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VÝPOČTOVÁ SIMULACE MECHANICKÝCH ZKOUŠEK IZOLOVANÝCH ŽIVOČIŠNÝCH BUNĚK
COMPUTATIONAL SIMULATION OF MECHANICAL TESTS OF ISOLATED ANIMAL CELLS

SHORT VERSION OF DOCTORAL THESIS
ZKRÁCENÁ VERZE DIZERTAČNÍ PRÁCE

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Abstrakt

Buňka tvoří složitý biologický systém vystavený mnoha mimobuněčným mechanickým podnětům. Hlubší pochopení jejího mechanického chování je důležité pro charakterizaci její odezvy v podmínkách zdraví i nemoci. Výpočtové modelování může rozšířit pochopení mechaniky buňky, která může přispívat k vytvoření vztahů mezi strukturou a funkcí různých typů buněk v různých stavech.

Za tímto účelem byly pomocí metody konečných prvků (MKP) vytvořeny dva bendotensegritní modely buňky v různých stavech: model vznášející se buňky pro analýzu její globální mechanické odezvy, jako je protažení nebo stlačení, a model buňky přilnuté k podložce, který vysvětluje odezvu buňky na lokální mechanické zatížení, jako třeba vtlačování hrotu při mikroskopii atomárních sil (AFM). Oba zachovávají základní principy tensegritních struktur jako je jejich předpětí a vzájemné ovlivnění mezi komponentami, ale prvky se mohou nezávisle pohybovat. Zahrnutí nedávno navržené bendotensegritní koncepce umožňuje těmto modelům brát v úvahu jak tahové, tak i ohybové namáhání mikrotubulů (MTs) a také zahrnout vlnitost intermediálních filament (IFs). Modely předpokládají, že jednotlivé složky cytoskeletu mohou měnit svůj tvar a uspořádání, aniž by při jejich odstranění došlo ke kolapsu celé buněčné struktury, a tak umožňují hodnotit mechanický příspěvek jednotlivých složek cytoskeletu k mechanice buňky.

Model vznášející se buňky napodobuje realisticky odezvu síla-deformace během protahování a stlačování buňky a obě odezvy ilustrují nelineární nárůst tuhosti s růstem mechanického zatížení. Výsledky simulací ukazují, že aktinová filamenta i mikrotubuly hrají klíčovou úlohu při určování tahové odezvy buňky, zatímco k její tlakové odezvě přispívají podstatně jen aktinová filamenta. Model buňky přilnuté k podložce dává odezvu síla-hloubka vtlačení ve dvou různých místech odpovídající nelineární odezvě zjištěné experimentálně při AFM. Výsledky simulací ukazují, že pro chování buňky je rozhodující místo vtlačení a její tuhost určují aktinová povrchová vrstva, mikrotubuly a cytoplazma.

Navržené modely umožňují cenný vhled do vzájemných souvislostí mechanických vlastností buněk, do mechanické úlohy komponent cytoskeletu jak individuálně, tak i ve vzájemné synergii a do deformace jádra buňky za různých podmínek mechanického zatížení. Tudíž tato práce přispívá k lepšímu pochopení mechaniky cytoskeletu zodpovědné za chování buňky, což naopak může napomáhat ve zkoumání různých patologických podmínek jako je rakovina a cévní choroby.

Klíčová slova: Cytoskelet, bendo-tensegritní struktura, modelování, metoda konečných prvků, biomechanika buňky, mechanotransdukce, tahová zkouška, tlaková zkouška, AFM (mikroskopie atomárních sil)

Abstract

A cell is complex biological system subjected to the myriad of extracellular mechanical stimuli. A deeper understanding of its mechanical behavior is important for the characterization of response in health and diseased conditions. Computational modeling can enhance the understanding of cell mechanics, which may contribute to establish structure-function relationships of different cell types in different states.

To achieve this, two finite element (FE) bendo-tensegrity models of a cell in different states are proposed: a suspended cell model elucidating the cell's response to global mechanical loads, such as elongation and compression and an adherent cell model explicating the cell's response to local mechanical load, such as indentation using atomic force microscopy (AFM). They keep the central principles of tensegrity such as prestress and interplay between components, but the elements are free to move independently of each other. Implementing the recently proposed bendo-tensegrity concept, these models take into account flexural (buckling) as well as tensional behavior of microtubules (MTs) and also incorporate the waviness of intermediate filaments (IFs). The models assume that individual cytoskeletal components can change form and organization without collapsing the entire cell structure when they are removed and thus, can evaluate the mechanical contribution of individual cytoskeletal components to the cell mechanics.

The suspended cell model mimics realistically the force-elongation response during cell stretching and the force-deformation response during cell compression, and both responses illustrate a non-linear increase in stiffness with mechanical loads. The simulation results demonstrate that actin filaments (AFs) and MTs both play a crucial role in defining the tensile response of cell, whereas AFs contribute substantially to the compressive response of cell. For adherent cell model, the force-indentation responses at two distinct locations are in accordance with the non-linear behavior of AFM experimental data. The simulation results exhibit that the indentation site dominates the cell behavior and for cell rigidity actin cortex (AC), MTs, and cytoplasm are essential.

The proposed models provide valuable insights into the interdependence of cellular mechanical properties, the mechanical role of cytoskeletal components individually and synergistically, and the nucleus deformation under different mechanical loading conditions. Therefore, this thesis contributes to the better understanding of the cytoskeletal mechanics, responsible for cell behavior, which in turn may aid in investigation of various pathological conditions like cancer and vascular diseases.

Keywords: Cytoskeleton, Bendo-tensegrity, Finite element modelling, Cell biomechanics, mechanotransduction, tensile test, compression test, AFM (atomic force microscopy)

Contents

1. INTRODUCTION

1.1. General background

Living cell is a universe unto itself and can be considered as one of the most complex form of matter. Study of mechanical behavior of living cells and their mechanical interactions with the extracellular space was the first step to understand their response to applied forces. Within living organisms, cells are constantly exposed to different mechanical stimuli and they respond to these stimuli by altering their morphology, function, and behavior. Novel experimental techniques that complement the robust computational approaches capable of modeling cell mechanical response at varying temporal and spatial scales provide new pathways to understand cell mechanics and mechanobiology. Studying cellular mechanics is of great importance for two main reasons: first, cells are continuously exposed to physical stresses and strains arising from external physical forces that determine health and function of human body [Lim et al. (2006)] and second, biomechanical investigations can provide quantitative information on the changes in cell mechanical properties through the progression of certain diseases.

1.2. Motivation

Cells convert different forms of energy and signals, maintain and modify their internal structure, and respond to extracellular stimuli. They possess structural properties attributed to the intracellular components that enable them to withstand physiological environment as well as mechanical stimuli within body. The coupling between the mechanical forces and biological processes is referred to as mechanobiology. Many *in vivo* and *in vitro* studies have proved the significance of mechanical load on different cellular processes such as cell growth, contractility, and apoptosis [Janmey et al. (1998)]. The mechanism by which cells transduce mechanical signals into biochemical responses is known as mechanotransduction, which is divided into two parts: the mechanical response and the biochemical response. Therefore, study of both intracellular load transfer mechanism and mechanotransduction is of high interest for better understanding of cell physiology. The cell forces, intracellular structures, and cell function are coupled phenomenon and their quantification using computational models will provide better understanding of their relationship.

1.3. Objective

The broad objective of this thesis is to investigate and model the mechanisms that determine the intracellular force propagation and the mechanical behavior of a cell and its structural components. More specifically formulated as follows:

- Investigate the cell response to distinct mechanical stimuli with global cell deformation by simulating mechanical tests such as tension and compression
- Investigate the cell response to local cell deformation by simulating AFM indentation test at distinct locations
- Investigate the mechanical contribution of cytoskeletal components to cell mechanics, individually and synergistically by simulating cytoskeleton disruption
- Investigate the effect of material properties of cellular components on cell's response by performing their parametric studies in all the three mechanical tests; for cytoskeletal components also investigate the effect of increase in their density

2. LITERATURE REVIEW

2.1. Cytoskeletal components

Living cells are highly complex structures consisting of large number of distinct structural components such as cytoskeleton, cell membrane (CM), nucleus, and cytoplasm. The cell functioning is mainly attributed to the structural stiffness and rheology of the cytoskeleton and its mechanical interaction with extracellular environment. The cytoskeletal network is composed of three types of filaments: AFs, MTs, and IFs, which are spread throughout the cytoplasm. Although distinct in properties, these filaments are interlinked to each other, to the nucleus, and the CM. Their structural arrangement is decisive for the response of cytoskeleton to both, external or internal mechanical stimuli. The actin-myosin contractility leads to the development of isometric tension or prestress in the cell, which is partly balanced by MTs and partly by the extracellular matrix (ECM) to which the cell is tethered [Ingber (1993)]. Thus, the cytoskeleton determines the mechanical characteristics of cell deformation needed for regulating different cellular processes.

2.2. Experimental methods for measuring cell mechanics

In the recent decade, the precise quantitative mechanical measurements of single living cells became feasible with the development of microrheological techniques. These techniques are broadly classified into two different classes: the passive measurement methods and the active measurement methods. The former examines the motion of particles introduced into the cell due to thermal fluctuations, while the latter involves direct application of forces to the cell. The active methods are further divided in two categories of experiments: those that apply mechanical stimulus to localized portion of the cell such as AFM [Pillarisetti et al. (2011)], magnetic twisting cytometry (MTC), etc. and those where a mechanical stimulus is applied to the entire cell such as microplate stretcher [Nagayama et al. (2006)], microplate manipulation [Ujihara et al. (2012), Nguyen et al. (2009)], etc. The passive methods may damage the cell and affect its interior by inducing changes in the cytoskeletal structure thus active methods are preferred over them.

