# Modelling the effects of cerebral microthrombi on tissue oxygenation and cell death

Yidan Xue1\*, Wahbi K. El-Bouri1,2, Tamás I. Józsa1 and Stephen J. Payne1

1 Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Oxford, UK

2 Liverpool Centre for Cardiovascular Science, Department of Cardiovascular and Metabolic Medicine, University of Liverpool, Liverpool, UK

\* Corresponding author. E-mail address: yidan.xue@eng.ox.ac.uk

**Abstract**

Thrombectomy, the mechanical removal of a clot, is the most common way to treat ischaemic stroke with large vessel occlusions. However, perfusion cannot always be restored after such an intervention. It has been hypothesised that the absence of reperfusion is at least partially due to the clot fragments that block the downstream vessels. In this paper, we present a new way of quantifying the effects of cerebral microthrombi on oxygen transport to tissue in terms of hypoxia and ischaemia. The oxygen transport was simulated with the Green’s function method on physiologically representative microvascular cubes, which was found independent of both microvascular geometry and length scale. The microthrombi occlusions were then simulated in the microvasculature, which were extravasated over time with a new thrombus extravasation model. The tissue hypoxic fraction was fitted as a sigmoidal function of vessel blockage fraction, which was then taken to be a function of time after the formation of microthrombi occlusions. A novel hypoxia-based 3-state cell death model was finally proposed to simulate the hypoxic tissue damage over time. Using the cell death model, the impact of a certain degree of microthrombi occlusions on tissue viability and microinfarct volume can be predicted over time. Quantifying the impact of microthrombi on oxygen transport and tissue death will play an important role in full brain models of ischaemic stroke and thrombectomy.

**Keywords**

Oxygen transport; Hypoxia; Cell death; Stroke; Thrombus

**Article type**: Full length article

# 1 Introduction

Stroke is one of the leading causes of death and disability in the world, while ischaemic stroke accounts for about 85% of cases (Johnson et al., 2019). During ischaemic stroke, large vessel occlusions lead to a significant reduction in cerebral blood flow (CBF) to regions of the brain and hence to brain tissue death (Dirnagl et al., 1999). Thrombectomy, the mechanical removal of a clot, is the most common surgical treatment to recanalize the vessel (Jovin et al., 2015). It remains unclear why complete reperfusion cannot always be achieved after mechanical recanalization. It has been hypothesised that the absence of reperfusion may be caused by downstream micro-occlusions, blood-brain barrier disruption or brain oedema (Molina, 2011). In this paper, we investigate the impact of microthrombi, which fragment off large clots during thrombectomy (Chueh et al., 2013, 2016; Molina, 2011), on tissue oxygenation and health.

Due to the limited resolution of current imaging techniques, it may not be possible to monitor microthrombi occlusions inside the human cerebral microvasculature, and hence to study the related clinical outcomes (Chueh et al., 2013; Gobin et al., 2004; van Veluw et al., 2017). Rodent models have thus been widely used to investigate cerebral micro-embolisms and micro-infarcts but these studies rely on assumed similarity between the human and rodent cerebral vasculature (Nishimura et al., 2007, 2010; Shih et al., 2013; van der Wijk et al., 2019). *In silico* modelling can be used as an alternative to study the effects of microthrombi on human brain tissues. Previous *in silico* microvasculature models include the capillary beds (El-Bouri & Payne, 2015; Linninger et al., 2013; Su et al., 2012) and penetrating vessels (El-Bouri & Payne, 2016; Linninger et al., 2013) generated from morphological data of the human cortex (Cassot et al., 2006, 2010; Lorthois et al., 2014). Recently, the effects of a penetrating vessel occlusion were simulated for the first time at a length scale comparable to that of MRI voxels, which can be directly validated against clinical images (El-Bouri & Payne, 2018).

The multidisciplinary INSIST (IN-Silico trials for treatment of acute Ischemic STroke, [www.insist-h2020.eu](http://www.insist-h2020.eu)) project aims to build a computational platform to evaluate medical interventions and devices for ischaemic stroke treatments (Konduri et al., 2020). As part of the project, virtual patients with organ-scale brain models are developed to simulate blood flow, oxygen transport and infarct progression during an ischaemic stroke (Józsa et al., 2021; Padmos et al., 2021). A model that simulates clot fragmentation and the resulting effects on perfusion and oxygen transport can be directly coupled with the current whole brain model to investigate the reasons for reperfusion failure after thrombectomy.

*In silico* models have been proposed to determine the impact of microthrombi occlusions on perfusion in the microvasculature (Cruz Hernández et al., 2019; El-Bouri et al., 2021; Schmid et al., 2021). Previously, we studied the effects of clot fragmentation on perfusion after thrombectomy (El-Bouri et al., 2021). The clot fragmentation and micro-emboli shower simulations were based on *in vitro* experimental data (Chueh et al., 2016). Blood flow was modelled inside statistically representative microvasculature models, including penetrating arterioles and capillaries (El-Bouri & Payne, 2015, 2016, 2018). The perfusion drops in microvascular voxels and cortical columns and their relationships with different blockage percentage were investigated. However, none of the aforementioned models simulate the impact of microthrombi on tissue oxygenation and cell death which is necessary to validate our *in silico* whole brain model against clinical imaging data from post-thrombectomy patients.

In this study, we examine the effects of micro-occlusions on oxygen transport to provide a direct link between occlusions and tissue response. To this end, the Green’s function method is used (Secomb et al., 2004). Thereafter, microvascular recovery is simulated by a new thrombus extravasation model (Grutzendler et al., 2014; Lam et al., 2010; van der Wijk et al., 2019). Additionally, a novel hypoxia-based cell death model is proposed to predict the tissue damage over time. These new models will enhance our organ-scale *in silico* brain model and enable future validation against clinical data.

# 2 Methods

## 2.1 Capillary networks and blood flow simulation

Statistically representative human capillary networks are used here to form the basis of the blood and oxygen transport simulations (El-Bouri & Payne, 2015). Blood flow was simulated through these networks using Poiseuille’s law assuming a constant haematocrit. The perfusion in each capillary cube was scaled to 55 ml/100g/min under healthy conditions. The methods describing the capillary networks and blood flow simulation can be found in Appendices A and B.

