Microfluidic approach of erythrocytes transport in diabetic retinopathy: microaneurysms and pro-coagulant endothelium activation.

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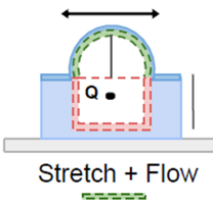
Context: Diabetes mellitus (DM) is a rapidly growing disease affecting over 420 million people worldwide (1). Chronic hyperglycemia modifies microcirculatory perfusion function, impacting blood cell properties and altering the topology and biology of microvessels' endothelium (the layer of cells of the vascular vessel in contact with blood).
While glycated hemoglobin (HbA1c) is used as a biomarker for DM (2), glucose also irreversibly alters membrane components in red blood cells (RBCs), leading to decreased RBC deformability (3-6). However, it is not yet fully understood which mechanical properties of the membrane are most affected by glycation (7) and how it affects RBC transport in the microcirculation.
Diabetic Retinopathy (DR) is an early chronic microvascular complication of hyperglycemia affecting all DM patients. DR is characterized by progressive alterations in the retinal microvasculature, leading to areas of retinal nonperfusion, increased vasopermeability, and pathologic intraocular proliferation of retinal vessels where often no RBCs can flow (8). The hydrodynamic mechanisms causing decreased perfusion in specific retinal regions and its relation to RBC deformability loss are not yet fully understood (9).
Abnormal outpouching regions of the retinal vessels, known as MicroAneurysms (MA), are a hallmark of early DR. The presence and severity of MAs determine the overall severity of DR, reducing vision due to the loss of endothelial barrier function, leading to leakage, retinal edema, and flow redistribution in the retinal network (10). The association between the physical and biological characteristics of individual MAs and perfusion attributes remains unclear for diabetic individuals.
Retinal MAs are highly dynamic lesions and some develop thrombi and block the vessel, leading to local revascularization with proliferative angiogenesis (11). The turnover rates of MAs can predict macular edema progression, but understanding how this relates to local RBCs perfusion alteration and endothelium activation by flow modification and by stretch induced by saccular inflation, is still lacking.

Objectives: The goal of the master project is to investigate glycation alterations on RBCs mechanics and the role it plays on transport in altered mimetic retinal networks, especially due to local micro-occlusions, MAs and induced proliferative angiogenesis. In continuation the thesis project will focus on exploring local pro-coagulant activation of endothelial cells (ECs) by glycation due to lower hydrodynamic shear conditions encountered in MAs and simultaneous high level of stretch imposed by periodic inflation of these saccular pouches.

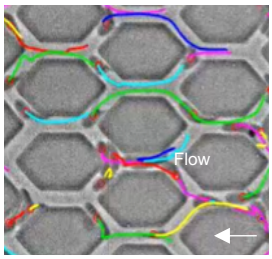
Approach: We will use microfluidics to look both at RBCs mechanics from DM donors and flow in complex circuitry of channels. The technique is well mastered by CBS and allowed to study many blood-flow related problems (12-16). For this thesis, we will also use a newly designed device (see right image), being developed during the thesis of A. Giannetti that can induce controlled shear flow and uniaxial stretching of confluent endothelial cells culture mimicking MA environment. Activation will be assessed by Elisa test and Western Blot as well as immunofluorescence following in particular upregulation of anticoagulant



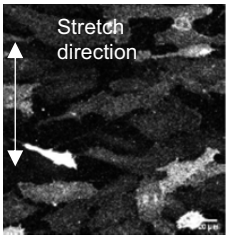
proteins such as Thrombomodulin (TM) and the endothelial protein C receptor (EPCR) and downregulation of the procoagulant protein von Willebrand factor (vWF) (17).

Tasks and expected results: **For the master**, we propose the following milestones:

1. Acquire and compare mechanical properties of RBCs from diabetic and healthy donors. Explore the impact of hyperglycemia on healthy RBCs with a high-throughput microfluidic approach to measure shear modulus and membrane viscosity on a large number of cells thanks to inverse computation performed at IMAG, team of S. Mendez.
2. Measure RBCs suspension transport in uniform microfluidic networks for diabetic and healthy blood samples. Treat healthy RBCs with glucose levels mimicking hyperglycemia. Use high-speed video microscopy to study RBC dynamics at bifurcations and junctions and their relationship with large scale resistance (see right image).



3. ***In continuation in Thesis:*** Explore the role of geometric heterogeneities by designing channels with increased levels of defect representing micro-Occlusions and MAs. Implement proliferative angiogenesis by random distribution of small connecting channels around these defects. Follow large scale hydrodynamic resistance and the local behavior of RBCs around these altered regions of flow and compare to the simulations realized in the team of Simon Mendez at IMAG
4. ***In continuation in Thesis:*** Perform confluent ECs cultures in a commercial cell stretcher to assess hyperglycemia on the onset of micro-thrombotic events. Look at the expression of TM, vWF and EPCR (see right image with TM expression). Microfluidic flow and stretch chambers will be used to investigate the role of shear stress decrease and stretch induced by MA growth on micro-thrombotic cues expression.



Feasibility: The student will receive training in microfluidic design, fabrication, and characterization from Andy Vinh Le. Arianna Giannetti's thesis on deep vein thrombosis will provide training in endothelial cell culture and the use of the new microfluidic stretching devices. The student will also receive training in immunofluorescence, confocal microscopy, Western Blot, and Elisa testing to study the effects of mechanical stimuli and glucose levels on endothelial cell response available in CBS.

References: (References from M. Abkarian in red)

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