## **Reviewer 420880\_File000009\_52530328**

* **Statement 2:** ‘Section 5 groups retinal oxygenation, retinitis pigmentosa and non-neovascular AMD. There is no stated rationale for doing so – it is as if the authors struggled to find a place for these topics in other sections and decided to group them together. Considerations on retinal oxygenation should be included in Section 4 or in a section on its own as a natural follow-up to considerations on retinal hemodynamics.’

**Response**: Retinal oxygenation, retinitis pigmentosa (RP) and non-neovascular AMD (non-nAMD) are a natural grouping since retinal oxygenation plays an important role in both RP (for the oxygen toxicity hypothesis) and non-nAMD (in drusen-driven hypoxia ). We have now made this rationale clearer in the text (**Section 5,pg. 21, ln.22**).

* **Recommendation 1:** The authors should write this review with Biomedical Engineers, Mathematicians, Physicists working in biomedical sciences and retinal physiologists in mind.
* **Recommendation 2:** I recommend narrowing the scope of the review to diseases involving vascular impairments only (it would be interesting to mention the proportion of ocular diseases that they represent in the general population). Diseases described in the review are already mainly of vascular nature, but the authors should explicitly state that it is their focus. Of all diseases mentioned by the authors only retinitis pigmentosa and non-neovascular AMD do not involve vascular pathologies (I would also include Glaucoma, but the authors do point out that increased IOP could affect blood supply to parts of the retina/optic nerve). I would therefore remove the paragraphs on retinitis pigmentosa and non-neovascular AMD (Sections 5.2 and 5.3) from the review.

**Reponse:**We feel that it is important to retain the sections on retinitis pigmentosa (RP) and non-neovascular AMD (non-nAMD) since they are significant areas of past and current retinal modelling work and since, as described above, they are intimately related to retinal oxygenation. RP has received more sustained modelling attention than any other retinal disease, while non-nAMD has received attention from some important studies and is ripe for future modelling work. Concerning the proportion of ocular diseases that are vascular impairments, we could not find a simple answer to this question so we will refrain from making conjunctures.

* **Recommendation 3:** I recommend replacing Section 3 by a very brief summary of modelling approaches relevant to in silico clinical trials (basically the ones that are mentioned in Section 4, 6 and 7). The textbook description of mathematical modelling concepts (such as ODEs, PDEs, or boundary conditions) is unnecessarily detailed and not really useful (it also lacks references).

**Response:** We have shorten this section and emphasized on some of the more relevant concepts (parameters and variables). Thank you for the suggestion.

* **Recommendation 4:** I recommend expanding Section 7 on in silico clinical trials significantly. Anything that can be learned from other organs and diseases should be included. More detailed descriptions on the validity of approaches implemented in the past, procedures applied for experimental validation of the trials, or even how regulatory bodies see these trials, would be extremely valuable. For instance, are they beneficial as pre-clinicial studies? Could they ever replace any phase of a clinical trial? It seems unlikely, but a discussion centred around these practical questions would be extremely valuable. Another important question is whether mathematical models described in the review are of any use for in silico clinical trials, and what approaches should be implemented in future studies to make them relevant. Including a discussion along these lines would tie all sections from the review together.

**Response**:

* **Recommendation 5:** Some sections are redundant, and the review would benefit from grouping them together. Specifically, Sections 2.2 and 6 overlap sufficiently to be merged. Parts of Sections 4.2 and 6.1-6.2 could also benefit from being merged.

**Response**: We chose not to merge sections 2.2 with section 6 as we thought that Section 2.2 offers additional information that may help a non-retinal specialist reader to understand the diseases described in-between. We are aware of the similitude between Sections 4.2 and 6. The rationale for keeping them separated is that Section 6 is meant to describe the intervention explicitly and study the effects of varying procedure parameters. On the other hand, Section 4.2 describes the interactions between drugs and metabolism (i.e., VEGF-antiVEGF bindings, *in vivo* drug parameters, treatment outcome) and the intervention is not explicitly modelled. Furthermore, models Section 4.2 are limited to the context of nAMD and DR while models in Section 6 may be more general, though a good proportion is specific to nAMD. Given the large amount of literature in both cases, we wanted to avoid having an inbalance by putting them together.

### Abstract

* **Line 21, “aging society… prevent sight loss”. Age is not a risk factor for all ocular diseases; this sentence is reductive.**
  + Thank you for the comment. This has been removed.
* **Line 23, “…scanning devices”. Recent advances in imaging technologies have been key to develop our understanding of retinal structures; they have however had a marginal contribution to our understanding of the specific physiology of the retina.**
  + Thank you for the comment. It has been clarified that understanding of the retinal physiology has only marginally been affected by the advances of scanning devices.
* **Line 36, “Despite rich in vivo data…”. This last paragraph is not necessary.**
  + This has been deleted.

