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Published in:
Journal of the Royal Society Interface

DOI:
10.1098/rsif.2019.0148

Citation for published version (APA):
van Rooij, B. J. M., Závodszky, G., Azizi Tarksalooyeh, V. W., & Hoekstra, A. G. (2019). Identifying the start of a platelet aggregate by the shear rate and the cell-depleted layer. Journal of the Royal Society Interface, 16(159), [20190148]. https://doi.org/10.1098/rsif.2019.0148

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Identifying the start of a platelet aggregate by the shear rate and the cell-depleted layer

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Computer simulations were performed to study the transport of red blood cells and platelets in high shear flows, mimicking earlier published in vitro experiments in microfluidic devices with high affinity for platelet aggregate formation. The goal is to understand and predict where thrombus formation starts. Additionally, the need of cell-based modelling in these microfluidic devices is demonstrated by comparing our results with macroscopic models, wherein blood is modelled as a continuous fluid. Hemocell, a cell-based blood flow simulation framework is used to investigate the transport physics in the microfluidic devices. The simulations show an enlarged cell-depleted layer at the site where a platelet aggregate forms in the experiments. In this enlarged cell-depleted layer, the probability to find a platelet is higher than in the rest of the microfluidic device. In addition, the shear rates are sufficiently high to allow for the von Willebrand factor to elongate in this region. We hypothesize that the enlarged cell-depleted layer combined with a sufficiently large platelet flux and sufficiently high shear rates result in a haemodynamic environment that is a preferred location for initial platelet aggregation.

1. Introduction

Arterial thrombosis occurs in vessels with a pathological shear rate (greater than 5000 s$^{-1}$) caused by for example a stenosed vessel due to atherosclerosis [1] or by disturbed flow around medical device [2]. Two other factors that are important for arterial thrombosis are a prothrombotic surface and prothrombotic blood chemistry (e.g. specific behaviour of von Willebrand factor (vWF) and platelets) [3]. In comparison with venous thrombosis, the typical coagulation cascade is less important in arterial thrombosis. The reason for this is that the flow is faster in arteries and the coagulation cascade happens on a longer timescale (minutes) than the aggregation of platelets (seconds) in these fast flows. Different types of clots can be distinguished in the venous and arterial system. Red blood cell-rich clots or red clots are mostly found in the veins and platelet-rich clot or white clots are found in the arteries. An increase in local shear rate can result in shear-induced platelet aggregation [2] which can lead to occlusion of the vessel or medical device. At high shear rates the vWF uncoils [4]. This uncoiling can happen when the vWF is free flowing in the blood or when it is bound to a prothrombotic surface (collagen). The shear rate threshold for the uncoiling of free-flowing vWF is around 5000 s$^{-1}$ [4] and for bounded vWF it is around 1000 s$^{-1}$ [5]. When the vWF is uncoiled, binding sites (A1 domain) become available for platelet receptors (GP Ib-V-IX or integrins), collagen and endothelial cells [6]. The vWF slows the platelets down to facilitate the binding of a platelet to collagen or platelet–integrin binding to vWF, forming a stable platelet aggregate [7].

In the last decade, many in vivo and in vitro experiments on the formation of a platelet aggregate in a vessel or microfluidic device with a stenosis were...
performed, e.g. [8–10] Nesbitt et al. [8] show that rapid changes in shear rate caused by stenosis or by the developing thrombus itself lead to the formation of a platelet aggregate. Westein et al. [10] extend this explanation by accounting for the importance of the vWF during platelet aggregation at post-stenosis sites. The influence of red blood cells (RBCs) on the formation of a platelet aggregate was investigated by Tovar-Lopez et al. [11] by dividing the flow in two fractions with different haematocrit. This study showed that the platelets present in the platelet aggregate came from 15 to 25% of the blood flow proximal to the stenosis. This addresses the importance of platelet margination in thrombus formation.

