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Oral Contributions

(ordered according to sessions)

Session 1: Mechanotransduction in the Vascular Wall

Mechanotransduction in Coronary Microvessels

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Cardiac contraction causes a rhythmic compression of the intramyocardial blood vessels. As a result, both transvascular pressure and flow are highly pulsatile, notably in sub-endocardial vessels. Such pulsatility affects regulation of both tone and structure of these vessels. We studied the behaviour of single vessels subjected to mechanical regimes mimicking the beating heart. Small arteries of ~100–200 μm were isolated from the porcine heart and cannulated. Vascular pressure and flow profiles were controlled independently by manipulating left and right cannula pressures. In acute experiments we found that pulsatile pressure causes endothelium-independent vasodilation. This was not associated with a reduction in intracellular calcium, and may reflect a direct effect on the contractile elements. Vessels became remarkably more sensitive to vasodilators under a pulsatile pressure profile. While steady flow caused flow-induced dilation, oscillating flow did not induce dilation at all. However, such dilation was uncovered in the presence of superoxide dismutase or the NADPH oxidase inhibitor, DPI. These data indicate that the pattern of flow sets the balance between NO and O_2^- production in the vessel wall. In cannulated vessels in organoid culture, inward remodeling occurred after sustained deep tone. E.g., inward remodeling was found in the absence but not in the presence of flow. Such remodeling was found to be related to activity of the cross-linking enzyme, tissue type transglutaminase.

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Mechanotransduction in Pulmonary Microvessels

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Both ventilation and perfusion exert mechanical forces – primarily vascular shear stress and circumferential stretch – on the pulmonary vascular endothelium. Increased circumferential stretch can result from increased vascular filling pressure or from mechanical forces associated with mechanical ventilation. This stretch activates endothelial cells along the tracheobronchial tree in a variety of ways such as an increase in intracellular calcium, expression of P-selectin on the plasma membrane, activation of eNOS and matrix metalloproteases (MMP) and release of endothelin. In general these alterations are pro-inflammatory and blocking P-selectin, integrins or MMPs prevents ventilation-associated leukocyte activation and/or

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Oxygen Saturation Measurement in Mesenteric Microvessels during Intravital Microscopy

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Introduction: Delivery and distribution of oxygen to the tissue is a central task of the microcirculation. A better understanding of supply, distribution and diffusion of oxygen under normal and abnormal conditions (such as hypoxia) in terminal vascular beds is therefore of fundamental relevance. A modified approach of spectral imaging allows measurement of oxygen saturation (SO_2) in microvessels during intravital microscopy. **Methods:** An intravital microscope connected to a monochromatic imaging system (T.I.L.L. Photonics, Gräfelfing, Germany) was used for trans-illumination screening of microvessels in vivo. Validation was performed using glass capillaries perfused with erythrocyte suspension. Hemoglobin absorption spectra in microvessels and glass capillaries were recorded. For larger diameters (35–50 μm), a wavelength range from 500 to 600 nm, and for small diameters (10–20 μm), a range from 410 to 440 nm was used. Microvessel SO_2 were calculated by fitting measured spectra to those of fully oxygenated and fully deoxygenated blood using a least square method. The calculated data were verified by blood gas analysis. In addition, data for determination of flow velocity and vessel diameter were collected. **Results:** In glass capillaries, SO_2 values predicted by the spectral imaging method correlate closely with data from blood gas analysis ($r = 0.997$). In microvessels, SO_2 levels for larger arterioles (approx. 35 μm) and venules (50–60 μm) of about 98% and 70%, respectively, were calculated. Upon local deoxygenation by superfusion with Na-dithionite, venular SO_2 levels decreased to 0%. **Conclusion:** Imaging systems, which are widely used to assess functional parameters like e.g. intracellular Ca^{2+}-concentration, can be used to measure SO_2 levels during intravital microscopy.

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Automated Assessment of Tumor Vascular Architecture in vivo – A New Technique

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Background: Tumor angiogenesis plays a key role in tumor growth and formation of metastasis. Quantitative analysis of tumor angiogenesis is therefore an indispensable prerequisite for the development of novel antivascular treatment strategies. Aim of our study was to establish a new automated analysis technique enabling non-invasive quantitative assessment of entire tumor vascular networks in vivo. **Methods:** Experiments were performed in the amelanotic melanoma A-MEL-3 implanted in transparent dorsal skinfold chamber of male Syrian golden hamster. At day 6 after tumor-cell implantation the tumor was two-dimensionally imaged by means of a computer controlled scanning-table combined with intravital microscopy using blue light trans-illumination. The entire vascular network was completely scanned step by step. Images were acquired with a magnification of 630× and digitized on-line. After conversion to binary images by a digital image analysis system (KS400, Zeiss, Germany), vessel density, vessel diameter and vessel area were automatically quantified online. For validation data have been correlated with results acquired by fluorescence microscopy after FITC-Dextran injection followed by off-line video analysis. **Results:** Up to 280 ROIs have been scanned in each tumor dependent on tumor size. Data of functional vessel density obtained by our new automated on-line technique correlated excellently with data obtained by conventional off-line frame by frame analysis ($r^2 = 0.97$). In addition, our technique is able to detect the heterogeneity of microcirculatory parameters within tumor vascular networks: we observed differences between the well-vascularized fast-proliferating tumor rim and the tumor centre with significantly lower functional vessel density (fvd at tumor rim: 217.1 [1/cm], centre: 81.0 [1/cm]). **Conclusion:** This new technique enables automated, quantitative assessment of entire tumor vascular networks and therefore provides a promising tool to characterize changes therein due to antivascular treatment regimes.

3-D Reconstruction of Tumor Vascular Networks

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Most solid tumors consist of optically dense tissue. Therefore intravital visualization of its complete vasculature with topological and morphological details is difficult. We propose a method to reconstruct tumor vascular networks using confocal intravital microscopy and a dedicated software system. **Methods:** 20,000 cells from a human squamous cell carcinoma xenograft line (FaDu) were injected in a skin fold window chamber of a nude mouse. After 10 days fluorescein dextran (2 Mda) was injected intravenously and imaging of the vasculature started directly afterwards. Vertical image stacks ('z-stacks'; ~40 images per stack; pixel size: 0.9 × 0.9 μm; z-distance: 3.3 μm) were recorded. The complete scan, scanning a tumor area of ~1.1 × 1.2 mm, took a total of 28 min. The image stacks were analyzed off-line using an application developed as an extension to the commercially available visualization and geometry reconstruction system AmiraDev. **Results:** Because the nonfluorescent leukocytes break the connectivity of the vessels a fully automatic reconstruction was not possible. Thus a semi-automatic application was used in which the user indicates the start and end point of a vessel segment. The software searches the most optimal centerline in 3D by minimizing its path length weighted by an energy function of the image intensity. Vessel diameters are estimated using spheres fitted along the centerline. Visual inspection allows to adjust the parameters of the algorithms. This way we obtained a reconstruction of a tumor vascular network which consists of 287 vascular segments, of which 76 are boundary segments, with a mean vascular diameter of 13.8 μm, total vascular length of 23 mm, and volume of 6.6e-3 mm^3. **Conclusions:** Reconstruction of tumor vascular networks in optically dense tissue with disorganized topology is possible using semi-automatic software. This application provides a useful tool to help understanding the mechanisms of tumor angiogenesis and growth.