S7: Hemorheology and blood coagulation

S7-1 Stress sweep tests on whole blood clots

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Not only the time required gaining certain stiffness, but also the response of a clot to deformation or shear stress, or its time-dependent response following certain pre-stresses provides information about clot performance and stability. Such quality can be tested in-vitro by oscillatory rheometry, where the clot can be stressed until it breaks. The elastic limit (yielding), the breakup stress, and the kind of plastic deformation the clot undergoes between these two limits can be analyzed. Clots prepared from plasma show higher elasticity and stress hardening if platelets are depleted. Addition of platelets stiffens the clot, but also reduces its elastic limit. Such clots appear brittle and break at a lower shear stress, possibly because their dense fiber network cannot withstand large deformation. Addition of erythrocytes decreases the strain-stiffening behavior. In contrast, addition of fibrinogen to whole blood clots increases both, strain softening, and strain hardening. By the fractal dimension of the fiber network, SEM images provide important information on the morphology of clots in order to understand the rheological behavior.

S7-2 The novel discovery of amyloid formation in fibrin(open) and how it affects hemorheology and blood coagulation

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Most chronic diseases are accompanied by long-term inflammation. Although typically mediated by 'inflammatory' cytokines, the origin of this inflammation is mostly unclear. Our group recently presented a novel explanation for the origin of this inflammation, and suggested that it is due to a (dormant) blood microbiome; and that this can shed highly inflammatory lipopolysaccharides (LPSs) and lipoteichoic acids (LTAs). It is also known that (most) inflammatory conditions are associated with gut dysbioses, and that these may be the site of constant replenishment of this (blood) microbiome and the resulting presence of LPS/LTA. Such inflammatory diseases are also accompanied by a hypercoagulable phenotype. We have also shown directly (using 6 different methods) that very low concentrations of LPS can affect the terminal stages of the coagulation properties of blood and plasma significantly, and that this may be mediated via a direct binding of LPS/LTA to a very small fraction (1 in 10^8) of fibrinogen monomers as assessed biophysically. In particular, we have shown, that during inflammation, fibrin adopts a ß-amyloid form, and thus that fibrin(ogen) is actually an amyloidogenic protein. LPS is also known to compromise the blood brain barrier (BBB), and is frequently used to induce Parkinson's disease (PD) and Alzheimer's disease (AD) symptoms in animal models. It has also been found inside the amyloid plagues in AD brains and there is evidence that it plays important roles in Type 2

diabetes (T2D). In this symposium talk, I will focus on the amyloid nature of fibrin(ogen) in various inflammatory conditions, its origins, and how it affects hemorheology and pathological clotting.

S7-3 Multiscale mechanics of fibrin networks

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Fibrin networks, the main structural component of blood clots, form a mechanical backbone that is highly extensible and that stiffens by several orders of magnitude when deformed. This remarkable behaviour appears to be crucial for the biological function of blood clots, who need to withstand the forces exerted by blood flow and the embedded cells.

Due to the complex supramolecular structure of fibrin, the molecular origin of this mechanical behaviour remains elusive. There are different molecular mechanisms that may contribute to fibrin's extensibility. For example, the fibrin monomers constituting the fibers have specific domains susceptible to unfold upon stretching, leading to an increase of the monomer length. Another possibility involves the unstructured alpha-C regions that act as flexible chains, linking adjacent protofibrils within each fibrin fiber.

I will present our recent efforts in understanding how the strain-stiffening behaviour of fibrin is linked to its molecular scale, based on small angle X-ray scattering (SAXS) and optical tweezer measurements. SAXS is an ideal method to quantify changes in the molecular packing order of fibrin fibers, and thus, the monomer length. We performed in situ SAXS measurements on fibrin networks under macroscopic shear, observing the load-induced changes in the internal structure of the fibrin fibers. To further elucidate the molecular mechanisms involved, we stretch isolated

S7-4 Study of blood clotting mechanism by rheological and electrorheological methods

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Blood clotting mechanism was investigated by means of the rheological and electrorheological methods at different flow conditions and under electric field. The study aims to evaluate the rate of coagulation of the forming clot by means of the rheological and electrical blood properties.

