1 Genesis of the project

It is sound knowledge in biophysics that a good model for blood is made up of a simple newtonian liquid and one-kind deformable particles [1]. However, such an approach turns to be not enough suitable for a complete description of blood circulation. In vivo observations, in fact, pointed out that red blood cell (RBC) velocity is slower than in in vitro experiments with glass capillary tubes of the same size [2] and an explanation for such an effect needs the model to embrace further details. The reason of the slowing down has been ascribed to a recently discovered network, shown in Fig.1: glycocalyx, an ensemble of polymers grafted to the inner surface of vessel walls (endothelium) [3]. The name “glycocalyx”explicitly refers to one of the major component of such an etherogeneous structure, glucose, that, together with proteins and other kinds of organic macromolecules, form the most relevant compounds (glycoproteins, glycoaminoglycans) this network is constituted by.

The first in vivo measurement of endothelium glycocalyx dates back to 1996 [4] and from then on increasingly refined experimental techniques [5] have settled that glycocalyx covers all mammalian blood vessels. Its width, ranging from 150nm to 400nm, is large compared to the thickness of the vessel wall itself, while it is no more then 10% of the tube diameter even in the smallest capillaries. Such proportions reveal how it forms a significant compartement devoted to vessel wall shielding, without constituting a relevant obstacle to blood flow.

However, although the importance of glycocalyx is beyond any doubt, the actual way it accomplishes for its functions is still under study. In the last decade, an increasing interest has developed around this topic, driven mostly by the potential medical applications: already published papers stress the connection between a glycocalyx reduction or lack and the onset of specific diseases (e.g. atherosclerosis [6], albuminuria [7]). Lots of effort have been thus invested in the identification of glycocalyx functions, but rigorous prooves still lack. Among the suggested hypotheses [3] the major ones are the following:

- sieve, regulating vessel permeability in allowing the passage only of molecules of a specific size;
- limiter of particle adhesion, inhibiting the attachment of flowing particles to the endothelial cell layer;
- mechanotransductor, transmitting the shear stress from the bulk of the vessel to the endothelial cells polymers are attached to.

Target of this project is to model glycocalyx dynamics and its interaction with in-blood flowing particles and RBCs. In this context the unknowns of the complex biological environment couple to the unknows of polymer brush behavior. The present work aims to contribute to the understanding of the wide branch of polymer brushes and propose a relatively simple model to examine their peculiar features under flow.
Figure 1: The endothelial glycocalyx layer: (a) cross-section of a rat left ventricular myocardial capillary coated with endothelial glycocalyx. Bar corresponds to $1 \mu m$ (image by electron microscopy from [8]); (b) diagram of the glycocalyx arrangement pointing out its typical bush-like shape (image from [3]).

2 A basic model for a polymer brush

Single polymers have been extensively studied and their characteristics are properly illustrated within the theoretical frames of several models. One of the most representative quantities is the size of a polymer chain, thought as “end-to-end distance” $R_{ee}$, namely the typical length scale between the two extreme monomers. In a statistical approach, for a free flexible chain of $N$ subunits of size $a$, $R_{ee}$ scales like the radius of gyration, $R_{gyr}$, defined as the root mean square distance between monomers:

$$R_{gyr} = \sqrt{\frac{1}{N} \sum_{k=1}^{N} (\vec{r}_k - <\vec{r}>)^2}, \quad (1)$$

where $\vec{r}_k$ is the $k$-th monomer position and $<\vec{r}>$ is the center of mass of the chain. Therefore, in good solvent conditions, the following relation holds:

$$R_{ee} \propto R_{gyr} \sim aN^{\nu} \quad (2)$$

with $\nu$ called Flory exponent. If the polymer is represented by an ideal chain, i.e. its subunits can occupy any position of space disregarding their neighbours, monomers are distributed as subsequent positions of a random walk and the Flory exponent is $\nu = 1/2$; if the model takes into account excluded volume interactions, monomers have not all the space available, since they must avoid each others, therefore their distribution follows a so-called self-avoiding random walk and the Flory exponent assumes the value $\nu = 3/5 \approx 0.588$. Let’s consider the second case and let’s now attach several polymers by one end to a flat substrate: two different regimes should be distinguished. As far as the distance $d$ between grafting point is such that $d \gg R_{gyr}$, the same statistical reasonings as before are valid to estimate $R_{ee}$, but if $d \leq R_{gyr}$ polymer chains would significantly overlap, therefore they stretch along the direction perpendicular to the grafting substrate to reach a balance between a maximal configurational entropy and a minimum cost in excluded volume interactions. This second regime of strong stretched chains actually defines the polymer brush condition and it is responsible of completely novel features for the system. It is now more convenient to introduce the grafting density $\sigma_{graft}$, such that $d = \sigma^{-1/2}$.