The shear rate and its gradients in the microfluidic devices used in those experiments were simulated by a model that represents blood as a continuous fluid. Many papers on experimental thrombosis have explored the haemodynamical parameters by computational fluid dynamics using continuous models [8–10,12,13]. A primary drawback of this method is that the influence of RBC is ignored. Given the fact that the size of the initial platelet aggregates is smaller than individual RBC, and that the scale of the microfluidic devices are typically only one order of magnitude larger than a red blood cell a full interpretation of the flow fields in these experimental set-ups would require computations that explicitly take the RBC and platelets into account. Such computations reproduce influential phenomena of blood, such as cell-depleted layers and platelet margination. The impact of RBC on the transport physics at the site of platelet aggregate initialization is not clearly understood yet. Tovar-Lopez et al. [11] hypothesize that collisions between RBCs and platelets are a key mechanism in the formation of the platelet aggregate in the lower shear zone behind the stenosis, but they did not further explore this hypothesis.

In this study, a cell-resolved model [14] that includes the behaviour of RBC and platelets is used. Many cell-resolved models have been developed in the last decade. The mostly used approaches for these models that can be found in the literature are the lattice Boltzmann method (LBM) in combination with the immersed boundary method (IBM) [14–17] and the dissipative particle method [18–25]. Additionally, the most popular material model for red blood cell deformation is the model of Fedosov et al. [19]. However, in this study, the model developed by Závodszky et al. [14] is used, since this model performs better at high shear rates and the transport physics in microfluidic devices that display very high shear rates (1000–40 000 s⁻¹) is investigated. The computational costs of the cell-resolved models are extremely high and therefore only small domains and short time spans can be studied with these models. Cell-based models can be used to study the suspension behaviour of blood with phenomena, such as the cell-depleted layer [26,27] and the margination of platelets [24,27,28]. The increased concentration of platelets at the side of the vessel wall is an important factor in arterial thrombosis. Additionally, these models can be of great value by developing experiments or optimizing microscale medical devices.

A direction of thrombosis research that recently gained more interest is the dynamics of the vWF that play a large role in high shear thrombosis. Rack et al. [29] and Lui et al. [30] included the vWF in their cell-based model to study the behaviour of this protein in the blood. Griffin et al. try to find new anti-thrombotic drugs in the form of nanoparticles that reduce the amount of uncoiling of vWF at high shear rates [31]. Additionally, Belyaev modelled the binding of platelets and vWF [32]. Furthermore, experimentalists try to understand the behaviour of the vWF better by designing experiments that specifically target high shear rate regimes [5,12]. Despite all the recent developments, the influence of RBC on the uncoiling of the vWF and in what haemodynamic environment this uncoiling happens is still unknown.

In this study, the transport of platelets in complex microfluidic geometries is investigated using computer simulations to shed further light on the role of haemodynamics and transport physics in initial platelet aggregation. For this, we rely on our cell-based blood flow simulation framework Hemocell [14]. The mechanical behaviour and transport properties of the RBC in Hemocell were thoroughly validated in Zavodszky et al. [14]. First, the cell-resolved flow dynamics of whole blood in flow chambers with stenosis is studied, i.e. the experiments of Tovar-Lopez et al. [9] are reproduced. Next, the thickness of the cell-depleted layers is investigated in detail and the platelet residence time as well as the platelet density in these layers are measured. The fluid shear rate and shear stresses are studied as well. Combining all this information, the aim is to better understand and predict where platelet aggregate formation starts. Additionally, the method used and results found in this study can be useful for designing future experiments and for optimizing the design of micro-medical devices.

2. Material and methods

2.1. Cell-based blood flow framework: Hemocell

Hemocell [14,33] is used to simulate the cell-resolved flow of blood in vitro experiments in which a platelet aggregate is formed. Within Hemocell, the blood plasma is modelled as an incompressible fluid by the LBM using the Palabos library [34,35]. The suspended RBC and platelets are modelled by a discrete element method (DEM) and fluid–structure coupling is achieved via the IBM. The RBC membrane model by Zavodszky et al. [14] is used to mimic the deformations of the RBC in the flow. The constitutive model for cells is validated by the optical tweezers stretching test of Mills et al. [36] and the wheeler shear test of Yao et al. [37]. In addition, the rheology of blood is validated using cell-free layer (CFL) measurements [38], velocity profile measurements [39] and the Pries curve [40]. This model was found to be more suitable to model red blood cell mechanics in high shear environments [14]. For further details on the material model and its validations the reader is referred to [14].

