Title of the Thesis: Cell Mechanics

Author: Ing. Katarína Mendová

Institution: Faculty of Mechanical Engineering, Czech Technical University in Prague

Department: Department of Mechanics, Biomechanics, and Mechatronics

Supervisor: prof. RNDr. Matej Daniel, Ph.D. Department of Mechanics, Biomechanics, and Mechatronics, Faculty of Mechanical Engineering, Czech Technical University in Prague

Field of Study: Biomechanics

The topic of the presented PhD thesis is highly relevant, as understanding of cell mechanics forms the basis for comprehending various biological processes and mechanisms. In a variety of scientific domains, including biology, medicine, drug discovery, tissue engineering, and many more, this knowledge is essential. Current research focuses on comprehending cellular illness mechanisms, such as mechanotransduction, which may help develop novel medications offering potential treatments for various conditions. To understand the cellular response to mechanical stimuli facilitates the development of scaffolds and materials that enable tissue growth and repair, ultimately resulting in enhanced therapeutic outcomes for degenerative illnesses and injuries. Gaining knowledge of these mechanisms at the mechanical level can help one better understand the basic biological roles that cells play and how they are regulated. Additionally, advances in biology and technology, such as microscopy, enable investigation of mechanical properties at the single-cell level, potentially leading to new discoveries.

The first part of the dissertation, which covers the issue of variability in cell mechanics and the requirement for standard references in mechanical testing. This is followed by a detailed literature review in the "State of Art" section, which covers cell structure, the role of liposomes as cell models, and various methods for preparing giant liposomes. This section establishes a solid foundation by highlighting the complexities and challenges in the field of cell mechanics. In the "Mechanical Testing of Cells and Vesicles" section, the dissertation provides a comprehensive overview of different force application and sensing techniques, including atomic force microscopy (AFM), nanoindentation, and micropipette aspiration. Each technique's principle, benefits, and drawbacks are clearly stated, facilitating comprehension of the experimental methodology.

The "Evaluation of Measurements" section reviews different models used to interpret mechanical testing data, such as the Hertz contact model, Overbeck model, and Canham-Helfrich model, offering a critical evaluation that helps in understanding how different models can affect the interpretation of cell mechanics data.

The section titled "Mechanical Properties of Cells and Vesicles" offers an analysis of the intrinsic mechanical characteristics of cells and vesicles, elucidating the factors that may impact these qualities and their potential range. The chapter 5 „Aims“ then provides description of the study goals, which center on the creation of mathematical models and standardized references. However, a clearly defined hypothesis that will be verified is missing.

The level of methodological rigor in the "Methods" section is excellent. It gives a thorough explanation of the invention of the liposome-producing microfluidic systems, how the liposomes are prepared, and the mechanical testing techniques used. This ensures clarity and reproducibility. I would higlight the complexity of knowledge applied, from the cell mechanics, mathematical analysis, CAD/CAM modeling, application of 3D printing and using different analytical tools to process results of the experimental testing. Applied research design and methods are appropriate for the study.

The study's conclusions, such as the effective creation of microfluidic devices and the outcomes of mechanical testing on liposomes, are presented in the "Results" section. The data is thoroughly analyzed, and the effectiveness of different models in interpreting this data is evaluated.

In the "Discussion" section written on six pages, the results are interpreted in the context of the existing literature, discussing the implications of the findings and the influence of different testing methods and data processing techniques on the mechanical properties of liposomes.

The dissertation ends with conclusion, highlighting the contributions made to the standardization of mechanical testing in cell mechanics, and offering recommendations for future research to strengthen the validity and relevance of the study's findings.

Among the dissertation's strengths are its comprehensive literature review, methodological rigor, innovative approach, and the development of mathematical models and novel models for testing the cell mechanics. The extensive review covers fundamental concepts and advanced techniques in cell mechanics, providing a solid background for the research. Detailed descriptions of experimental setups and procedures ensure that the research is reproducible and transparent. The use of liposomes as standard references in mechanical testing is a novel and valuable contribution, addressing a significant gap in the field. The mathematical modeling adds depth and robustness to the research findings. However, there are areas for future improvement. Although the dissertation admits that mechanical qualities can vary, it should investigate the causes of this variability more and suggest other methods to reduce it. A more in-depth discussion on the limitations of the mathematical models used, along with potential improvements or alternative models, could strengthen the findings. The suggestions for future research could also be extended to include more specific experimental setups or alternative methods for standardizing mechanical testing.

It is important to draw attention to the potential interdisciplinary applications of this research in addition to the thorough analysis and discussion that are already included in the

dissertation. The knowledge gathered by studying cell mechanics at such a fine scale has applications beyond the immediate subject of biomechanics, such as in the study of cancer, where abnormal cells mechanical characteristics are very different from healthy cells. This research has the potential to enable more precise diagnosis and individualized treatment regimens by creating uniform references and techniques. Moreover, the innovative use of liposomes could inspire similar approaches in other areas of cell biology, promoting a more integrated understanding of cellular functions and interactions. Future research building on this work could explore the dynamic aspects of cell mechanics over time, providing a more comprehensive picture of cellular behavior under various physiological conditions. This could involve real-time monitoring of cellular responses to mechanical stimuli, offering new avenues for research in cellular adaptation and resilience. All things considered, the dissertation not only makes a substantial contribution to the state of knowledge in cell mechanics today, but it also opens the door for future advancements and applications in a variety of scientific fields.

On the formal side, the work is written at an excellent level, some a few grammatical mistakes do not detract from the overall high level of the work. Author reviewed 137 literature sources with precise citing of the references.

To sum up, the dissertation represents a significant advancement in the science of biomechanics. It presents a thorough examination of the mechanical characteristics of cells, suggests creative ways to standardize mechanical testing, and offers insightful information by using rigorous methodological techniques and mathematical modeling. The study's conclusions and suggested models fill a vital gap in the field's standardization of references by providing a strong framework for further research in cell mechanics.

Questions:

Recent studies shows, that low-magnitude, low-frequency (LMLF) vibrational stimulation can affect the cells growth (e.g. increasing the bone mass). Could your models and mechanical testing modalities using liposomes as cell models explain this process of bone mass formation?

There is demand for more comprehensive cell models, which will reflect the properties of another cell structures. Where research aims from the point of view of experimental models complexity?

Based on the above facts, I recommend the thesis for defense and after its successful defense, and answering all questions, **I propose to grant the author of the work a Ph.D (philosophiae doctor).**

Reviewer Information

Name: prof. Ing. Radovan Hudák, PhD.

Position: Head of Department and Head of Institute

Institution: Department of Biomedical Engineering and Measurement, Institute for Special Engineering Processologies, Faculty of Mechanical Engineering, Technical University of Košice

Signature: