Monitoring cell migration and wound healing.

APPLICATION NOTE

CELL IMAGING WITH THE SPARK® MULTIMODE READER PLATFORM.

TECAN.

INTRODUCTION

Cell migration is a central process in the development and maintenance of multicellular organisms, with tissue formation during embryonic development, wound healing and immune responses all requiring the orchestrated movement and assembly of cells. Abnormalities in cell migration are associated with various diseases – including cancer, atherosclerosis and arthritis – and so understanding cell migration and the biological events that trigger the movement of cells from one location to the other is a key focus of cell-based research.
In this context, imaging-based analysis methods are highly valuable tools for the investigation of cell migration.
While image analysis using visual microscopy is a long-standing and reliable method in cell biology, it is very time-consuming and laborious. Automated imaging and analysis of cell samples greatly facilitate the experimental workflow and increases the throughput while minimizing experiment-to-experiment variations caused by variable starting conditions. Live imaging-based readouts can be used for walkaway monitoring of cell growth and health during long-term experiments.

Tecan's Spark instrument platform has an integrated bright-field cell imaging module that enables label-free and real-time assessment of cell confluence in microplate wells. The reader is able to detect cell-covered areas in 6- to 96-well plate formats and calculate the relative confluence ratio based on the analysed area. The confluence ratio can also be used to normalize other cell-associated signals (e.g. the cellular ATP content) to the number of cells in the sample. The Spark's confluence-imaging function allows the study of various different forms of cell migration – such as chemotaxis, transmigration and tissue invasion, as well as the analysis of the metastatic potential of tumor cells.

The Radius™ 96-well Cell Migration Assay is a so-called 'gap-closure' assay system that uses a defined cell-free area that can be repopulated by cells and monitored in real-time by visual or automated microscopy.
Unlike Boyden chamber assays which can only be analysed at endpoint, the Radius assay uses proprietary cell culture plates with a carefully-defined, circular spot created with biocompatible hydrogel (Radius™ gel) at the center of each well. When cells are seeded in the well, they will attach everywhere except the gel spot, creating a cell-free zone. Following cell seeding the gel spot is removed, allowing migratory cells to move across the area and close the gap. This 'artificial wound' in the cell monolayer can be used to study cell migration, cell proliferation and wound closure. Due to the gap's well-defined area and position inside the well, this method is a much more consistent alternative to conventional scratch assays.

This application note describes the application of the Spark 20M's imaging capabilities for the monitoring of cell migration in the context of wound healing, using the Radius™ 96-well Cell Migration Assay as a test system. Furthermore, this note describes the correlation between cellular confluence and intracellular ATP content as an indicator of cell viability and metabolism.

MATERIALS AND METHODS

Migration

The Radius 96-well Cell Migration Assay (Cell Biolabs, CBA-126) was tested in combination with the Spark 20M's confluence imaging function to monitor the cell migration and repopulation of the cell-free area over a period of 30h. The kit was used according to the manufacturer's instructions [1]. Two biliary tract cancer cell lines ("CL1" and "CL2") were used. CL1 is known to have negligible migration properties, whereas CL2 is known to show high migration activity. The cells were cultured in DMEM high glucose supplemented with L-glutamine, penicillin/streptomycin, sodium pyruvate and HEPES + 10% fetal calf serum (FCS). For the migration experiments, the cells were transferred into FCS-free medium to minimize cell division and be able to monitor only migration and wound healing activity instead. The cells were then seeded into transparent Radius 96-well Cell Migration Assay microplates (supplied with the kit) at an initial density of $3x10^4$ cells/well (the optimal seeding density was predetermined) and left to adhere over night. The inserts of the Radius plates were removed at the beginning of the experiments, and the repopulation of the cell-free area by migration cells was monitored over a period of 30 hours.
The migration assay was performed in two ways: a manual and an automated/kinetic setup.

1. **Manual:** The test plate was kept inside a conventional CO_2 incubator between the measurements to provide a suitable temperature and gas atmosphere, and transferred to the instrument for the readouts at 0, 2, 4, 6, 8, 24 and 30h.