Whole human normal blood conserved with CPD-A_1 solution was used for experiments. The process of clotting was initiated in vitro and the kinetics of clot formation, viscous properties of blood clot were evaluated at a steady flow conditions. The experiments were carried out by the rotational viscometer Low Shear 30 (LS30) Contraves as a base unit, connected with a

specially developed conductivity measurement device. Blood viscosity and conductivity changes with the evolution of the coagulation process and under shear were measured simultaneously.

We found that the complete coagulation process until clot formation could be divided into a stage of an initial coagulation process, following by an intensive coagulation. During the initial period a gradual increase of the apparent viscosity and a decrease of conductivity in parallel were observed. During the intensive coagulation the viscosity growth function (viscosity vs. time) at a constant shear rate has been determined and an exponential growth to 16 000 mPa.s÷60 000 mPa.s was established at shear rates from 0.0175 s^{-1} to 1,25 s^{-1} after recalcification solution addition. Both stages were characterized by a decrease in the conductivity in parallel. The kinetics of clot formation is dependent on the intensity of flow too.

It was found that the apparent viscosity of the stored blood has been elevated during conservation. Hemocoagulation kinetics research demonstrated a decrease of the blood clotting time during storage period.

S7-5 Influence of polymeric nanoparticles on the kinetics of coagulation of conserved blood

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Interactions of nanoparticles with the blood coagulation system can be beneficial or adverse depending on the intended use of a nanomaterial. Nanoparticles can be engineered to be procoagulant or anticoagulant or to carry drugs to intervene in other pathological conditions in which coagulation is a concern. The basic moment of coagulation process is plasma fibrinogen transformation in structural fibrin. As a result of coagulation process the blood structure has been changed in complicated net formation and from here its rheological and electrical properties vary too. The study aims to provide an overview of the role of the polymeric nanoparticle solutions in determining interactions with components of the coagulation system and on the kinetics of coagulation of whole human conserved blood induced in vitro with 2% aqueous solution. Poly(acrylic acid) macromolecules of different architecture and molecular weight were used: (i) a new core-shell type star polymer whose interior forms hyperbranched polystyrene bear-ing arms of poly(acrylic acid) with molecular weight M_n = 56 920 Da and (ii) linear polyacrylic chains with average molecular weights M_n = 6000, 20000, and 225000 Da. The polymers dissolved in physiological solution with weight concentrations 1 mg/ml and 0.2 mg/ml were used for the experiments. Blood samples in the presence and absence (the control) of nanoparticles were measured using a rotational viscometer Contraves Low Shear 30 (LS 30) at a steady flow at shear rate from 0.0237 to 94.5 s^{-1} and temperature 37° C. A method, based on the measurement of dielectric properties of

dispersed sys-tems in Couette viscometric blood flow was applied and blood conductivity under the same flow conditions.

S7-6 What are conditions defining blood clot properties in some disorders

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Background. Routinely blood clot properties can be assayed by thromboelastography (TEG) with calculation of shear modulus (G, d/sc) as one of elastic constants.

Patients and Methods. Clot firmness (CF, normal values of 3,6-8,5) was assayed in 59 patient with atrial fibrillation (AF), in 115 patients with myeloproliferative neoplasms (MPN), in 96 patients with chronic cerebrovascular diseases comorbided with Ph-negative MPNs (CCVD+ MPN), in 174 patients with acute ischemic stroke (AIS), and in 96 patients followed up within 12 months after acute ischemic stroke (After AIS). Additional testing was performed for blood rheology, and with 98 biomarkers reflecting coagulation, anticoagulation, platelets, vascular wall, angiogenesis, fibrinolysis, inflammation, etc. Non-parametric statistics and multivariate analysis has performed for obtained data.

Results. Gender differences in CF were not found. CF has correlated with hematocrit, WBC and blood viscosity at the range of 50 to 300 1/s. CF was the highest in AF and above the norm, the smallest in MPN, and additionally showed significant differences between groups CCVD+MPN, and AIS, and After the AIS.

The influencing parameters pattern proved to be specific: blood cells for AF, blood cells, platelets aggregation and angiogenesis factors for CCVD+MPN, clotting factors, inflammation and fibrinolysis for AIS, fibrinolysis, renal function and inflammation for 'after AIS'. These factors have jointed in CF pattern for MPN.

Conclusion. We conclude that blood clot properties has differences depending to disorder. Smaller CF are typical for more severe morbidity which has a resistance to therapy and causes generally worst outcomes.