Our intention is to model flexible chains in the limit of strong stretching. Chains would be grafted to the infinite walls of a slit pore three-dimensional channel. To have a detailed point of
view on the length scale of polymers we choose a particle simulation model. Each chain would be constituted by \( N \) particles, connected by finite extensible nonlinear elastic (FENE) potential. Excluded volume interactions and the good solvent condition will be taken into account, as illustrated in the next section.

2.1 The code

The simulation method should reproduce phenomena happening both on the scale of polymers and on the scale of hydrodynamics (hydrodynamics interactions are expected to play a central role in the dynamics of the system). Such a method has been chosen to be Dissipative Particle Dynamics (DPD). It is basically a coarse-graining of Molecular Dynamics (MD) \cite{9}: while the object evolving according to MD reproduces the atomistic dynamics, the DPD particle represents a certain amount of real molecules, therefore it covers bigger timescales and bigger length scales than the usual ones recovered by MD, becoming a more suitable model to account for hydrodynamic interactions. Forces acting on particles, therefore, cannot be fitted by molecular potential but have to be gauged in consequence of the coarse-graining. Validation of our DPD code can be supported by simulations in bare channels, extracting the velocity profile and checking it is the expected Poiseuille parabola.

Part of the thousand and thousand particles contained in the simulated channel represents a simple liquid (water), the remaining part form the dense layer of grafted linear chains. The anchor points are randomly distributed, according to the imposed grafting density \( \sigma_{\text{graft}} \).

A DPD basic code adopts three forces, acting within a same distance range \( r_c \) between beads: conservative, \( \vec{F}_C(r) \), dissipative, \( \vec{F}_D(r) \), and stochastic ones, \( \vec{F}_S(r) \). These are all pair forces, as guarantee of momentum conservation. The conservative force has the following expression:

\[
\vec{F}_{ij}^C = \begin{cases} 
  a_{ij} \left( 1 - \frac{r_{ij}}{r_c} \right) \vec{r}_{ij} & r_{ij} \leq r_c, \\
  0 & r_{ij} > r_c 
\end{cases}
\]

with the definitions \( \vec{r}_{ij} = \vec{r}_i - \vec{r}_j \) for the vector distance between the i-th and j-th particle, \( r_{ij} = |\vec{r}_{ij}| \) and \( \vec{r} = \vec{r}_{ij}/r_{ij} \). It is a conservative soft-core repulsive force. The choice for the soft-core potential is justified by the “blob” nature of DPD particles that, containing (in the atomistic perspective) also empty space, should have the possibility to overlap; the choice for the repulsive effect has basis in liquid theories, stating that the specific structure of a liquid is fundamentally determined by the repulsive part of the intermolecular potential (while the attractive part can be treated as perturbation). The constant \( a_{ij} \) is a measure of the force between two completely overlapping particles (the potential corresponding to Eq.\( (3) \) does never diverge even at \( r_{ij} = 0 \)). The value for the parameter \( a_{ij} \) for solvent-solvent interaction is estimated from the adimensional compressibility of the simulated liquid; we set the same value also for the monomer-monomer interaction therefore, as already mentioned in the previous section, our simple chains are not ideal ones, but experience an excluded volume interaction; for solvent-monomer interaction we fix a lower value for \( a_{ij} \) and that way a softer repulsion favouring the contact between monomer and solvent will fulfill the good solvent condition.

The two other extra forces account for the loss of details in the coarse-graining procedure, namely the huge number of collisions occurring between real molecules and constituting the microscopic foundation of transport properties, such as viscosity. The dissipative force takes the form:

\[
\vec{F}_{ij}^D = -\gamma w_D(r_{ij})(\dot{r}_{ij} \cdot \vec{r}_{ij})\dot{r}_{ij},
\]

where the standard choice in literature for the “weight function” \( w_D(r_{ij}) \) defining the cut radius of interaction is:

\[
w_D(r_{ij}) = \begin{cases} 
  \left( 1 - \frac{r_{ij}}{r_c} \right) \dot{r}_{ij} & r_{ij} \leq r_c, \\
  0 & r_{ij} > r_c
\end{cases}
\]
Equation (4) accounts for the energy loss, introducing a friction among particles proportional to the relative velocity $\vec{v}_{ij} = \vec{v}_i - \vec{v}_j$ and to a factor $\gamma$ that will depend on the temperature $T$. Since the $F^C(r)$ and $F^D(r)$ forces could reach an equilibrium that freezes the system, a random force is also necessary:

$$F^S_{ij} = \sigma w^{R}(r_{ij}) \theta_{ij}(\Delta t) - \frac{1}{2} \hat{r}_{ij}$$

where $\sigma$ is related to temperature, $w^{S}(r_{ij})$ is a weight function and $\theta_{ij}$ is a random number extracted from a gaussian distribution having the following characteristics:

- zero averaged: $\langle \theta_{ij}(t) \rangle = 0$
- independent in time and among couples: $\langle \theta_{ij}(t) \theta_{lm}(t') \rangle = (\delta_{il} \delta_{jm} + \delta_{im} \delta_{jl}) \delta(t - t')$.

