Shear rate behaviour within \textit{in vitro} thrombotic geometries: height and surface curvature dependence.

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Abstract

The flow of blood past thrombi, situated within an \textit{in vitro} geometry, is computed via the solution to the three-dimensional (3D) Navier–Stokes equations. The thrombotic geometry is derived from microscopy measurements of thrombotic geometries within \textit{in vitro} experiments which the \textit{in silico} experiment reflects. The flow within the numerical domain is solved using the OpenFOAM package to provide an solution of the steady state and incompressible Navier–Stokes equations. Previous work (Butler et al, 2012) explored the shear rate variations that occur in a simplified thrombotic geometry. Here additional flow complexity is introduced through the complexity of the thrombus geometry. Localised fluctuations of the thrombus surface are shown to generate significant variations in the shear–rate the thrombus surface experiences. Convex and exposed surfaces are shown to have the highest shear rates. In addition to the concavity the height above the microchannel floor is shown to correlate strongly with increased shear rate.

Introduction

Thrombosis, the development of blood clots within the vasculature, presents a significant clinical risk of myocardial infarction, cerebral and pulmonary embolism, and other diseases. Studies have long shown that there is an interaction between vascular diseases and the dynamics of blood flow (for a review see [7]). However, the quantification of flow effects on thrombus growth is more recent. In general a thrombus is an aggregate of blood cells and proteins bound to a blood vessel wall. The structure of the thrombus under arterial conditions is dominated by platelets. As a result the growth of a thrombus, under arterial conditions, is dependent on the aggregation, adhesion and activation of platelets [9]. An important question may be posed; after initial formation of a thrombus what interactions mediate platelet adhesion and activation after isolation of the injury site?

In addition to the biochemical processes that occur, early studies [1] showed shear force dependent platelet functionality at pathological shear rates ($\gamma \geq 10000$). Note that within these experiments $\gamma \geq 10000$ for a substantial period of time Further research revealed that shear rate correlates with the adhesive potential of platelets [11]. However, recent research, particularly that of Nesbitt et al. [9], has shown a causal relationship between shear–force and the initial adhesion and recruitment of a platelet to a thrombus. Critically the physical recruitment occurs in the absence of chemical agonists previously thought to be required for recruitment [6]. It was also theorized that spatial ‘micro–gradients’ of shear are required for shear mediated platelet aggregation [9, 13].

The current state of the art method for studying platelet adhesion \textit{in vitro} can be found summarized in [8]. Importantly we note that there is no consideration of the non-linear effects of the Navier–Stokes equations on shear rate. Shear rate is approximated globally by Poiseuille flow: $\gamma_{pw} = \frac{6U}{Pw}$, where $U$ is the mean flow velocity, $H$ is the height of the channel and $\gamma_{pw}$ shear rate at the wall of a channel presuming Poiseuille flow conditions. Our previous work [2, 3, 4] explored a thrombus analogue with a simplified geometry that has shown that changing the thrombus size and $\gamma_{pw}$ significantly changes both the amplitude of shear rate and the topology of shear rate on the surface of the thrombus. In this paper we seek to tightly couple our \textit{in silico} experiments with \textit{in vitro} experiments. Flow will be computed around thrombotic geometries derived thrombotic geometries from the experiments previously presented within [13].

Methodology

The thrombotic geometry considered by this study was derived from the experiments conducted with [13]. The first part of this section will discuss the \textit{in vitro} model and the acquisition of the thrombotic geometry. The later parts will discuss the numerical techniques for the fluid flow and required post–processing techniques.

The \textit{in vitro} fluid flow is based on that of a high aspect ratio microchannel. Here the channel as aspect ratio (AR) where:

$$AR = \frac{W}{H}$$

(1)

where $W$ is the width of the channel and $H$ the height. The length $L$ of these channels is such that $L : H$ is 500 : 1 for this
The Reynolds number for the system is defined as for channel
flow being left to right across the page. Identifiable thrombi are
marked by red arrows. Image courtesy of Dr. Elham Tolouei,
from the experiments reported in [13].

