S19: Interactions of blood cells / tissue engineering

S19-1 Long-term prognosis of coronary microvascular dysfunction

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Exertional angina with possible signs of ischemia in stress testing and with exclusion of relevant coronary disease or complete lack of it is one of the main clinical presentation of patients with coronary microvascular dysfunction.

Though the etiology of this disease, which is not obligatory associated with a cardiac disease - not even coexisting cardiovascular risk factors - , is not fully understood, there has been several approaches of categorizing this entity.

The lack of a systematic and uniform diagnostic approach and the lack of monitored follow-up visits in the setting of controlled trials leads to difficulties in the determination of the prevalence of the disease, and thus, the impact and prognosis of it.

However, there has been promising new tools, e.g. CMR- or PET-using approaches, to diagnose the coronary microvascular dysfunction, to complement the contemporary golden standard of invasive coronary angiography and measurement of, e.g. mediator-induced coronary flow reserve.

Long-term data of patients with suspected or diagnosed coronary microvascular dysfunction show a higher rate of adverse cardiovascular events and a worse outcome.

S19-2 AD-MSCs change their morphology and secretion profile as a response to changes in substrates' elastic properties in combination with inflammatory stimuli

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Background: Mesenchymal stem cells (MSCs) are often deployed to augment wound healing and regeneration and are delivered *in vivo* utilizing various types of biomaterials. As a result, within their delivery vehicle, there are exposed to an inflammatory environment. However, the effect of different mechanical properties on MSCs' respone is still unclear. Objective: Evaluating the effect of different mechanical properties of cell culture substrates during inflammatory conditions on adipose derived (AD)-MSCs. Methods: A glycidylmethacrylate (GMA)-gelatin based hydrogel system with adjustable mechanical properties was utilized . This system allowed to use GMA-gelatin with identical functionalization degree, while mechanical properties were adjusted by shortening the biopolymer chain length. AD-MSC were cultured on gelatin-based films (3 mm) with different G' moduli (approx. ranging 400 to 1400 Pa) and subjected to different concentrations of interferon γ (IFN-γ). Results: Hydrogels with various elastic moduli were synthesized and analyzed using rheology and AFM. All

hydrogel substrates supported cell viability and spreading, while the mechanical properties affected F-actin rearrangement. For stiffer gels, a higher degree of cell stretching and formation of F-actin stress fibers was observed in the absence of inflammatory stimuli. In contrast, AD-MSCs growing on softer substrates without an inflammatory stimulus exhibited a looser network of F- actin. Only in the presence of IFN-γ AD-MSCs were stimulated to form denser stress fibers. Moreover, VEGF secretion in response to different concentrations of IFN- γ was increased by substrate stiffness. Conclusion: The potential of an innovative *in vitro* platform for simulating an inflammatory ECM-mimetic environment was established with adjustable mechanical properties. Substrate elasticity was shown to influence cell expansion and attachment but also the secretion of VEGF under inflammatory condition. Current findings indicate that the mechanical microenvironment affects AD-MSC response towards inflammatory stimuli. This should be taken into account when designing biomaterials to deliver stem cells for tissue healing and regeneration.

S19-3 Thrombogenicity testing of polymers: round-robin study to assess inter-center variability

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Clinical use of cardiovascular devices is continuously increasing along with concerns on the thrombogenicity of such implants. The lack of standardization in the in vitro testing of implant materials has led to a situation, where definite specifications about test panels and protocols are lacking and inter-study comparisons are rather impossible. Here, we report about a prospective, randomized and double-blind multicenter trial demonstrating that standardization of in vitro test protocols allows a reproducible assessment of platelet adhesion and activation from platelet rich plasma as indicators of the thrombogenicity of cardiovascular implant materials. The stringent standardization of a static platelet adhesion test resulted in a laboratory independent scoring of the polymers; the materials were evaluated in all laboratories in the same order for their thrombogenicity. While poly(dimethyl siloxane) showed very reduced platelet adhesion and activation, the density of cells and the degree of activation were highest on poly(tetrafluoro ethylene). Polyethylene terephthalate showed intermediate values. The results of this study reveal that inter-laboratory and inter-study comparisons of the thrombogenicity testing of blood-contacting biomaterials can be achieved by a stringent standardization of the test protocol [1]. Future perspectives for the standardization/harmonization in the in vitro testing of implant materials will be discussed.

[1] S. Braune, C. Sperling, M. F. Maitz, U. Steinseifer, J. Clauser, B. Hiebl, S. Krajewski, H.

