27/28th International Conference
ENGINEERING MECHANICS 2022
Milovy, Czech Republic, May 9 –12, 2022
Paper **#60,** pp. 297–300, doi: 10.21495/51-2-297

THE IMPACT OF THE MITOCHONDRIAL NETWORK ON THE MECHANICAL RESPONSE OF DISEASED ANIMAL CELL

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Abstract: *Understanding the cell mechanics during cancer progression is potentially relevant for cancer diagnosis and treatment. As a consequence of metabolic reprogramming in cancer cells, mitochondrial fusion and fission are altered which causes a reorganization in the mitochondrial network. A relation between such reorganization and the cell response to mechanical stimuli calls for further investigation because mitochondria could thus prove to be a significant therapeutic target. A computational simulation of an indentation test using a structural cell model incorporating a realistic mitochondrial network indicates a significant impact of mitochondria on the overall response of the cell.*

Keywords: Mitochondria, Structural cell model, Mechanical response, Indentation test, Cancer cell.

1. Introduction

Understanding the cell mechanics during cancer progression could be the key milestone transitioning into the capability to either detect the affected tissue, treat the already-formed carcinomas or solid tumors, or even prevent the initiation and progression of cancer. It is known that the metabolism of the cell is being altered significantly due to cancer, but similarly important changes occur in the inner arrangement of organelles and the cell mechanical response is therefore affected.

The motility of the cell reportedly increases in primary stages which is a highly energetically-demanding process and results in a decrease in overall cell stiffness (Alibert, 2017) accompanied by a complex inner reorganization. Energy generation for the whole cell is a basic function of mitochondria whose division and fusion occur continuously in equilibrium throughout the cell lifetime (Okamoto, 2005). Cancer induces an imbalance in this vital process (Pustylnikov, 2018) which is tied with changes in mitochondrial metabolism. As a result, cancer cells may contain branched, longer mitochondrial filaments (Alirol, 2006), whereas the mitochondrial network of a healthy cell comprises shorter mitochondria distributed through the cytoplasm. In contrast to decreased cell stiffness in primary stages, in vitro prostate cancer cells from secondary site show increased cell stiffness (Raudenska, 2019) and develop further mitochondrial reorganization, especially in a perinuclear region due to increased mitochondrial fusion.

Mitochondrial arrangement within a cell is based on complex interactions with other organelles, especially with cytoskeletal microtubules that allow for mitochondrial movement (Woods, 2016). Apart from this important binding, mitochondria also form direct points of contact for example with the nucleus, actin filaments, or plasma membrane (Desai, 2020, Moore, 2018, Tepikin, 2018).

Computational modeling can provide answers to whether or not the mitochondrial reorganization directly leads to varying cell stiffness. To date, no evidence of mitochondrial contribution to the cell stiffness has been presented as most of the computational studies in the field focus on cytoskeleton, for example, (Xue, 2013) or (Lili, 2019), a load-bearing structure of the cell. The cytoskeleton is considered to play a decisive role in cell stiffness (further assessed for example in Jakka, 2022) and is significantly remodeled during various pathological processes. Nonetheless, the contribution of the cytoskeleton to the stiffness of the cell

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has been researched widely and its role in cancer has been described in some detail which is another reason why we consider the investigation of mitochondrial contribution to cell mechanical response a current and relevant topic. To achieve a basic understanding of the role of mitochondria in cell stiffness, a hybrid computational model has been formulated and investigated under the indentation loading conditions.

2. Methods

Microscopy

The investigated cell line is a human prostate cancer cell line PC-3 derived from the fourth-grade adenocarcinoma. Individual cells were cultivated in an adherent state as in (Raudenska, 2019) allowing for advantageous direct stiffness analysis using Atomic Force Microscopy (AFM).

General morphology parameters (height and radius of the cell and nucleus) were measured using holographic microscope Nanolive 3DCX and confocal microscope Zeiss LSM880. Confocal microscopy using the MitoTracker CMXRos staining provided us with the tools for scanning the irregular mitochondrial arrangement (see Figure 1) which could subsequently be incorporated into the realistic computational model. Geometry parameters are summarized in Table 1 (the thickness of mitochondria measured using electron microscopy).

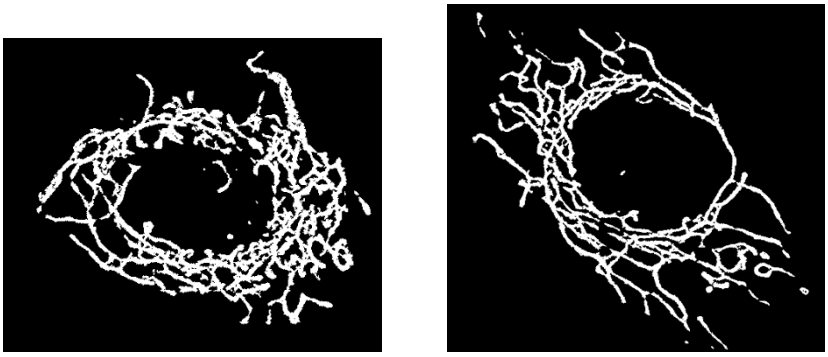

Fig. 1: Mitochondrial arrangement in a PC-3 cell line.

Tab. 1: Geometry parameters of a PC-3 cell line, all dimensions in [μm].