2. **Automated:** The entire measurement was performed inside the Spark reader, using the instrument's environmental control features to create an automated workflow and maintain 5% CO_2 and 37°C throughout the entire measurement period.

Parameter	Setting
Measurement	Cell Confluence
Readout mode	Kinetic
Duration	60 cycles
Interval	30 min
Pattern	central
Settle time	0 ms
Data analysis	activated
Well border detection	none

Table 1: Measurement settings for cell migration experiments

The measurement settings for the automated/kinetic cell migration experiments are summarized in table 1. In the manual setup, each measurement was performed in endpoint mode instead of kinetic mode. For the automated/kinetic setup, the confluence was recorded over the entire experimental period of 30 hours, with one measurement point every 30 minutes. The confluence pattern was set to 'Central', meaning that a central picture from the center of each well was taken and analyzed. Importantly, the 'Central' pattern is only useful when the area of interest, i.e. the cell-free spot, is located in the middle of the well. The measurement/imaging area can not be repositioned within the well, and the detected confluence value only reflects the percentage of cell-covered area in the analysed spot(s). The confluence values calculated based on the pictures were then evaluated as an inversely proportional indicator of cell migration, with the initial (0h) value assumed to represent a 100% cell-free area.

Correlation of confluence and cellular ATP content

The second aim of this study was to correlate the confluence signals with the ATP content of the cells. In this context, the CellTiter-Glo® Luminescent Cell Viability Assay (Promega, G7571) was used to detect and quantify the ATP levels in the cells. The assay is a homogeneous method of determining the number of viable cells in culture based on quantitation of their ATP level, as an indicator of metabolically active, viable cells present in the sample [2]. Again, two biliary tract cancer cell lines ("CL3" and "CL4") were used and seeded into 96-well plates at a range of initial cell numbers per well (25000, 12500, 6750, 3125 and 1563 cells/well). After 24, 30, 48 and 50h, the confluence in each well was measured using the 'Whole well' pattern, immediately followed by the ATP quantification assay in the same wells. The results were evaluated by calculating the luminescence/confluence ratio, with outliers identified and removed based on the Grubbs outlier detection test.

RESULTS

Migration / Wound Healing

The expected migration pattern of the two different cell lines could be clearly seen using the confluence function of the Spark 20M (figure 1). While CL1 showed no or only negligible migration, CL2 exhibited continuous repopulation of the cell-free area over the analysis period.

