Immunofluorescence Staining of HUVEC in 3D in the µ-Slide Chemotaxis

Related Topics:
AN 34: Chemotaxis of HUVEC in 2D and 3D

Keywords:
Chemotaxis, 3D cell migration, immunofluorescence staining, immunostaining, antibody, fluorescence microscopy, imaging, Matrigel, gel, HUVEC, endothelial cells, nucleus, F-actin, VE-cadherin

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1. General Information

This is a detailed protocol for immunostaining of HUVEC (human umbilical vein endothelial cells) embedded in Matrigel® after performing a 3D chemotaxis experiment in the µ-Slide Chemotaxis. After chemotactic cell migration towards 10% FCS in Matrigel®, morphological characteristics of directed cell migration were investigated by immunocytochemical staining.

More general information about the handling of the slide, preparation of Matrigel®, experimental planning, and troubleshooting of chemotaxis experiments with HUVEC is provided in Application Note 34 “Chemotaxis of HUVEC in 2D and 3D”.

2. Material

Material	Manufacturer	Cat. Nr.
µ-Slide Chemotaxis	ibidi GmbH	80322
Matrigel	BD Biosciences	356231
Paraformaldehyde	Polysciences, Inc.	00380
Triton X-100	Sigma-Aldrich	T8787
1x PBS	Sigma-Aldrich	D8537
Mouse anti-VE-Cadherin antibody	Santa Cruz Biotechnology	SC-9989
Goat anti-mouse IgG (H+L), Alexa Fluor 680	Invitrogen	A-21057
Rhodamine-phalloidin	Invitrogen	R415
Hoechst 33342	Sigma-Aldrich	14533

3. Methods

3D chemotaxis experiments were performed as described in AN 34. Briefly, HUVEC were seeded in 50% Matrigel® and filled into a µ-Slide Chemotaxis before they were allowed to migrate for 24 hours along a 10% FCS gradient. Morphological characteristics of endothelial cells after directed migration in Matrigel® were investigated using immunostaining. Staining of HUVECs was directly carried out in the µ-Slide Chemotaxis. During all steps of the staining protocol, 60 µl of the respective solution was added into each of the two large reservoirs. During incubation, all plugs were kept closed in order to not disturb diffusion through the observation channel. Unless otherwise stated, all steps were carried out at room temperature.

1) Remove the plugs from the large reservoirs, and aspirate the medium. Leave plugs in the central channel during aspiration and wash steps.
2) Wash with 1x PBS → 3x 10 min
3) Fix cells and matrix proteins with 4% paraformaldehyde → 1x 40 min
4) Wash with 1x PBS → 2x 10 min
5) Permeabilize cells with 0.2% Triton X-100/PBS → 1x 20 min
6) Wash with 1x PBS → 6x 10 min
7) Block non-specific antigens with 1% BSA/PBS → overnight
8) Wash with 1x PBS → 6x 10 min
9) Add primary antibody: monoclonal anti-VE cadherin, mouse (1:100 in 1% BSA/PBS) → 1x 24 h at 4°C
10) Wash with 1x PBS → 6x 10 min
11) Add secondary antibody: goat anti-mouse IgG (H+L), Alexa Fluor 680 (1:50 in 1% BSA/PBS) → overnight at 4°C
12) Wash with 1x PBS → 6x 10 min
13) Add staining mixture → 1x 40 min
    i) Rhodamine-phalloidin to stain actin filaments (1:400 in PBS)
    ii) Hoechst 33342 to stain nuclei (1:100, 0.5 µg/ml in PBS)
14) Wash with 1x PBS → 6x 10 min
15) Leave last PBS in reservoirs and start imaging.

Immunofluorescent images were obtained by Leica SP8 SMD confocal laser scanning microscope equipped with 63x HC PL APO 1.2 NA water objective. 405 nm UV-laser was used to excite Hoechst 33342, 594 nm HeNe-laser for rhodamine-phalloidin, and 638 nm HeNe-laser for Alexa Fluor 680.

Important Note: compared to standard staining protocols the incubation times needed for washing and staining steps are longer. This is to ensure sufficient diffusion of antibodies through the gel.

4. Results

Immunostaining of HUVEC in a Matrigel® matrix showed that adjacent cell groups preferentially formed cell clusters and migrated as collectives. A few cells were observed with migration as singles.

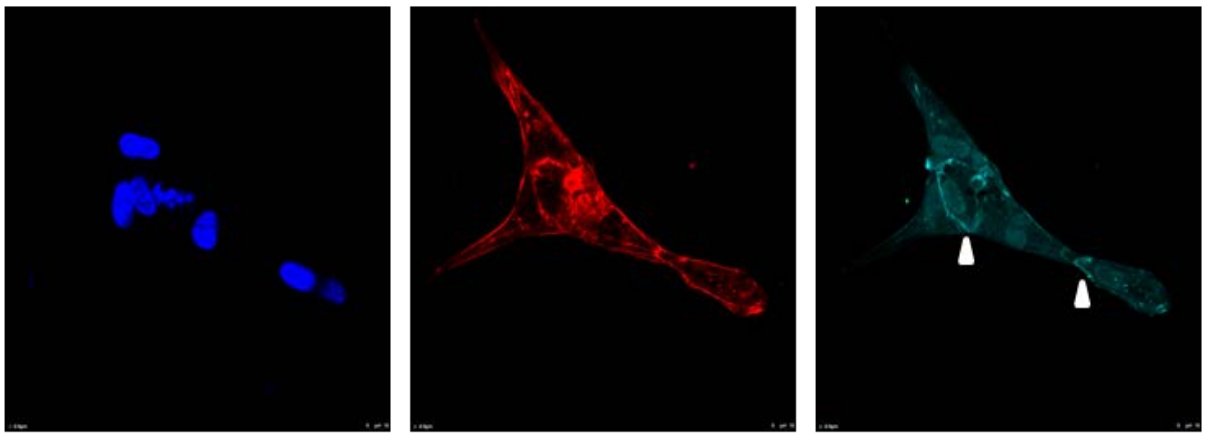

Fig 1: A cell cluster showing multicellular chemotaxis of HUVEC in 50% Matrigel®. After fixation, cells were stained for cell nuclei (blue), F-actin (red), and VE-cadherin (cyan). White arrowheads denote VE-mediated cell-cell contacts.

When the cells were migrating as individuals, the cell body was elongated and showed a mesenchymal spindle-shaped morphology. Cells exhibited a front-rear polarity with a characteristic leading edge showing a small lamella and protrusions in direction to the gradient.
When organized in multicellular units, the cell clusters exhibited a group polarization with clear front-rear asymmetry (Fig 1). Here, one or several leader cells were involved in formation of the leading edge, which exhibited an elongated protrusion or a broad lamella. Successive cells in deeper regions of migration complexes were not polarized. However, these cells characteristically retained VE-cadherin-mediated cell-cell contacts (Fig 1).