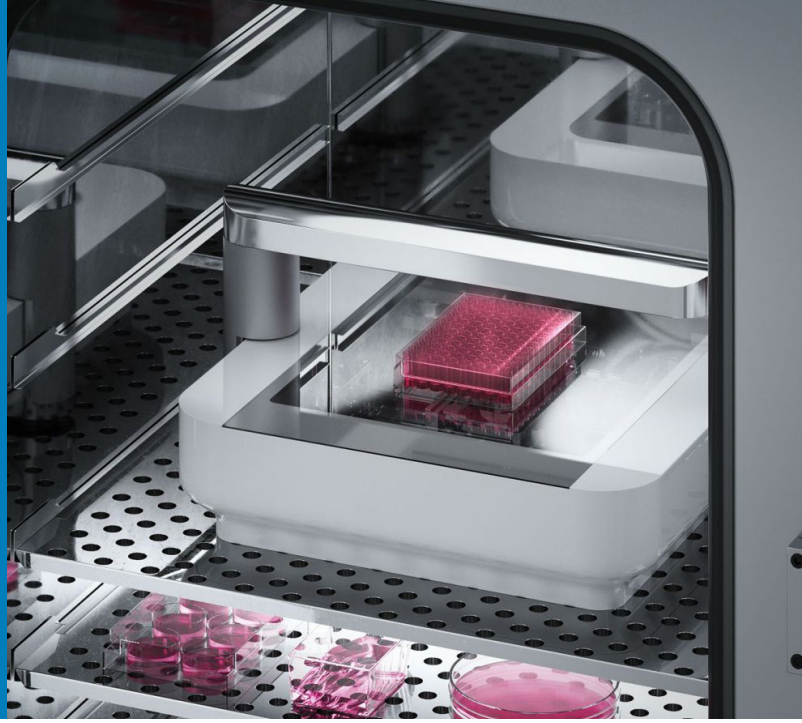
Optimizing a fibroblast scratch assay protocol using live-cell imaging

Dr. Simone Riis Porsborg, Aalborg University, Denmark.
Written by Dr. Inge Thijssen-van Loosdregt, CytoSMART Technologies, the Netherlands

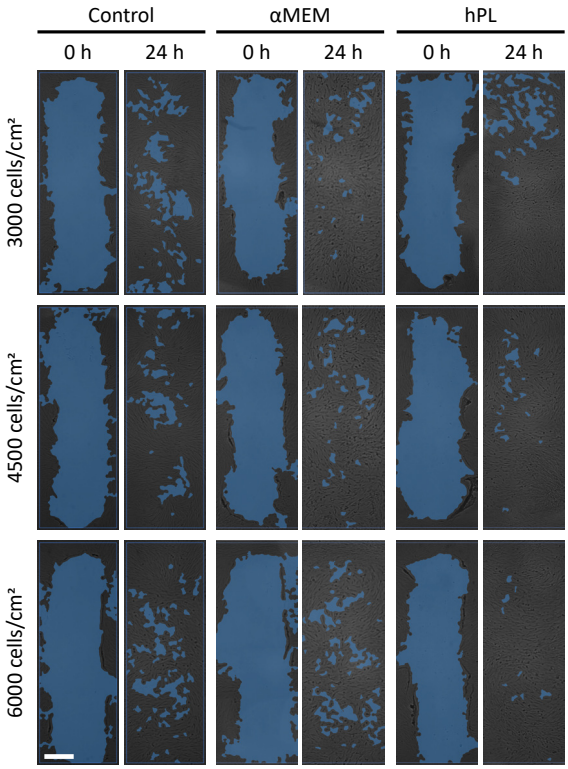
Case study: Dr. Simone Riis Porsborg from Aalborg University has used automated brightfield live-cell imaging to optimize her fibroblasts scratch assay protocol.



Introduction

Wound healing is a dynamic process involving many different cells and chemokines. In case the wound healing process is perturbed, chronic wounds with insufficient healing may arise. Novel therapies are currently being developed to treat chronic wounds. One of these potential therapies is the use of adipose-derived stem cells (ADSCs) to promote the wound healing process[1,2]. Since it is still unclear via what mechanisms ADSCs promote the wound healing process, *in vitro* studies need to be performed to investigate this into detail. One of the questions that are currently unanswered, is whether the chemokines produced by ADSCs affect fibroblast migration speed. This can be investigated by examining the effect of ADSC-conditioned media on the scratch closure of fibroblasts in a scratch assay setup. However, before this type of experiment can be performed, some challenges need to be overcome. One of these challenges is that ADSCs and fibroblasts are cultured in different types of media. Next to this, in order to be able to use ADSCs as a cell therapy agent in the future, the commonly used Fetal Calf Serum (FCS) needs to be replaced by human Platelet Lysate (hPL) as the latter is approved for clinical use. Lastly, the fibroblast seeding density needs to be optimized to ensure proper collective cell migration before the scratch assay can be performed.

Automated brightfield live-cell imaging is a convenient tool to quickly investigate the effect of different culture conditions on scratch closure. The CytoSMART Omni, for example, automatically scans and analyzes the scratch area of complete well plates. This case study provides a proof-of-concept for rapid evaluation of many different culture conditions on collective cell migration.