2.3. Cytoskeleton disruption

One of the ways to investigate the mechanical role of cytoskeletal components, individually and synergistically is by performing the disruption study using cytoskeleton-disruptor chemical drugs. Some of the most commonly used drugs to selectively disrupt the individual cytoskeletal components are: cytochalasin-D to destabilize the actin networks, nocodazole to destabilize the MTs, and acrylamide that disrupts the IFs. For various cell types, disruption of all cytoskeletal components showed decrease in the cell reaction force when measured using different experimental techniques such as MTC [Wang (1998)] and AFM [Charras and Horton (2002a)].

2.4. Cell mechanics modeling approaches

Computational modeling provides better control over the form and organization of individual cytoskeletal components, thus it can be used to unravel the potential mechanism of cell responses to distinct mechanical stimuli [Ghaffari et al. (2016), Xue et al. (2015), Dowling et al. (2014), Barreto et al. (2013), Kardas et al. (2013), Bursa et al. (2012, 2006), Ujihara et al. (2012, 2010), Maurin et al. (2008), Unnikrishnnan et al. (2007), Cañadas et al. (2006, 2003, 2002), McGarry et al. (2004)]. The existing computational modeling approaches for cell mechanics are broadly classified into two

categories: the continuum approaches and the microstructural approaches.

The continuum approaches are employed when the smallest length scale of interest is much larger than the space over which the structures and properties of the cell vary significantly. The continuum mechanics uses coarse-graining approach to localize the microscopic stress-strain relationships, which then yields a constitutive relationship and deformation description of the material that can be applied at macroscopic scale [Lim et al. (2006)]. These are broadly classified as liquid drop models and the material models such as elastic, viscoelastic, biphasic, and active continua.

The microstructural approaches consider the cytoskeleton as the critical component in cell mechanics. One of the most frequently used models in this class is the cellular tensegrity model that envisioned cytoskeleton as an interconnected network of cables in tension representing AFs and struts in compression representing MTs [Ingber (1993)]. This model has successfully predicted viscosity modules of the cytoskeleton [Cañadas et al. (2006, 2003, 2002)] as well as experimentally observed features of cell mechanical behavior such as strain hardening [Stamenović et al. (1996)]. However, this model does not take into account other cellular components such as nucleus, cytoplasm, and CM.

For more reliable formulation of cell mechanical behavior, the hybrid modeling approach using FE analysis has been put forward by McGarry et al. (2004). Following the same approach, a more complex cell model of 210-members tensegrity structure has been proposed by Bursa et al. (2012, 2006), successfully simulating both tensile and AFM indentation tests.

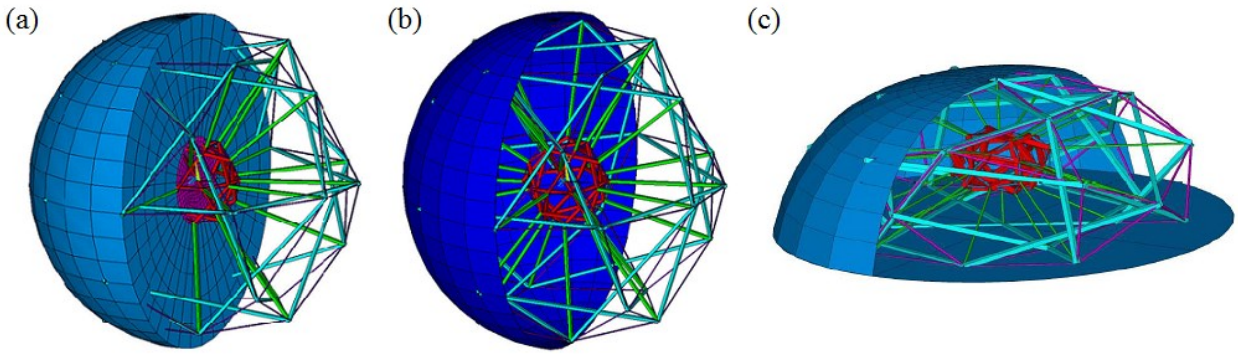

Fig. 2.1: Tensegrity FE model of suspended cell by Bursa et al. (2012, 2006), including (a) solid elements representing nucleus (purple), cytoplasm (blue) and (b) discrete elements representing cytoskeleton structure, where AFs (purple), MTs (light blue), and IFs (green) connected to the nucleoskeleton (red) and shell for CM (dark blue) and (c) modified adherent cell model of the same.

The suspended cell model depicted in Figs. 2.1a and 2.1b was based on the realistic shape of cell and includes all cytoskeletal components. Further, it was modified to adherent cell model (Fig. 2.1c) to simulate AFM indentation test, details of its creation can be found in [Lebiš (2007)]. Some of the recent models in this category are multi-structural model [Barreto et al. (2013)], self-stabilizing tensegrity structure based model [Kardas et al. (2013)], spring network cell model [Ujihara et al. (2010)], granular cell model [Maurin et al. (2008)], etc. None of these models take into account the active cell responses, where the cytoskeletal fiber undergoes polymerization and depolymerization during loading.

Recent studies of active approaches have successfully overcome this drawback by incorporating the cell's inherent active nature in computational modeling, such as bio-chemo-mechanical model [Deshpande et al. (2006)], the dynamic stochastic model [De et al. (2007)], a kinematic model

[Kaunas et al. (2009)], etc. Although recent models of this class are equipped with the formulations to explain both passive and active responses of cells, they do not elucidate the contribution of other cytoskeletal components such as MTs, IFs, etc.

2.5. Summary

The lack of consensus on the observed biophysical and biomechanical cellular responses from diverse single-cell simulation techniques thus calls for a novel structural model. This model should aim at describing the cell mechanical structure-function relationship to predict the non-linear behavior of cell. Also, it should be compatible with intracellular mechanical signaling pathways that may contribute towards better understanding of mechanotransduction. Therefore, the objective of this thesis is to develop more generic FE cell model to predict the cell mechanical properties during different experimental techniques for various cell types and at the same time, describe the passive response of intracellular components.

3. FINITE ELEMENT BENDO-TENSEGRITY MODEL OF SUSPENDED CELL

3.1. Hypothesis

Recently, the concept of "bendo-tensegrity" was proposed by Mehrbod et al. (2011) suggesting a modification to contemporary cytoskeletal tensegrity models that takes into account the flexural response of MTs. In the current study implementing this concept with hybrid modeling approach, a FE bendo-tensegrity model of suspended cell is proposed. The hypothesis of this work is that the proposed bendo-tensegrity model of suspended cell can describe the cellular structural behavior and determine cell's global response to distinct mechanical stimuli. It is not only important to study the cell response to global deformation of extracellular environment but also if it responds differently depending on the stimulus. For this, two mechanical tests are simulated with the proposed model: tensile test with micropipettes describing the cell tensile response and compression test with microplates characterizing the cell compressive response. This work is aiming towards the study of mechanical role of individual cytoskeletal components in intracellular force propagation and the quantitative characterization of nucleus deformation.

3.2. Material and methods

3.2.1. FE model formulation

In situ microscopic observations of cell shape in suspended state as well as images of distributions of cytoskeletal proteins were referred to create the three-dimensional (3D) suspended cell model and the architecture of its cytoskeletal components in ANSYS. The suspended cell model encompasses the nucleus and cytoplasm surrounded by the CM (Fig. 3.1a) and cytoskeletal components like AFs, MTs, and IFs (Fig. 3.1b). For the proposed model implementing the hybrid modeling approach, the continuum parts (nucleus, cytoplasm) were modeled using continuous (volume) elements circumscribed by a thin layer of shell elements (representing CM) and the cytoskeletal components were modeled using discrete (beam or truss) elements.

The shape of this cell model was defined as spherical with diameter (D) of 32.264 μm, taken from one of the experimental measurements by Nagayama et al. (2006). Both cytoplasm and nucleus were modeled with eight-node hexahedral isoparametric elements. A thin flexible layer circumscribing the cytoplasm referred to as the CM was modeled with four-node quadrilateral shell elements on the outer surface of the cytoplasm, with thickness of 0.01 μm [Rand (1964)] and no bending stiffness.

In a real cell, MTs of unequal lengths originate from the centrosome located near the nucleus and emanate outward through the cytoplasm till the cortex where they interact with other cytoskeletal filaments at focal adhesions (FAs). It is now evident that MTs do not have compression-only behavior but they appear highly curved (buckled) in living cells under no external load. This indicates that compressive forces in MTs induce substantial bending solely by the action of prestressed AFs; this is referred to as the "bendo-tensegrity" concept [Mehrbod and Mofrad (2011)]. Implementing this concept, the MTs of varying curvature were modeled using beam elements, originating from a single node near the nucleus (representing the centrosome) and further extending till the FAs to form a star-like shape. Every FA was connected to the centrosome with

only one MT and it was ensured that they do not penetrate the nucleus.

IFs are scattered throughout the intracellular space, connecting the FAs to the nucleus and creating a dense network in perinuclear region that stabilizes the nucleus at the center of cell. To incorporate their waviness, the IFs were modeled as truss elements resisting only tensile loads under elongations higher than 20% and all the IFs were equally strained for simplification. To mimic their real structural arrangement, they were modeled tangentially to the nucleus thus mimicking a dense network in perinuclear region. Each FA was connected to the nucleus via at least two IFs.

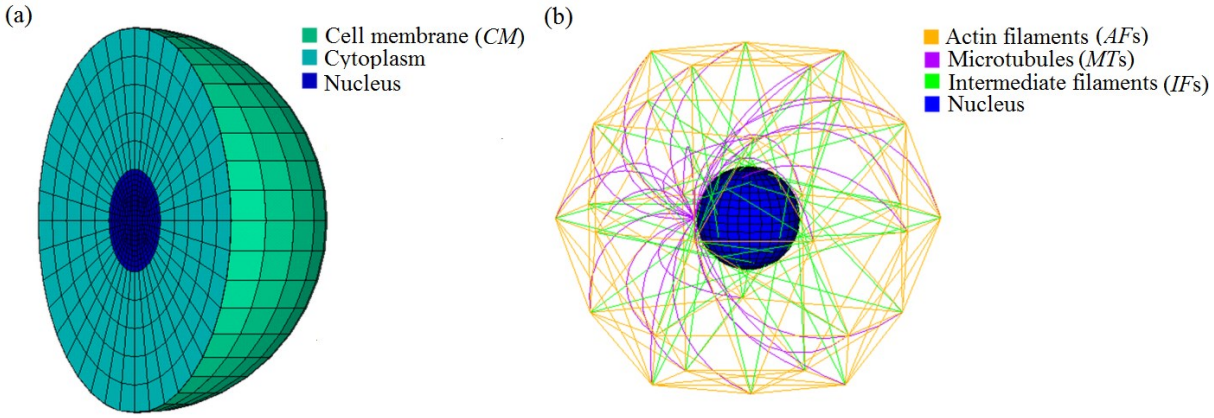

Fig. 3.1: For suspended cell model: (a) sections of continuous (volume) elements and (b) structural arrangement of cytoskeletal components with respect to the nucleus.

