Okolab s.r.l.
Via Giuseppe di Prisco, 152
80040 Ottaviano NA - Italy
Phone: +39 081 8062624; fax: +39 081 8764410
VAT: IT04506341215
www.oko-lab.com

A New Barrier Test to Characterize Cell Migration

An application of *In Vitro* Live Cell Imaging by microscope incubation with the Oko-Light Time-Lapse Station

Lanzaro Luca, PhD[1] and Remco van Horssen, PhD[2]
[1]OKO-Lab, Italy and [2]Department of Cell Biology, Nijmegen Centre for Molecular Life Sciences (NCMLS), Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Cell migration is a key event in a number of (patho-)physiological processes, ranging from embryonic morphogenesis to cancer metastasis. The development of *in vitro* assays is essential in order to improve the still limited understanding of the mechanisms underlying cell motility. The novel *in vitro* barrier test system from van Horssen et al. (*J. Cell. Biochem.* 99: 1536–1552, 2006) allows quantitative evaluation of the effects of growth factors and extracellular matrix components in combination with cell morphology on cell migration from confluent monolayers without inflicting damage to cells and underlying substratum, such as in the wound healing assay. The Oko-light workstation is the ideal microscopy platform to carry out live imaging based assays, such as the *in vitro* barrier test. In fact, cells can be maintained under optimal culture conditions for up to several weeks while allowing time-lapse image recording under bright field or fluorescence microscopy. The extended live imaging capability and thermal stability of the Oko-light workstation, coupled with the Oko-Vision Time Lapse and Image Toolbox software, provides a powerful, yet user-friendly experimental tool for successfully implementing the *in vitro* barrier test towards new and exciting insights into migration patterns, cell morphology and the accompanying molecular alterations at the cellular level.

Fig. 1: On the Left: Effects of extracellular matrix components and growth factors on the migration of human umbilical endothelial cells migrating along clean matrixes for 24 hours. Dashed lines indicate migration front at start of experiment. On the right: Oko-Light Time-Lapse Station.

Introduction

Cell migration is crucial in a virtually all (patho-)physiological processes, ranging from embryonic morphogenesis to cancer metastasis. In many cell types, migration is an active process based on a complex, partly understood locomotion machinery (as opposed to passive convective transport of non-adherent cells, such as in blood flow). Cell motion is driven by actin-polymerization resulting in protrusion of the cell body in the leading direction through adhesion to the surrounding extracellular matrix (ECM). It is a dynamic process where the advancing of the leading edge is accompanied by the detachment of the trailing part of the cell body.
At a larger spatial scale, cell motion can be described as random or directional, the latter being elicited by the presence of some anisotropy in the underlying substratum (e.g., fiber orientation in contact guidance or a chemical gradient in chemotaxis). Directional migration is characterized by a sustained cell polarization. Even when cells show random migration, the nature of the microenvironment plays a crucial role in cell motion, which is determined by ECM composition, growth factors, chemokines and other cell types, and physiological parameters such as pH and pO2.

Besides the basic scientific interest, the investigation of factors affecting cell migration is of special relevance in biomedical applications due to the implication of cell migration in several pathologies and in the body's essential repair processes. Thus, researchers hope to find new and more efficient therapeutic approaches based on a better understanding of the motion-associated cellular and molecular interactions. Areas of application include, for example, nerve regeneration in neurology, wound healing in dermatology and tumor metastasis in oncology.
In this context, *in vitro* studies are gaining further impulse thanks to the ongoing development of new protocols and assays which allow to test live cell behavior in a quantitative fashion through direct cell observation in time-lapse experiments. An example is the potential application of re-implanted stem cells for tissue repair in cardiovascular diseases, where *in vitro* studies offer the advantage of analyzing cell differentiation and development under precisely defined and reproducible parameters. Individual growth factors or extracellular matrix components can be easily and selectively varied to examine their influence on cellular development.

The development of novel *in vitro* assays based on direct optical observation of living cells relies on the availability of sophisticated experimental systems enabling extended sample incubation under the microscope. Today, the Oko-light workstation provides the researchers with the ability of developing novel *in vitro* assays or to implement existing ones in a user-friendly software environment.

In Vitro Characterization of Cell Migration Using the Barrier Test

Van Horssen et al. (*J. Cell. Biochem.* 99: 1536–1552, 2006) used the Oko-Light workstation as the platform for the quantitative characterization of specific migration patterns in the barrier test they developed at the Erasmus MC, The Netherlands. The barrier test enables study of undamaged monolayers of cells migrating along untouched and well defined matrixes. Human endothelial cells isolated from the umbilical cord, microvascular endothelial cells and fibroblasts were analyzed. The initial focus of the experiments lay in determining the influence of the extracellular matrix components Gelatin (Gel), fibronectin (FN) and Collagen-I (Col-I) on migration induced by vascular

endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and lysophosphatidic acid (LPA) in a comparative set up with the widely used wound healing assay.

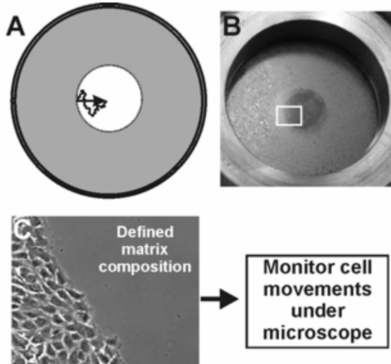

Fig. 2: The Van Horssen et al., 2006, in vitro Barrier Assay to study cell migration. A: schematic representation showing the two compartments, one with cells (gray) and a cell-free area (white). Curved line indicates migration path and arrow indicates cell displacement, parameters obtained from migration movies. B: Picture of Barrier assay, cells are prevented to grow in the middle area. C: Microscope image (rectangle in B) showing migration front facing clean, defined matrix.

