

CYTONOTE Application Note

Automated continuous monitoring of cell migration (Scratch assay) for senescence study

Introduction

Cell migration is a central process in the development and maintenance of multicellular organisms. Tissue formation during embryonic development, wound healing and immune responses all require the orchestrated movement of cells in particular directions to specific locations. Cells often migrate in response to specific external signals, including chemical and mechanical signals. Errors during this process have serious consequences, including intellectual disability, vascular disease, tumor formation and metastasis. An understanding of the mechanism by which cells migrate may lead to the development of novel therapeutic strategies for controlling, for example, invasive tumor cells. Cell migration and invasion play a role in many normal and pathological processes including immune responses, embryonic development, angiogenesis, regeneration, tumor metastasis and wound healing.

In vitro experiments, Scratch assay is the primary method for assessing cell migration and cell invasion. In this application note, we will describe this test through measurements with the CYTONOTE.

Material & methods

1. The CYTONOTE

The CYTONOTE is able to perform measurements inside the incubator and it recognizes cells without any labelling. The HORUS software automatically calculates cell number, cell saturation, cell area, cell concentration, cell morphology ... Unlike a conventional microscope, the absence of focus in the CYTONOTE allows an extremely wide field of view.

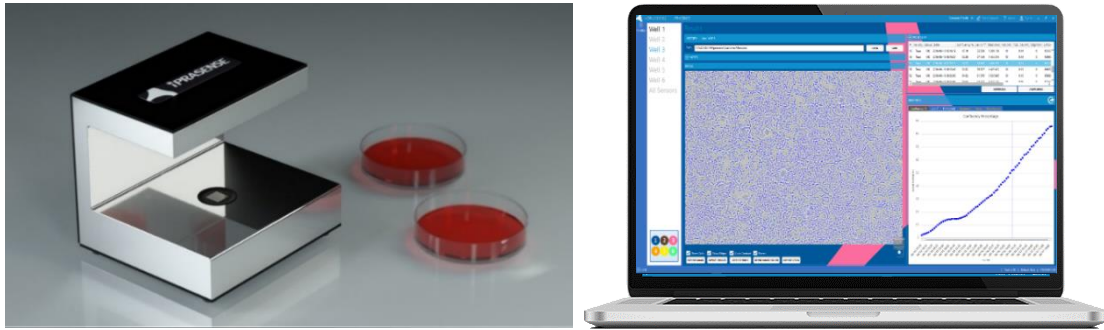


Figure 1: CYTONOTE 1W on the left and HORUS software on the right.

2. Cell migration assay

The CYTONOTE provides complete images and data. Scratch assay assesses cell migration and cell invasion (A). Scratch assay quantifies cell migration by a carpet of cells (so 100% confluent), make a scar in the middle of the carpet with pipetting (B) and see how long it closes. This method is used in particular for tumor cells, for research on cancers and cellular invasions. The CYTONOTE records the healing of scratches in real time and also calculates the percentage of closure of scratches (C) and the migration rate of cells (D) for example.

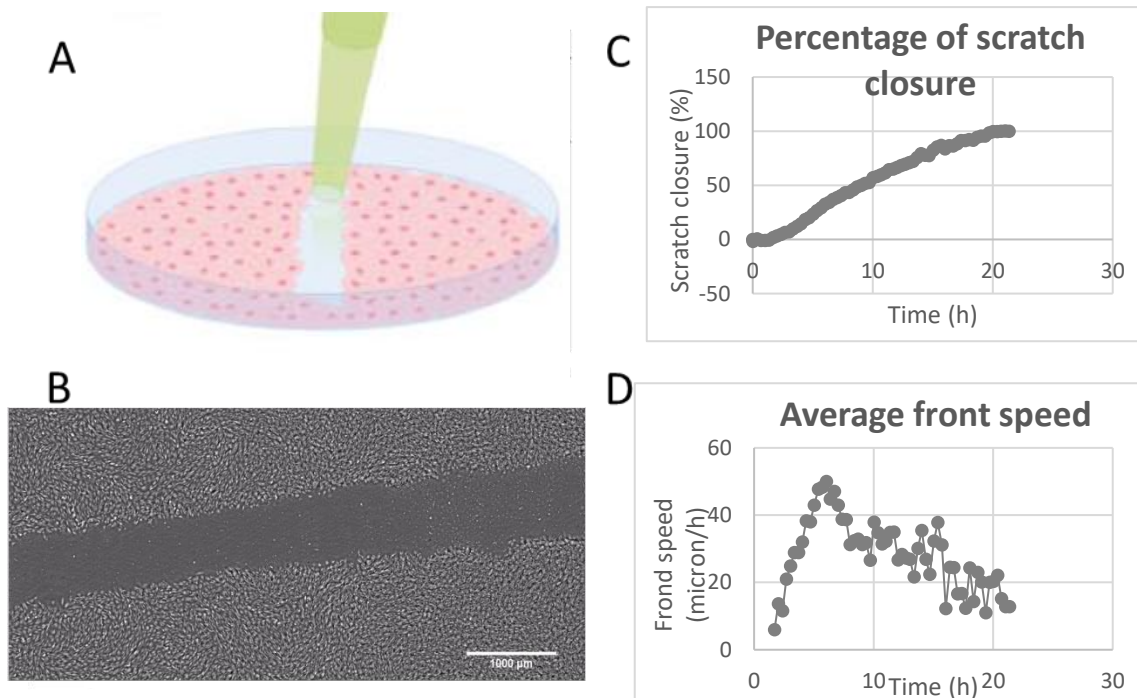


Figure 2: Scratch assay

(A) the monolayer was scraped with a p200 micropipette tip in a straight line to create a cell-free “scratch”