For cell in suspended state, a dense network of thin AFs localized beneath the CM plays a vital role in maintaining the cell shape. This framework was created by employing the geometrical shape of icosidodecahedron with its vertices representing FAs. Thin AFs filaments were modeled as truss elements that resist only tensile loads. AFs are internally prestressed; to achieve this in the proposed models, experimentally measured prestrain of 24% [Kojima et al. (1994)] was assigned to them generating the initial force (prestress) essential for cell shape stability. For simplification, this prestrain was equal in all AFs of the model. To achieve the synergistic effect of cytoskeletal components, the elements representing AFs, MTs, and IFs were connected by sharing the same end nodes at the CM representing FAs.

3.2.2. Material properties

Homogenous, isotropic, and linear elastic material properties were considered for all the cytoskeletal components; their elastic parameters taken from the literature are summarized in Table 3.1.

Table 3.1: Elastic properties of discrete components of suspended cell model

Cell component	Elastic modulus, E (Pa)	Poisson's ratio, υ	Diameter (nm)
Microtubules (MTs) [Gittes et al. (1993)]	1.2×10^9	0.3	(outer/inner) 25/17
Actin filaments (AFs) [Gittes et al. (1993)]	2.6×10^9	0.3	7
Intermediate filaments (IFs)[Bertaud et al. (2010)]	7.6×10^6	0.3	10
Actin bundles (ABs) [Deguchi et al. (2005)]	**0.34×10^6**	**0.3**	**250**

Note: The cell component in bold is included in adherent cell model instead of AFs

For the elasticity of cell components modeled using continuous elements, however, a Neo-Hookean

hyperelastic incompressible description was used with shear modulus being the only material parameter (Table 3.2).

Table 3.2: Hyperelastic properties of continuous components of suspended cell model

Cell component	Elastic modulus, E (Pa)	Calculated shear modulus, G (Pa)
Cytoplasm [Caille et al. (2002)]	0.5×10^3	0.17×10^3
Nucleus [Caille et al. (2002)]	5×10^3	1.7×10^3
Cell membrane (CM) [Rand (1964)]	1×10^6	0.33×10^6
Actin cortex (AC) [Stricker et al. (2010)]	**2×10^3**	**0.67×10^3**

Note: The cell component in bold is included in adherent cell model instead of CM

3.2.3. Loads and boundary conditions

The tensile test of a suspended cell with rigid micropipettes was simulated to investigate the cell response to stretching. The simulation was performed in several steps (Fig. 3.2 a-c), mimicking the experiment [Nagayama et al. (2006)].

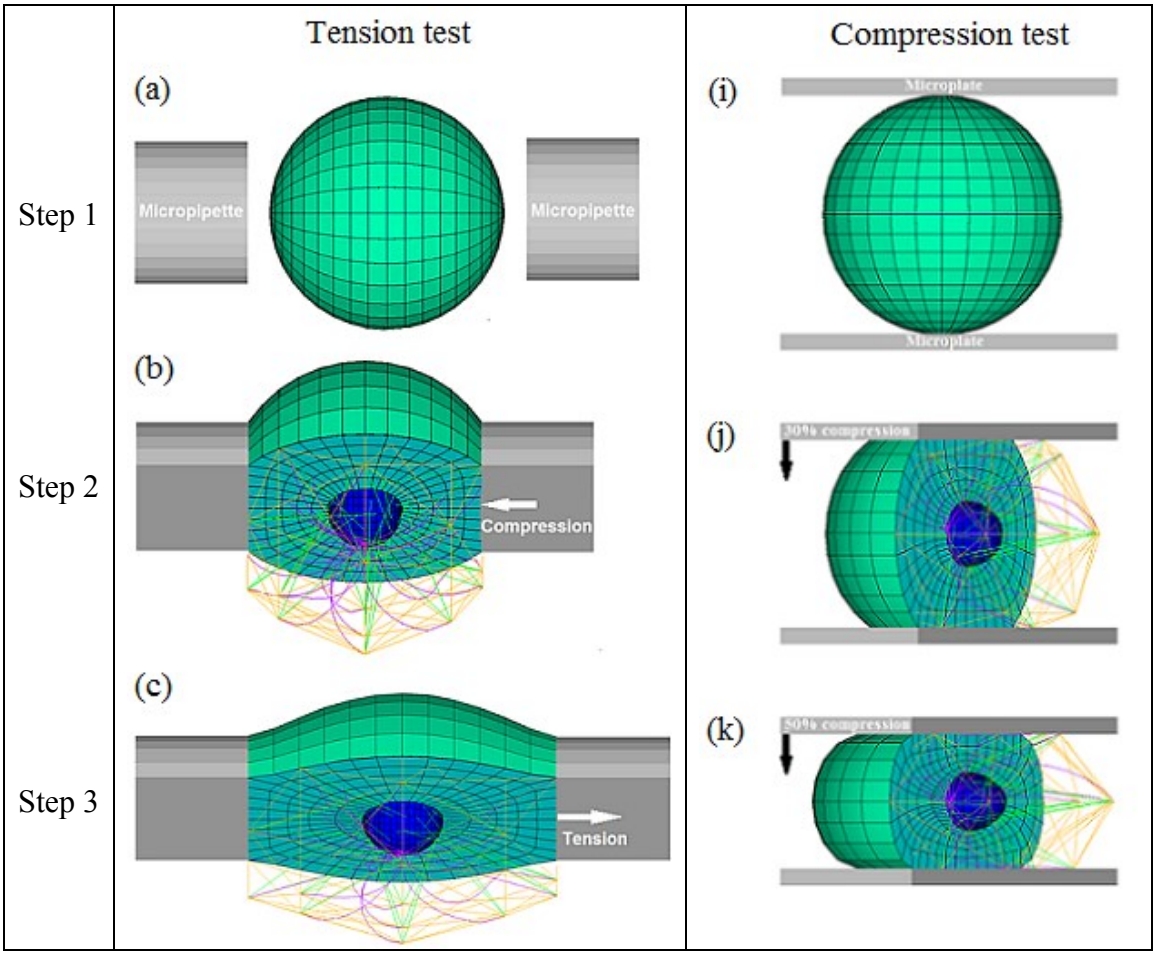

Fig. 3.2: Sectional views of the suspended cell model during consecutive steps in simulation of tensile test: (a) spherical cell and micropipettes, (b) compressing the cell against fixed micropipette, and (c) stretching the cell with movable micropipette. Sectional views of the suspended cell model during consecutive steps in simulation of compression test: (i) spherical cell and microplates, compressing the cell with the movable microplate to (j) 30% and (k) 50% deformation against the fixed microplate.

In the first step, contact between the spherical cell and both micropipettes was established by compressing the cell (with the left micropipette being fixed). The contact was set as bonded in the program to enable transmission of tensile forces in the following steps of the simulation. In the next step, the cell was elongated to achieve zero reaction forces in the micropipettes; this shape serves then as the initial (unloaded) state of the cell. In the final step, the displacement that corresponds to cell stretching in the tensile test was applied to the nodes of the movable micropipette. The reaction force was assessed as the sum of forces at the nodes of the contact surface between the cell and the movable micropipette.

The compression test of a suspended cell model with rigid microplates was simulated to investigate the cell response to compression. The simulation was performed in several successive steps (Fig. 3.2 i-k), mimicking the experiment [Nguyen et al. (2009)]. The spherical shape of the cell serves as the initial state. First, the contact was established between the cell and both microplates without cell deformation. The contact setting as bonded was kept in the program from the tensile test simulation. The cell was then compressed against the fixed microplate (bottom) by applying vertical displacements to the nodes of the movable microplate to achieve successively 10%, 30%, and 50% deformation of the cell. The reaction force was evaluated as the sum of forces at nodes of the contact surfaces between the cell and the movable microplate.

3.2.4. Parametric studies

For the proposed model, parametric studies were performed considering the material properties of different cell components as parameters. The cell model incorporating all discrete and continuous elements with the material properties specified in Table 3.1 and 3.2 are referred to as the control model. To illustrate the influence of the material parameters on cell mechanical response, their values were increased and decreased by 50% of the values used for the control model. In addition, eight different cell models were created by totally removing one or more of the cytoskeletal components to investigate their contribution to cell mechanical response. In simulation of tensile test, the reaction force of altered cell models was compared with that from the control model for 6.3 μm elongation and in simulation of compression test similarly, the reaction force of the altered cell models was compared with that from the control model for 50% deformation. In the current study, effect of increase in the density of cytoskeletal filaments included in the model was analyzed in terms of overall cell reaction force by varying their number. Additional AFs and IFs were created in the cell interior with different orientation than earlier. More MTs were created originating from the centrosome with their end nodes being chosen randomly at the CM. To investigate a similar effect of CM thickness on the overall cell reaction force its value was increased and decreased with respect to the control value.

3.3. Results of simulated tension and compression tests

3.3.1. Validation of the proposed model

The force-elongation curve calculated from tensile test simulation is in good agreement with the non-linear responses of the experimental curves obtained from the tensile test of cultured aortic smooth muscle cells (SMCs) [Nagayama et al. (2006)], as depicted in Fig. 3.3 and thus validates the proposed bendo-tensegrity model of a suspended cell. The slope of the simulated force-elongation curve increased with increase in cell stretching, as observed experimentally [Nagayama et al.