2.2. Experiments from the literature

In this paper, two experiments from the literature are examined in more detail using Hemocell. First, the microfluidic experiment of Tavar-Lopez et al. [9] is reproduced. The geometry of the experiment as used in our simulations is shown in figure 1a. In this experiment, the development of a platelet aggregate in time is studied using a microfluidic channel with a microcontraction. Different contraction/expansion angles α of 90, 60 and 30°, that obstruct 80% of the microfluidic device, are investigated. In the numerical reproduction of the experimental set-up, Tovar-Lopez et al. [9] applied a continuous Newtonian fluid model to the geometry and they reported shear rates at 1 μm from the wall. In this work, the blood flow in these geometries was studied using a continuous fluid model, to reproduce Tovar-Lopez’s results, and Hemocell with periodic boundary conditions in
containing $O(10^4)$ cells, we need to execute the simulations on parallel supercomputers. Models like Hemocell [14]. Also, as these simulations typically contain $O(10^3)$ cells, we need to execute the simulations on parallel supercomputers. We typically ran the $60\degree$ microcontraction simulation with 8444 RBCs and 711 platelets on 256 cores of the supercomputer Cartesius (Amsterdam, The Netherlands) (SURFsara, Cartesius) for 5 days.

For the bead assay experiment, two simulations with different initial velocities were performed. Shear rates of 675 s$^{-1}$ and 1875 s$^{-1}$ were applied on the velocity boundary at the top plane of the domain. A discharge haematocrit of 36% and the kinematic blood viscosity in the simulation where blood is modelled as a continuous fluid is set to 3.3 m$^2$ s$^{-1}$. The simulations with blood modelled as a continuous fluid and blood modelled as a suspension are driven with the same flow rate. The contraction angles $\alpha$ (figure 1a) are chosen the same as in the experiment [9]: 30, 60 and $90\degree$. In the cell-resolved simulations, the platelets and RBC are initially randomly distributed with a platelet to RBC number ratio of 1:10, and we allow for stable cell distribution during a period of 20 ms that is needed to reach a steady state in viscosity [14].

The random distribution of RBC and platelets is performed by a packing algorithm build in Hemocell [14]. Note that these simulations, simulating blood flow at high shear rates and haematocrit of 36% are at the upper limit of what is currently achievable with cell-based models like Hemocell [14]. Also, as these simulations typically contain $O(10^3)$ cells, we need to execute the simulations on parallel supercomputers. We typically ran the $60\degree$ microcontraction simulation with 8444 RBCs and 711 platelets on 256 cores of the supercomputer Cartesius (Amsterdam, The Netherlands) (SURFsara, Cartesius) for 5 days.

For the bead assay experiment, two simulations with different initial velocities were performed. Shear rates of 675 s$^{-1}$ and 1875 s$^{-1}$ were applied on the velocity boundary at the top plane of the domain. A discharge haematocrit of 25% and sphere diameters of 2 and 15 $\mu$m were used. The platelets are fully marginated at the start of the simulation. The bead assay computation with the large bead contained 598 RBCs and 68 platelets and was computed on 64 cores of Cartesius (SURFsara, Cartesius) for 10 days. The small bead assay computation contained 637 RBCs and 73 platelets and was computed on 64 cores for 10 days.

The parameters for the blood plasma and for the red blood cell model are the same for both experiments and these can be found in the electronic supplementary material.

2.3. Simulation parameters

For the microcontraction simulations, approximately the same input parameters as those used by Tovor-Lopez et al. [9] are used here. The volumetric flow rate of approximately $16 \mu$l min$^{-1}$ is obtained in our simulations by driving the flow with the corresponding body force $F_w$ (figure 1a). The flow rate for the $60\degree$ case is $17 \mu$l min$^{-1}$. The discharge haematocrit is 36% and the kinematic blood viscosity in the simulation where blood is modelled as a continuous fluid is set to 3.3 m$^2$ s$^{-1}$. The simulations with blood modelled as a continuous fluid and blood modelled as a suspension are driven with the same flow rate. The contraction angles $\alpha$ (figure 1a) are chosen the same as in the experiment [9]: 30, 60 and $90\degree$. In the cell-resolved simulations, the platelets and RBC are initially randomly distributed with a platelet to RBC number ratio of 1:10, and we allow for stable cell distribution during a period of 20 ms that is needed to reach a steady state in viscosity [14].

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The parameters for the blood plasma and for the red blood cell model are the same for both experiments and these can be found in the electronic supplementary material.