## 2.2 Oxygen transport simulation

The oxygen delivery from microvasculature to tissue was simulated using the well-established Green’s function method (Hsu & Secomb, 1989; Secomb et al., 2004). In Green’s function simulations, oxygen transport and metabolism depend on the partial pressure of oxygen (PO2) both in the blood vessels and the tissue. The oxygen transport was simulated by an integration of oxygen strength field generated from each blood vessel acting on each cubic tissue region. The cerebral metabolic rate of oxygen (CMRO2) was assumed to follow a Michaelis-Menten relationship dependent upon tissue PO2. The relationship between the blood oxygen saturation and the blood PO2 was assumed to be described by a Hill equation. The governing equations of these models are summarised in Appendix C. All of the model parameters remained the same as previously used (Secomb et al., 2004), except for the maximum CMRO2, which was set as based on human data (Payne & Lucas, 2018) and the haematocrit was assumed to be a constant value of 0.45 which has been used previously to simulate the blood flow (El-Bouri & Payne, 2015). This assumption will be re-examined below.

The inlet PO2 (PO2, in)was then varied from 13 mmHg to 50 mmHg in steps of 1 mmHg in each capillary cube, in order to explore a wide range of values. After running the simulation, PO2 and oxygen consumption were analysed to obtain the average tissue PO2, hypoxic fraction (taken here to be the fraction of tissue at a value of PO2 below 10 mmHg) (Georgakopoulou et al., 2021), CMRO2 and oxygen extraction fraction (OEF, fraction of blood oxygen taken by tissue). An example capillary cube and its Green’s function solutions under both normal and hypoxic conditions are shown in Fig. 1.

## 2.3 Micro-thrombi simulation inside the microvasculature

To mimic the presence of micro-thrombi inside the microvasculature, random occlusions were introduced in selected vessels of a 375-micron capillary network. A specified number of vessels was occluded to give a blockage fraction ranging from 1% to 20% in steps of 1% (rounded to the closest integer). At each blockage fraction, 5 different simulations were carried out with PO2, in set to be 40 mmHg (Sakadžić et al., 2014). Hence a total of 100 micro-blockage simulations have been run in this study.

In order to maintain network periodicity, the vessels connected to boundary nodes were excluded from occlusions. In addition, any occlusion simulation that divided the network into multiple isolated parts was disregarded to keep the connectivity, so that the conductance matrix (Eq. B2) remained invertible and the flow could be solved with the existing algorithm (El-Bouri & Payne, 2015). For the same purpose, the maximum blockage fraction was set as 20%.

After randomly blocking the vessels, the perfusion was simulated with the same pressure drop along the capillary cube as applied under healthy conditions, based on the assumption of normal cerebral autoregulation (Payne, 2016). The same Green’s function simulation and data post-processing were performed as described earlier.

## 2.4 Thrombus extravasation simulation

After the initial blockage, the blockage fraction was assumed to decay exponentially with time due to thrombus extravasation (Grutzendler et al., 2014; Lam et al., 2010). The blockage fraction thus can be written as

Equation 1

where is the initial blockage fraction and is the time constant. A recent extravasation study in a rat model (van der Wijk et al., 2019) was used for fitting the parameter as shown in Figure 2. The value of 1/day was found using 4 data points at 0, 1, 7, and 28 days. It should be noted that the thrombus extravasation model here is fitted with animal data (a rat model) due to the lack of human data. We thus assume that thrombus extravasation is similar in the rat and the human brain (Grutzendler et al., 2014), although it would be straightforward to adapt this model for human brain tissue should additional data become available.

## 2.5 Three-state cell death model

A new hypoxia-based 3-state cell death model is proposed here based on a similar concept from previous work studying hyperthermic cell death (O’Neill et al., 2011). Although this is a significant simplification to the complex mechanisms that govern the response to hypoxia, we chose this type of model as it has been proven successful in modelling hyperthermia and is readily suitable for experimental validation. In the proposed model (Eq. 2), there are three compartments representing the fraction of cells at each point in space and time that are in one of three states: alive (*A*), vulnerable (*V*) and dead (*D*), where . We assume that the process from *A* to *V* is reversible, but that from *V* to *D* is irreversible:

Equation 2

Equation 3

Equation 4

Here is the forward and the backward rate constant, both of which are assumed to depend solely on the hypoxic fraction (*H*) so that

Equation 5

Equation 6

where 1/s and 1/s represent forward and backward rates at zero hypoxia, respectively. These two constants will be further justified in Appendix D. Based on the Green’s function simulations, the hypoxic fraction can be expressed as a sigmoidal function of blockage fraction (Section 3.3).

## 2.6 Numerical procedure

The linear equations governing the blood flow were solved in MATLAB R2019b (MathWorks, USA). The oxygen diffusion-reaction equations were simulated by Green’s function methods using the publicly available implementation, written in C++[[1]](#footnote-2) and recently applied to oxygen delivery (Celaya-Alcala et al., 2021; Secomb et al., 2020). Every Green’s function simulation in this manuscript was conducted under an overall convergence tolerance of . Curve fitting (non-linear least square optimization in SciPy), ordinary differential equations solving (integrate library in SciPy) and post-processing were carried out in Python (Virtanen et al., 2020).

# 3 Results

## 3.1 Capillary networks and Green’s function method solutions

One example of a 375-micron capillary network cube is shown in Fig. 1a, where the vessel diameter is represented by the line thickness. Figures 1b and 1c display the tissue PO2 distributions generated by the Green’s function method inside the same network under normal and hypoxic conditions respectively. Figures 1e and 1f show the tissue oxygenation and the positions of intersecting vessels on the centre plane (Fig. 1d). Tissue PO2 is distributed heterogeneously but is closely correlated with the geometry of the microvasculature.