(2006), Ujihara et al. (2010)].

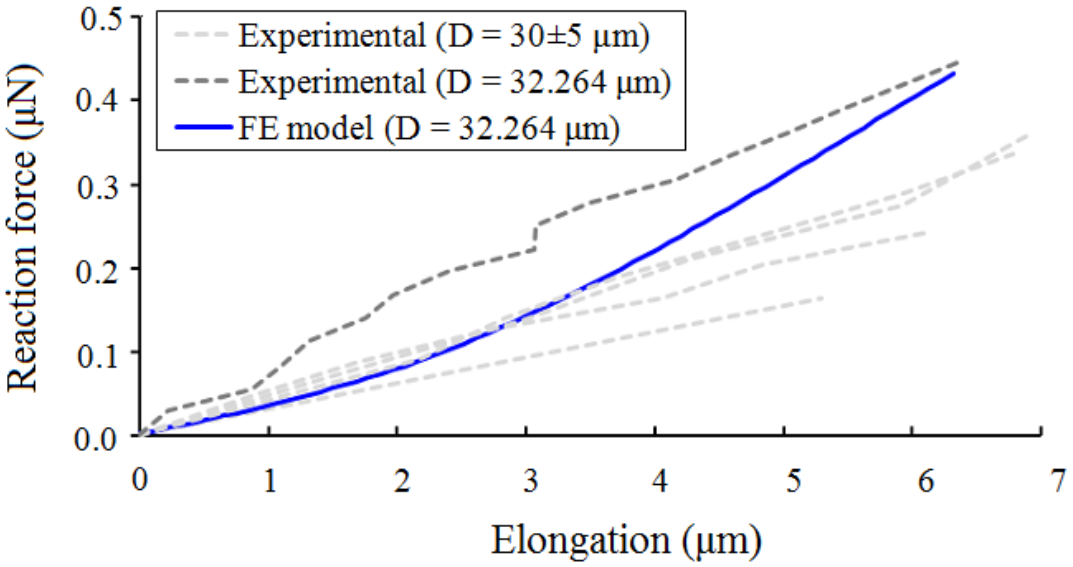

Fig. 3.3: Comparison of simulated force-elongation curve with the experimental curves taken from a study by Nagayama et al. (2006), measuring the tensile properties of cultured aortic SMCs of diameter (*D)* using a cell tensile tester.

The force-deformation curve calculated from compression test simulation is in good agreement with the non-linear response of the experimental curve obtained from the compressive test of a single chondrocyte [Nguyen et al. (2009)], as depicted in Fig. 3.4 and thus validates the proposed bendo-tensegrity model of a suspended cell.

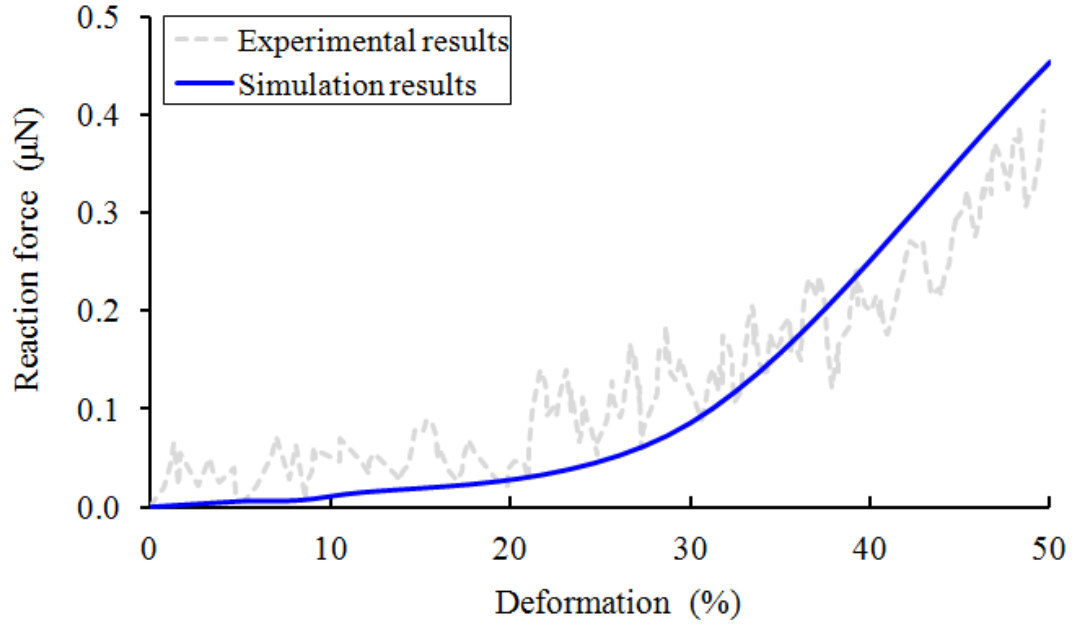

Fig. 3.4: Comparison of simulated force-deformation curve with the experimental curves taken from a study by Nguyen et al. (2009), investigating the biomechanical properties of a single chondrocyte using a micromanipulation technique.

The slope of the simulated force-deformation curve increased with increase in cell compression, similar to that observed in the experiments [Nguyen et al. (2009), Ujihara et al. (2012)].

3.3.2. Predictions of deformation inside the cell

During simulation of tensile test, the nucleus appears elongated in the direction of stretch concomitant of cell elongation (Fig. 3.5a), analogous to that observed in experiments [Nagayama et

al. (2006), Ujihara et al. (2010)]. During cell stretching some of the MTs were straightened out, while others remain bended, similar to that observed experimently [Nagayama et al. (2008)]. MTs that were straightened out and aligned in the direction of stretch resist tensile forces and generate high stresses, while the ones that were bended due to compressive forces generate low negative stresses (Fig. 3.5b); this effect highlights the influence of the shape of this component on cell deformation.

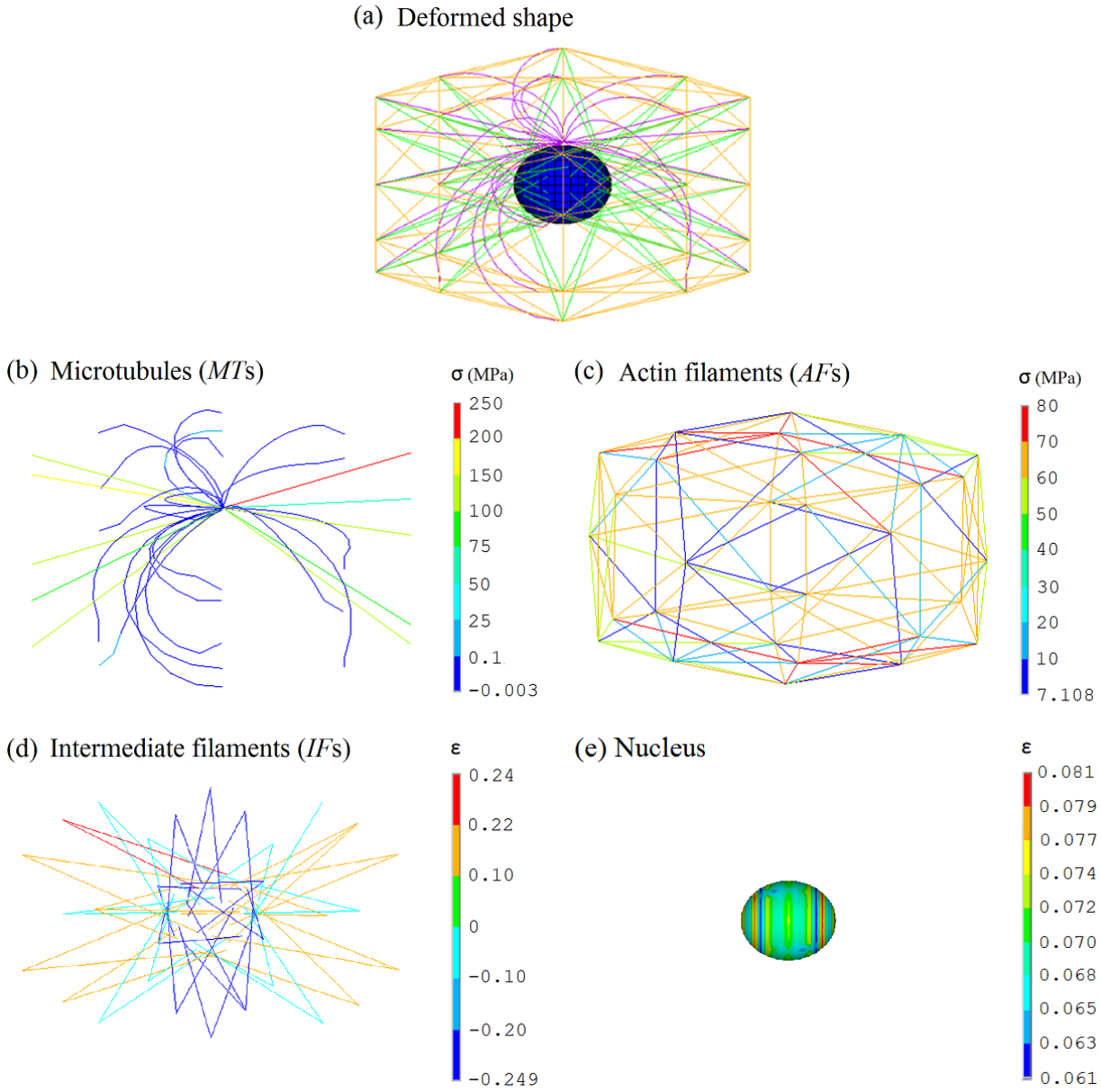

Fig. 3.5: Simulation results of 6.3 μm cell elongation: (a) deformed shape of the cytoskeletal components and nucleus; distribution of axial stress in the discrete elements representing (b) MTs and (c) AFs; (d) distribution of axial strain in the discrete elements representing IFs; (e) distribution of first principal strain in the continuous elements representing nucleus.