2.4. Cell-free layer and haematocrit

The time-averaged CFL is obtained by measuring the local concentration of RBC in the domain averaged over all time steps. If the local concentration is lower than a certain threshold, this is defined as the CFL. The threshold used in this study is 10% of the volume fraction.

The region of interest (ROI) in the bead assay simulations in which the platelet count per second, residence time of a platelet and shear rate are measured is the area from the bottom plate to 3 $\mu$m above this plate for the small bead (2 $\mu$m, figure 2a) and the area from the bottom plate to 16 $\mu$m above this plate for the large bead (15 $\mu$m, figure 2b).

The residence time of platelet $\tau$ is defined as the time it takes for a platelet to move a diameter away from the current position in space

$$\tau = \frac{D_{plt}}{V_f},$$

with $D_{plt}$ the diameter of platelet (2 $\mu$m) and $V_f$ the time-averaged fluid velocity at the position of the platelet.

3. Results

In the following sections, the results of our study of the transport physics in devices that favour platelet aggregate formation are presented. We start with the microcontraction experiment in which we investigate the difference between continuous and cell-resolved modelling. Second, we study the haemodynamic environment in which the platelet aggregate starts to form in the bead assay experiment.

3.1. Continuous versus cell-resolved blood flow modelling

In this subsection, the shear rates and shear stresses of the microcontraction for blood modelled as a continuous fluid and blood modelled as a suspension are compared. In our simulations, the microcontraction is perfused either with a continuous fluid or a suspension of RBC and platelets in blood plasma. The resulting shear rates and stresses of the fluid (as defined in the electronic supplementary material) that a platelet or vWF will feel when it moves 1 mm
from the wall of the microfluidic device are shown in figure 3a,b, respectively.

Figure 3c shows that for the 60° microcontraction simulation a 10% higher shear rate was found when the blood was simulated as a suspension (Ht = 36%). The increase in shear rate was even more pronounced in the simulation with a haematocrit of 44% (data not shown). We approximately reproduced the shear rates as those reported by Tovar-Lopez et al. [9] in our continuous fluid simulations.

However, the shear stress is significantly higher in the continuous fluid simulation in comparison to the cell-based simulation. This can be explained by the fact that the shear stress was obtained in the CFL. In this layer, the local viscosity is thrice as low as in the continuous fluid simulation which leads to a higher shear stress in the continuous simulation.

3.2. An enlarged cell-depleted layer

In our simulation of the experiment of Tavor-Lopez et al. [9], we observed an enlarged CFL at the location where the platelet aggregate started to form. A visualization of this simulation is presented in figure 4. The yellow arrow points to the position where the platelet aggregate approximately started to form as reported by Tovar-Lopez et al. [9]. At this place, the cell-depleted layer becomes larger. Such an enlarged CFL was observed for all three geometries used in the experiment of Tavor-Lopez (see electronic supplementary material).

The averaged cell-depleted layer was also larger in the bead assay simulation with the largest sphere (15 μm), which for a haematocrit of 25% is shown in green in figure 5a. The smaller sphere with a diameter of 2 μm is completely embedded in the CFL as shown in figure 5b.

3.3. Platelet count per second and platelet residence time

In this section, the platelet count per second and residence time of a platelet in the CFLs of the bead assay experiment are obtained. Figures 6 and 7 show the projection to two dimensions of the platelet count per second and the projection of the residence time of platelets for the small and the large bead with a diameter of 2 μm and 15 μm, respectively. For the largest sphere, the projection covers the slices from the bottom of the microfluidic device up to the maximum cell-depleted layer size (see dashed line in the inset image in figure 6a). What is interesting in these data is that around the sphere the platelet count and residence time are slightly higher than in the rest of the domain. The small sphere shows a different behaviour, because it is so small such that it is fully emerged in the CFL at the bottom of the device.

There is no pattern visible in the 2D projections in figures 6a and 7b for the platelet count per second and residence time, respectively. For this smaller sphere, all values in the cell-depleted layer are taken into account as is shown in the inset images. When the shear rate on the upper plane of the geometry is increased in case of the largest sphere, the increased platelet count and residence time around the sphere become more pronounced (figure 8). At various positions (e.g. at (x, y) = (50, 8) for the 2 μm bead), a peak in platelet count is observable. These peaks are caused by platelets rolling slowly on the bottom wall. This effect is more pronounced in the 2 μm bead case, because the ROI is smaller in that case. The effect is reduced when a higher shear rate is applied to the top plane. In order to reduce this effect, the simulations need to be run for a longer period of time, however, this is computationally very expensive.