Study. This geometry is shown diagrammatically within figure
1. These geometries the standard methodology for considering
shear dependent platelet adhesion and are used widely (for ex-
ample see [6, 9, 11] and others). The channel employed in this
case has $H = 200 \mu m$ and here we are examining the cases where
the flow rate is set such that $\gamma_{pw} = 1800$ presuming there is no
influence of the growing thrombi on the channel wall. Within
this channel a thrombus is introduced as a protuberance from
the lower wall into the channel, this is shown within figure 1 as
the red mass within the channel. Within this study eight indi-
vidual thrombi are separately introduced into their own numer-
cal channel. They are introduced such that the centroid of the
thrombus (of the cross section at the channel wall) is located
in the lower wall reflecting the paradigm of Nesbitt et al. [9] where
the number across the channel height $N$ where the edge length
$\Delta z = \frac{H}{N}$. Cubic mesh and the thrombus surface approxima-
tion are merged using the process described within [5] to create a
numerical approximation of the channel including the thrombotic
grouping.

**Numerical technique**

This study considers blood as an incompressible, Newtonian
fluid. The flow is considered to be unsteady within the previ-
ously described channel. This approximation yields the Navier–
Stokes equations to be solved as

\begin{equation}
\frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla P + \nu \nabla^2 \mathbf{u},
\end{equation}

\begin{equation}
\nabla \cdot \mathbf{u} = 0,
\end{equation}

where $\nu$ is the kinematic viscosity, $\mathbf{u}$ is the velocity vector
field and $P$ is the kinematic pressure field. Here the Open
FOAM® numerical solver is employed to solve the incompress-
ible Navier–Stokes equations to a steady state using the SIM-
PLe algorithm [12]. The algorithm operates over a arbitrary
polyhedral finite volume mesh maintaining second order accu-
sity in space with the advection operator employing the QUICK
scheme. Both a domain size study and a grid resolution study
were performed for the computational mesh described previ-
ously. A mesh was chosen where $L = 12$ and $\frac{H}{N} = 20$ as the
point where the error was less than 0.1% with respect to the
largest domain size and highest resolution cases, respectively
(data not shown).

**Boundary conditions**

There are three separate types of boundary conditions: The input
to the channel, the output from the channel and the solid
boundaries. The solid boundaries include the side, upper and
lowers walls in addition to the thrombus surface. The solid
boundaries have a Dirichlet boundary condition for velocity
$\mathbf{u} = 0$, and a Neumann boundary condition for pressure. The
outlet has a Neumann boundary condition for velocity and a
Dirchelet pressure condition $P = 0$. The inlet boundary has a
Neumann pressure condition in addition to the prescription of the
analytic solution to laminar flow in a rectangular channel
(see [2]).

**Results and Discussion**

All of the thrombi are solved to a steady state with residuals of
$\mathbf{u}$ and $P$ are less than $10^{-10}$. Figure 3 examines two thrombi
that have been selected to be representative of the set of thrombi
considered. Each sub-figure (3a and 3b) considers a particular
thrombus, where the left hand and centre frames display $\chi$. Ex-
amining the centre frame for each thrombus, where fluid flows
from left to right, as the fluid approaches the thrombus on the
channel wall, the shear rate decreases from $\chi = 1$ towards $\chi = 0$
at the interface between the lower wall and the thrombus sur-
face. The low shear region surrounding the thrombus and on
the lower wall reflects the paradigm of Nesbitt et al. [9] where

- **Figure 2:** Bright field microscopy images of a partial in vitro
  microchannel. The vertical extent of the tiled images is ap-
 proximately wall–to–wall across the width of the channel, with
  flow being left to right across the page. Identifiable thrombi are
  marked by red arrows. Image courtesy of Dr. Elham Tolouei,
  from the experiments reported in [13].