The randomly oriented AFs were likely to be aligned in the direction of stretch and this passive realignment gradually increased the overall reaction force of the cell, causing the force-elongation curve to be non-linear [Ujihara et al. (2010)]. High stresses were observed also in AFs (Fig. 3.5c) that were aligned in the direction of stretch and resist tensile forces. IFs aligned in the direction of stretch were linearized from their assumed initial waviness and exhibited high strains, while low strains were observed in the ones localized in the central region indicating that they remain wavy

(Fig. 3.5d). A symmetrical and uniform strain distribution pattern was observed in the nucleus (Fig. 3.5e) with a maximum deformation of 8% (first principal strain).

During simulation of compression test, the nucleus appears elongated perpendicularly to the loading direction concomitant of cell compression (Fig. 3.6a), analogous to that observed in experiments [Ujihara et al. (2012), Caille et al. (2002)]. MTs localized in the central region perpendicular to the direction of loading were straightened, while the others remain bended. MTs that were straightened and aligned in the direction perpendicular to compression resist tensile forces and generate low positive stresses, while those ones that were bended due to compressive forces generate low negative stresses (Fig. 3.6b); this effect highlights the influence of the shape of MTs on cell deformation.

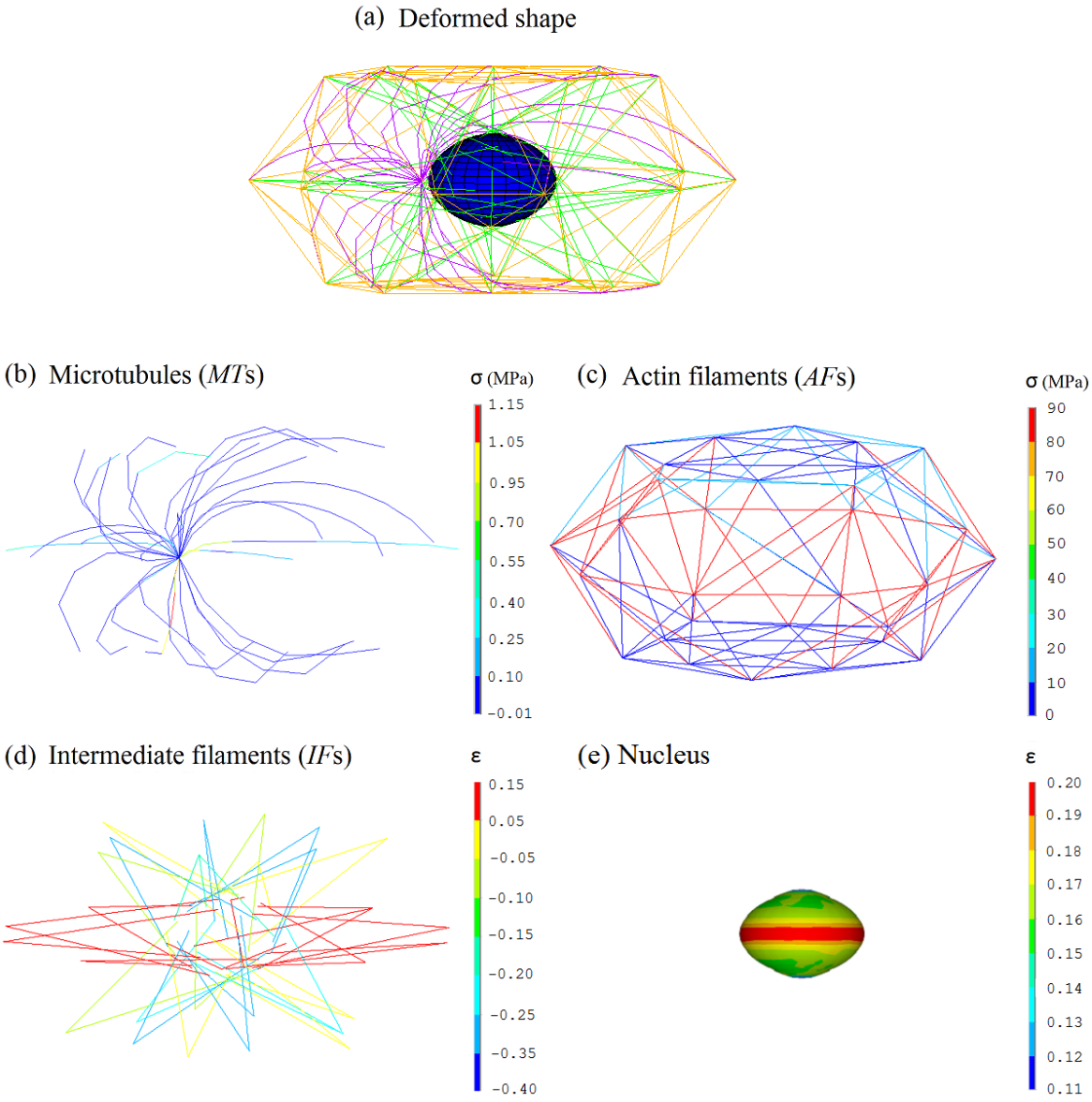

Fig. 3.6: Simulation results of 50% cell compression: (a) deformed shape of the cytoskeletal components and nucleus; distribution of axial stress in the discrete elements representing (b) MTs and (c) AFs; (d) distribution of axial strain in the discrete elements representing IFs; (e) distribution of first principal strain in the continuous elements representing nucleus.

The randomly oriented AFs and IFs were likely to be aligned passively in the direction perpendicular to loading. The filaments aligned in the loading direction were compressed, whereas the perpendicular ones were stretched. AFs reoriented perpendicularly to the loading direction resist

tensile forces and generate high stresses (Fig. 3.6c) consequently increasing stiffness of the entire cell. However, IFs that reoriented perpendicularly to the direction of compression were slightly uncoiled from their assumed initial waviness and exhibited positive strains with no stresses induced, while negative strains were observed in those ones aligned in the compression direction indicating that they remained wavy (Fig. 3.6d). A symmetrical and non-uniform strain distribution pattern concentrated at equatorial region was observed in the nucleus (Fig. 3.6e) with a maximum deformation of 20%.

3.3.3. Mechanical contribution of cytoskeletal components

Figure 3.7 illustrates the results of parametric studies investigating the mechanical contribution of individual cytoskeletal components to the overall reaction force of cell models to distinct mechanical stimuli, such as stretching and compression. The role of each cytoskeletal component in resisting global deformation is investigated via removal of each cytoskeletal component from the control model.

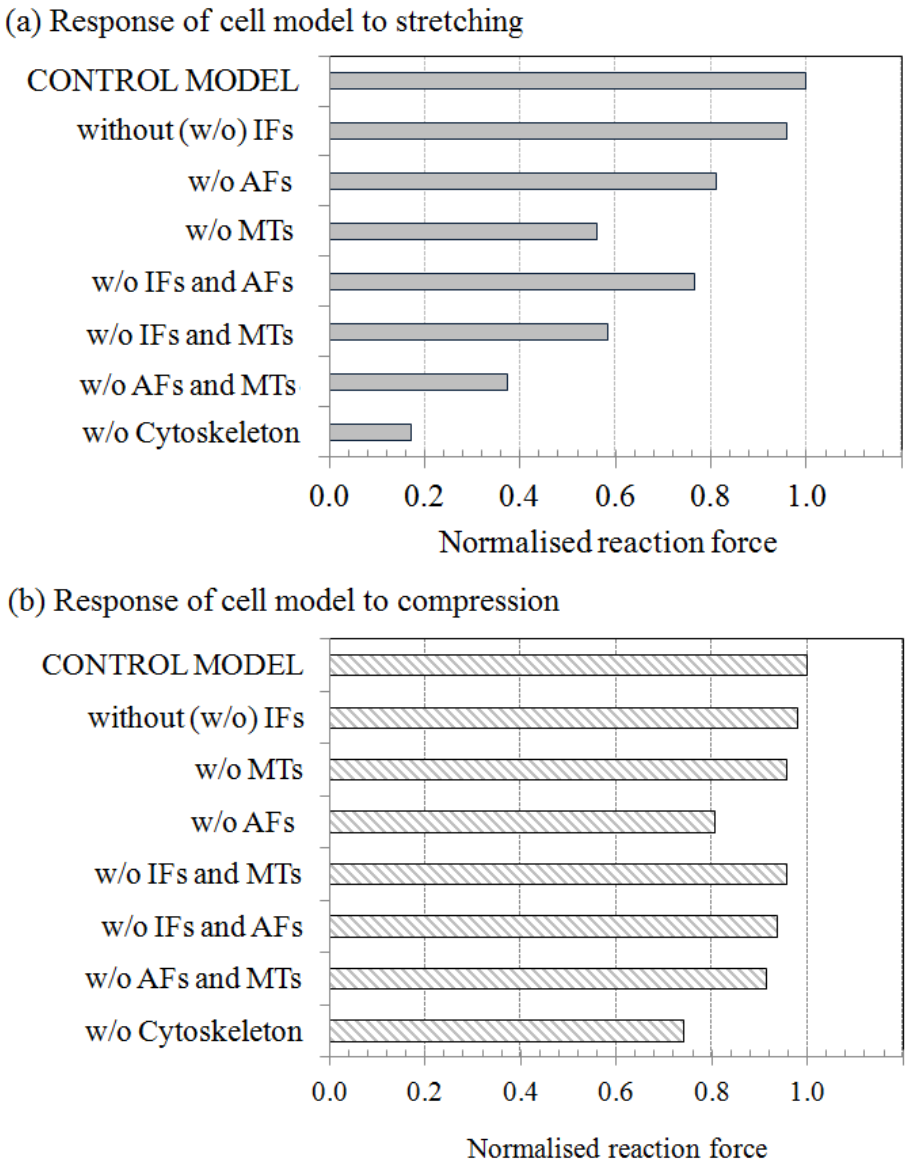

Fig. 3.7: Contribution of the cytoskeletal components individually and in mutual combination to response of cell model during (a) stretching and (b) compression, highlighting their synergistic effect. The reaction force of different cell models is normalized with respect to that from the control model.