3.4. Shear rate and shear stresses

The shear rate of the fluid is measured in the bead assay experiment to study the possibility of the uncoiling of the vWF coated on the surface of the bead. The shear stress and shear rate of the fluid are further detailed in the electronic supplementary material. In figure 9, the shear rate is projected on the bottom of the microfluidic device for the case with an shear rate of 675 s⁻¹ on the top plane of the domain for the smallest and largest sphere. It can be seen that the shear rates on the side of the sphere in case of the largest sphere (1200 s⁻¹) are higher in comparison to the rest of the domain (600 s⁻¹). In case of the smallest sphere, this difference can be neglected, since the shear rate is high everywhere in the CFL (1000 s⁻¹). When a shear rate of 1875 s⁻¹ is applied to the top plane a higher shear rate (4000 s⁻¹) is obtained for the larger sphere (figure 10) compared to the case in which a shear rate of 675 s⁻¹ was applied.

4. Discussion

We studied the influence of RBC on haemodynamic parameters in microfluidic devices to better understand how the transport physics influence platelet aggregation. The uniqueness of our study is that we used a cell-based blood flow framework [14] to predict the relevant haemodynamic parameters instead of a continuous blood flow model that has been used in the literature so far. One major difference that we observed is the difference in shear rate and shear stress obtained from continuous and suspension based blood flow simulations. This difference was surprisingly high and suggests that modelling blood as a continuous fluid is not suitable, directly. A continuous blood fluid model should be adopted to include a CFL and a haematocrit, to obtain the shear stresses and shear rates in microfluidic devices close to surfaces. The continuous fluid model is a good estimate of the order magnitude of shear rate in a microfluidic experiment, but exact shear rates cannot be derived from such simulations. Additionally, the shear stresses close to the wall in a continuous fluid were almost twice as high. The haematocrit of the blood was not the same everywhere, it was higher in the middle of the channel and close to zero in the vicinity of surfaces. This leads to a plug flow behaviour in the bulk of the channel, where shear stresses are low, and increased shear rates in the cell-depleted layers that act as a lubrication layer. Additionally, this
migration of RBC to the middle and the presence of a CFL result in a lower local viscosity close to the channel surfaces. This explains why the shear stress is overestimated when it is modelled as a continuous fluid. This finding supports the statement of Diamond et al. [41] that cell-based blood flow simulations are necessary when studying the thickness of the CFL, the bulk of the blood, the drift and accumulation of platelets into the cell-depleted layer and enhanced platelet diffusivity. Most of these factors are important in platelet aggregation.
Secondly, we observed an enlarged CFL in both experiments of Tovar-Lopez et al. [9] and Nesbitt et al. [8]. In the microcontraction simulations, we obtained a CFL just after the stenosis. The enlarged CFL can be explained by the mechanism of Tovar-Lopez et al. [11]. They hypothesize that the wall lift effect decreases at the microcontraction.
and the shear-gradient lift force pushes cells to the wall. The platelets are influenced minimally by this shear gradient, as it results in negligible lift forces, thus they can maintain their trajectories. RBC are bigger and deform easier, they will be lifted from the wall and experience a change in their trajectories. Platelets can bind to vWF factor which is adhered to the PDMS surface of the microfluidic device by the Vroman effect [42], the blood plasma proteins that are most mobile will attach first to the PDMS and will be exchanged between the vWF and the glycoprotein Ib factor to uncoil. Additionally, platelets are likely to be in the area of the high shear rate, since the platelet count, we obtained is high in these areas. Moreover, the residence time is larger than the time it takes to form a bond between the area of the high shear rate, since the platelet count, we obtained is high in these areas. Moreover, the residence time is larger than the time it takes to form a bond between the PLTs and fibrinogen were still present.

