

# Automated monitoring of metabolic activity and differentiation of human mesenchymal stem cells.

Application Note

REAL-TIME MEASUREMENTS WITH THE SPARK® MULTIMODE READER



## INTRODUCTION

Detailed analysis of human stem cells and the cellular processes underlying differentiation of these cells towards specialized cell types offers the potential for the specific and targeted treatment of diseases by cell-based therapies, also called regenerative medicine.

Human mesenchymal stem cells (MSC) are multipotent adult stem cells with a high capacity for self-renewal originating from perivascular progenitor cells [1]. Using appropriate differentiation media, MSC can be differentiated *in vitro* towards adipocytes, chondrocytes, neurons and osteoblasts [2].

This application note describes the results of a study aimed at identifying phenotypic changes during MSC differentiation by automated, real-time analysis using the Spark 10M multimode reader and several well-established and reliable cell-based assays for the assessment and quantification of cell viability, cytotoxicity and apoptosis developed by PromoCell.

Tecan's Spark multimode microplate reader platform offers tailor-made solutions to suit virtually any life science research application. It has a variety of functions and features for cell-based analyses, including an automated cell imaging module and environmental control features such as CO<sub>2</sub>, temperature and humidity control right inside the reader.

## MATERIAL & METHODS

### Stem cell culture and differentiation

Human mesenchymal stem cells from adipose tissue (hMSC-AT, PromoCell C-12977) were propagated in MSC Growth Medium DXF (PromoCell, C-28019) on cell culture vessels pre-coated for 20 min at 37°C with 10 µg/ml of human fibronectin (PromoCell, C-43060) in HEPES Buffered Saline Solution (PromoCell, C-40020). For differentiation, MSC were resuspended at 2-5 x 10<sup>4</sup> cells/ml in DXF medium and 100 µl per well were plated on black 96-well plates with µClear bottom (Greiner Bio-One, 655 087). For counting, vessel coating, seeding and growth of MSC a Spark 10M multimode plate reader was used, equipped with cell chip adapter, injector system and humidity cassette. After adhesion of MSC, DXF medium was replaced by 200 µl/well of the following ready-to-use differentiation media:

- adipogenic (PromoCell C-28016)
- osteogenic (PromoCell C-28013)
- chondrogenic (PromoCell C-28012)
- neurogenic (PromoCell C-28015)

Differentiation of MSC was monitored for up to 60 hours. Cells were seeded into the microplate and left to adhere over night. The plate was then placed into the Humidity Cassette, with the fluid reservoirs of the plate filled with deionized water. To avoid edge effects due to evaporation, the outer wells of the plate were filled only with culture medium without cells. The Spark 10M was set to 37°C and 5% CO<sub>2</sub>. Cell differentiation was monitored by automated measurement of absorbance, cell confluence, luminescence or fluorescence at defined intervals.

### Reagents and measurement

The Fluorometric Cell Viability Kit I (Resazurin; PromoCell, PK-CA707-30025-0) was used for viability assessment. The assay is based on the conversion of resazurin to its fluorescent product resorufin by a chemical reduction in living cells. The ready-to-use resazurin solution was added directly to the culture medium at a 1:20 dilution. Blank wells consisting of culture medium without cells, but with resazurin, were included.

For analysis of cytotoxicity, the LDH Cytotoxicity Kit I (PromoCell, PK-CA577-K314) was used to detect lactate dehydrogenase (LDH) enzyme activity in cell culture supernatant as an indicator for necrotic cell death.

Adenosine triphosphate (ATP) levels were quantified using the Bioluminescent Cell Viability Kit I (ATP, PromoCell, PK-CA577-K254-200). All kits were used according to the manufacturer's instructions.

## RESULTS

To observe tissue-specific morphological changes and expression of cell surface markers differentiation of human MSC *in vitro* has to be monitored/investigated over several days to weeks [3]. However, subtle changes in the cells' phenotype, e.g. metabolic activity, are expected to occur already early upon the onset of differentiation. Thus, we wondered if we could reveal those changes by real-time measurement already hours after exposing MSC to the various differentiation media.

In order to do so, MSC were subjected to adipogenic, osteogenic, chondrogenic or neurogenic differentiation and resazurin, a non-toxic, cell-permeable non-fluorescent blue dye, was added directly to the culture media. As a result of the reducing power of living cells, resazurin is converted to the red-fluorescent product resorufin, thus, offering a measurement for cell viability or cell metabolism. To enable online measurement, the experiment was performed by propagating MSC within the Spark 10M multimode plate reader for the total length of the experiment.