During cell elongation of 6.3 µm, the maximum reaction force of the cell model without cytoskeleton was 5.82 times lower than the control model (Fig. 3.7a), emphasizing their pivotal role in characterizing the tensile properties of cell. The reaction force of the cell model without IFs was slightly less than the reaction force of the control model indicating their minimal contribution to cell stiffness at low strains (below 20%). Compared to the reaction force of the control model, removal of AFs reduced the reaction force of cell model by one fifth, whereas removal of MTs reduced it to approximately half, suggesting that MTs resist intracellular tension generated by AFs. Moreover, removal of both AFs and MTs drastically reduced the reaction force of cell model making it more compliant and highlighting thus their synergistic effect. Therefore, AFs and MTs play crucial roles in maintaining cell stiffness during stretching, similar to that observed in experiment by Nagayama et al. (2008).

During 50% cell compression, the maximum reaction force of the cell model without cytoskeleton was 1.35 times lower than the control model (Fig. 3.7b), highlighting their role in characterizing the compressive properties of cell. When MTs were removed the model showed an approximately twofold decrease in the cell reaction force compared to the model with IFs being removed. Compared to the reaction force of the control model, removal of AFs reduced the reaction force of the cell model by one fifth. Even though AFs are tension bearing elements, they play a vital role in maintaining the cell stiffness during compression, similar to that observed experimentally by Ujihara et al. (2012). Additionally during simulation of both tests, the cell models created without one or more cytoskeletal components were also inadequate to withstand the cell forces, underlining their synergistic effect.

3.3.4. Parametric variation of material properties

Figure 3.8 depicts the results of parametric studies investigating the effect of varying modulus of elasticity of individual cellular components on the cell response to stretching and compression. During these studies, the prestress in AFs was retained constant. It was observed that the reaction force of the model was highly sensitive to the variation of Young's modulus of certain cellular components.

During simulation of tensile test, the variation of Young's modulus of both AFs and MTs substantially affected the cell reaction force (Fig. 3.8a). A 50% decrease in Young's modulus of MTs was not realized due to problems with convergence and thus, only 25% decrease in the same was considered. Among continuum elements, regardless of small thickness the change in Young's modulus of CM had significant influence on the overall cell reaction force followed by cytoplasm. The parametric studies revealed that during stretching the overall cell reaction force was relatively insensitive to the changes in elastic modulus of IFs and nucleus. Although not presented in Fig. 3.8a, simulation results have demonstrated that inclusion of the compressibility for both nucleus and cytoplasm, did not much affect the overall cell reaction force.

During simulation of compression test, the variation of Young's modulus of AFs considerably affected the cell reaction force (Fig. 3.8b). Among continuum elements, cytoplasm with its large volume had significant influence on the cell reaction force followed by CM regardless of its small thickness. On the other hand, the cell model sensitivity to elastic modulus of the nucleus was limited that can be associated to its small volume. The parametric study revealed that the cell

reaction force was relatively insensitive to the changes in Young's modulus of both MTs and IFs.

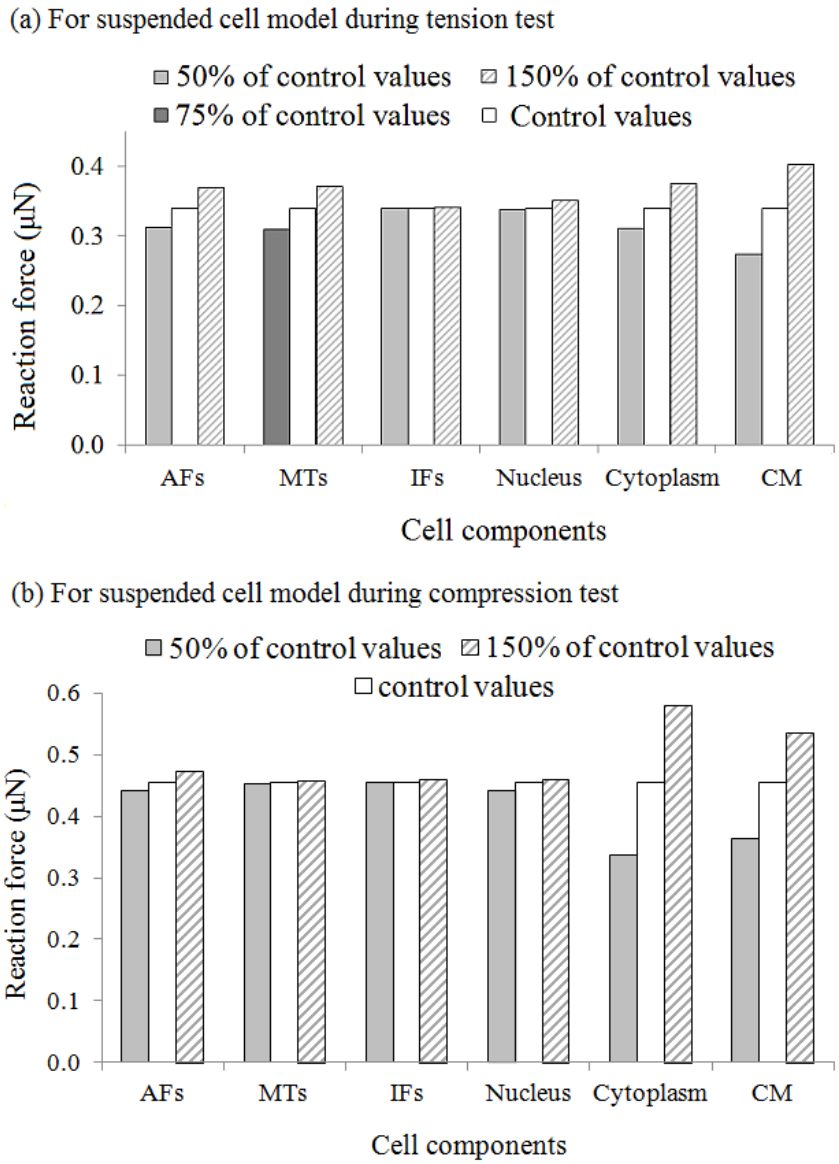

Fig. 3.8: The effect of varying elastic modulus of individual cell components from the control values (Table 3.1 and 3.2) on the overall cell reaction force during (a) stretching and (b) compression.

Although not presented in Fig. 3.8b, simulation results have demonstrated that inclusion of cytoplasm compressibility minimally affected the overall cell reaction force.

3.3.5. Effect of increase in the density of cytoskeletal components

Measuring the number of cytoskeletal filaments experimentally is one of the most difficult parameters however, effect of increase in the density of these filaments can be determined computationally. During cell elongation, additional AFs created in the cell interior got aligned in the direction of stretch and increased the cell stiffness. In the same way, additional MTs orientated in the loading direction were straightened to resist tensile forces and thus substantially increased the overall cell reaction force. Increase in the density of elements representing IFs did not show much variation in cell reaction force, suggesting low impact of these filaments on the cell stiffness due to their waviness. On the other hand, during cell compression, additional AFs created in cell interior increased the overall cell reaction force, whereas increase in the density of both MTs and IFs did

not show much variation. Both during cell elongation and cell compression, variation of the thickness of CM affected the overall cell reaction force substantially.

It has been observed that the influence of increase in the number of cytoskeletal components shows an effect similar to that of increase in their elastic modulus. A similar effect occurring for CM suggests that its behavior is dominated by tension (membrane stresses) rather than by bending (bending stiffness of a shell is proportional to the 3rd power of its thickness).

3.4. Summary of suspended cell model

The proposed bendo-tensegrity model of suspended cell describes the response of cytoskeletal components and cell as a whole to distinct mechanical stimuli (elongation and compression), thus satisfying the initial hypothesis. The proposed model predicted the relation between cellular mechanical response and stress/strain distributions within the specific cytoskeletal components under different loading conditions. For simulation of both tests, it provides quantitative characterization of nucleus deformation.

The tensile test simulations demonstrated that AFs and MTs play a crucial role in cell stiffness including its increase with stretching, while the compression test simulations revealed that although being tension-bearing elements, AFs contribute significantly to the compressive response of a cell. Thus, the proposed bendo-tensegrity model identifies the cytoskeletal components that influence the global mechanical response of suspended cell depending on the type of mechanical loading.

Parametric studies of material properties of cell components demonstrated that cell response to stretching was highly affected by changes in elastic modulus of AFs, MTs, CM, and cytoplasm. Likewise, cell response to compression was highly affected by variations of elastic modulus of AFs, CM, and cytoplasm. Similar results were obtained from parametric studies of increase in the density of cytoskeletal components: the cell response to both stimuli was sensitive to increase in the density of AFs and variations of the thickness of CM, but only the cell response to stretching was sensitive to increase in the density of MTs.

Thus, the proposed suspended cell model based on the bendo-tensegrity concept provides new insights into the interdependence of cellular mechanical properties, the mechanical role of components of cytoskeleton, and the nucleus deformation under different mechanical loading conditions.

4. FINITE ELEMENT MODIFIED BENDO-TENSEGRITY MODEL OF ADHERENT CELL

4.1. Hypothesis

The hypothesis of this study is that the proposed bendo-tensegrity model of adherent cell modified from the suspended cell model (chapter 3) can describe the cellular structural behavior and determine cell's local response to extracellular deformation. The proposed model can not only study the cell's response to local surface deformation initiated by some extracellular body but also if it responds differently depending on the deformation location. To achieve this, indentation using AFM is simulated with the proposed model indenting at two distinct locations at the apex (between the FAs above the nucleus) and at a receptor (at FA away from the nucleus). This study is focusing on the mechanical role of each cytoskeletal component in the intracellular force propagation and the quantitative characterization of the deformation of nucleus.

4.2. Material and methods

4.2.1. FE model formulation

In this study, the simulation of AFM indentation test with the proposed FE adherent cell model was performed and the results obtained were compared with corresponding experimental results. *In situ* microscopic observations of cell shape in adherent state (Fig. 4.1a) as well as images of distributions of cytoskeletal proteins were referred to create a 3D adherent cell model (Fig. 4.1b) and the architecture of its cytoskeletal components. The adherent cell model is slightly different from the suspended cell model (section 3.2.1), it encompasses the nucleus and cytoplasm enclosed by the AC (Fig. 4.1c) and cytoskeletal components like ABs, MTs, and IFs (Fig. 4.1d).

For this model, the size and approximate geometry was based on the rules described by McGarry et al. (2004). The model geometry was semi-ellipsoidal with the radius of 19 μm (semi-major axis in both front and top views), height of 8 μm (semi-minor axis in front view), and half-width of 12.66 μm (semi-minor axis in top view). Based on the experimental observations [Caille et al. (2002)], nucleus was also modeled ellipsoidal. It is positioned at the center of the top view at the height of 2 μm from the substrate with radius of 2.5 μm (semi-major axis) and height of 3 μm (semi-minor axis of 1.5 μm). Due to the geometric complexity of cell configuration, both cytoplasm and nucleus were meshed with four-node tetrahedral solid elements. Using analogous approach as presented by Barreto et al. (2013), a thin layer of actin-gel at the cell surface referred to as AC was modeled with four-node quadrilateral shell elements of 0.2 μm thickness [Unnikrishnan et al. (2007)] and no bending stiffness. Here, the CM was not explicitly considered as it is thinner than the adjacent AC and therefore its minor contribution to resisting local deformation was included in the shell stiffness dominated by AC.

For the proposed adherent cell model, the morphological representation and spatial distribution of both MTs and IFs were retained analogous to that of the suspended cell model (see section 3.2.1). In contrast, however in a cell adhered to a rigid substrate, thick ABs were observed localized at the cell periphery running almost uniformly in the longitudinal direction [Barreto et al. (2013), Deguchi et al. (2005)].

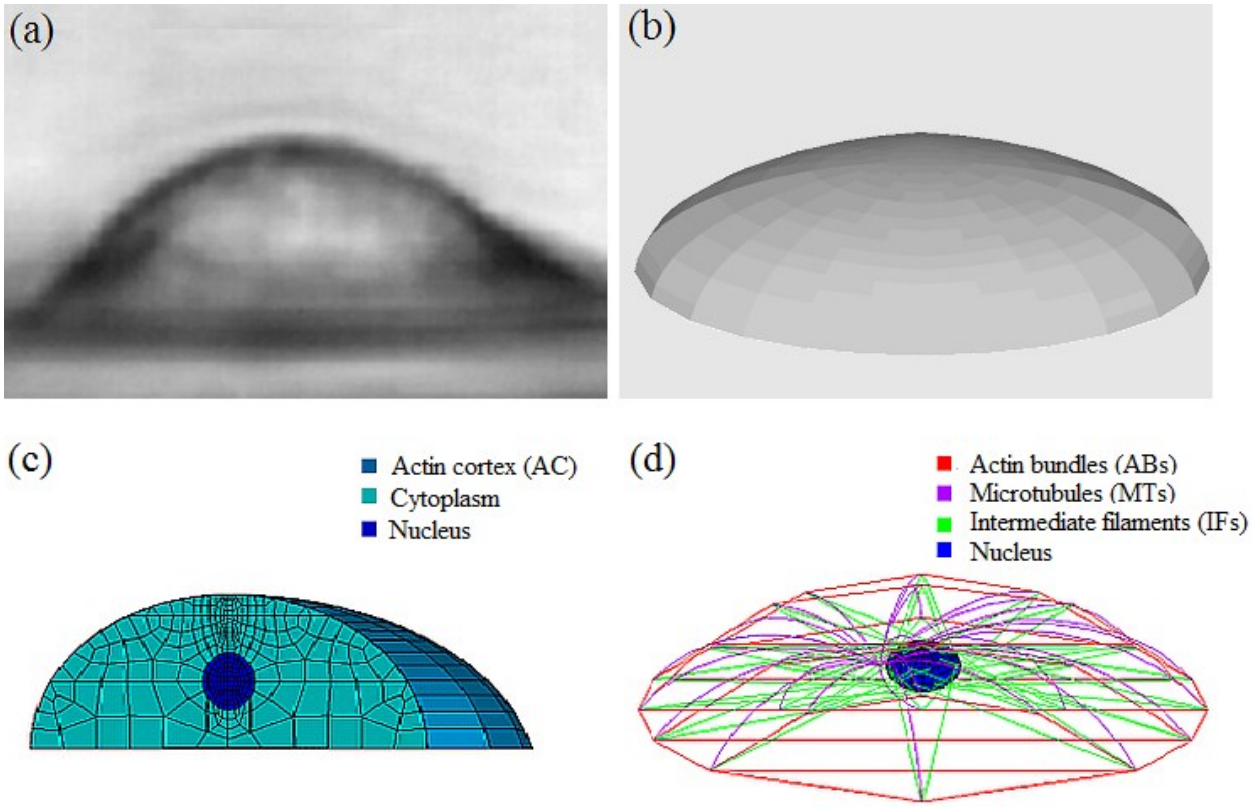

Fig. 4.1: (a) Typical images of an adherent cell on a rigid microplate [Thoumine et al. (1999)] and (b) its corresponding proposed FE model with (c) sections of continuous (volume) elements and (d) structural arrangement of cytoskeletal components with respect to the nucleus.

These bundles were modeled using truss elements that resist only tensile loads and arranged along the AC with both ends anchored to it at FAs together with elements representing MTs and IFs to achieve their synergistic effect. ABs are internally prestressed; to achieve this in the proposed model, the prestress caused by 24% of prestrain [Deguchi et al. (2005)] was assigned to them generating the initial force essential for cell shape stability. For simplification, all ABs in the model were prestressed equally.

4.2.2. Material properties

Material properties have been set identical with the suspended cell model presented in chapter 3 and differences are highlighted in bold in Tables 3.1 and 3.2. Due to the difficulty of obtaining elastic parameters of cellular components from a single cell type, those employed in proposed models were acquired from literature for distinct cell types measured using various experimental techniques.

4.2.3. Loads and boundary conditions

Indentation of a rigid AFM tip into a cell adhered to a rigid substrate was simulated to investigate the cell's response to indentation. A rigid paraboloid indenter with the tip curvature radius of 200 nm was modeled above the cell in line with nucleus (Fig. 4.2), to exert a stimulus and obtain the reaction force in the assigned pilot node. By prescribing a vertical shift to the pilot node, the tip was advanced towards the cell to penetrate it after having come into contact. Corresponding to indentation depth in AFM experiments, the vertical displacement of 2.5 μm was applied to the tip.

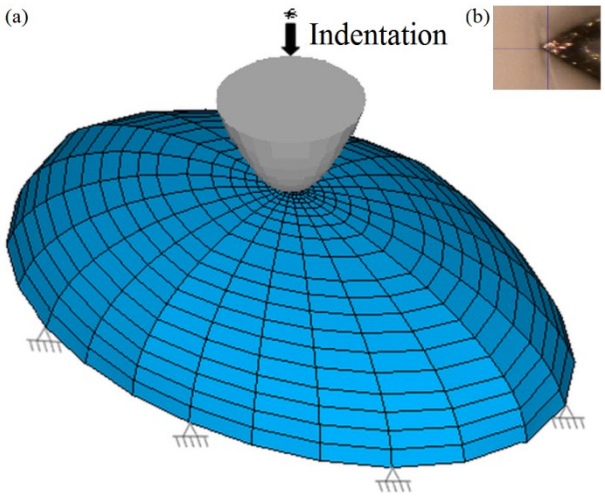

Fig. 4.2: (a) FE model of an adherent cell in the indentation test, with constraints and displacement load applied at the tip acting on the top of the cell. (b) Snapshot of a cell during AFM indentation experiment (top view) [Hemmer (2008)].

For a non-linear contact problem of tip-cell interaction the augmented Lagrangean algorithm was considered. For all nodes at the bottom of the cell a constrained boundary condition was prescribed, simulating a cell adhered to a rigid substrate.

4.2.4. Parametric studies

For the proposed model, parametric studies were performed considering the material properties of different cell components as parameters. The cell model incorporating all discrete and continuous elements with the material properties mentioned in Tables 3.1 and 3.2 are referred to as the control model. To illustrate the influence of the material parameters on cell mechanical response, their values were increased and decreased by 50% of the values used for the control model. In addition, ten different cell models were created by totally removing one, two, or more of the cytoskeletal components to investigate their mechanical contribution. The reaction force of altered cell models was compared with that from the control model under 1 μm indentation. In the current study, effect of increase in the density of cytoskeletal filaments present in the model was analyzed in terms of overall cell reaction force by varying their number. Additional ABs were created at the cell periphery as well as in the cell interior with different orientation than earlier. The number of MTs originating from the centrosome was increased with their end nodes being chosen randomly at the AC. The density of IFs was increased by creating more IFs in the cell interior making their network denser in the perinuclear region. To investigate a similar effect of AC thickness on the overall cell reaction force its value was increased and decreased with respect to the control value.

4.3. Results of simulated AFM indentation test

4.3.1. Validation of the proposed model

The force-indentation curves calculated from simulations of AFM tip indenting the adherent cell model at the apex (Fig. 4.3a) and at a receptor distant from the apex (Fig. 4.3b) are depicted in Fig. 4.3c. Both results lie within the variation range of the experimental curves obtained with AFM indentation of embryonic stem cells (ESCs) [Pillarisetti et al. (2011)], which thus validates the

proposed bendo-tensegrity model of an adherent cell. The indentation test was simulated at two distinct locations on the cell, to explore the significance of its structural inhomogeneity that could be one of the reasons for scattering of experimental curves [Ohara et al. (2000)].