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S19-4 The controversial origin of pericytes - implications for cell-based therapies

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Pericytes isolated from quiescent tissue were demonstrated to be a specialized subpopulation of mesenchymal stem cells and promising candidates for therapeutic angiogenesis applications. However, cell-based therapies of ischemic diseases have not resulted in significant long-term improvement. Just recently pericytes from a hematopoietic origin were observed in embryonic skin. Additionally, a pericyte sub-population expressing leukocyte and monocyte markers was described during adult angiogenesis in vivo. Since mesenchymal stem cells do not express hematopoietic markers by definition, the latter cell type might represent an alternative hematopoietic pericyte population relevant to angiogenesis.

We therefore sourced blood-derived angiogenic cells (BDACs) from monocytes that closely resembled hematopoietic pericytes in vitro, which had only been observed in vivo thus far. BDACs displayed many pericytic features (PDGFRb and NG2), while expressing leukocyte markers CD45, CD11b. They enhanced angiogenesis in vitro and in vivo and accelerated revascularization and functional tissue regeneration in a pre-clinical model of critical limb ischemia. Comparison between BDACs and mesenchymal pericytes in functional in vitro assays revealed that in direct co-culture BDACs enhanced, while mesenchymal pericytes impaired endothelial sprouting.

We therefore concluded that BDACs (while resembling hematopoietic pericytes) enhanced early stages of angiogenesis, while mesenchymal pericytes were responsible for blood vessel maturation and homeostasis. Since the formation of new blood vessels is crucial during therapeutic angiogenesis, hematopoietic pericytes (and therefore BDACs) might offer an advantageous addition or alternative for cell-based therapies.

S19-5 A facile way to achieve biomimetic laminin networks on substrates

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BACKGROUND: Laminin (LAM), a major glycoprotein of the basement membrane, has the tendency to form networks in vitro and in vivo by self-assembly, and plays crucial biological roles such as cellular adhesion, polarization and apoptosis.(1) LAM coatings are used widely for induced pluripotent stem cell (iPSC) maintenance and differentiation.(2) Substantial efforts have focused on the selection of the right type of LAM subtype for iPSC culture.

However, only limited attention has been given to controlling the formation of LAM layers and enhancing their presentation to cells at the material interface.
PURPOSE: Here, we employ Langmuir-Schaefer (LS) method, where LAM forms networks by self-assembly at the air-water (A-W) interface. This layer is subsequently transferred by horizontal touching onto a planar substrate. This approach is scalable to coat large areas of substrates by suitable choice of vessel and substrate dimensions and multilayer coatings can be produced.
MATERIALS AND METHODS: LAM-1 (Sigma) was obtained from mouse tumor and the LAM layer was produced in a circular trough (KSV-NIMA) or in the wells of standard six-well culture plate (Corning). Polarization-modulation infrared reflection-absorption spectroscopy (PM-IRRAS; KSV-NIMA) and Atomic force microscope (AFM; JPK instruments) were used to characterize the LS films.
RESULTS: LAM forms networks on a subphase with pH 4 and 100 mM NaCl in any vessel, even in wells of six well plate. AFM micrographs showed that controlled LAM networks were transferred on Si-wafers by LS method. Finally, the stepwise increase of LAM amount with multilayer LS deposition on gold was confirmed by PM-IRRAS.
CONCLUSION: LS technique is a facile tool to equip substrates with defined LAM layers for applications such as iPSC culture.

S19-6 Medical compression stockings reduce hypertension of nailfold capillaries at the toe of patients with chronic venous insufficiency

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In five patients who suffered from chronic venous insufficiency clinical stage C4 (n=3) and C6 (n=2) the capillary blood pressure was measured twice by means of invasive direct cannulation of nailfold capillaries of the toe. During one measurement course the patients wore below knee medical compression stockings (40 mmHg) during the other they did not have compression therapy. With the patient in supine position, the CP was investigated by the servo-nulling technique under resting conditions and under dynamic conditions: the calf-muscle/ankle joint venous pump was simulated by means of inflating a blood pressure cuff, which surrounded the mid lower leg, to 60 mmHg for 60 s. Results: The simulated calf-muscle contraction induced a steep increase of CP with 5.65 mmHg/s (Q1 5.27 mmHg/s, Q3 5.92 mmHg/s), which was significantly (p=0.013) reduced by MCS to 2.47 mmHg/s (Q1 1.65 mmHg/s, Q3 3.0 mmHg/s). Time needed to reach the max. CP was 11.35 s, which was lengthened by MCS to 23.4 s (p=0.134). Conclusion: Compression therapy prevents capillary hypertension, the major hemodynamic reason for the development of advanced stages of chronic venous insufficiency which are defined by skin disease like hyperpigmentation, lipodermatosclerosis and ulcer.