Imaging and Evaluation of Wound Healing with the Cellavista System

Application Note
AN-B003-XIV-04

Introduction

Wound healing is a complex phenomenon conducted by numerous cells interacting with each other and their environment when cell-cell contact is disrupted.
To study this process *in vitro* a scratch or an artificial gap is created within a confluent monolayer of cells. The wound is then closed by a combination of cell proliferation and migration within a time course of several hours to days depending on the cell type and size of the scratch. This recovery is usually tracked with time lapse microscopy or can be monitored by fixing samples at different points in time.
In general, wound healing assays are easy to set up and are rather inexpensive. Thus, they are a convenient method to study various processes like cell polarization, cell migration and matrix remodeling. Furthermore, the role of certain factors or substances in wound healing or even pathologic processes as cancer cell metastasis and invasiveness of tissues can be analysed. The challenge of this method is, to find a reliable method to track the wound healing process and to analyze the generated images in a quick and accurate way. The Cellavista provides high-end automated microscopy and image analysis and, when combined with the Ibidi incubation system, also a way of performing wound healing and other live cell applications in enhanced throughput. Furthermore the high image quality allows documentation and evaluation of cell morphology.

Fig. 1: Ibidi chamber integrated in the Cellavista System

Here we present a wound healing assay performed with Caco-2, human epithelial colorectal adenocarcinoma cells, to demonstrate the basic principle of a wound healing assay. Wound healing was monitored continuously over two days in the Ibidi chamber integrated in the Cellavista System.

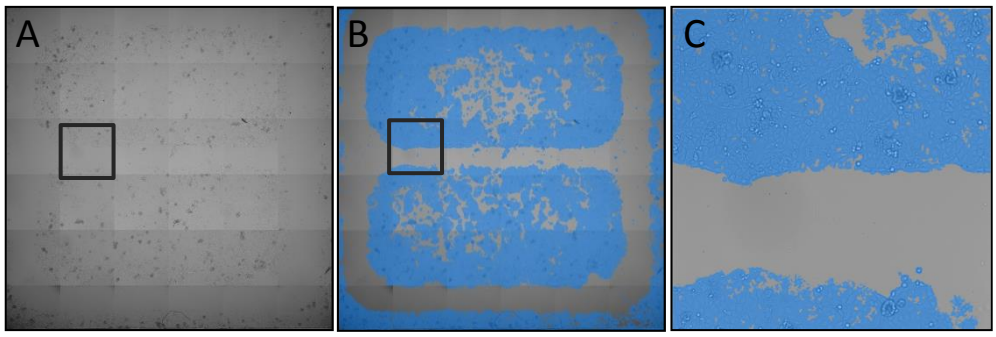

Fig. 2: Overview of imaged well and processing results

One whole well of a 8-well slide has been imaged by time lapse microscopy. **A**: Overview of one whole well (6x6 images) at the start of the wound healing assay. The black box marks the detail which is magnified in image C. **B:** Overview of the same well as in A with the cell area identified by the Cellavista software in blue. **C:** Zoom into details depicted in A and B.

Results

We imaged closure of a Caco-2 epithelial cell layer over 48 hours, taking images every 30 minutes. The area covered by cells was detected and quantified with the Cellavista software. Results were exported into csv format.

The central cell free gap between the cell layer narrows at a steady speed and is nearly closed after 44 hours (Fig. 3 and 4).

The high quality images of the Cellavista allow detailed analysis of the mechanism of the gap closure process. Here we checked whether cell migration or proliferation takes place. We observed that cells of the leading edge of the wound stay in close contact to each other and do not seem to protrude into the open well area during wound closure.