Materials and methods

Fibroblasts were seeded in a 96-well plate at a density of 3000, 4500 or 6000 cells/cm^2. After 3 days of culture, the samples were scratched using the AutoScratch (BioTek) and culture medium (DMEM with 10% FCS) was replaced with either culture medium (control), αMEM with 10% FCS (αMEM) or αMem with 5% hPL (hPL). The well plate was subsequently placed on the CytoSMART Omni inside a culture incubator (37°C and 5% CO_2). The entire plate was scanned every 3 hours for 24 hours. Images were uploaded to the CytoSMART cloud where the scratch area was automatically analyzed. The scratch area was subsequently used to calculate the relative scratch closure over time. Differences in scratch closure were analyzed using a two-way ANOVA followed by a Bonferroni post-hoc test in Prism (GraphPad).

Figure 1. *Analysis of the scratch area at 0 and 24 h indicated by the blue overlay on brightfield images. Rows represent different seeding densities and columns different media compositions (Control: DMEM + 10% FCS, αMEM: αMEM + 10% FCS, hPL: αMEM + 5% hPL). Scale bar represents 500 µm.*

Results

The amount of scratch closure over time was similar for the three seeding densities tested for each medium condition (Fig. 1 and 2). No differences were found between the αMEM and control groups. Only at 18 h, the scratch closure of the hPL samples was higher compared to the αMEM samples at a seeding density of 6000 cells/cm^2 (Fig. 2). From 15 h onwards, the scratch closure of the hPL groups with a seeding density of 3000 and 6000 cells/cm^2 was higher compared to the control groups, except for the 3000 cells/cm^2 group at 24 h.

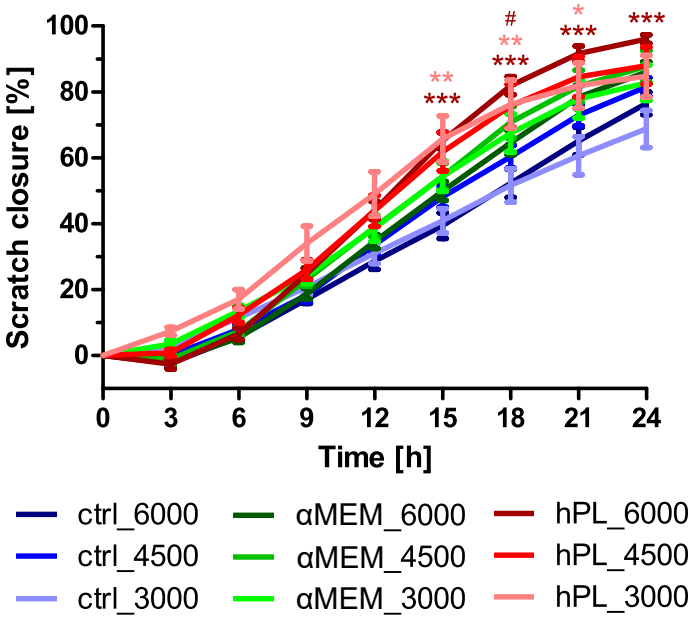


Figure 2. *Increase in percentage scratch closure over time (mean ± standard error of mean; n=8-12). *: significantly higher than control at the same seeding density and #: significantly higher than αMEM at the same seeding density. # and *: p<0.05, **: p<0.01, and ***: p<0.001.*

Discussion

Fast and easy optimization of culture conditions in scratch assays allows researchers to save time and effort in the protocol optimization phase of their research. In this proof-of-concept study the effect of different culture conditions on fibroblast scratch closure was investigated. The automated image capture and analysis by the CytoSMART Omni easily provided insight into the effect of seeding density and medium composition on fibroblast migration. No differences were found between the different seeding densities used, indicating a seeding density of 3000 cells/cm2 is sufficient to study collective cell migration. More importantly, using αMEM instead of DMEM did not lead to any differences in fibroblast scratch closure. The majority of the samples treated with αMEM supplemented with hPL instead of FCS even had an increased scratch closure compared to the control samples. This indicates that in future experiments, the effect of ADSC-conditioned medium on fibroblast migration can safely be investigated using αMEM supplemented with hPL as fibroblast culture medium.

Concluding, in this study, we have demonstrated that the CytoSMART Omni can be used to quickly and efficiently optimize cell culture conditions for scratch assays.

Acknowledgements

We would like to thank Simone Riis Porsborg (Aalborg University, Denmark) for performing the experiments and providing the videos, images and data.

References

1. Zou M, Liu S, Sun Z, et al. Insights into the role of adipose - derived stem cells: Wound healing and clinical regenerative potential. J Cell Physiol. 2021;236(4):2290-2297. doi:10.1002/jcp.30019
2. Alemzadeh E, Oryan A, Mohammadi AA. Hyaluronic acid hydrogel loaded by adipose stem cells enhances wound healing by modulating IL - 1β, TGF - β1, and bFGF in burn wound model in rat. J Biomed Mater Res B Appl Biomater. 2020;108(2):555-567. doi:10.1002/jbm.b.34411