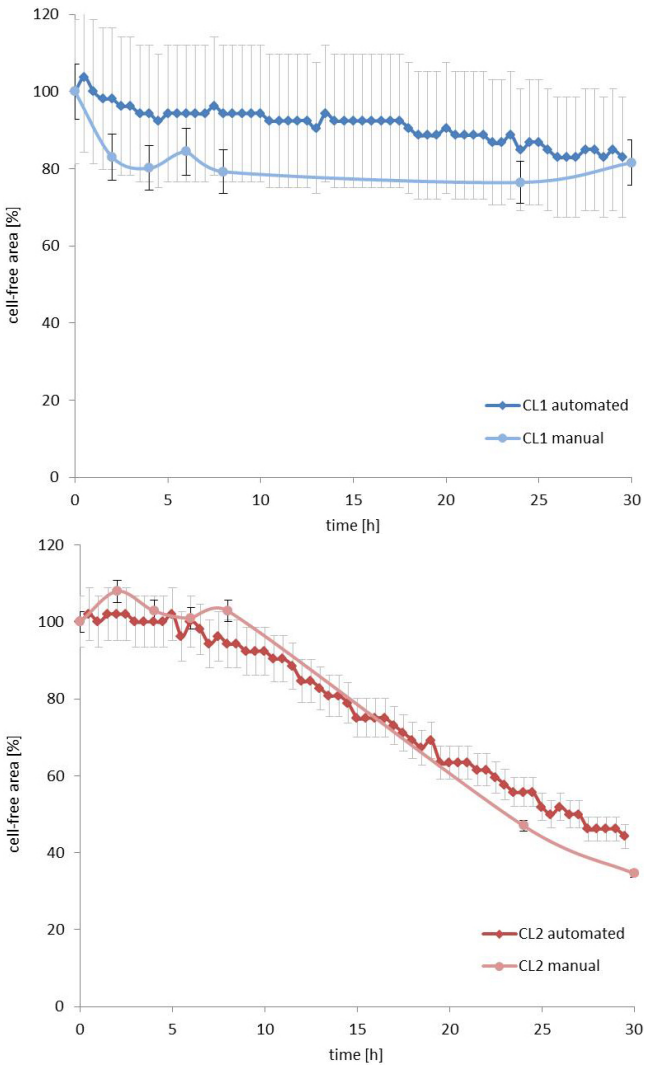

Figure 1: Dynamics of cell-free area repopulation by CL1 and CL2 (shown as percentage of cell-free area), comparing manual vs. automated handling of both cell lines.

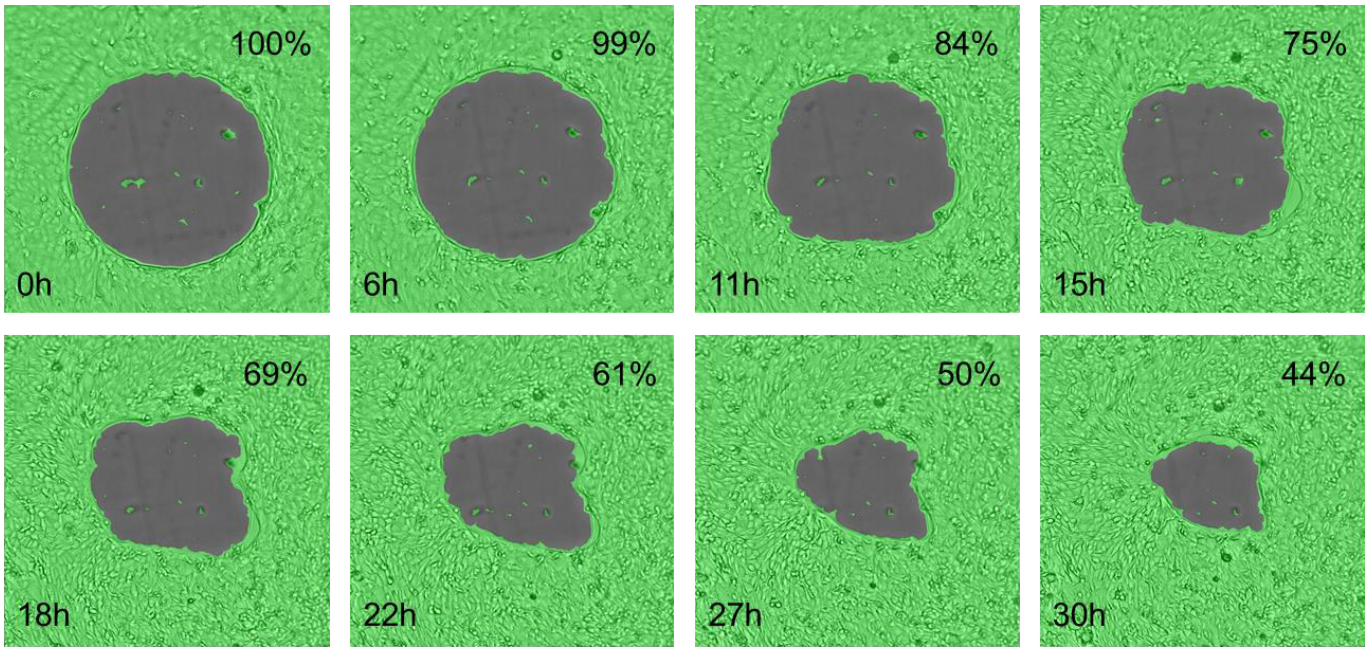

Figure 2: Gap closure by CL2 (biliary tract cancer cell line with high migrating potential) over 30h.