(B) scratch scar of human fibroblast cells in Petri dish (video is available on Youtube “Wound healing” <https://www.youtube.com/watch?v=F4-YFK0gHZs>;

(C) real-time quantitative scratch closure

(D) real-time quantitative scratch cell edge migration rate (micro / h)

3. Cells

For the experiment, human epidermal keratinocytes were cultured in Petri dish of 35 mm² until confluence in an incubator at 37 °C with 95% humidity. Then, the monolayer was scraped in a straight line to create a cell-free “scratch”. Keratinocytes in the scratch assay were first treated with mitomycin C (10 µg/ml) to inhibit cell proliferation while preserving viability and ability to migrate.

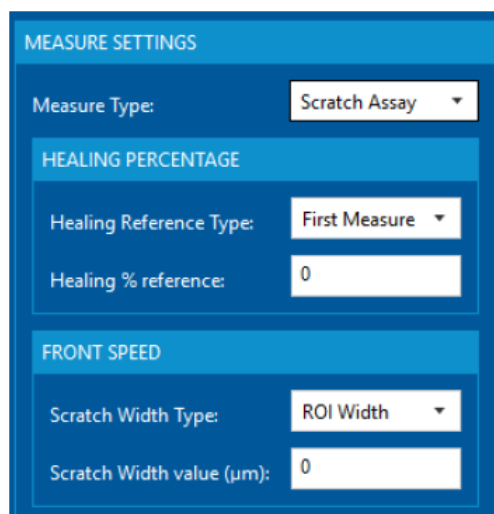


Figure 3: Measure setting in Scratch assay by HORUS software

Results

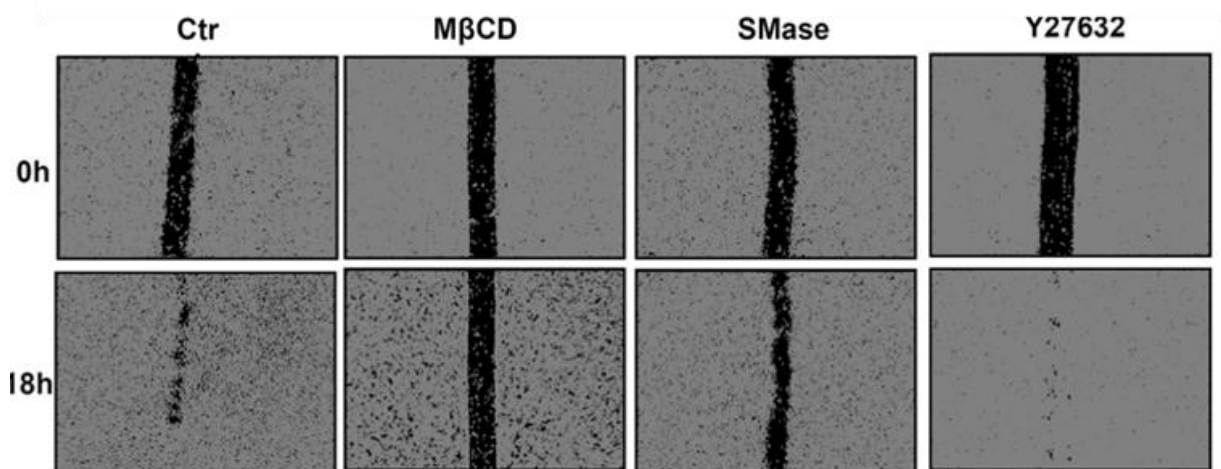


Figure 4: Images of Scratch assay with 4 conditions

Same size scratches of same keratinocytes were treated with different medications (M β CD: methyl- β -cyclodextrin treatment for cholesterol depletion, SMase: *Bacillus cereus* sphingomyelinase treatment for sphingomyelin depletion, Y27632: compound for treatment with Rho kinase inhibitor) at 0h. And the scratches were closed at 18 h.

While cholesterol depletion by M β CD (7.5 mM) abolishes keratinocyte migration, degradation of sphingomyelin in keratinocyte plasma membrane by incubation of cells with SMase (5 mU/ml) clearly slows down the process. Conversely, Y27632 which preserves the organization of sphingomyelin rich submicrometric domains during senescence is well known to simultaneously enhance cell migration, suggesting again possible links between sphingomyelin organization in keratinocyte membranes and their propensity to migrate. We observe the ability of keratinocytes to migrate decreases in senescent cells as well as in cells treated to deplete cholesterol or sphingomyelin in membranes.

Conclusion

The CYTONOTE allows to analyse cell migration ability (such as tumor cell invasion ability) using time-lapse imaging. Real-time monitoring provides constant quality control for the cells allowing the user to add a compound at good timing for example. The distance of cell migration is measured by the HORUS software in real-time recording.

Reference

Online version available at <https://www.iprasense.com/migration-assay/>

Mound A, et al. *Non-senescent keratinocytes organize in plasma membrane submicrometric lipid domains enriched in sphingomyelin and involved in re-epithelialization*. *Biochim Biophys Acta*. 2017 Sep;1862(9):958-971
<https://www.sciencedirect.com/science/article/pii/S138819811730104X?via%3Dihub>



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