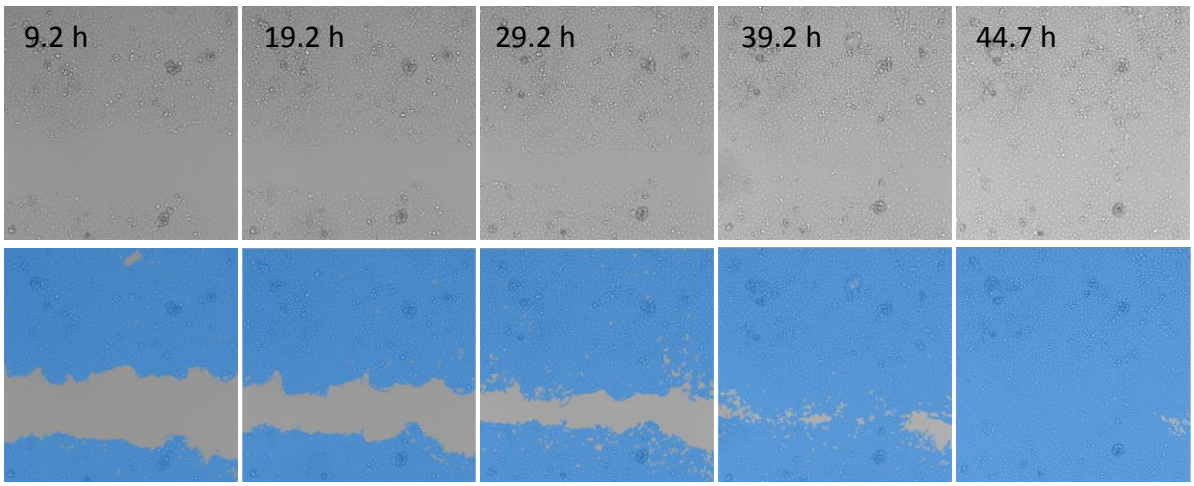

Fig. 3: Images of wound closure over time
Images of the same detail of cells at different points in time with (bottom row) and without (top row) image analysis results in blue. The area covered by cells has been detected with the Cellavista cell confluence operator.

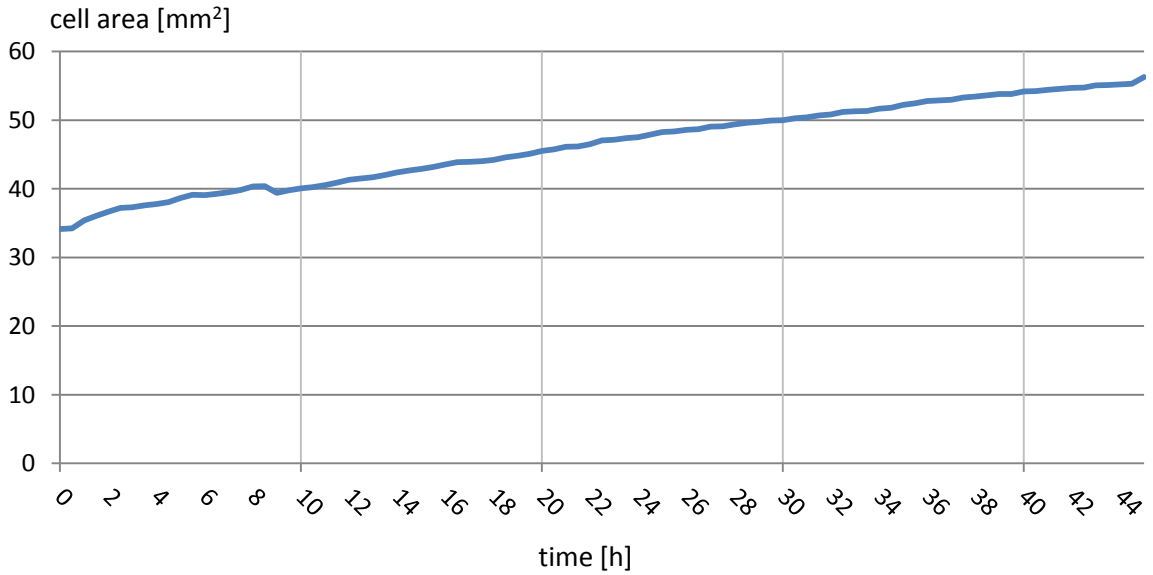

Fig. 4: Change in Caco-2 cell area over time.
Data from wound healing assay determined with the Cellavista cell confluence operator.

Only apoptotic cell bodies and cell aggregates appear in cell free regions. When both parts of the damaged cell layers are approaching each other only very few single cells protrude into the open space (Fig. 5 A). Noteworthy, also changes in cell morphology are visible as large cells appear in the confluent cell layer (Fig. 5 B).

Hence, considering the duration of the process and the lack of protruding cells during the whole process, we suggest that mainly cell proliferation and not cell migration is taking place to close the artificial wound.

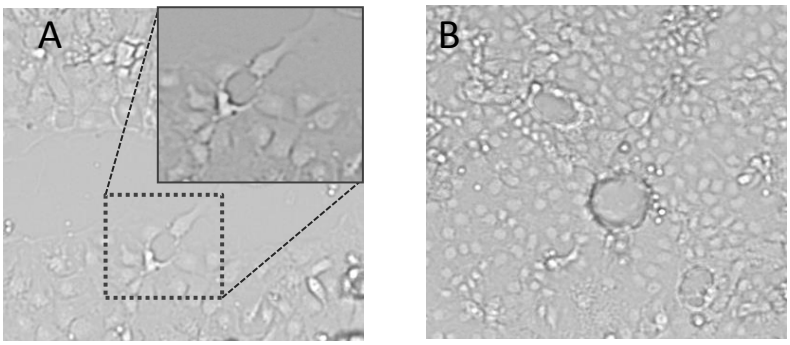

Fig. 5: Protruding cell from the leading edge of the cell layer (A) and detail of cell morphology (B)
A: A single protruding cell is apparent when both cell layers are in close proximity to each other (black box= zoom in).
B: Confluent Caco-2 cell layer showing morphological differences between individual cells.