The cells are seeded on a round coverslip coated with either Gel, FN or Col-I. Cells are prevented to attach and proliferate in the inner circular region of the coverslip by applying a growth barrier during the test start phase and thus creating a two compartment set up (Fig. 2). Hence, initially cells grow on the outer, open surface only, the inner area being not available at this time. Only after the barrier has been removed upon cell confluency, the cells start growing and migrating towards the center of the coverslip. Looking at Fig.2A, the freehand line indicates the hypothetical path of an individual cell (not to scale). The total path traveled is integrated. The migration to the centre of the test area is defined as the effective path (cell displacement, arrow in Fig.2A) and represents the measure for the directionality of cell movement. The speed, direction and morphology of the motile cells are influenced by the specific substrate of the central area as well as by the presence of additional growth factors, which can be added at some point. Exact, quantitative evaluation of the various relevant migration parameters can be carried out based on time-lapse observations of the trajectories of selected cells.
In summary, the observed migration patterns show that FN and Col-I both stimulate motion of endothelial cells. However, the cell movement induced by FN appeared to be more or less random with lower net displacement over the time course of the experiment. The addition of bFGF and VEGF growth factors compensated this effect (Fig. 1). In addition, VEGF caused a higher directional movement of endothelial cells. In contrast, FN increased cell motility of fibroblasts. This effect can be further increased by bFGF. By using various FN concentrations a dose-dependent effect on endothelial cells has been observed. The tests also quantitatively document for the first time the intimate interrelationship between matrix components and growth factors. These findings were less pronounced or even absent when wound healing assays were used indicating again the benefit of using defined matrix composition. Thus, a precisely matched combination of these factors is responsible for regulating directed cell migration and determines their patterns. Further studies are still needed to complete this picture.

Advances in Live Cell Imaging: the Oko-Light workstation

As opposed to the discontinuous sample observations which can be performed by using a conventional, bench top incubator, the Oko-Light workstation allows one to maintain cells under optimal culture conditions for up to several weeks while allowing time-lapse monitoring and image recording under bright field or fluorescence microscopy. The system is the result of the sound and long-standing technical expertise of OKOLAB, which is among the pioneers in the area of microscope incubation. Indeed, the Oko-Light workstation (see Fig. 1) has been developed with an application-oriented interdisciplinary approach by bringing the company engineers in close contact with researchers from biology and medicine areas to ensure both product performance and easiness of use. The Oko-Light workstation is the result of a careful work of integration among the following components:
1) The OKOLAB microscope incubator, which is available in different configurations (stage, cage and electric) and endowed with a variety of adapters for the most popular culture dishes, including Okolab round steel chambers.
2) A research-grade inverted microscope which can be selected among the most popular microscopy brands and can be used in fluorescent or bright field illumination modes.
3) Motorized microscope control, both of X-Y microscope stage and focus system (or just the latter if automated sample positioning is not needed), and of shutters for fluorescence imaging.
4) OkoVision Imaging Software. The former module is used to set the parameters of a time-lapse experiment (number of fields to be imaged, number of iteration, time difference between two consecutive acquisitions, and so on) and to run the experiment by controlling the operation of the motorized stages and shutters. The software features a sophisticated array of additional tools, including autofocus, addition or removal of a field in the course of the experiment, integration of several video cameras, acquisition of 3D stacks and mosaic of images. The Image Toolbox module is used to review the experiment by displaying the recorded images in different formats (single and multiple images as a function of time and position) and by creating and saving movies of selected image fields. The module can be also used to analyze images and adjust the contrast. An image database has been recently added to the Image Toolbox in order to navigate among different experiments and facilitate image recording on DVD or CD.
In conclusion, these components are integrated into a sophisticated and easily operated platform providing the user with a number of decisive advantages: a high degree of flexibility in the choice of culture vessel formats; outstanding temperature accuracy; a cell-friendly environment with high humidity content and long-term reliability. Computer controlled time-lapse studies of complex migration processes are thus possible with a minimum effort after only a brief familiarization period.

References:

Remco van Horssen, Niels Galjart, Joost A.P. Rens, Alexander M.M. Eggermont, and Timo L.M. ten Hagen. "Differential Effects of Matrix and Growth Factors on Endothelial and Fibroblast Motility: Application of a Modified Cell Migration Assay", *J. Cell. Biochem.* 99: 1536–1552, 2006.

Correspondence for Barrier Assay:
Remco van Horssen, PhD
Department of Cell Biology
Nijmegen Centre for Molecular Life Sciences (NCMLS), Radboud University Nijmegen Medical Centre
phone: +31 24 3614673
mail to: r.vanhorssen@ncmls.ru.nl

Contact:

Lanzaro Luca, PhD
Sales and Marketing Manager

Okolab s.r.l.
Via G. di Prisco 152 - 80040
Ottaviano, NA, ITALY
phone: +39 081 8062624
mobile:+39 3489680717
fax: +39 081 8764410
website: http://www.oko-lab.com
mail to: lanzaro@oko-lab.com