DEVELOPMENT OF A NEW METHOD FOR PERMEABILITY MEASUREMENTS

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Background: Increased endothelial cell permeability plays an important role in the pathomechanism of several immunologically relevant diseases, for example in hereditary angioedema, allergy, or sepsis. Numerous methods are known for measuring the permeability changes, such as the protein transport through transwell inserts, impedance measurement, or immunostaining of adhesion molecules, just to mention a few. These methods are widely accepted and used, but expensive, unreliable or labor-intensive. To overcome these problems, we aimed to develop a cheap, simple and highly reliable technique, which allows to test several samples simultaneously.

Methods: In our experiments, we used confluent human umbilical vein endothelial cell (HUVEC) culture as a model. HUVECs were prepared from fresh umbilical cords, and were kept in AIM-V medium, completed with FCS, EGF and bFGF. VE-cadherin, PECAM-1 and ZO-1 adhesion molecules were stained with the corresponding primary antibodies, followed by fluorescently labeled secondary antibodies. Samples were observed and images were recorded using an Olympus IX-81 inverted fluorescence microscope mounted with an Olympus XM10 digital camera.

Results: HUVECs were grown on 96 well plates precoated with biotinylated gelatin. After treating with a given agonist, we added a strepatvidin-Alexa 488 (a straptavidin conjugated fluorescent dye) to each well, which tightly binds to the biotinylated gelatin in the gaps between endothelial cells. After paraformaldehyde fixation, paracellular gaps were visualized using fluorescent microscopy. To evaluate these images, we performed quantitative image analysis to define the size of the stained area on each photograph. The new method was validated by known permeability-increasing factors; thrombin and bradykinin, which increased the size of the stained area as expected. To confirm our permeability test results, we stained key adhesion molecules (VE-cadherin, PECAM-1, ZO-1) after thrombin and bradykinin treatment, which showed that endothelial cells became separated from each other.

Conclusion: Consistent with the literature data, thrombin and bradykinin increased endothelial permeability in our novel permeability test. We could also confirm our results by immunostaining of adhesion molecules, therefore we found the new method suitable for permeability measurements. Based on our results, we can conclude that we successfully developed a new, simple, cheap and high throughput method, which can be used in a wide variety of experiments assessing permeability.

I would like to present our results on a poster.