Conclusions

Performing wound healing assays with an automated microscopy and image analysis system permits the accurate capture and evaluation of image data and is especially useful for high throughput assays.

The Cellavista imaging System offers automatic image acquisition with the option for an individual focus for each image or well. If working with microplates, which can vary in quality (e.g. variations in bottom thickness or rippled plate bottom), this focus is essential to be able to obtain analyzable images. The following evaluation of image data can be done simultaneously to data acquisition or at a later point of time.

In these experiments the Cellavista confluence operator has been used to detect and calculate the area covered by cells. The data can also be reprocessed with different operators or parameter settings If required.

In the Cellavista System brightfield can also be combined and evaluated with fluorescence imaging in up to six different fluorescence channels. This can be useful if wound healing assays are extended so that e.g. fluorescent reporter proteins mark cells with certain characteristics or modifications.

In general the analysis and evaluation of brightfield is more challenging than fluorescence images, as objects or cells can vary in their morphology, brightness and contrast. Although, Caco-2 cells show only little contrast as apparent in Fig. 3 and 5 the Cellavista System can image, automatically detect and evaluate individual cells or areas. High quality images also enable visual analysis of cell morphology. Data can directly be displayed in tables and diagrams or can be conveniently exported as csv or pdf format.

Material and Methods

Materials:

- Adherent epithelia forming cells, here Caco-2 cells
- DMEM/Ham's F12 1:1 with 5 % fetal bovine serum and 1 % Penicillin/Streptomycin
- Phosphate Buffered Saline (w/o Ca^{2+}, w/o Mg^{2+})
- Trypsin 0.25 %
- Device to scratch or insert to form a gap in the cell layer
- 8-well object slide
- Cellavista imaging System
- Ibidi heating and gas incubation chamber

Method:

Cells were trypsinized to detach and transferred to the culture dish, which contained a bio-silicone insert forming a gap of about 500 µm width. Cells were grown to a confluence of about 90 % over night in the incubator. The next day the insert was carefully removed with a forceps.

Subsequent to transferring the object slide to the heating and gas incubation system the whole chamber was put into the Cellavista microscopy System. The chamber was incubated at 37°C within an environment of 5 % CO_2 and 90 % relative humidity.

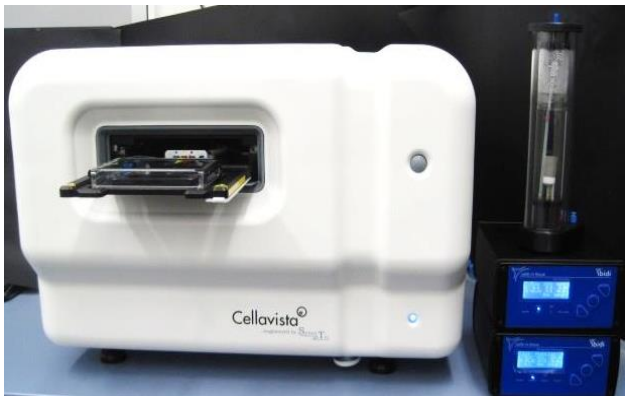

Fig. 6: Cellavista imaging System with integrated incubation chamber
Overview of the Cellavista System with integrated Ibidi chamber, heating and gas controller elements to the right.

The measurement including brightfield illumination and autofocus was setup once at the beginning. Also the number of images taken per well was defined. For data evaluation a magnification with either a 4x or 10x objective is sufficient. Here we imaged the whole well (≈1 cm^2), resulting in 6x6 images with the 10x objective.

Images of all wells were taken automatically (software driven) by defining the time interval of images taken (here 0.5 h) and the length of measurement (here 48 h). Therefore 96 individual measurements were performed.

Data were processed with the cell confluence operator, measuring the cell covered area (Fig. 3 and 4). Measurement and evaluation of the images can be performed e.g. for the whole sample tray or for either one or a selection of wells from the plate, depending on the layout of the wound healing assay. In this assay we processed one whole well composed of 36 images (Fig. 2 A and B) in 96 measurements. With the Cellavista System data can directly be generated and visualized in diagrams or can be exported as csv format.

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