REAL-TIME PLATELET OBSERVATION SETUP FOR IN-VITRO HEMOCOMPATIBILITY TESTING

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Introduction

Poor hemocompatibility of artificial materials is still a bottleneck in medical engineering. A current approach is the microstructuring of substrate surfaces to reduce platelet adhesion as an initialization of thrombus formation. Although the advantage has already been demonstrated [1-3], the underlying mechanism is still not fully understood. The aim of this study is to investigate the mechanism by analyzing the flow behavior of platelets in a systematic study including in-vitro testing as well as a computational study addressed by Raveleau et al.

Particle Image or Particle Tracking Velocimetry (PIV or PTV) are the gold standard for flow visualization, analyzing movement of particles in a fluid in a wide range of applications. This abstract presents the preliminary unpublished outcomes of a real-time platelet observation setup using µPTV with platelets as tracer particles for in-vitro hemocompatibility testing including the protocols to produce the test fluid, the design and manufacture of a flow chamber and the application in the µPTV setup.

Methods & Results

The test fluid is prepared using platelets isolated from citrated porcine whole blood by gel filtration and buffered in calcium-free PBS. The cells are then stained with 1,1'-Dihexadecyl-3,3,3',3'-Tetramethylindocarbocyanine Perchlorate (DilC16), resulting in homogeneous fluorescence staining of the cell membranes without compensating platelet function.

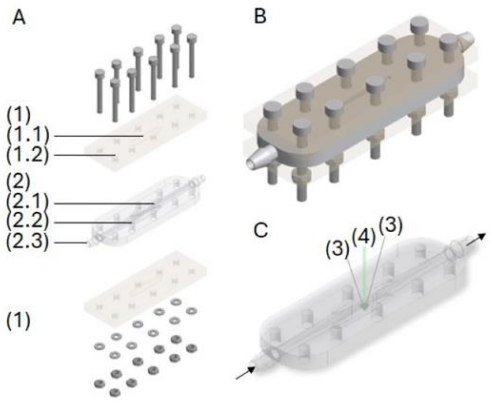

Figure 1: The design of a flow chamber for the novel application in a µPTV setup

(A) The flow chamber consists of (1) two identical sample plates and (2) a semi-closed flow channel. (1) The sample plates are PMMA sheets designed with (1.1) a sealing groove and (1.2) passage holes, whereas the sample can be permanently fixed in the area within the sealing groove. (2) The flow channel is a one-step 3D-print using KeySplint Soft® clear, as a translucent, semi-flexible material. It has a continuous transition from a circular inlet to (2.1) a rectangular channel at the sample area with openings at the top and bottom to the sample plates to allow uniform light entry and exit for µPTV measurement. (2.2) 1 mm from the opening, a sealing lip is placed, (B) which makes it possible to seal the flow chamber only by clamping the sample plates to the channel with screws. (2.3) The inlet and outlet were designed with male barb connectors integrated into the device. (C) In the µPTV application, the stained platelets in the flow are stimulated with (4) a 532 nm laser at the sample area. The light emitted by the cells is recorded in 400 x magnification with (3) 4000 Hz stereo cameras using a 575 nm filter. To compensate for the difference in refractive index between flow chamber materials and the fluid, a calibration of the refractive shift through the sample plate was performed.

In conclusion a proof-of-concept was demonstrated showing the recording of the flow pattern over a surface and status recordings of the total deposition of platelets in the context of dynamic in-vitro hemocompatibility testing.

Discussion

Using the novel µPTV test setup, the impact of microstructures on platelet flow behaviour can be systematically investigated in the future. The results will allow for improving the hemocompatibility of blood contacting medical devices by specifically tailored structured surfaces.

References

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