease along the *in vitro* culture. They are proteinogenic amino-acids, thus they get incorporated in proteins under biosynthetic conditions, such as, in 3D culture and differentiation where, there is a great demand for extracellular matrix (ECM) protein synthesis to keep the pellet stability and its functionality.[5]
As a result, through this new analytical approach, we observed the variation in levels of metabolites, in agreement with known biological activity.

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