### Section 2

* **Figure 1, caption. Should a reference to Wikipedia be made there? I would imagine that the name of the users who shared the images is sufficient. Mentioning Wikipedia in a scientific article may be frowned upon. The authors could use figure from open papers as an alternative.**
  + Thank you for the remark. The figure has been remade with images from open access sources.
* **Page 5, Line 53. “Surround” should be replaced by “surrounded”.**
  + The typo has been corrected.
* **Page 6, Line 34, “hexagonal”. My understanding is that choriocapillaris functional lobules do not have to be hexagonal. I would replace the sentence by “Each of these arterioles inserts in the interior of a domain delineated by a set of draining venules surrounding it”.**
  + Thank you, the suggested sentence replaced the inaccurate description of lobules.
* **Page 6, Line 41, “diffusion across BrM and the RPE”. Movement of molecules across the RPE occurs through various mechanisms, including active transport. I would replace “diffusion” by “transport” or “transfers”.**
  + “Diffusion” was replace by “transport”. Thank you.
* **Page 6, Line 49, “For this reason”. The reason here is (grammatically) not obvious.**
  + The paragraph (p.6 last paragraph) was reordered for clarity. Thank you.
* **Page 7, Line 6, “at the core”. Non neovascular AMD is driven by many known factors (age, genetics,environment), and disruption of oxygen perfusion remains, arguably, a hypothetical one.**
  + The sentence was replaced by “As another example of retinal fragility, excess oxygen or oxygen deprivation have been hypothesised to help drive retinal degeneration in the diseases retinitis pigmentosa (RP) and non-nAMD respectively, as described in Section 5.” (p.7 l.1).

### Section 3

* **Most of this section is superfluous and unnecessarily detailed. It lacks references. Without context or relevant examples, this section is simply a textbook description of concepts used in mathematical modelling. I recommend removing it altogether and replacing it by a section describing the underlying principles of models mentioned in Section 4.1.1, in addition to PK/PD and other models described in Section 4.2.**
  + The sections “Differential equations” and “Deterministic and stochastic models, continuum and discrete models” were removed. More details and examples were added for model parameters and variables.

### Section 4

* **This section is one of the stronger ones. I would integrate the disease-specific descriptions that makes up most of the introduction of the section in the main text and use them as applications of the core concepts listed. They could also be grouped into a “phenotypic manifestation of impaired retinal haemodynamics” subsection**
  + Thank you for the suggestion. The disease descriptions were moved to the main text, in the appropriate subsections/paragraphs.
* **Page 12, Lines 59-61. Specify that current imaging techniques only allow to visualise large to intermediate-sized retinal vessels. In addition, devices allowing for the measurement of oxygen saturation remain experimental and are not broadly available.**
  + Both remarks have been added in the paragraph p.10 l.56 .
* **Figure 4, caption. Specify that the exponent translating the fractal aspect of the retinal vasculature is experimentally measured, and maybe provide a typical value.**
  + Both comments were added in the caption. Thank you.
* **Page 18, Line 52, “challenge for clinicians.” A reference is needed here.**
  + **“**Eyes showing little to no improvement in their condition are referred to as non-responsive and present a real challenge for clinicians.”   
    replaced by   
    “Indeed, in both DR and nAMD, the real-world visual outcome of anti-VEGF therapy has been showed numerous time to be inferior to those reported by controlled clinical trials”. References added.

### Section 5

* **I recommend removing retinitis pigmentosa and non-neovascular AMD entirely. A section on retinal oxygenation and technical challenges that it is associated with (from both a modelling and experimental point of view) would be useful.**
  + We feel that it is important to retain the sections on retinitis pigmentosa (RP) and non-neovascular AMD (non-nAMD) since they are significant areas of past and current retinal modelling work and since retinal oxygenation plays an important role in both – for the oxygen toxicity hypothesis in RP and drusen-driven hypoxia in non-nAMD.
* **Page 20, Line 51, “hungry”. Replace by “highest rate of oxygen consumption per mass”, or something similar.**
  + Changed to *‘The retina has one of the highest rates of oxygen consumption per unit of tissue mass in the body…’ (*p.21 l.27)

### Section 6

* **Page 26, Section 6.3. Why group together subretinal, periocular and systemic administration? The rationale for doing so should be laid down.**

This choice was made based on the limited clinical uses and number models for those three administration routes, as stated in the first paragraph:  
 “Therefore, systemic or intravenous administration of drugs for treatment of retinal pathologies remain uncommon. Accordingly, few modelling works have been published on the matter.”