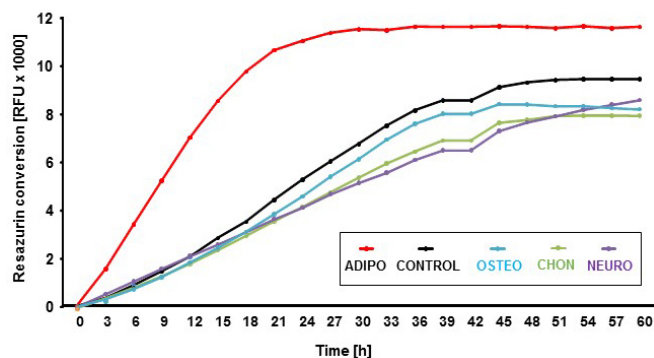


Figure 1: Automated analysis of metabolic activity during differentiation of stem cells.

Compared to all other conditions applied, MSC in adipogenic differentiation medium already showed a significant increase in resazurin conversion three hours after induction, which constantly increased for more than 24 hours before reaching a plateau (Figure 1). Relative to MSC which had been left in non-differentiating DXF medium, osteo-, chondro- or neurogenic differentiation conditions showed a slight decrease in resazurin conversion, reaching a plateau-phase by around 48 hours. This result shows that the change in the phenotype of MSC after induction of differentiation is already apparent within few hours.

In order to investigate if the difference in resazurin conversion reflects changes in cell viability or cell metabolism, the presence of LDH activity in MSC culture supernatant was analyzed as a measure for ruptured (necrotic) cells. Interestingly, a clear indication for cytotoxicity during MSC differentiation could be seen, with the osteogenic differentiation condition being most toxic (Figure 2). However, the cells in adipogenic differentiation medium with the highest rate of resazurin conversion (Figure 1) did not show lowest cytotoxicity.

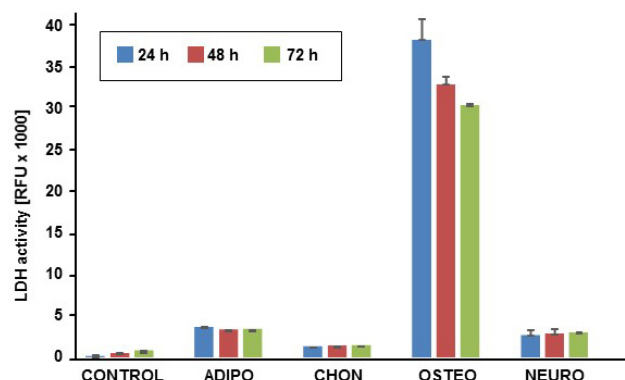


Figure 2: Cytotoxicity measured during stem cell differentiation.

Thus, differences in cell viability did not explain the difference in resazurin conversion. It was therefore investigated if differences in resazurin conversion reflect changes in metabolic activity during differentiation. For this purpose, the ATP levels during MSC differentiation were measured. Indeed, the adipogenic differentiation condition showed the highest ATP content, followed by non-differentiated MSC (Figure 3). This result correlated very well with the resazurin measurement (Figure 1), indicating that changes in metabolic activity are an early event during differentiation of stem cells.

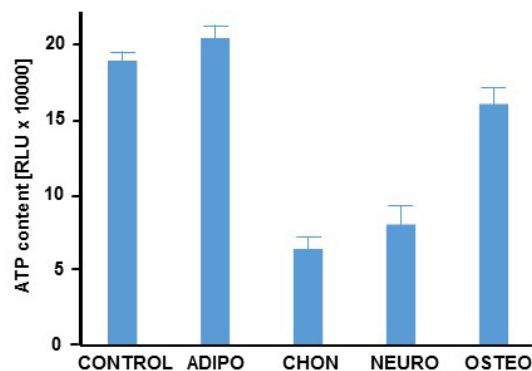


Figure 3: ATP levels during differentiation of stem cells.

## DISCUSSION / CONCLUSION

Based on the finding summarized above, it could be demonstrated that already early after induction of differentiation towards adipocytes, human MSC show a substantial elevation of cell metabolism, indicated by the cells' reducing power and production of ATP. In contrast, osteogenic, chondrogenic and neurogenic differentiation significantly reduce the metabolic activity of MSC. Of note, *in vitro* differentiation of MSC with ready-to-use cell culture media works reliable and robust. Cell counting, plating and culture of stem cells for several days within the Spark multimode reader was convenient and reliable and, thus, provided a good basis for the automated multi-parameter measurement of cell characteristics. Furthermore, addition of resazurin to the culture medium for up to 60 hours proved to be very sensitive and non-toxic to stem cells.

## ACKNOWLEDGEMENTS

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## REFERENCES

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2. da Silva Meirelles L, Caplan AI, Nardi NB., Stem Cells 2008; 26(9):2287–99.
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## ABBREVIATIONS

ATP	adenosine triphosphate
LDH	lactate dehydrogenase
MSC	mesenchymal stem cells

## About the authors

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