## 3.2 Independence of oxygen transport on microvascular geometry and length scale

Figure 3 shows average tissue PO2 and hypoxic fraction (fraction of tissue with PO2 below 10 mmHg) for values of PO2, in in the range 13 to 50 mmHg in the capillary cubes at two sizes (375 μm and 625 μm). Average tissue PO2 varies approximately linearly with the PO2, in within this range. The hypoxic fraction however has a strongly non-linear relationship with PO2, in.

Figure 4 presents the values of CMRO2 and OEF in the same range of PO2, in in the capillary cubes at the two sizes. Changing PO2, in from 50 to 13 mmHg is followed by a nonlinear CMRO2 decrease alongside OEF increase to compensate oxygen shortage.

The small standard deviation of the results between the different capillary cubes indicates that the oxygen transport at the microvasculature scale is largely independent of the specific geometry of the individual capillary networks, as long as the geometrical properties of the networks are maintained (see error bars in Figs. 3 and 4). The standard deviation is smaller in the 625-micron cubes than in the 375-micron cubes indicating that the effect of microvascular topology and geometry on the oxygen transport becomes increasingly negligible in a larger simulation domain. The small difference between the results in cubes with two different sizes (Figs. 3a and 3b; Figs. 4a and 4b) suggests that our oxygen transport simulation is length scale independent above a few hundred microns. This finding agrees with results for permeability (El-Bouri & Payne, 2015).

We set the hypoxic fraction threshold to be 10 mmHg (Georgakopoulou et al., 2021). The effects of hypoxic threshold on hypoxic fraction are shown in Fig. 5. The general sigmoidal shape of hypoxic fraction against PO2, in is not changed under different hypoxic thresholds. However, the curve will move horizontally when the hypoxic threshold varies. These results indicate that the hypoxic threshold should be matched with the experimental study carefully when fitting the parameters and validating the simulation results.

## 3.3 Microthrombi have significant impact on tissue hypoxia beyond 10% vessel occlusions

Figure 6 presents the change in perfusion and hypoxic fraction when the vessels in the capillary network are blocked to mimic the presence of micro-thrombi. Since the pressure drop along the capillary cube under occlusion conditions is the same as applied under healthy conditions, the perfusion change (Fig. 6a) is thus only due to the increased network resistance caused by microthrombi. Relative perfusion decreases linearly with the increasing blockage fraction in this range, which agrees with previous simulations (Cruz Hernández et al., 2019; El-Bouri et al., 2021). The slope of decrease in this study is -3.1 %/%, which is comparable to -2.5 %/% (Cruz Hernández et al., 2019) and -3.2 %/% (El-Bouri et al., 2021). In Figure 6b, the hypoxic fraction remains almost unchanged until the blockage fraction reaches 0.1. Beyond this threshold, the hypoxic fraction increases steeply. To describe the results of the simulations in a form suitable for larger-scale simulations, we characterized the results for hypoxic fraction, which was fitted to a sigmoidal relationship dependent upon the blockage fraction (*B*) as:

Equation 7

where a = 30.71 and b = -5.35 are two constants derived from curve fitting.

Note that at a 10% blockage fraction, there are 56 microthrombi inside a 375-micron capillary cube with a total volume of mL, assuming that each vessel is blocked by a single spherical thrombus with diameter equal to the diameter of the corresponding vessel. This results in a microthrombi volume of mL per mL cerebral microvascular volume. If we assume that there are micro-embolisms in a territory of 100 mL fed by middle cerebral artery, the total microthrombi volume will be mL. Assuming that the volume of a thrombus is 0.17 mL (Baek et al., 2017) and that 25% of clot fragments are transported into capillary networks, the total microthrombi volume will be mL, which is about 2,600 times more than the volume predicted from blocked capillaries. These results indicate that the microthrombi in larger vessels must be considered to match the thrombus volume, due to the third power of radius in sphere volume. The results of thrombus volume here thus seem to provide a lower limit of the volume required to give a 10% micro-embolism blockage fraction. When considering blockage of both capillary beds and upstream larger vessels, tissue hypoxia will result from a combined effect of micro-occlusions and ischaemia, which would appear likely to be the real clinical situation based on thrombus volume calculations.

## 3.4 Cell viability with increasing micro-embolism

Figure 7 shows the cell viability predicted from the proposed 3-state cell death model in different scenarios. In these six example predictions, the initial blockage fraction ranges from 0 to 25% with a step of 5% in order to demonstrate the model response under different conditions. When there is no occlusion in the capillary network (Fig. 7a), the cell viability maintains near 100%. The small gap between alive cell fraction and 100% is negligible over the time scale of usual clinical practice.

With a small initial blockage fraction of 5% (Fig. 7b), most of the cells remain alive due to small hypoxic fraction. This can be well explained by the interconnected nature of the topology of the cerebral microvasculature, which gives good resistance to mild microthrombi occlusions. As the blockage fraction increases to 20% (Figs. 7c, 7d and 7e), there is a substantial change in model behaviour. The sharp increase in cell death speed and final cell death fraction is caused by the sigmoidal dependence of hypoxic fraction with blockage fraction (Fig. 6b). The blockage fraction of 10% thus appears to be a threshold deciding the final cell death fraction. Beyond the threshold, a further increase in blockage fraction contributes little to the model behaviour (Fig. 7f), since most of the tissue regions have become hypoxic (below 10 mmHg, based on our definition). Figure 8 shows a sensitivity analysis on cell death model parameters, further discussed in Appendix D.

# 4 Discussion

In this paper, we present a new way to quantify and to analyse the effects of cerebral microthrombi on oxygen transport and tissue viability for the first time. The oxygen transport inside the cerebral microvasculature is strongly influenced by the network geometry and thus leads to a heterogeneous distribution of oxygen levels. However, the overall feature of cerebral oxygen transport in a representative elementary cube is found to be essentially largely independent of the microvascular geometry, which is in line with our previous study on cerebral blood flow (El-Bouri & Payne, 2015). In addition, the oxygen transport results are found to be independent of length scale. The vessel blockage fraction has a linear impact on perfusion, which agrees with previous simulations (Cruz Hernández et al., 2019; El-Bouri et al., 2021). However, the relationship between blockage fraction and hypoxic fraction is found to be strongly non-linear, modelled here with a sigmoidal function. With the thrombus extravasation model and the cell death model, the impact of a certain number of microthrombi on tissue viability can be predicted at a given time after the formation of such microthrombi.

