Subject: Evaluation of the Yogesh Deepak Bansod’s Thesis

Analysis of the thesis:
Yogesh Deepak Bansod has made his doctoral thesis under the direction of Prof. Jiří Burša and the co-direction of PhD Stanislav Polzer at the Brno University of Technology – Institute Of Solid Mechanics, Mechatronics And Biomechanics – Faculty Of Mechanical Engineering. The field concerns the so-called Computational Simulations of Mechanical Tests of Isolated Animal Cells by using two novel FE-bendon-tensegrity models. The manuscript is divided in 6 chapters.

The first chapter (i.e., Introduction) is devoted to the recall of the context and the challenge of this work: to propose an accurate biomechanical modelling of individual cells by considering their variable complexity in function of their environment in the aim to analyse the intracellular force propagation secondary to an external mechanical loading (that is related to the cell mechanotransduction). This chapter exposes well the present scientific question: how to propose a biomechanical model of such a complex material that takes into account its micro-structure, able to consider various cell situations (in particular in adhesion versus in suspension) and to explore the specific role of the main cellular sub-domains governing mechanical response to different local or global loading? The organisation of the manuscript is here well exposed. This chapter is well referenced.

The second chapter concerns the literature review. The eukaryote cell and its major mechanical components are well describe, as well as its mechanical behaviour and both the main topical experiments and the more recent models developed to study it. Even if some information are incomplete (for example, Optical Tweezers are not only used to apply global stresses to non adherent cells as it it stated in page 15, but also to apply local loading to adherent cells; see for instance the published works such as V. M. Laurent, S. Hénon, E. Planus, et al., 2002, Assessment of Mechanical Properties of Adherent Living Cells by Bead Micromanipulation: Comparison of Magnetic Twisting Cytometry vs Optical Tweezers, J Biomech Eng 124(4), 408-421, and many others…), this chapter is well referenced and is useful for the reader to set present problematic. Yogesh Deepak Bansod shows the interest in developing more complex cell models able to consider at the same time the continuous cell components (in particular the cytoplasm) and the structural ones (the pre-stressed cytoskeleton) and to take into account the effects of microtubule buckling. However, the author forgets published works in his literature review, e.g. M. F. Coughlin and D. Stamenović, 1997, A Tensegrity Structure With Buckling Compression Elements: Application to Cell Mechanics, J. Appl. Mech 64(3), 480-486, where a tensegrity model was developed to precisely study the flexural feature of microtubules and it implication in cell mechanics (Stamenović’s group has already published more studies concerning thi buckling of microtubules by using tensegrity concept). Furthermore, this part does not explain why AFM, MS and MD are the three experiments chosen here to apply present model. Moreover, the author does not expose why his models, described in the following chapters, do not take into account cell viscosity whereas he shows its importance. In spite of these imperfections, this chapter exposes well the main objective of this thesis i.e. to develop two new hybrid (continuum-tensegrity) cell models, one devoted to study non adherent cells and the other for adherent cells, that consider also the flexural behaviour of compressive elements representing microtubules.
Chapter three describes precisely both the FE model and connected tensegrity structure of the proposed model developed to analyse the mechanisms responsible for suspended cell deformation. The architectural organisation of the major structural components (actin fibres, actin bundles, microtubules, intermediate filaments) and their links (or absence of links) with cell membrane, cytoplasm and nucleus, are here well described. Quantitative data are also available, such as mechanical properties of each constituent of the model. The boundary conditions and parametric analysis are well done. My main criticism here concerns the idea that a cell tested by MS is characteristic of suspended cells. Indeed, when one uses MS, the first steps of the experiments consists precisely in permit the chosen cell to adhere to the micro-pipettes. Without this initial step (that can last some hours), so without such cell adhesion, it would not be possible to submit the cell to tensile tests. As a result, my opinion is that the appropriate model to use in the aim to analyse MS experiments is not present suspended cell model but present adherent cell model described in the following chapter. Moreover, some cell photographs chosen in this chapter (fig. 3.2 and 3.3) represent in reality adherent cells and can not illustrate the structure of suspended cell cytoskeleton. Indeed, as it is well shown by Yogesh Deepak Bansod in the two first chapters, the cytoskeleton organisation (and the corresponding mechanical behaviour) of an eukaryote cell differs considerably in function of its state of adherence, in particular for the two “extreme” conditions studied here: suspended cell and flat adherent cell on a planar surface. On the other side, the use of MD technique seems in total accordance with the objective consisting in investigate suspended cell mechanics. The agreement between experimental and numerical results seems good and the parametric study interesting. A little criticism here: it is a pity the elastic modulus calculated for the model during compression MD tests (E ~ 1.109 kPa) is not compared to experimental estimation. Moreover, there is not mapping of the level of stresses or strains within the cytoplasm, whereas it is the made for AFM tests (chapter 4) for adherent cells, making impossible the comparison between suspended and adherent cytoplasm under external loading, predicted by the models. Similarly, while a cell elasticity modulus is computed for suspended cell model, this is not the case for adherent cell, preventing every comparative study.

The fourth chapter describes the equivalent hybrid tensegrity-continuum model of adherent cell. Once again, the components, theirs links, the mechanical properties, the parametric variations and the boundary conditions are well explained. The simulation of AFM tests is well adapted to the analysis of adherent cell mechanics. The model and the numerical data are also well described here. Results obtained are again interesting even if they reveal some paradoxes in particular about the mechanical implication of actin bundles and microtubules, as exposed in question 1 bellow.

The two following chapters (5 and 6) are devoted to the discussion and the conclusion of this interesting thesis. The good agreement between the numerical and experimental results is well analysed and shows the importance in using cell models that consider at the same time its continuous part and its internal structures (cytoskeleton, nucleus-cytoskeleton), including some specific features (microtubule buckling, tensed actin assemblies, ...). All these positive aspects underline the good quality of the work performed by Yogesh Deepak Bansod during his thesis. Some present limitations are also given associated to some ideas in the aim to improve the model accordingly (by considering cell viscosity, cytoskeleton filament polymerisation/depolymerisation, ...). However, some questions remain (questions 2 to 5 bellow).

Moreover, a short version of the thesis is also available and brings the relevant information on the complete version.
Questions of the reviewer:

1. Results shown in fig. 4.8 (p. 72) seem to indicate that the stress fibres could have a “negligible” role in the value of cell elasticity modulus, whereas fig. 4.10 (p. 74) shows that its implication could be important (it is the opposite concerning the role of MT). Could the author attempt an explanation of such a paradox?

2. Why the author did not perform pre-stress variations in the simulation whereas he recalls the importance of cell tension in the literature review and the tensegrity structures are particularly adapted to such analysis? This could be interesting to compare with previously studies based on tensegrity models.

3. How the physical contact between cytoskeleton filaments during large cell deformation (in particular for suspended cells) is mechanically taken into account? And what is the corresponding numerical strategy used here to manage it? Indeed, with such a high number of filaments organised with such architectural organisation, cytoskeleton filaments (microtubules and intermediate filaments in particular) may be in contact during such large deformations.

4. Why the idea written p.85 about the future consideration of cell viscosity concerns only the continuous components and not also the structural ones? Indeed, it has already be shown that cytoskeleton filaments have a viscoelastic behaviour and some previously published studies have also shown the great role of the cytoskeleton (probably mainly actin assemblies) in the overall viscosity properties of cells.

5. The perspectives of this work do not show anything about three-dimensional adhesion. Indeed, it is largely admitted that in situ cells are mainly adherent to a 3D surrounding matrix. Moreover, experimental observations made on cells adhering to a 3D substratum seem to fail in exhibiting stress fibres, indicating that the architecture of the cytoskeleton structure may differ greatly in 3D environment regardless planar matrix. Could the author should say some words about this 3D aspect and the corresponding developments needed to present models?

Conclusion of the evaluation:
The work done by Yogesh Deepak Bansod at the Brno University of Technology concerns a very difficult task. Nevertheless, both the statement of the theory developed here and the related examples of possible applications show that this thesis constitutes a significant work in the general field of cell biomechanics and a useful basis to further analysis of cell mechanotransduction, as it is shown by his numerous publications.

As a result, I highly recommend this doctoral thesis for defence.

Patrick Cañadas, PhD