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Opponent's dissertation review

Jakka Veera Venkata Satya, M.Sc.

Computational Simulation of Mechanical Behaviour of Endothelial Cells

Relevance of the topic

Given the fact the flow mechanics in vessels changes during atherosclerosis, the author of this thesis focuses on modelling of physiologically relevant stresses on individual endothelial cells. In this study, five mechanical tests of single cell were simulated and in three cases i.e., tensile test of suspended cell with micropipettes, compression test of suspended cell with microplates, and compression of adherent cells. Models were compared with corresponding published experimental data. The author also investigated the mechanical response of the flat cell within the endothelium layer under physiological conditions in arterial wall and the cell response in debonding during cyclic stretches using simulations. Compared to published approaches, the models consider more complex properties of cytoskeletal fibers and thus may provide more biological relevance – they consider flexural (buckling) as well as tensional/compressional behavior of microtubules and also incorporate the waviness of intermediate filaments. I particularly appreciate the analysis of the influence of individual cytoskeletal components on the mechanical properties of cells. This is a biologically very interesting topic, experimentally (microscopically) detectable only with significant limitations. Nevertheless, in the last decade, thanks to the massive expansion of advanced microscopy methods, there has been a significant expansion of cell mechanobiology. However, the cited literature does not reflect this significantly. Despite all this, the author has demonstrated the ability to grasp a very interdisciplinary work where even biological aspects are discussed correctly and in a rational context with modelling.

Objectives of the dissertation and their fulfilment

Based on the objectives of the dissertation mentioned in the thesis, it can be concluded that the objectives of the dissertation have been met. These were in particular:

(1) To investigate and to model the mechanisms that determine the intracellular force propagation and the mechanical behaviour of endothelium cell and its structural components.
(2) To investigate the cell response to distinct global mechanical stimuli by simulating mechanical behaviour of isolated endothelial cell such as: o tension and compression of a suspended endothelial cell for validation, compression for adherent and flat endothelial cells, and shear of the flat endothelial cell.
(3) To investigate the mechanical contribution of cytoskeletal components to cell mechanics, individually and synergistically by simulating disruption of cytoskeleton and its components.
(4) To investigate the mechanical response of the flat cell within the endothelium layer under physiological conditions in arterial wall.
(5) To investigate the cell response in debonding during cyclic stretches using 3-D finite element simulations. For this purpose, the created 3-D finite element model will be expanded by cohesive elements capable to simulate gradual debonding from the substrates under cyclic load.

Regarding the publication activity the author published two papers in impacted journals (BioMed Research International, IF 3.41, and Computers in Biology and Medicine, IF 4.58) and in four conference papers, all of these are part of the work, which is adequate for a PhD student.

The work process, the specific contribution and the methodology used.

Finite element models enable us to investigate mechanical factors not only at the level of the arterial wall but also at the level of individual cells. Similarly, these approaches were used by other authors to model mechanical properties of cells. Mr. Jakka Veera Venkata Satya, M.Sc. therefore employed a suitable methodology. The usage of bendo-tensegrity model (or specifically the hybrid models also reflecting the nucleus and the membrane) enable the author high degree of complexity in the modeling of the actual cell, therefore might well reflect the in situ biological conditions.
Author relevantly models the cell response based on global cellular stressing with tensile and compressive test experiments which are also routinely used to asses cell elastic properties in the biological experiments.
However, due to the reliance solely on experimental data from published studies to validate the modelling, there are limitations for the author. Therefore, the design of the models (and their limitations) always corresponds to published data; for example, Section 5.1.2 is based on a study where the cell diameter differs from that modelled. Although this is addressed by the author by normalization, these steps can be a source of inaccuracy. Similarly, the author assumes that actin fibers are with both ends anchored to it at focal adhesions together with elements representing microtubules and intermediate filaments, which is too reductionist from a biologist's point of view. As a biologist, however, I cannot assess the feasibility of a model with more complexity and thus for many biological applications the current approach may be more than sufficient.

Significance for the further development of the discipline and practice

The work is concerned with a currently widely researched topic with great potential for future application far beyond the technical field of the author. Specifically, cell biology and mechanobiology might greatly benefit from the modeling approaches and build on them. The author also demonstrated a significant contribution in the modeling by increasing the model complexity with actual tubulin and intermediate filament properties.
One of the aims of the studies mention to investigate the mechanisms determining intracellular force propagation and the motivation of the study mention mechanotransduction mechanisms. Mechanotransduction is relevant for various physiological and pathological cellular processes. Given the importance of this process in atherosclerosis development (which author mentions). I miss more emphasis on this cellular process in my work. Developing a line connecting modelling with mechanotransduction could take the whole topic much further and open up other fields of study. However, I understand the limitation preventing greater consideration of mechanotransduction caused by the author's reliance on already published data

Formal editing of the dissertation

The work would benefit from a greater amount of attention during the final inspection. Inaccuracies then unnecessarily reduce the overall impression of the study and impair its comprehension.

- Legends to figures and tables are often not sufficiently explanatory, for a number of figures there is no correspondence between the axis labels and the figure legends
- Figure 5.24 it seem fig a and b are identical, Fig. 5.30 lacks description of axes and color code.
- Fig 3.7B and 38B are identical,
- on a page 19 there is a missing part of the sentence.
- Lists of citations might be done sequentially ([12-15]), instead of "[12]; [13]; [14]; [15] (...)"
- 5.2.1 subchapter is “Simulation of suspended cells in compression (…)” despite the chapter 5.2 title is “Simulations of adherent cell model”

Comments and questions about the work

- On what basis is it evident that the effect of intermediate filaments on the mechanical properties of the cell is manifested only at large strains (above 20%) and is thus in contrast to the effect of actin filaments?

- According to simulations, 5% axial strain applied on the substate is sufficient to induce cell debonding. Is such strain observable in arteries without any atherosclerotic lesions, or rather in strictly in vessels with severe atherosclerosis?
- In the additional ideas and future works the author mention the viscoelastic nature of the biological material and thus the importance of the timescale; what times are relevant for the viscosity of large vessels? In the order of milliseconds, seconds, or longer?
- Further to the comment about relying on the results of published studies: if it were possible to rely on a mechanobiology lab, what parameters experimentally measured on cells could refine or advance the modeling possibilities?
- Cytoskeletal components, actin in particular, is characterized by high levels of dynamic instability and constant remodeling; is there approach to consider this in (some, more advanced) models?

Theses

The thesis provides sufficient information about the content of the doctoral thesis.

Conclusions

I evaluate the submitted work positively. Jakka Veera Venkata Satya, M.Sc. has demonstrated adequate knowledge in the field, ability to work scientifically, participate in the work of the team, but also independently solve the assigned problem. positively from the point of view of a biologist and physician, I appreciate the author's ability to accurately discuss the terminology of a field significantly beyond his field. The thesis meets the substantive and formal requirements for doctoral dissertations and therefore I recommend this thesis for defense and after its successful course

I recommend to award Mr. Jakka Veera Venkata Satya, M.Sc. degree of Ph.D. in Engineering Mechanics

Brno, 2nd May 2022 MUDr. Jaromír Gumulec, Ph.D.