### Section 7

**I recommend expanding Section 7 on in silico clinical trials significantly. See general comments above.**

## **Reviewer ‘2420880\_File000010\_53246314’**

### Specific comments

* **p1 It is quite unusual to have an abstract of this length split over so many paragraphs**
  + The last paragraph has been removed.Thank you
* **p4, l13 Can the authors be more specific about how many clinical trials succeed - perhaps quantify what is needed to prove effectiveness - this gives a benchmark for what is then needed from the models**
  + Thank you for the suggestion. The success rates and adequate references explaining failures of clinical trials have been added to the sentence. **(p.4 l.18).**
* **p4, l21 Perhaps give a clearer definition of what is meant by an in silico clinical trial at this stage (ch 7 is much too late)- how does it differ to a regular clinical trial and is there scope for a hybrid approach? And how is computer aided development different?**
  + A quick definition was added at the beginning of the paragraph in addition to reference to section 7 (p.4 l.27). Computer aided development referred to in-silico models/trials. The term has been removed to avoid confusion.
* **p4, l47 It might help to give more detail about retinal layers here. We are told that the macula is oval shaped, but then only given one dimension.**
  + A brief description of cell types found in the retina and their functions has been added (p.4 l.54).
  + The size of the macula is now described as: “5mm wide at its widest”.
* **p4, l52 Are conditions more likely to present at the macula, or are they simply detected sooner because their influence on vision is more significant and so present at clinic more rapidly?**
  + This is a good question, thank you. In the absence of a definitive answer, “The macula is also an area where a number of pathological conditions present” was changed to “As such, pathologies are most damaging to visual acuity when affecting the macula.” (p.4 l.12)
* **p5, l51 picked up->transduced? What kind of cells do you mean? I found the term ‘enabling vision’ a little weak - perhaps describe how signals are interpreted by the thalamus/visual cortex**
  + More detail on the cells transmitting information between photoreceptors and the brain has been added (p4. l.53). However, the interpretation of signals by the visual cortex seems out of scope for the paper and lies outside of the authors’ expertise as well so is omitted.
* **p6, l1 wide->diameter**
  + Corrected. Thank you.
* **1p6, l7 The term choroid appears without introduction, but is then more fully described a few paragraphs later. Perhaps rearrange.**
  + A brief definition of the choroid was added (p.5 l.26) with reference to the paragraph below. Thank you.
* **p7, l24 How can the production of aqueous humor be lowered? It may be that the authors are trying to link to the paragraph immediately below, but this is not clear.**
  + A sentence was added describing the surgical procedure (p.7 l.37). Thank you for the comment.
* **p7 l51 The discussion of stem cells is a little vague - perhaps say more about the specific type of stem cells that differentiate into RPE etc**
  + The paragraph on stem cells was added for completeness and since no modelling work seems to have been done on retinal stem cell therapy, we thought it was not necessary to expend on it. With that said, we added that it is human or mouse pluripotent stem cells that are taken to differentiate into RPE or photoreceptor (with references).(p.8 l.47)
* **p8 l41 For this audience I might suggest first including a discussion of independent and dependent variables, along with examples, before discussing in silico simulations**
  + A paragraph “Variables” was added (p.9 l.20). The superfluous details on differential equations were removed and examples added.
* **p9, l16 For this audience I think it would be worth saying more about what parameters are, and why they appear in a model. And how the number of parameters can become very large, and need to be tuned to the physiological range**
  + The section on “Model parameters” was extended to answer those comments and includes more examples (p.9 l.47).
* **p9, l28 I feel you can say more here about the required number of boundary conditions and the number of initial conditions for a given model.**
  + This section was moved to the “model parameters” section. Because a description of mathematical concepts was deemed unnecessary for the target audience, it was removed.
* **p9, l51 The discussion of the slope of a curve is a little confusing, as it is not clear how the ‘curve’ relates to the variables - perhaps just omit this sentence in brackets**
  + This was removed along with the section.
* **p10, l21 I didn’t quite get how lumped parameter models differ from compartmental models**
  + The distinction is too thin and given the target audience, it is probably better to not make the distinction. We only use the term compartmental model in the second draft. Thank you.
* **p10, l51 Perhaps say more here about the statistical technique of model emulation - using a simpler model to guide searches of the parameter space, which can then be supplemented with final simulations of the full model**
  + Thank you for the suggestion. However, the section was removed because deemed superfluous for the audience.
* **p11, l1 The review does not really say anything about statistical modelling approaches - using machine learning on large data sets to learn a statistical (as opposed to a mathematical) model**
  + The distinction between the two was added in Section 3 (p.9 l.9).
* **p11, l37 photorectors->photoreceptors**
  + Corrected. Thank you.