While the migration behavior over the total analysis period shows similar dynamics for manual and automated handling, the latter has the advantage that no data points are lost due to overnight gaps where the operator is not present to perform the manual plate handling. Automated handling therefore provides consistent and gap-free analysis of the cells' migration dynamics.

Hours	Av. conf.	SD	% cell-free area	% cell-free area (norm.)
0	82.7	1.15	17.3	100
6	82.8	1.15	17.2	99
11	85.3	1.15	14.7	84
15	87.0	1.73	13.0	75
18	88.0	2.00	12.0	69
22	89.4	2.52	10.6	61
27	91.3	2.52	8.7	50
30	92.3	3.21	7.7	44

Table 2: Confluence / migration dynamics and calculated cell-free area of CL2 (selected data points)

The continuous repopulation of the cell-free area can be clearly seen in figure 2 where the time course of gap closure is documented. Over the 30h analysis period, repopulation of more than half of the initial cell-free area could be observed. The cell-free area was observed to get progressively smaller with increasing cell migration into the circle. In figures 1 and 2, the initial value (0h) was assumed to represent 100% cell-free area. All other values are given in relation to the initial situation (also see table 2).

The extent of migration was determined by plotting the inverse confluence value detected by the SparkControl™ software in the selected measurement area, i.e. the single picture in the well center (table 2). For an exact determination of the gap area, the use of third-party image analysis software, such as ImageJ, is recommended.

Correlation of confluence and cellular ATP content

Figure 3 shows the correlation between confluence and ATP-based luminescence over a period of 54h using different initial cell numbers at the time of seeding. Both signals exhibited similar dynamics over time, indicating the progressive growth of the cells which is characterized by both an increase of the cellular ATP content and increasing cell density on the well bottom. With cell numbers below 3000 cells/well the confluence values were outside of the recommended range (<10%), so it is advisable to use higher cell numbers for reliable experiments. From 3125 cells/well upwards a good correlation between ATP signal and confluence values could be observed, indicating that it is possible to normalize the results using confluence measurements instead of the more laborious cell number determination by measuring the ATP content.

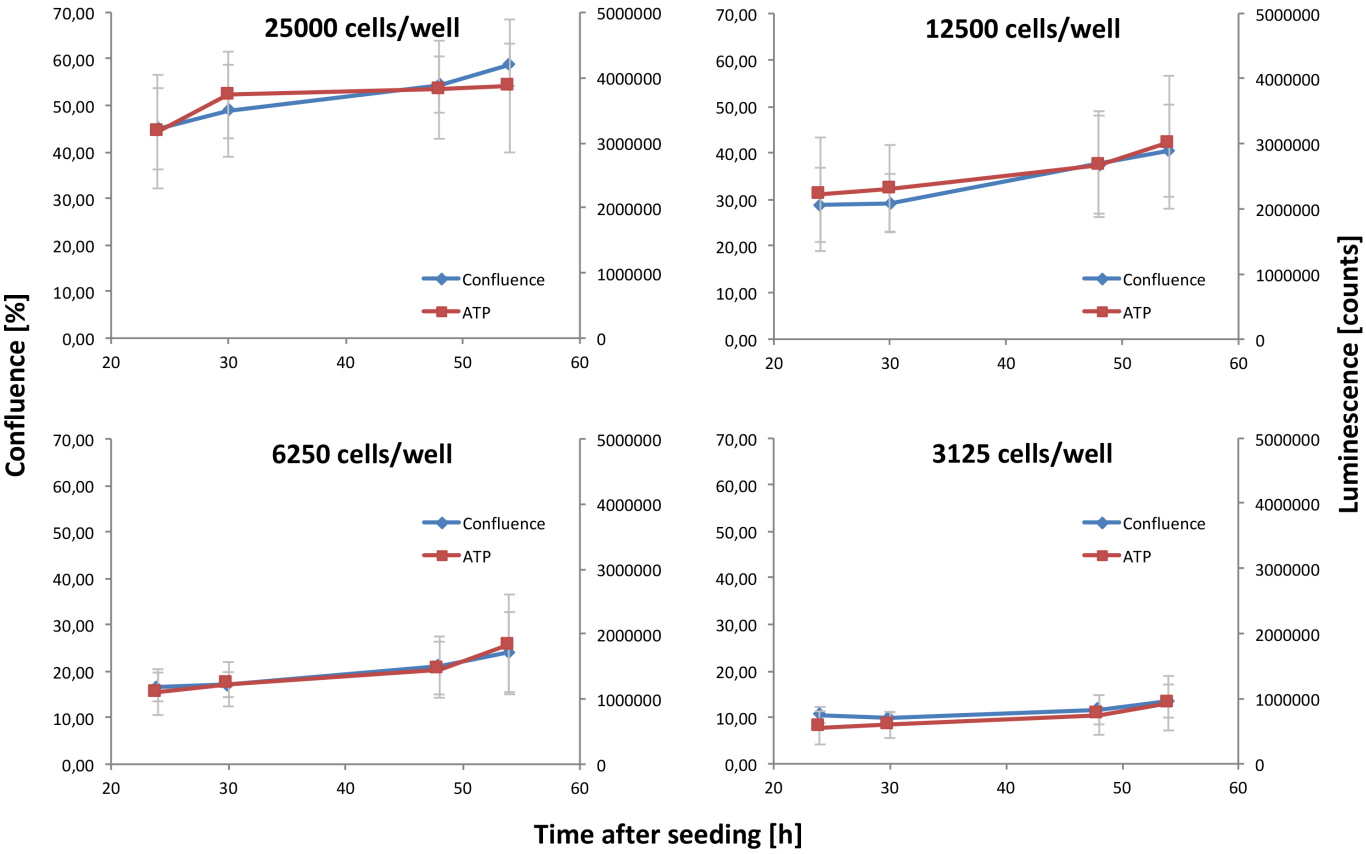

Figure 3: Correlation of confluence and ATP-based luminescence signals at different cell numbers.

CONCLUSION

The results of this study show that the Spark reader platform is ideally suited to the measurement of cell migration and wound healing assays such as the Radius 96-well Cell Migration Assay. The instrument's brightfield imaging-based confluence function enables the high-quality and label-free real-time monitoring of cell movement, allowing the dynamics of gap closure and wound healing to be quantified and followed over time. The reader's SparkControl software makes it easy to quantify cell confluence right in the microplate wells, with user-selectable patterns for analysis of different areas within the well and automated well border detection for any type of microplate.

The ability to correlate confluence with cellular ATP content further simplifies cell-based assay workflows by allowing complete walk-away automation of experiments. As the confluence measurement is label-free and does not interfere with any other cellular characteristics or properties, it can be performed continuously over the entire experimental period, providing a simple and reliable alternative to more traditional, costly and/or laborious techniques for the determination of the population size/mass of cells of interest.

REFERENCES

[1] Radius™ 96-Well Cell Migration Assay, Product Manual, CBA-126, Cell Biolabs Inc., San Diego, USA

[2] CellTiter-Glo Luminescent Cell Viability Assay, Technical Bulletin 288, 03/15, Promega, Madison, WI, USA

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ABBREVIATIONS

ATP	adenosine triphosphate
CL	cell line
DMEM	Dulbecco's modified Eagles medium
EM	emission
EX	excitation
FCS	fetal calf serum
GFP	green fluorescent protein
PBS	phosphate-buffered saline
RFU	relative fluorescent units
SD	standard deviation

About the author

Dr Katrin Flatscher is an application scientist at Tecan Austria. She studied molecular biology at the University of Salzburg and focused on cell biology and immunology research during her PhD. She joined Tecan in 2007 and has been involved in the development of the Infinite as well as the Spark reader series.

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