

External Examiner's Report for the Doctoral Thesis Entitled

## **Computational Simulations of Mechanical Tests of Isolated Animal Cells**

submitted in November 2016 by

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in partial satisfaction of the requirements for the degree Doctor of Philosophy in the Ph.D. programme Applied Sciences in Engineering at the Faculty of Mechanical Engineering, Brno University of Technology

Cells do not only sustain and generate mechanical forces, but they also do sense them, transmit them and react to them. Concepts and methods of mechanics have, accordingly, proved to be particularly useful in our endeavour to understand the structure and function of cells, as well as their interaction with the surroundings. Moreover, deviations in mechanical properties of cells and their environment are symptomatic of pathological processes; this fact might be of diagnostic use. Although new experimental techniques together with advanced simulation approaches provided us with an insight into the mechanics of cells, our understanding is far from complete due to immense complexity of living matter. In this context, detailed models of cell mechanics, which is the topic of the thesis by Mr. Bansod, are of great interest thanks to their anticipated potential to explain experimentally obtained data and also to predict mechanical response of cells.

Author of the thesis focussed on studying the contribution of cytoskeletal components to the mechanical response of a cell by use of bendo-tensegrity model. For this purpose, he implemented two passive mechanical models of a cell in the commercially available engineering software. These models were subsequently analysed for deformation response such as stretching and compression between microplates or indentation by the Atomic Force Microscope. Parametric analysis of the contribution of individual components was performed too. Results of the thesis confirm the generally accepted view that cytoskeletal components affect mechanical properties of a cell.

The main contribution of this thesis is the implementation of buckling of microtubules into the tensegrity-based mechanical model of a cell. Regrettably, the consequences of this addition are neither satisfactorily discussed nor comprehensively compared with classical tensegrity models. I also have some concerns about the design of presented model. The model assumes connection of microtubules, actin filaments and intermediate filaments in the same node. I am afraid this is too simplistic assumption, which determines the behaviour of the model. Unfortunately, the results obtained are poorly explained and no deeper insight into the behaviour of the model is presented (why, for example, is the reaction force of the cell lower in the absence of actin filaments than in the absence of actin filaments and microtubules?). This is in sharp contrast with the fact that the results obtained are described with unnecessary details (for instance, 4 digits' accuracy in the case of an *ad-hoc* model). Another substantial weakness of the research presented in this thesis is the fact that it was not accompanied with an experiment or, at least based on and verified upon detailed experimental data including morphological details. Comparison with few reports by other groups is rather insufficient. Having contact with contemporary experimental approaches to cell mechanics, the author may learn that these methods are dynamic (for instance the stiffness mapping by the AFM) and that dynamic response of the model might be of great interest nowadays. In this context, the damping of cytoskeletal movement by cytosol, very likely absent in the presented model, emerges as an important parameter.

Besides above stated major objections, I also have following critical remarks: Biological details should be referenced by primary or reviewing sources, but definitely not by secondary engineering works which oftentimes describe these details very inaccurately. As a result, the thesis presents oversimplified view of the cytoskeleton which is far from current comprehension of the topic. The properties of cytoskeletal components and other compartments, as reviewed in Table 2.2, 3.2 and 3.3, contain factual error on polymerization dynamics of actin filaments and microtubules, omit crucial role of microtubules in motility, and are based on obsolete experiments (from 1993 or even 1964). There are also terminological errors such as confusion between G-actin and F-actin or

naming protofilaments as “columns”. Also the assumption of homogeneous, linear and isotropic properties of cytoskeletal components (section 3.3) is at variance with current knowledge. It has been shown, for instance, that mechanical properties of microtubules are highly anisotropic and length-dependent. Another omitted fact is the natural variance of mechanical parameters of cytoskeletal components which is given by posttranslational modifications or interaction with counterions, cytoskeleton associated proteins, and other compounds. Moreover, the properties of cytosol were taken from a reference which does not distinguish mechanical properties of the cytosol from properties of the cytoskeleton which in result may have introduced an error into the model.

In light of above summarized imperfections and inaccuracies, most importantly the oversimplification of biophysical reality and unsatisfactory design of research questions, I have doubts about the potential of this work to attract greater interest from the scientific community.

The thesis is written in good English with only few typos and grammatical errors. Unfortunately, the author extensively uses abbreviations which eventually reduces the accessibility of the text. Moreover, abbreviations are often introduced only in the list of abbreviations and not in the text itself upon their first appearance. The typographic layout is well arranged with the exception of figures' captions, which are not distinct from the body of the main text.

The research presented in the thesis was published in one conference paper indexed in a recognized database and a number of papers (mainly literature reviews) published in irretrievable conference proceedings and journals. One paper is currently under review in an internationally recognized peer-reviewed journal. The absence of a manuscript accepted for journal publication at the time of submitting the thesis should come under scrutiny during the presentation of the thesis. All relevant publications by Mr. Bansod are presented as an appendix to his thesis. The short version of the doctoral thesis includes all required parts and covers all essential sections of the body of the doctoral thesis.

Although I have plenty of critical comments, I have to conclude that (i) the objectives of the thesis, as stated in section 1.3, were accomplished, and that the thesis (ii) complies with the requirements for a dissertation as stated by the law of the Czech Republic, and (iii) is therefore suitable for presentation with the aim of receiving the degree Doctor of Philosophy (Ph.D.).



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## Conflict of interest statement

I wish to declare that I have no conflict of interest with the author of the thesis, having never collaborated or published joint work with him or anyone else from his lab.