The periodic, statistically representative microvasculature cubes used here remove the need to reconstruct the vessel geometries in detail and to set the boundary conditions. The cubes can also be connected to represent a larger microvascular domain due to its periodicity, which has been demonstrated in recent studies (El-Bouri & Payne, 2018; Gkontra et al., 2019). The Green’s function method significantly reduces the computational costs of solving the oxygen transport in a complex network by transforming 3D diffusion equations into a 1D Green’s function. This study implements the Green’s function method in statistically representative capillary networks based on human morphometric data (Cassot et al., 2006), which provides a pathway to investigate oxygen transport in the human microvasculature with limited experimental data and much reduced computing power.

The prediction of irreversible tissue damage is always a tricky task, which is usually neglected in cerebral metabolism models (Aubert & Costalat, 2005; Cloutier et al., 2009; Orlowski et al., 2011). Here we implemented a 3-state cell death model, a similar form of which was used in a previous hyperthermic cell death study (O’Neill et al., 2011). The model has the advantage of modelling the highly non-linear process of tissue damage. In this study, temperature-related rate constants in the 3-state model are replaced with hypoxia-related rate constants so that the cell death rate is positively correlated with tissue hypoxic fraction and the forward pathway will shut down as hypoxia is relieved by the extravasation of microthrombi.

The primary advantage of this workflow is its possibility to scale up the local behaviour inside a capillary cube to the tissue scale easily, and to couple with existing models at both a cortical column scale (El-Bouri & Payne, 2016; El-Bouri & Payne, 2018; El-Bouri et al., 2021) and a whole brain scale (Józsa et al., 2021; Padmos et al., 2021), based on the independence of oxygen transport on the microvascular geometry and length scale of the capillary cubes (Section 3.2).

One limitation of this study is the assumption of no oxygen supply from larger vessels like arterioles, however, the oxygen transport from vessels to tissue happens over several vessel generations (Vovenko, 1999; Boas et al., 2008; Sakadžić et al., 2014; Payne & Lucas, 2018). The oxygen transport from larger vessels cannot be simulated with the current capillary cubes, which limits the length scale at which this model can be used. In future studies, the oxygen transport from larger vessels could be modelled when the microvascular model is coupled with the penetrating vessel models (El-Bouri & Payne, 2016), as has previously been performed with blood flow (El-Bouri & Payne, 2018).

Another limitation is the partial use of animal data and parameters, when this study is intended to be applied on human cerebral microvasculature. Rat data have been used to derive the parameters in the Hill equation for blood oxygen saturation in the Green’s function method (Secomb et al., 2004) and to fit the thrombus extravasation model (van der Wijk et al., 2019). Here we assume that these physiological relationships or processes are very similar in human and rats. However, once more human data become available, these models can be easily updated.

Additionally, constant haematocrit in the capillary networks is assumed in this study. However, the haematocrit is not evenly distributed at microvascular bifurcations or trifurcations due to phase separation (Pries et al., 1989; Gould & Linninger, 2015; Gould et al., 2017; Secomb, 2017; Hartung et al., 2018, 2021). The variance of intravascular haematocrit in each blood vessel can have effects on blood flow (viscosity) and oxygen transport (oxygen carrying capacity) at a local scale, although at the length scales studied here, previous studies have indicated that this effect is likely to be second order (Su, 2012; El-Bouri & Payne, 2015).

The cell death model used in this paper is a significant simplification of the real physiological process of tissue damage. Although it may lack a detailed physiological basis, the presented cell death model is one of the simplest models which can describe a highly nonlinear scenario with only 2 parameters. It thus can prevent over-fitting, since the temporal data from experiments are very limited. However, the quantitative conclusions cannot be achieved until fitting of the model parameters with the experimental data has been attempted, which will be the next step.

Lastly, the models proposed in this study are purely passive, although local perfusion and oxygen transport is actively and tightly controlled (Payne, 2017). In addition, this paper focuses on the averaged properties inside the capillary cubes, while neglecting the heterogeneous distribution of hypoxic regions (Hartung et al., 2021). These topics will be the subjects of future studies.

In summary, this paper has applied the Green’s function method on periodic, statistically representative human capillary networks to investigate the oxygen transport inside the cerebral microvasculature. With a thrombus extravasation model and a novel 3-state cell death model, the effect of microthrombi on oxygen transport and hypoxic cell death can be simulated. Such an approach will be a key part to the prediction of tissue damage in response to microthrombi.

# Declaration of Competing Interest

There are no conflicts of interest.

# Acknowledgement

This work was partially funded by the European Union's Horizon 2020 research and innovation programme, the INSIST project, under grant agreement No 777072.

# Figures

A picture containing graphical user interface

Description automatically generated

Figure 1. 3D visualisation of an example capillary network (a) and the tissue oxygenation inside it solved by the Green's function method under normal condition (b) and hypoxic condition (c). A horizontal plane (d) is placed in the centre of the capillary network and the tissue oxygenation on the plane is plotted under both normal (e) and hypoxic (f) conditions. The black crosses indicate the intersecting points between the centrelines of blood vessels and the plane.



Figure 2. The extravasation of thrombi from occluded vessels over time. The experimental data (van der Wijk et al., 2019) are taken from a rat model.

Chart, line chart, histogram

Description automatically generated

Figure 3. Hypoxic fraction and average tissue PO2 in the capillary networks based on cube sizes of 375 μm (a) and 625 μm (b). The error bar shows the standard deviation of the results among 10 different cubes.

Chart, histogram

Description automatically generated

Figure 4. Oxygen extraction fraction and oxygen consumption rate in the capillary networks based on cube sizes of 375 μm (a) and 625 μm (b). The error bar shows the standard deviation of the results among 10 different cubes.



Figure 5. The average hypoxic fraction in 10 capillary networks based on cube sizes of 375 μm against PO2, in under hypoxic thresholds from 6 to 14 mmHg.