- **Extended details of the biological setup are beyond the scope of this paper and are discussed fully within [13] and the references contained within. Here we briefly describe the details relevant to this study. The walls of the microchannel are coated with a thrombogenic material to allow thrombi to develop on the interior surface. A syringe pump is used to generate a steady state flow rate of whole blood until the thrombi within the microchannel reach a steady state size. At this point a fixative agent is used to fix the thrombi to the surface within the channel, allowing the profiles of the thrombi to be digitised at a later time.**

- **Results and Discussion:**
  - All of the thrombi are solved to a steady state with residuals of $\mathbf{u}$ and $P$ are less than $10^{-10}$. Figure 3 examines two thrombi that have been selected to be representative of the set of thrombi considered. Each sub-figure (3a and 3b) considers a particular thrombus, where the left hand and centre frames display $\chi$. Examining the centre frame for each thrombus, where fluid flows from left to right, as the fluid approaches the thrombus on the channel wall, the shear rate decreases from $\chi = 1$ towards $\chi = 0$ at the interface between the lower wall and the thrombus surface. The low shear region surrounding the thrombus and on the lower wall reflects the paradigm of Nesbitt et al. [9] where
low shear regions at the rear of the thrombus are the platelet aggregation zones. Here we see that the surface topology of the thrombus is complex with many pockets and protuberances across the surface. The high protuberances directly exposed to the flow appear to have higher \( \chi \) than the concave pockets which appear to have \( \chi \ll 1 \).

The right hand frame of figures 3a and 3b displays the surface curvature of the thrombus surface. Here it is seen that the regions of high shear correlate with convex surfaces. The nature of the three dimensional surface creates a difficult in understanding the correlation between surface curvature, shear rate, and position on the surface. Figure 4 is an attempt to reduce the data such that this is possible. A set of bivariate bins, with respect to both curvature, \( \kappa \), and height on the thrombus (as a fraction of channel height), \( h \), are established. Every mesh vertex on the surface of the thrombus is allocated to a bin based on \( \kappa \) and \( h \). If one surface mesh vertices exist within a bin the coloring is based on the the mean of \( \chi \) for the vertices in the bin. Note that negative and positive \( \kappa \) represent convex and concave regions, respectively. Here the strong correlation between height on the thrombus surface and \( \chi \) is clear. Large \( \chi \) is focused on a region where \( h \) is large \( h \) and \( \kappa < 0 \). It is noted that for \( h \approx \max(h) \) that \( \chi \) is high for both positive and negative \( \kappa \). We note that for both thrombi there is a region at a lower \( h \) and \( \kappa < 0 \) where large \( \chi \) is observed.

The results of figures 3 and 4 paint a picture: Points protruding into the flow have high \( \chi \). The thrombus is acting as a bluff body and consequently the surfaces on the thrombus which are ‘exposed’ to the flow have high \( \chi \). Figure 5 summarised the effect of \( \kappa \) across a number of surfaces. Here a clear trend is seen: regions of high shear are highly convex. Here a gross estimation of where high \( \chi \) regions occur may be made from \( \kappa \) and \( h \).

The link between \( \kappa \) and \( \chi \) can be elucidated by considering the vorticity generated as the flow follows around a curved surface. Figure 3: Contours of \( \chi \) and \( \kappa \) on the thrombus surface and the surrounding microchannel floor. The left two frames are of \( \chi \). Colouring spectrum: blue, white and red, represents \( \chi = 0, \chi = 1 \) and \( \chi = 8 \) respectively. Flow is from bottom left to top right and left to right, in the left and middle frames, respectively. The right hand frame is colored by \( \kappa \) with the same view as the left hand frame.

Conclusions

This numerical investigation has explored the dynamics of shear rate, surrounding a thrombotic geometry within an in vitro environment. Global behaviour has been maintained with previous in vitro and in silico studies, yet, highly complex \( \chi \) behaviour is observed on the thrombus surface. The complex behaviour has been shown to correlate with the surface curvature and height - potentially allowing a simplified model of \( \chi \) to be formed.
Figure 4: Map of $\chi$ with respect to $\kappa$ and $h$. Bivariate bins (with respect to $\kappa$ and $h$) are established. Points are binned and if a set of points exists within the bin the shear rate is averaged and that point is displayed on the map.

Figure 5: Plots of curvature versus shear rate on the thrombus surface. Each point on the surface is binned based on the shear rate. The curvature values within the bin are averaged. Each coloured curve represents one of the thrombi simulated in this study.

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References