Of course $\theta_{ij} = \theta_{ji}$ for the momentum conservation. To be noted that $F^D(r)$ and $F^S(r)$ also act as thermostat.

It has been proved by Español and Warren [10] by use of the fluctuation-dissipation theorem that in order to reproduce the correct probability distribution of the NVT ensemble the dissipative and random forces has to verified two constraints:

$$w^{D}(r_{ij}) = [w^{R}(r_{ij})]^2 \quad \text{and} \quad \gamma = \frac{\sigma^2}{2k_BT}. \quad (7)$$

### 3 Polymer brush at equilibrium

At equilibrium the brush can be characterize by mean of its density profile [11], basically counting how many monomers are, on average, at a certain distance from the wall. The profile should be the result of the balance between configurational entropy and the excluded volume limiting the total number of possible configurations the single chain can assume. The resulting brush height is expected to scale as

$$h_b \sim N \sigma_{\text{graft}}^{1/3} \quad (8)$$

according to Milner’s theory [12].

Analyses on this regard have already been performed, nevertheless a systematic study is still lacking.

![Figure 2: Typical density profiles of a polymer brush at equilibrium for a fixed chain length, $N = 16$, and various grafting densities $\sigma_{\text{graft}}$. The qualitative behavior spans from a parabolic profile to more step-like ones, as chains are forced to stand straighter.](image)

Typical density profiles are shown in Fig.2 for a fixed chain length: increasing the grafting density, the distribution of monomers change from parabolic to step-like, as chains, subdued to excluded volume interactions, are forced to stand straighter.
4 Polymer brush under flow

Nowadays there is no consensus on the effect of shear flow on a network of grafted polymers: following Ref.[13] the flow is expected to compress the brush, while according to Ref.[14] it is expected to swollen it. Moreover, recent experiments performed at the LIPhy reveals how the dynamics of such a network under flow is not trivial at all. In fact, looking at the velocity profile of water flowing inside glass microtubes, they found that the presence of a polymer brush slows down the flow as if the channel was effectively reduced of about twice the brush thickness [15]. It seems that the polymer brush influences the flow up till a bigger distance than the one indicated by its height $h_b$. Reason for such an effect will be investigated under two perspectives:

- possibility of flow reversal.
  Recent numerical simulation of polymer brush in polymer melts [16] have brought the attention on a novel phenomenon arising at the interface between the outer fringe of a brush and the melt, namely the inversion of flow. It could be responsible for the increase of the dissipation of energy by the flowing liquid.

- polydispersity.
  A more realistic model for a polymer brush would not be constituted by equal chains, but rather by a distribution of chains of different length. It has to be clarified if such a polydispersity, given the same $h_b$, can lead to smaller fluid velocities with respect to a homodisperse brush.

4.1 Interaction with solid beads

The same set up and the same numerical method (DPD) will be used in order to detect possible repulsive or trapping effects for single in-blood flowing particles due to the presence of the grafted polymers. Such a kind of free beads should mimic the “bad lipids” (LDL=low density lipoproteins) whose adhesion to the endothelial surface layer constitutes the first stage of atherosclerosis.

4.2 Interaction of polymer brush with RBCs

A vesicle (a drop surrounded by a bilayer membrane) can be a good approximation for a RBC, since it reproduces the major feature of these cells, namely their high deformability [17]. Embedding such a particle in our system, already including polymers, could be a demanding task because of the well-distinct length scales involved: the brush reaches at most 400nm of thickness, while a RBC has a size of about 8µm. Moreover a vesicle has free boundaries, namely that its shape is not known a priori, but it is determined by the interplay between the local flow and its membrane features, like shear and bending elasticities. Computational approaches are always challenging. A two-dimensional code solving the Stokes equation by means of the Boundary Integral method is available, already validated for a dilute suspension of vesicle. Polymer brush could be inserted as threadlike membranes. It would allow the matching between the continuous and the discrete point of view.

As further step, several vesicles could be insert: it is known that RBCs can interact and form aggregates [18], but it is not yet known how the features of these aggregates change in presence of a polymer brush.

References


