Design and Validation of Parallel Flow Chamber to Support Real-Time Visualization

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Abstract

Microparticles are cell-derived vesicles, of 0.2-1 µm diameter, that have been linked to the acceleration of thrombus growth due to their procoagulant activity.[1] In order for microparticles to assist in thrombotic events, they must first deposit. Real time visualization of microparticle deposition will provide greater understanding behind their deposition mechanism. Specific attention is directed towards designing a flow chamber that is compatible with the confocal microscope to allow for real-time visualization. A flow field that introduces flow conditions that encourage microparticle deposition is crucial to the design. The flow field was tested using computational fluid dynamics, CFD.

Background

Microparticles circulate throughout the blood and microparticles expressing tissue factor have been found in thrombin clots. Because microparticles support thrombin generation, they can be used as biomarkers for thrombotic events. Microparticles must deposit in order to expedite the clotting cascade. The knowledge of how microparticles deposit and which flow conditions are responsible can be crucial in determining patient risk for cardiovascular diseases, including stroke, pathological thrombosis. Previous computational research performed by C. Hall, analyzing MP deposition in non-parallel flow regimes, has cited impaction as being the primary mechanism behind microparticle deposition.[2] Real time visualization will provide experimental data to assist in determining the mechanism by which microparticles deposit in pathological flow conditions. Pathological flow conditions can result in recirculation zones where microparticles get trapped and eventually deposit (Figure 1). This flow pattern of recirculation can result downstream from vessel narrowing, stenosis. Introducing this flow pattern, through a reverse step flow chamber, and visualizing microparticle deposition in real-time, can aid in confirming this mechanism of deposition.

The deposition of microparticles is visualized using confocal microscopy. Microparticles can be identified from the clots in which they incorporated through fluorescence labeling. Platelets in whole blood are mepacrine labeled, fluorescing green, and MPs are labeled with PKH-26 , fluorescing red, before adding them to the whole blood to them be perfused through the chamber (Figure 2). As MPs and platelets adhere, the height will increase. With the desired scanning speed, the means of deposition of MPs will be determined by their presence in multiple image stacks.

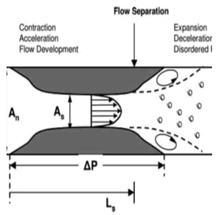

Figure 1. Downstream flow characteristics occurring from a narrowing to an expansion

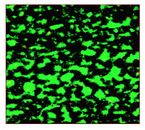

Figure 2. Image of MPs (red) and platelets (green) from confocal microscope

Materials and Methods

Design Requirements for Flow Chamber

Requirement	Specification
1. Flow chamber must be compatible with Leica TCS SP 8 confocal microscope stage	1.1 Must weigh less than 200 g 1.2 Must not exceed dimensions of stage insert
2. Must allow for visualization of clot formation at the site of disturbed flow	Visualization through a glass coverslip (no. 1) using a 63 or 100x oil immersion objective
3. Must produce desired disturbed flow conditions while minimizing volume of blood	Perfuse blood through chamber for 10 minutes using no more than 100 mL of blood.
4. Disturbed flow conditions must encourage microparticle deposition	A minimum of 5% of particles injected must deposit in intended, viewable location

- Flow chamber was designed using Solidworks (Dassault Systèmes).

Verification of Flow Field using Computational Fluid Dynamics

- Selected dimensions of flow field to match specifications. Designed reverse vertical step and curved stenosis using CFD-GEOM (ESI Group, Paris, France).
- Performed mesh sensitivity test to select appropriate mesh fineness.
- Performed flow simulations at 5, 7, and 10 ml/min using CFD-ACE+ (ESI Group, Paris, France).
- Assumed blood flow to be:
  - Laminar
  - Newtonian
  - Incompressible
- Injected particles of various sizes and densities with flow and employed Lagrangian tracking. Particle sizes and densities reflect those of microparticles (0.51 µm and 0.2 µm) and platelets (2 µm).[3]

Table 1. Particle Sizes and Densities Tested

Size (µm)	Density (kg/m^3)
0.51	1060
	1080
0.20	1060
	1080
2.0	1060
	1080

- Added user subroutine to indicate location of particle deposition.
- Used CFD-View (ESI Group, Paris, France) to analyze flow characteristics and ensure negative z-direction velocity vectors, indicative of recirculation, as well as to view particle deposition.