Chart, line chart

Description automatically generated

Figure 6. The perfusion (a) and hypoxic fraction (b) as functions of microthrombi blockage fraction.



Figure 7. Predicted cell viability and hypoxic fraction over 7 days with different initial microthrombi blockage fraction.



Figure 8. Final dead fraction at the end of Day 7 under different initial blockage fractions and combination of rate constants in the 3-state cell death model. The forward and backward rate constants used in the paper are indicated as the red cross.

# Appendix A: Capillary networks

The statistically representative human capillary networks (El-Bouri & Payne, 2015) were generated as detailed by Su et al. (2012). The artificial networks match morphometrically the human cerebral capillaries, including connectivity, vessel density, length and diameter distributions (Cassot et al., 2006).In order to demonstrate the independence of oxygen transport results on length scales, two randomised capillary cubes were selected (375 μm and 625 μm) both within the convergence range of the permeability tensor (El-Bouri & Payne, 2015). At each size, 10 cubes were used to investigate the dependence of oxygen transport on the capillary network.

# Appendix B: Blood flow simulation

The same method was used here as in previous studies to calculate the blood flow in each vessel (El-Bouri & Payne, 2015). Poiseuille flow was assumed for each blood vessel and periodic boundary conditions were assigned for the vessel boundary nodes on the cube surface. With the periodic boundary conditions, the blood inflow (outflow) at the boundary node is equal to the outflow (inflow) at the relative node on the opposite cube surface. The baseline value of perfusion in each cube was scaled to 55 ml/100g/min under healthy conditions, by adjusting the pressure difference, where perfusion was calculated as the ratio of the inlet volumetric blood flow rate and the cube volume divided by tissue density (taken here to be 1.05 g/cm3) (Nelson et al., 1971; Payne, 2017).

In general, blood flow is governed by the Navier-Stokes equations. Under the assumption of steady-state, axisymmetric and fully-developed flow along the direction of the blood vessels, the blood flow inside the capillary network can be represented by Hagen-Poiseuille equation:

Equation B

where is pressure, is flow rate, is the vessel length, is the vessel radius, is the flow conductance and is the apparent blood viscosity, which is an empirical equation of vessel diameter and haematocrit (Pries et al., 1992, 1994). The details of implementing the empirical equations in the capillary network can be found in Su (2012).

Since there is a linear relationship between pressure drop and flow rate in each vessel segment, the flow characteristics inside the capillary network can be described by the conductance matrix as:

Equation B2

where and are boundary nodes of capillary segments and is the flow rate from to . is equal to , if there is no connection between node and node . Due to flow conservation at each node, the pressure at each capillary segment node inside the network can be solved as:

Equation B3

where is the source term at each node. The flow in each segment can thus be solved using Eq. B2.

# Appendix C: Governing equations for Green’s function method and oxygen reaction-diffusion models

The Green’s function method has been introduced in detail in previous studies (Hsu & Secomb, 1989; Secomb et al., 2004). The oxygen transport in brain tissue can be simplified as a reaction-diffusion equation:

Equation C1

where is the diffusion coefficient, is the tissue oxygen solubility and is the partial pressure of oxygen and is the metabolic rate. According to potential theory, the steady-state diffusion of oxygen is rewritten as a Green’s function between every point source of capillary segments and every point sink evenly distributed in the tissue as:

Equation C

where is the Green’s function, is a three-dimensional Dirac delta function, is a point inside the tissue and is a point source on the capillary surface. In an infinite domain, the Green’s function can be solved as:

Equation C

By integrating the oxygen potentials from all sources (surfaces of all capillary segments), the oxygenation of each tissue point can be calculated as:

Equation C

where is the total tissue-vessel boundaries, is the distribution of the source strengths on these boundaries and is a constant updated in each iteration.

In this study, 25 tissue points were placed along each dimension of the capillary cubes to give good spatial resolution (Figs. 1). The spacings of tissue points are 15 μm (375 μm cubes) and 25 μm (625 μm cubes), which match the range of 10-30 μm used in a previous study (Secomb et al., 2004). The maximum capillary vessel segment length required by the algorithm was set as 100 microns, which ensured that any vessel segment longer than 100 microns would be divided into smaller segments.

The consumption of each tissue point is represented by a Michaelis-Menten relationship with oxygenation:

Equation C

where is the maximum metabolic rate of oxygen and is the Michaelis-Menten constant.

The oxygen transport in blood vessels follow the 1D mass transport equation. The blood oxygen concentration can be expressed as:

Equation C

where is the blood oxygen solubility, is blood PO2, is the haemoglobin-bound oxygen capacity in fully saturated red blood cells, is the haematocrit and is the oxygen saturation of haemoglobin. The oxygen saturation of haemoglobin follows a Hill equation with partial pressure of oxygen as:

Equation C

where is the Hill equation exponent and is PO2 at half maximal haemoglobin saturation. All the Green’s function parameters are the same as previously used (Secomb et al., 2004), except for values of maximum metabolic rate of oxygen and haematocrit that are quoted in the main text.

# Appendix D: Sensitivity analysis for the cell death model

Figure 8 shows a sensitivity analysis on the forward rate constant () and the backward rate constant () in the cell death model, which are currently unknown and will be fitted with the experimental data (Georgakopoulou et al., 2021) in a future study. The final dead fraction at the end of Day 7 with forward and backward rate constants in the range to 1/s is plotted for a range of different initial blockage fractions. The combination of rate constants selected for use in this paper is indicated with the red cross. The clear and narrow boundary between the yellow region (high dead fraction) and the blue region (low dead fraction) indicates that there is a distinct threshold for the combination of two rate constants when simulating the final tissue damage. This threshold can be explained by the thrombus extravasation model (Eq. 1) where there is no tissue damage when the forward death rate, which is proportional to the forward rate constant and hypoxic fraction, is much smaller than the extravasation rate under a certain initial blockage fraction. The threshold can also be explained by the relationship between the two time constants. When there is a much larger backward rate than forward rate, there is also no tissue damage due to many more cells being saved from the vulnerable compartment than dying. This can be observed as the blue regions at the top left corner of each plot in Fig. 8. These all contribute to the highly non-linear behaviour of the cell death model.

**References**

Aubert, A., & Costalat, R. (2005). Interaction between astrocytes and neurons studied using a mathematical model of compartmentalized energy metabolism. *Journal of Cerebral Blood Flow and Metabolism*, *25*(11), 1476–1490. https://doi.org/10.1038/sj.jcbfm.9600144

Baek, J.-H., Yoo, J., Song, D., Kim, Y. D., Nam, H. S., Kim, B. M., Kim, D. J., Lee, H. S., & Heo, J. H. (2017). Predictive value of thrombus volume for recanalization in stent retriever thrombectomy. *Scientific Reports*, *7*(1), 15938. https://doi.org/10.1038/s41598-017-16274-9

Boas, D. A., Jones, S. R., Devor, A., Huppert, T. J., & Dale, A. M. (2008). A vascular anatomical network model of the spatio-temporal response to brain activation. *NeuroImage*, *40*(3), 1116–1129. https://doi.org/10.1016/j.neuroimage.2007.12.061

Cassot, F., Lauwers, F., Fouard, C., Prohaska, S., & Lauwers-Cances, V. (2006). A novel three-dimensional computer-assisted method for a quantitative study of microvascular networks of the human cerebral cortex. *Microcirculation*, *13*(1), 1–18. https://doi.org/10.1080/10739680500383407

Cassot, F., Lauwers, F., Lorthois, S., Puwanarajah, P., Cances-Lauwers, V., & Duvernoy, H. (2010). Branching patterns for arterioles and venules of the human cerebral cortex. *Brain Research*, *1313*, 62–78. https://doi.org/10.1016/j.brainres.2009.12.007

Celaya-Alcala, J. T., Lee, G. V., Smith, A. F., Li, B., Sakadžić, S., Boas, D. A., & Secomb, T. W. (2020). Simulation of oxygen transport and estimation of tissue perfusion in extensive microvascular networks: Application to cerebral cortex. *Journal of Cerebral Blood Flow & Metabolism*, 0271678X2092710. https://doi.org/10.1177/0271678X20927100

Chueh, J. Y., Kühn, A. L., Puri, A. S., Wilson, S. D., Wakhloo, A. K., & Gounis, M. J. (2013). Reduction in distal emboli with proximal flow control during mechanical thrombectomy: A quantitative in vitro study. *Stroke*, *44*(5), 1396–1401. https://doi.org/10.1161/STROKEAHA.111.670463

Chueh, J. Y., Puri, A. S., Wakhloo, A. K., & Gounis, M. J. (2016). Risk of distal embolization with stent retriever thrombectomy and ADAPT. *Journal of NeuroInterventional Surgery*, *8*(2), 197–202. https://doi.org/10.1136/neurintsurg-2014-011491

Cloutier, M., Bolger, F. B., Lowry, J. P., & Wellstead, P. (2009). An integrative dynamic model of brain energy metabolism using in vivo neurochemical measurements. *Journal of Computational Neuroscience*, *27*(3), 391–414. https://doi.org/10.1007/s10827-009-0152-8

Cruz Hernández, J. C., Bracko, O., Kersbergen, C. J., Muse, V., Haft-Javaherian, M., Berg, M., Park, L., Vinarcsik, L. K., Ivasyk, I., Rivera, D. A., Kang, Y., Cortes-Canteli, M., Peyrounette, M., Doyeux, V., Smith, A., Zhou, J., Otte, G., Beverly, J. D., Davenport, E., … Schaffer, C. B. (2019). Neutrophil adhesion in brain capillaries reduces cortical blood flow and impairs memory function in Alzheimer’s disease mouse models. *Nature Neuroscience*, *22*(3), 413–420. https://doi.org/10.1038/s41593-018-0329-4

Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: An integrated view. *Trends in Neurosciences*, *22*(9), 391–397. https://doi.org/10.1016/S0166-2236(99)01401-0

El-Bouri, W. K., MacGowan, A., Józsa, T. I., Gounis, M. J., & Payne, S. J. (2021). Modelling the impact of clot fragmentation on the microcirculation after thrombectomy. *PLoS Computational Biology*, *17*(3). https://doi.org/10.1371/journal.pcbi.1008515

El-Bouri, W. K., & Payne, S. J. (2015). Multi-scale homogenization of blood flow in 3-dimensional human cerebral microvascular networks. *Journal of Theoretical Biology*, *380*, 40–47. https://doi.org/10.1016/j.jtbi.2015.05.011

El-Bouri, W. K., & Payne, S. J. (2016). A statistical model of the penetrating arterioles and venules in the human cerebral cortex. *Microcirculation*, *23*(7), 580–590. https://doi.org/10.1111/micc.12318

El-Bouri, W. K., & Payne, S. J. (2018). Investigating the effects of a penetrating vessel occlusion with a multi-scale microvasculature model of the human cerebral cortex. *NeuroImage*, *172*(January), 94–106. https://doi.org/10.1016/j.neuroimage.2018.01.049

Georgakopoulou, T., van der Wijk, A.-E., Bakker, E. N. T. P., & VanBavel, E. (2021). Recovery of Hypoxic Regions in a Rat Model of Microembolism. *Journal of Stroke and Cerebrovascular Diseases*, *30*(6), 105739. https://doi.org/10.1016/j.jstrokecerebrovasdis.2021.105739

Gkontra, P., El‐Bouri, W. K., Norton, K., Santos, A., Popel, A. S., Payne, S. J., & Arroyo, A. G. (2019). Dynamic Changes in Microvascular Flow Conductivity and Perfusion After Myocardial Infarction Shown by Image‐Based Modeling. *Journal of the American Heart Association*, *8*(7). https://doi.org/10.1161/JAHA.118.011058

Gobin, Y. P., Starkman, S., Duckwiler, G. R., Grobelny, T., Kidwell, C. S., Jahan, R., Pile-Spellman, J., Segal, A., Vinuela, F., & Saver, J. L. (2004). MERCI 1: A phase 1 study of mechanical embolus removal in cerebral ischemia. *Stroke*, *35*(12), 2848–2853. https://doi.org/10.1161/01.STR.0000147718.12954.60