* **p11, l42 The statement around glaucoma is a little vague. Clarify the direction of the pressure gradient around the optic nerve and its role in glaucoma**
  + The gradient is compressing the optic nerve. This was added to the sentence (p15 l.44). Thank you.
* **p13, l1 Viscous fluids such as blood are modelled...->The flow of viscous fluids, such as blood, is modelled...**
  + The suggested correction has been added. Thank you.
* **p13, l7 The description of laminar flow is rather simplistic - there are many other laminar profiles than just parabolic**
  + Indeed. This sentence was removed from the text.
* **p14, all There are many more sophisticated models for blood flow rather than just a viscous Newtonian fluid - perhaps more could be said about this**
  + Following a reviewer’s comment, we tried to keep mathematical/physical content to a minimum as it is not relevant for the broader audience (retinal specialist for who the content is too complex or engineer/mathematicians for who these explanation might be too simple).
* **p15, l44 I found the sentence ‘Good agreement with data validated the use of artificial networks’ a bit vague - can more be said about this approach - how extensively was the approach validated and why can we be sure the artificial networks are a good approach - how many different metrics were compared**
  + The problematic sentence was removed given that those models belong more to the section on Retinal Oxygenation.
* **p16, l34 Can you say why the in series configuration is more appropriate - can you explain why this would be the most appropriate arrangement of the vessels?**
  + A potential explanation was added (p.13 l.50). However, it is by no mean a definitive answer, only the interpretation of a single modelling work.
* **p16, l38 Is CC defined in the main text? Perhaps I just missed it!**
  + CC was defined page 6 of the first draft in “2.1.2 Blood supply to the retina”.
* **p17, l29 I found the statement ‘yielding significantly different results for each combination of parameters’ a bit vague - are these parameters all chosen in the physiological range? I find it hard to believe how ‘significantly’ different they can be?**
  + This sentence was removed and replaced by: “*showed noticeable differences in the haemodynamic response of the retina of the virtual populations tested (namely, normo-, hypo- and hypertensive populations).”* in the *Summary, future directions and prospects for in silico clinical trials* subsection (p.17 l.34).
* **p17, l53 How does the model show that surgery increases macular blood flow - again the statements are a bit vague - make clear how the surgical protocol is captured by the model, and how the observation is validated by clinical or experimental data**
  + Explanations were added (p.16 l.34): “*The model differentiate macular and peripheral vascular compartments, with the vascular resistance of the latest assumed to be increasing after surgery. This increased resistance forces blood flow to be redirected towards the macula.”*
* **p19, l25 The values of α need units**
  + Indeed. However, the mention to alpha was removed as it seemed more logical to mention drug half-life times instead.
* **p19, l39 I find it hard to understand how a model can show quantitative agreement without determining unknown parameters - please clarify this statement**
  + This was clarified, thank you.
* **p20, l5 Why is suprachoroidal injection not suitable for delivery? Please clarify.**
  + This was clarified p.19 l.24.
* **p20, l33 Is it really a surprise that the model shows good agreement with data once the parameters have been fitted? Please clarify. And why did the spatial model show better performance?**
  + This has been removed as part of a wider reworking of the section. Thank you.
* **p23, l7 Section 5.3 transitions to a number of very short paragraphs - reads more like a list rather than a coherent narrative**
  + We have condensed the text into two paragraphs.
* **p25, l15 I don’t get how the maximal principal stress is the normal stress on a plane subject to no shear stress? The maximal principal stress is the largest eigenvalue of the stress tensor, which could have normal and shear components. Please clarify.**
  + Indeed. This was replaced by: “*, a stress component extensively used to predict fractures.”*
* **p25, l26 Is it clear why the higher movement amplitude hastens the spread of drug - steady streaming or some other secondary flow?**
  + This may not be clear. We added a reference and explained that this may be due to increases of secondary flow velocity with movement amplitude.(p.26 l.50)
* **p25, l32 A difference in timescale between minutes and days is enormous - can more be said here about why it could be so vast? Secondary flows? Shear enhanced diffusion?**
  + We added that, as reported in the original paper, this was due to advection becoming the principal transport mechanism over diffusion (p.26 l.48).
* **p27, l29 Can you say more about the protocols and checks needed to be able to trust the outcome from an in silico clinical trial? Have the drug regulatory bodies (eg FDA in USA) set any rules? How do they compare to those for a regular clinical trial?**
* **p29, l8 Can the authors comment on why no authors have applied the paradigm to the retina? Or why VPs have not been used in the literature? Again these statements are a bit vague, and rather detract from the thrust of the paper.**
* **p30, l17 There are other ways to validate a mathematical model rather than just direct comparison to an analytical solution - eg. systematic model reduction and parameter asymptotics, model problems, validation against simulations of others,...**
* **p30, l50 Aknowlegement->Acknowledgement**
  + This was corrected, thank you.