Results and Discussion

The flow chamber (Figure 3) designed fits the Leica TCS SP 8 confocal microscope seen in the assembly of the flow chamber and the stage insert (Figure 4). The polycarbonate base sits flush with the stage insert allowing for better visualization as the focal plane is closer to the objective. Device meets requirement 1 and 2.

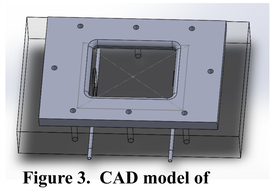

Figure 3. CAD model of flow chamber

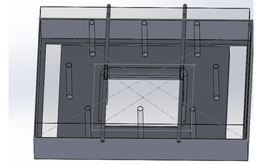

Figure 4. CAD model of flow chamber placed in confocal microscope stage insert

The dimensions of the reverse vertical step flow field were determined based on specifications (Figure 5). A 10:1 ratio for length to width was used to reduce the effect of the walls on the flow pattern. A coarse mesh of about 370,000 cells was made as well as a fine mesh of over 1 million cells (Figure 6). A mesh sensitivity study comparing the height of the recirculation zone, as well the reattachment point, between the two models was performed. No significant difference between the two models was found. For all CFD simulations, the coarse version was used.

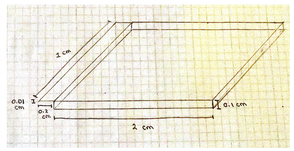

Figure 5. Dimensions of flow field

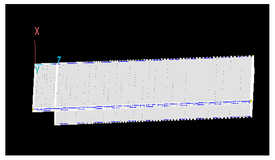

Figure 6. Mesh of reverse vertical step flow field

Disturbed flow conditions were evident from the max flow rate of 10 ml/min all the way down to 5 ml/min, satisfying design requirement 3. The largest recirculation zone, indicated by a negative velocity in the z direction was at 10 ml/min with only a slight recirculation zone at the lowest flow rate, 5 ml/min (Figure 7).

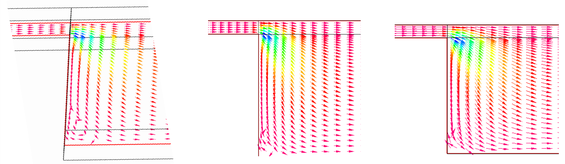

Figure 7. Velocity vectors at 10, 7, and 5 ml/min flow rates (left to right). Recirculation zones evident at each flow rate.

Particles of different sizes and densities, reflecting literature values on microparticle and platelet sizes and densities, were injected with the flow. The particle-fluid interaction was assumed to be uncoupled. Mass flow rate was calculated under the assumption that there are about 5000 particles per µL of blood.

Results and Discussion

Despite recirculation zones, relatively few particles deposited in that region. Most particles deposited right before the expansion (Figure 8).

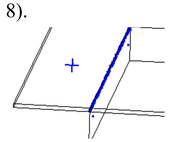

Figure 8. Deposition of particles upstream from expansion

This occurred for all various particle sizes and densities tested (Table 1). These findings were not consistent with literature, as a reverse step flow field, of similar dimensions and flow parameters, noticed deposition of spheres at 0.2 µm and 0.5 µm when used experimentally[4]. In an attempt to remedy this problem, the geometry of the reverse step was altered to be a curved stenosis, with the curvature modeling a sine wave. It was speculated that the abrupt expansion may be a potential reason for premature deposition. The same methods were used to set up the CFD simulation for the curved stenosis. However, there was no particle deposition evident with this flow field geometry even at the flow rate of 10 ml/min, with the greatest instance of disturbed flow. The device failed to meet design requirement 4.

Conclusions

The design of the flow chamber met requirements to support real time visualization. However, the design failed to produce any substantial microparticle deposition. Continued efforts will be made to alter the flow field design to introduce disturbed flow that results in a greater amount of deposition. Because of the devices compatibility with the confocal microscope, the chamber will still be manufactured and a parallel plate flow field will be used instead. Although this will not provide insight into the behavior of microparticles in pathological flow conditions, it can still provide valuable insight.

References

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