Head-Mounted Operating Microscopy System for Angiogenesis Research

Summary

The HET-CAM (Hen Egg Test-Chorioallantoic Membrane) angiogenesis test system was standardised for validation by use of a head-mounted operating microscope for both experimental procedures and digital documentation.

Introduction

Angiogenesis is defined as the sprouting of new vessels from the pre-existing vascular system. It is a vital process during embryonic development as well as in adult reproductive tissues, during wound healing and in disease states like chronic inflammation. Additionally tumors need it for growth and development. Because angiogenesis is a key factor in both health and disease its regulation is a current research topic with great impact.

HET-CAM Assay

The avian CAM, outermost extraembryonic lining of the eggshell membrane in a fertilized chicken egg, develops by fusion of chorion and allantoic membrane and shows an extremely rich vascular network. The CAM supports the extraembryonic respiratory capillaries, serves as ionic transporter for sodium/ chloride and calcium and as excretory system. Because the CAM undergoes very rapid growth, e.g. increasing 20- fold in size from incubation day five to six, it is used as screening assay for rapid identification of substances that inhibit/ enhance vessel growth. Advantages are that it is a complete tissue, well vascularised, but lacking sensory innervation. For experimental set up the shell around the egg´s air cell is removed and the developing CAM is allegorated. Test samples are placed directly onto the membrane in vivo. Low costs, simplicity and possibility of continuous test site observation make the CAM test system interesting for angiogenesis research, toxicology, cell/tumor transplantation and biomaterial testing, to name just a few. As the CAM is not innervated, the HETCAM test represents an alternative model to animal testing if terminated prior to incubation day 11. Validation of the method
has shown an acceptable correlation between HET-CAM results and data from the Draize animal eye irritation test. The controversial discussion on animal tests, particularly on the Draize rabbit eye scheme, is increasing in both public opinion and the scientific community.

However, for technical reasons the CAM assay´s quantitative/qualitative interpretation is difficult, timeconsuming and not very reproducible. To establish a Standard Operating Procedure (SOP), CAM dissection and specimens application procedure protocols as well as the technical equipment must be standardised. The grade of experience in interpretation and registration of experimental effects in this sensitive biological assay is crucial for the final conclusion, but general documentation options do not suffice.

**Varioscope M5 Microscope**

A broad range of activities in medicine, research and technology demands superior vision for experimental procedures and documentation. Miniature head-mounted operating microscopes like the Varioscope M5 tested by the authors enables the user to analyse objects difficult to access with the required magnification and operating distance and to manipulate them precisely. Usually the brain constructs the third dimension in vision from the disparity between the experimenter´s eyes images, which is re-corrected permanently. The parallax control is automatically performed by the Varioscope leading to less tiring microscopy. Automatic sensors detect the object continously and adjust the optics, documentation is digitally performed from the experimenter´s visual angle, zoom is stagelessly variable, the pivoting radius is 72 °. This kind of optical vision enhancement is used in operating theatres/dental clinics and manufacture/quality control of precision components.

**Application**
Today HET-CAM test procedures, documentation and interpretation are usually performed macroscopically with a common hand-held camera. This modus makes it difficult to recognise fine structures and tiny cavities, especially with the naked eye. Another option is to transport the eggs to a stationary microscope-image analysis system. This routine risks generation of artefacts due to bleeding, membrane rips, contamination with bacteria/yeasts and cooling the in vivo test system below the critical incubation temperature of 37 °C. Reproducibility of generated data is therefore often unsatisfactory. Head-mounted microscopy systems offer improved resolution and can also be used in class I/II safety cabinets, minimising the risk of contamination and permitting testing of substances potentially hazardous for the staff. Bulkiness of conventional operating microscopes did not allow such applications. The automatic parallax control is a serious advantage, because the experimenter is not obliged to stay in a firm position as with microscope or loupes and at the same time gets a three-dimensional image at a large field of vision. Documentation for data evaluation or training purposes is performed simultaneously. Advanced imaging methods are proving to be indispensable for studying angiogenesis in the laboratory and clinic, providing noninvasive, functionally relevant images of angiogenesis in animals/humans. They can pinpoint sites of angiogenesis, determine the amount of blood vessel growth, characterise functional abnormalities of vessels, assess vascular heterogeneity and elucidate features that distinguish angiogenic blood vessels from normal blood vessels. Varioscope M5 allows standardisation of CAM experimental procedures following Good Laboratory Practice (GLP) rules.

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