From the literature, we know that the binding time between the vWF and the glycoprotein Ibα is the lowest known in biology, faster than 10 μs [44]. As mentioned in the introduction, platelets can bind to vWF when the protein is uncoiled which happens above a shear rate of 5000 s⁻¹ [3], standard literature agrees that vasoconstriction happens upon vessel damage in haemostasis; however, they are not clear about the position in the vessel at which the vasoconstriction happens. Zucker [43] studied vasoconstriction in vivo in rats and found out that the vessel constricts downstream of the damaged site in the vessel. Together with our constricted vessel simulations it can be explained why vasoconstriction is happening before the damaged site and not at the site of the damage. However, this needs further investigations. Our findings are solely based and argument based on observed transport properties in our simulations. Our next step is to add platelet binding to the vessel wall and to each other to study the initial platelet aggregation in more detail, in our fully resolved cell-based blood flow model.

The increased platelet count per second and high residence time in regions with a high shear rate is our third main result. We were only interested in the platelets that are present in the CFL, because Tovar-Lopez et al. [11] demonstrated that platelets that contribute to the platelet aggregate are coming from the 15 to 25% of the blood stream proximal to the stenosis. When they depleted this layer from platelets, no platelet aggregate was formed. However, the plasma proteins vWF and fibrinogen were still present.

Figure 9. Two-dimensional projection of the averaged shear rate in the bottom layer of the flow chamber (ROI) of the bead assay simulation of the largest sphere (a) and smallest sphere (b) (γ = 675 s⁻¹). The position of the sphere is marked by the black circle. The simulation details are shown in the inset image. The dashed line gives the upper limit of the layers in which the shear rate is averaged. (Online version in colour.)

Figure 10. Two-dimensional projection of the averaged shear rate in the bottom layer of the flow chamber (ROI) of the bead assay simulation (γ = 1875 s⁻¹). The position of the sphere is marked by the black circle. The simulation details are shown in the inset image. The dashed line gives the upper limit of the layers in which the shear rate is averaged. (Online version in colour.)

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ones. Our hypothesis is that platelets stop rolling in the area behind the sphere due to lower shear rates and therefore they will have time to form stronger bonds with integrins.

As mentioned above, the vWF plays an important role in the formation of the platelet aggregate in high shear conditions [10]. In both experiments, we found that the shear rate is high enough to elongate the vWF that is bound to the sphere or the microcontraction wall, as explained above the shear rate should be higher than 1000 s⁻¹ [5]. This elongation will take place in the CFL. It is possible that the CFL in the uncoiling of the vWF is important as it provides a high shear rate zone. When the vWF is elongated, platelets can bind to it and an aggregate platelet is formed as explained above. The formation of an enlarged CFL is supported by a stenosis or as a result of vasocostriction, the thrombus itself, but also for example, by an atherosclerotic plaque or the struts of a stent.

Together these results provide relevant new insights into the haemodynamic environment in which a platelet aggregate starts to form. We conclude that the CFL together with an area of lower shear and an area of high shear upstream will in the presence of the vWF lead to the formation of a platelet aggregate. Nesbitt et al. [8] already concluded from their experiment that a local microgradient in shear rate, caused by a change in geometry or the thrombus itself, promotes platelet aggregate. However, we cannot compare our results directly to the results of Nesbitt et al. [8], since we are not modelling the platelet adhesion and aggregation. This will be the next step in our model development.

5. Conclusion

In this study, we investigated the influence of the flow dynamics on the initialization of a platelet aggregate. Based on the simulations, we conclude that an enlarged cell-depleted layer together with a high shear area in front of the CFL identifies the location where a platelet aggregate starts to form. We hypothesize that this cell-depleted layer is important for vWF to uncoil and form a platelet aggregate. Additionally, cell-based blood flow simulations are important when researchers want to assess the details of local haemodynamic parameters in their microfluidic devices, such as shear stress and shear rate.

Data accessibility. This article has no additional data. Authors’ contributions. B.J.M.v.R. performed the research, carried out the simulations and wrote the paper. V.W.A.T. helped with technical issues of Hemocell and revised the manuscript. G.Z. and A.G.H. conceived and supervised the research and revised the manuscript. All authors approved the final version of the manuscript.

Competing interests. We declare we have no competing interests. Funding. This work was supported by the European Union Horizon 2020 research and innovation programme under grant agreement no. 675451, the CompBioMed project. All authors are funded by CompBioMed. The use of supercomputer facilities in this work was sponsored by NWO Exacte Wetenschappen (Physical Sciences).

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