Gould, I. G., & Linninger, A. A. (2015). Hematocrit Distribution and Tissue Oxygenation in Large Microcirculatory Networks. *Microcirculation*, *22*(1), 1–18. https://doi.org/10.1111/micc.12156

Gould, I. G., Tsai, P., Kleinfeld, D., & Linninger, A. (2017). The capillary bed offers the largest hemodynamic resistance to the cortical blood supply. *Journal of Cerebral Blood Flow and Metabolism*, *37*(1), 52–68. https://doi.org/10.1177/0271678X16671146

Grutzendler, J., Murikinati, S., Hiner, B., Ji, L., Lam, C. K., Yoo, T., Gupta, S., Hafler, B. P., Adelman, R. A., Yuan, P., & Rodriguez, G. (2014). Angiophagy Prevents Early Embolus Washout But Recanalizes Microvessels Through Embolus Extravasation. *Science Translational Medicine*, *6*(226), 226ra31-226ra31. https://doi.org/10.1126/scitranslmed.3006585

Hartung, G., Badr, S., Moeini, M., Lesage, F., Kleinfeld, D., Alaraj, A., & Linninger, A. (2021). Voxelized simulation of cerebral oxygen perfusion elucidates hypoxia in aged mouse cortex. *PLOS Computational Biology*, *17*(1), e1008584. https://doi.org/10.1371/journal.pcbi.1008584

Hartung, G., Vesel, C., Morley, R., Alaraj, A., Sled, J., Kleinfeld, D., & Linninger, A. (2018). Simulations of blood as a suspension predicts a depth dependent hematocrit in the circulation throughout the cerebral cortex. *PLOS Computational Biology*, *14*(11), e1006549. https://doi.org/10.1371/journal.pcbi.1006549

Hsu, R., & Secomb, T. W. (1989). A Green’s function method for analysis of oxygen delivery to tissue by microvascular networks. *Mathematical Biosciences*, *96*(1), 61–78. https://doi.org/10.1016/0025-5564(89)90083-7

Johnson, C. O., Nguyen, M., Roth, G. A., Nichols, E., Alam, T., Abate, D., Abd-Allah, F., Abdelalim, A., Abraha, H. N., Abu-Rmeileh, N. M., Adebayo, O. M., Adeoye, A. M., Agarwal, G., Agrawal, S., Aichour, A. N., Aichour, I., Aichour, M. T. E., Alahdab, F., Ali, R., … Murray, C. J. L. (2019). Global, regional, and national burden of stroke, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *18*(5), 439–458. https://doi.org/10.1016/S1474-4422(19)30034-1

Jovin, T. G., Chamorro, A., Cobo, E., de Miquel, M. A., Molina, C. A., Rovira, A., San Román, L., Serena, J., Abilleira, S., Ribó, M., Millán, M., Urra, X., Cardona, P., López-Cancio, E., Tomasello, A., Castaño, C., Blasco, J., Aja, L., Dorado, L., … Dávalos, A. (2015). Thrombectomy within 8 Hours after Symptom Onset in Ischemic Stroke. *New England Journal of Medicine*, *372*(24), 2296–2306. https://doi.org/10.1056/nejmoa1503780

Józsa, T. I., Padmos, R. M., Samuels, N., El-Bouri, W. K., Hoekstra, A. G., & Payne, S. J. (2021). A porous circulation model of the human brain for in silico clinical trials in ischaemic stroke. *Interface Focus*, *11*(1), 20190127. https://doi.org/10.1098/rsfs.2019.0127

Konduri, P. R., Marquering, H. A., van Bavel, E. E., Hoekstra, A., & Majoie, C. B. L. M. (2020). In-Silico Trials for Treatment of Acute Ischemic Stroke. *Frontiers in Neurology*, *11*(September), 1–8. https://doi.org/10.3389/fneur.2020.558125

Lam, C. K., Yoo, T., Hiner, B., Liu, Z., & Grutzendler, J. (2010). Embolus extravasation is an alternative mechanism for cerebral microvascular recanalization. *Nature*, *465*(7297), 478–482. https://doi.org/10.1038/nature09001

Linninger, A. A., Gould, I. G., Marinnan, T., Hsu, C. Y., Chojecki, M., & Alaraj, A. (2013). Cerebral microcirculation and oxygen tension in the human secondary cortex. *Annals of Biomedical Engineering*, *41*(11), 2264–2284. https://doi.org/10.1007/s10439-013-0828-0

Lorthois, S., Lauwers, F., & Cassot, F. (2014). Tortuosity and other vessel attributes for arterioles and venules of the human cerebral cortex. *Microvascular Research*, *91*, 99–109. https://doi.org/10.1016/j.mvr.2013.11.003

Molina, C. A. (2011). Reperfusion Therapies for Acute Ischemic Stroke: Current Pharmacological and Mechanical Approaches. *Stroke*, *42*(1, Supplement 1), S16–S19. https://doi.org/10.1161/STROKEAHA.110.598763

Nelson, S. R., Mantz, M. L., & Maxwell, J. A. (1971). Use of specific gravity in the measurement of cerebral edema. *Journal of Applied Physiology*, *30*(2), 268–271. https://doi.org/10.1152/jappl.1971.30.2.268

Nishimura, N., Rosidi, N. L., Iadecola, C., & Schaffer, C. B. (2010). Limitations of collateral flow after occlusion of a single cortical penetrating arteriole. *Journal of Cerebral Blood Flow and Metabolism*, *30*(12), 1914–1927. https://doi.org/10.1038/jcbfm.2010.157

Nishimura, N., Schaffer, C. B., Friedman, B., Lyden, P. D., & Kleinfeld, D. (2007). Penetrating arterioles are a bottleneck in the perfusion of neocortex. *Proceedings of the National Academy of Sciences*, *104*(1), 365–370. https://doi.org/10.1073/pnas.0609551104

O’Neill, D. P., Peng, T., Stiegler, P., Mayrhauser, U., Koestenbauer, S., Tscheliessnigg, K., & Payne, S. J. (2011). A three-state mathematical model of hyperthermic cell death. *Annals of Biomedical Engineering*, *39*(1), 570–579. https://doi.org/10.1007/s10439-010-0177-1

Orlowski, P., Chappell, M., Park, C. S., Grau, V., & Payne, S. (2011). Modelling of pH dynamics in brain cells after stroke. *Interface Focus*, *1*(3), 408–416. https://doi.org/10.1098/rsfs.2010.0025

Padmos, R. M., Józsa, T. I., El-Bouri, W. K., Konduri, P. R., Payne, S. J., & Hoekstra, A. G. (2021). Coupling one-dimensional arterial blood flow to three-dimensional tissue perfusion models for in silico trials of acute ischaemic stroke. *Interface Focus*, *11*(1), 20190125. https://doi.org/10.1098/rsfs.2019.0125

Payne, S. (2016). *Cerebral Autoregulation*. Springer International Publishing. https://doi.org/10.1007/978-3-319-31784-7

Payne, S. J. (2017). *Cerebral Blood Flow and Metabolism*. WORLD SCIENTIFIC. https://doi.org/10.1142/10463

Payne, S. J., & Lucas, C. (2018). Oxygen delivery from the cerebral microvasculature to tissue is governed by a single time constant of approximately 6 seconds. *Microcirculation*, *25*(2), e12428. https://doi.org/10.1111/micc.12428

Pries, A. R., Ley, K., Claassen, M., & Gaehtgens, P. (1989). Red cell distribution at microvascular bifurcations. *Microvascular Research*, *38*(1), 81–101. https://doi.org/10.1016/0026-2862(89)90018-6

Pries, A. R., Neuhaus, D., & Gaehtgens, P. (1992). Blood viscosity in tube flow: dependence on diameter and hematocrit. *American Journal of Physiology-Heart and Circulatory Physiology*, *263*(6), H1770–H1778. https://doi.org/10.1152/ajpheart.1992.263.6.H1770

Pries, A. R., Secomb, T. W., Geßner, T., Sperandio, M. B., Gross, J. F., & Gaehtgens, P. (1994). Resistance to blood flow in microvessels in vivo. *Circulation Research*, *75*(5), 904–915. https://doi.org/10.1161/01.RES.75.5.904

Sakadžić, S., Mandeville, E. T., Gagnon, L., Musacchia, J. J., Yaseen, M. A., Yucel, M. A., Lefebvre, J., Lesage, F., Dale, A. M., Eikermann-Haerter, K., Ayata, C., Srinivasan, V. J., Lo, E. H., Devor, A., & Boas, D. A. (2014). Large arteriolar component of oxygen delivery implies a safe margin of oxygen supply to cerebral tissue. *Nature Communications*, *5*(1), 5734. https://doi.org/10.1038/ncomms6734

Schmid, F., Conti, G., Jenny, P., & Weber, B. (2021). The severity of microstrokes depends on local vascular topology and baseline perfusion. *ELife*, *10*. https://doi.org/10.7554/eLife.60208

Secomb, T. W. (2017). Blood Flow in the Microcirculation. *Annual Review of Fluid Mechanics*, *49*(1), 443–461. https://doi.org/10.1146/annurev-fluid-010816-060302

Secomb, T. W., Bullock, K. V, Boas, D. A., & Sakadžić, S. (2020). The mass transfer coefficient for oxygen transport from blood to tissue in cerebral cortex. *Journal of Cerebral Blood Flow & Metabolism*, *40*(8), 1634–1646. https://doi.org/10.1177/0271678X19870068

Secomb, T. W., Hsu, R., Park, E. Y. H., & Dewhirst, M. W. (2004). Green’s function methods for analysis of oxygen delivery to tissue by microvascular networks. *Annals of Biomedical Engineering*, *32*(11), 1519–1529. https://doi.org/10.1114/B:ABME.0000049036.08817.44

Shih, A. Y., Blinder, P., Tsai, P. S., Friedman, B., Stanley, G., Lyden, P. D., & Kleinfeld, D. (2013). The smallest stroke: Occlusion of one penetrating vessel leads to infarction and a cognitive deficit. *Nature Neuroscience*, *16*(1), 55–63. https://doi.org/10.1038/nn.3278

Su, S.-W. (2012). *Modelling Blood Flow and Oxygen Transport in the Human Cerebral Cortex*. DPhil Thesis.

Su, S.-W., Catherall, M., & Payne, S. (2012). The Influence of Network Structure on the Transport of Blood in the Human Cerebral Microvasculature. *Microcirculation*, *19*(2), 175–187. https://doi.org/10.1111/j.1549-8719.2011.00148.x

van der Wijk, A.-E., Lachkar, N., de Vos, J., Grootemaat, A. E., van der Wel, N. N., Hordijk, P. L., Bakker, E. N. T. P., & VanBavel, E. (2019). Extravasation of Microspheres in a Rat Model of Silent Brain Infarcts. *Stroke*, *50*(6), 1590–1594. https://doi.org/10.1161/STROKEAHA.119.024975

van Veluw, S. J., Shih, A. Y., Smith, E. E., Chen, C., Schneider, J. A., Wardlaw, J. M., Greenberg, S. M., & Biessels, G. J. (2017). Detection, risk factors, and functional consequences of cerebral microinfarcts. *The Lancet Neurology*, *16*(9), 730–740. https://doi.org/10.1016/S1474-4422(17)30196-5

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., … van Mulbregt, P. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods*, *17*(3), 261–272. https://doi.org/10.1038/s41592-019-0686-2

Vovenko, E. (1999). Distribution of oxygen tension on the surface of arterioles, capillaries and venules of brain cortex and in tissue in normoxia: An experimental study on rats. *Pflugers Archiv European Journal of Physiology*, *437*(4), 617–623. https://doi.org/10.1007/s004240050825

1. https://physiology.arizona.edu/people/secomb/greens [↑](#footnote-ref-2)