	Whole cell	Nucleus
Long axis a (base xz plane)	40.4	16.7
Short axis b (base xz plane)	20.3	10.6
Height (y axis)	9.09	4.61

	Membrane	Mitochondria
Thickness	0.010	0.234

Atomic Force Microscopy (AFM)

Measurement of mechanical properties and fluorescence was carried out on NanoWizard™ 3 Atomic force microscope (JPK-Bruker, Berlin, Germany). AFM was operated in force curve Quantitative imaging (QI) mode using a soft tetrahedral Nitra-tall cantilever (Applied NanoStructures, Mountain View, US). Mechanical properties were calculated using the Hertz-Sneddon model in JPK Data Processing software.

Computational modeling

The impact of mitochondria on the mechanical response of the cell was investigated by the means of hybrid structural modeling. At first, the segmented mitochondria were processed in MitoGraph tool (Harwig, 2018) and subsequently transformed into a network of bent beam elements with constant thickness and curvature. Apart from incorporating a realistic mitochondrial network, the model comprises other mechanically significant components as identified in (Bansod, 2018, Jakka, 2021): nucleus, cytoplasm and cell membrane. While it may not comply with the observations, we idealized the overall cell shape as a spherical segment and similarly, the nucleus has been considered an axisymmetrical ellipsoid (refer to Figure 1). All of the components have been linked together by interconnecting the displacements of both ends of each mitochondrion as well as 1, 2 or 3 other points along the beam with continuous parts (more in Results).

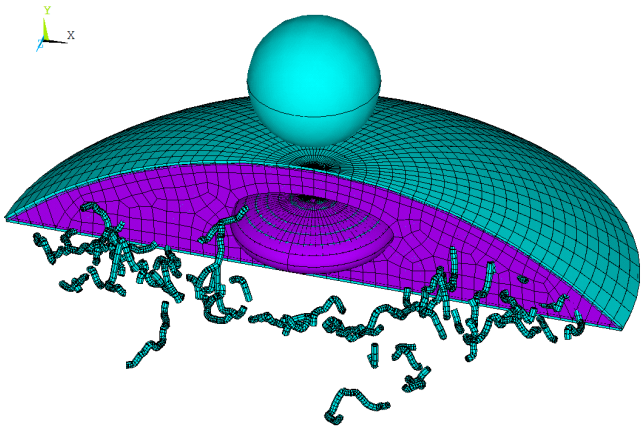

Fig. 1: A hybrid computational model incorporating nucleus, cytoplasm, membrane and a realistic mitochondrial network displayed with a spherical indenter for a simulation of indentation test.

Due to a significant lack of information about the material properties of the organelles, values originating from the literature were reviewed and adjusted to correspond with the data from AFM. We assumed isotropic linear elasticity of the discrete elements (i.e. mitochondria) and non-linear hyperelasticity of the continuous elements (nucleus, cytoplasm and membrane). All of the components are considered incompressible, for further details refer to Table 2 below.

Tab. 2: Mechanical properties of individual cell components adapted from (Jakka, 2021) to correspond with the experimental force-indentation curve. All dimensions in [MPa].

	Elastic modulus	**Shear modulus**
Nucleus	2.5×10^{-3}	8.5×10^{-4}
Cytoplasm	1.3×10^{-4}	4.3×10^{-5}
Membrane	0.50	0.17

	Elastic modulus
Mitochondria (discrete, linearly elastic)	0.10

A model comprising continuous components only (used later as a reference model) was loaded with rigid body indentor displacements. Multiple indentation points distributed evenly over the cell body have been probed in compliance with standard AFM testing. The importance of mitochondria could not be assessed by a single-point indentation because the cell mechanical response is dominated by the nucleus in the central region.

3. Results

As already mentioned, the mitochondrial arrangement is significantly influenced by cancer which causes an increase in the number of interactions with other cellular components (Desai, 2020). We evaluated the impact of this phenomenon by increasing the number of points (binding sites) that tie each mitochondrion to the cytoplasm (see Table 3). Already at 3 binding sites, the response of the cell increased significantly, confirming the link between mitochondria reorganization and the altered cell stiffness. Following the model with 2 binding sites, we further manipulated other mitochondrial parameters possibly influenced by cancer (see Table 4).

Tab. 3: The increase in the cell stiffness dependent on the number of binding sites of each mitochondrion in the cytoplasm.

Number of binding sites	**Percentage increase in cell stiffness**
1 bond	+1.6%
2 bonds*	+4.7%
3 bonds	+11.9%
** Reference model for Table 4*	

Tab. 4: Further increase in the cell mechanical response due to altered mitochondrial parameters.

Percentage change in:	**Diameter**		**Elastic modulus**	
	150%	200%	150%	200%
Percentage change in cell stiffness	+6.0%	+7.3%	+5.0%	+5.5%

4. Discussion

The presented computational model shows a significant impact of mitochondria depending on the number of binding sites in the cytoplasm. We expect that the binding sites with cytoskeletal components may be even more important as the cytoskeleton forms a major part of the cell stiffness. Further investigation of cancer cell mechanics requires a novel approach incorporating mitochondria together with cytoskeleton accompanied by an extensive laboratory study of the cell inner arrangement. Uncovering the alterations in both cell stiffness and its inner arrangement may present us with new methods for detecting cancer in time for successful medical intervention.

Acknowledgments

The study was supported by the project of BUT No. FSI-S-20-6175.

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