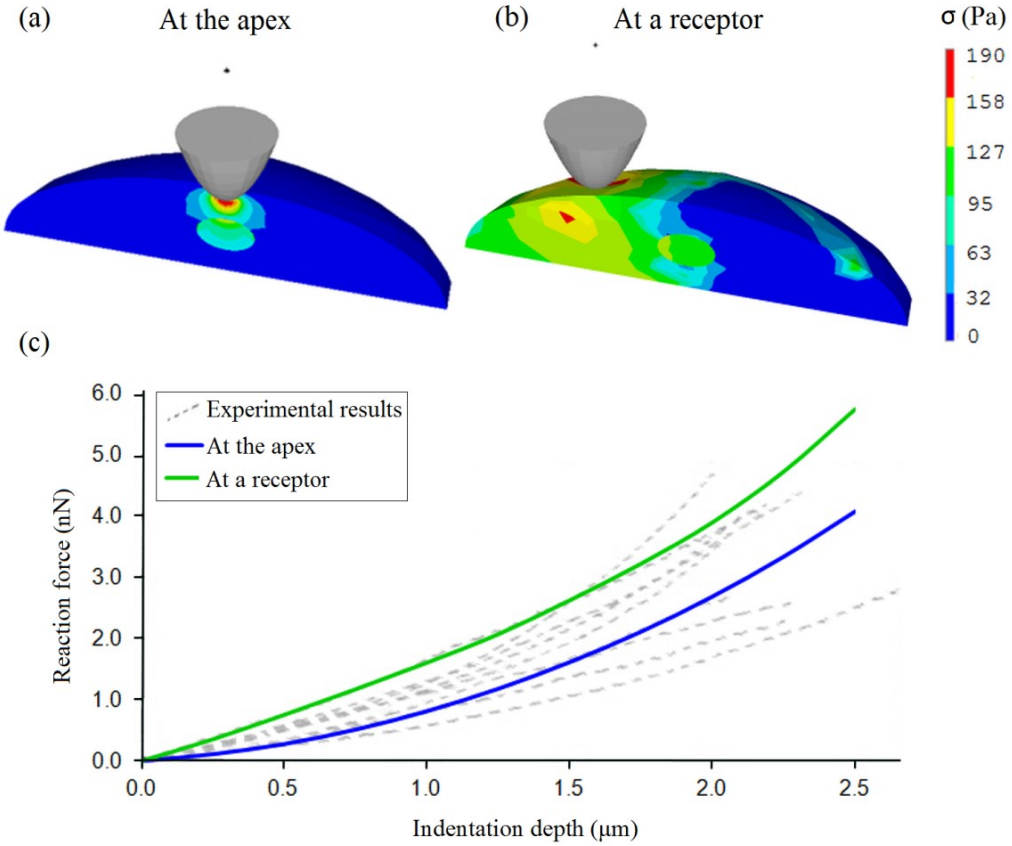

Fig. 4.3: Sectional views comparing the contour plots of von Misses stress in cytoplasm during AFM simulation for indentation depth of 1 μm at (a) the apex and (b) a receptor; (c) comparison of simulated force-indentation curves with the experimental curves taken from a study by Pillarisetti et al. (2011), measuring the stiffness of ESCs using AFM indentation.

For indentation depth of 2.5 μm, the reaction force predicted by the model at the apex was 4.05 nN and at a receptor was 5.72 nN, thus illustrating approximately 40% stiffer response when indenting at a receptor compared to the apex. This can be explained by the direct transmission of indenting force to cytoskeleton that is much stiffer than the surrounding cytoplasm. Therefore, these results exhibit that the indentation site dominates the force-indentation curve which corresponds to the experimental observations [Ohara et al. (2000)]. A remarkable difference in stress (von Misses) distribution pattern within the cell was observed when indenting at the apex compared to a receptor, underlining the synergistic effect of cytoskeletal components on localized load transmission to the distant parts of the cell known as action-at-a-distance effect [Wang et al. (2005)]. For both simulations, stiffening of force-indentation curve with increase in indentation depth was observed.

4.3.2. Predictions of deformation inside the cell

The proposed model can predict cellular mechanical response to indentation and stress/strain distributions within the specific cytoskeletal components. Figure 4.4a exhibits the deformed shape of cell during indentation simulation at a receptor. The model predicted low stresses in MTs, which can be explained by their flexural behavior. MTs that were compressed due to localized load generated low negative stresses (Fig. 4.4b), while those that were stretched resisting intracellular

tension generated low positive stresses. Higher stresses were observed in ABs (Fig. 4.4c) compared to MTs, highlighting the effect of this component on the localized cell deformation although they do not propagate compressive forces due to the level of prestress they are subjected to.

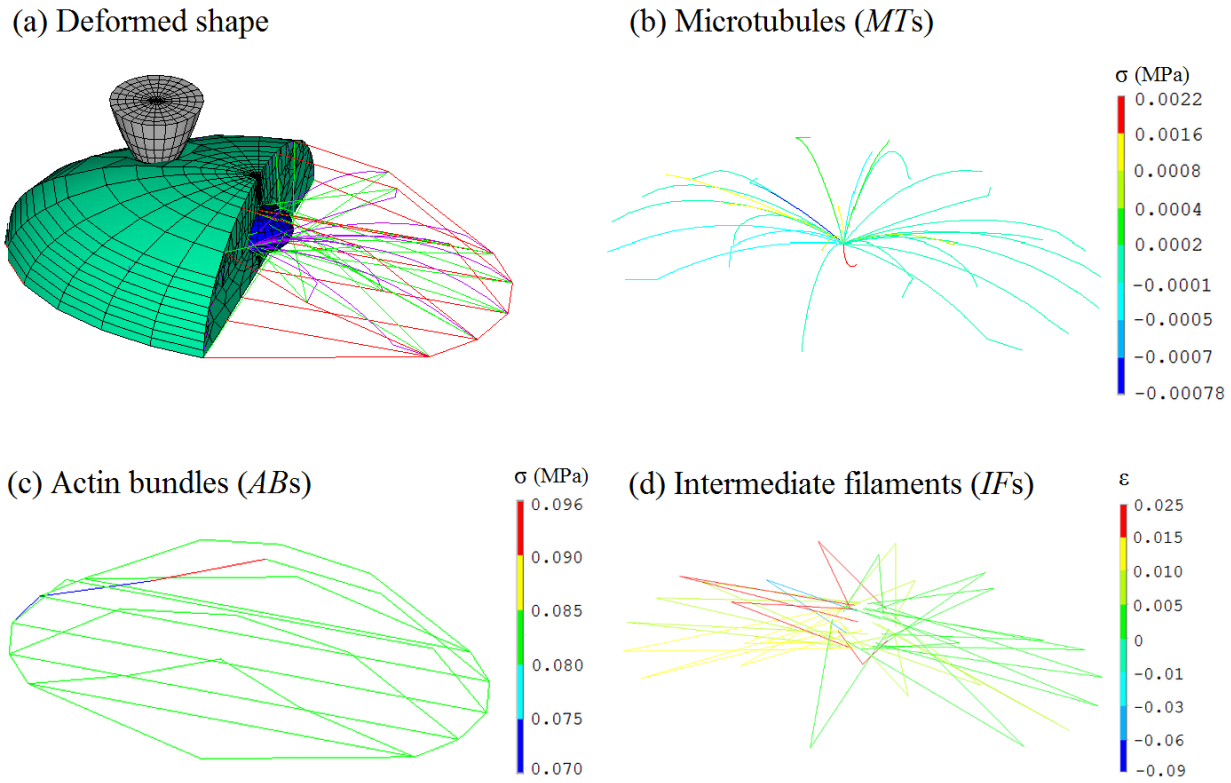

Fig. 4.4: Simulation results for indentation depth of 2.5 µm at a receptor: (a) deformed shape of cell; distribution of axial stress in the discrete elements representing (b) MTs and (c) ABs; (d) distribution of axial strain in the discrete elements representing IFs.

For IFs strains are shown instead of stresses, because stresses in them are zero due to their high waviness. IFs localized around a receptor were slightly uncoiled from their assumed initial waviness and exhibited high strains, while the remaining ones showed low strains indicating that they continue to remain wavy (Fig. 4.4d). For the same indentation depth of 2.5 µm, the nucleus deformation was about 4% when indenting cell at the apex in line with the nucleus (Fig. 4.5a), whereas it was about 0.08% when indenting cell at a receptor away from the nucleus (Fig. 4.5b); this highlights the dominance of indentation site on nucleus deformation.

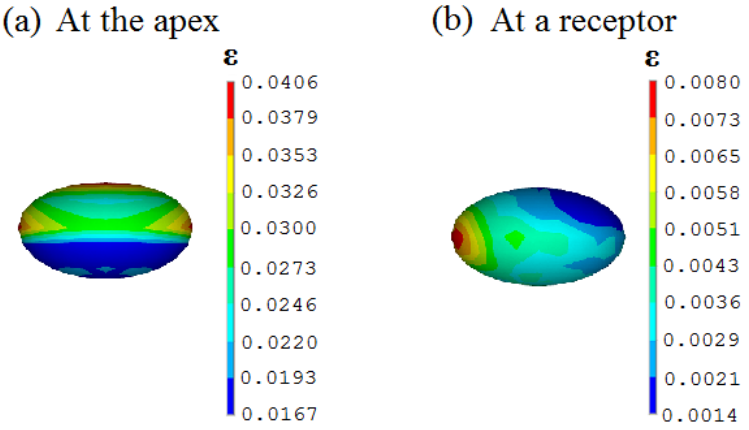

Fig. 4.5: Distribution of first principal strain in the elements representing nucleus when indenting cell at (a) the apex and (b) a receptor.

This quantitative characterization of nucleus deformations could be hypothetically decisive for mechanotransduction. In both simulations a non-uniform strain distribution pattern was observed, symmetrical and concentrated at the top center region in the former, whereas non-symmetrical and concentrated at the side region (facing indentation location) in the latter.

4.3.3. Mechanical contribution of cytoskeletal components

Figure 4.6 illustrates the results of parametric studies investigating the mechanical contribution of individual cytoskeletal components to the overall cell reaction force during indentation. The role of each cytoskeletal component in cell's response to indentation was investigated via removal of one or more cytoskeletal components from the control model.

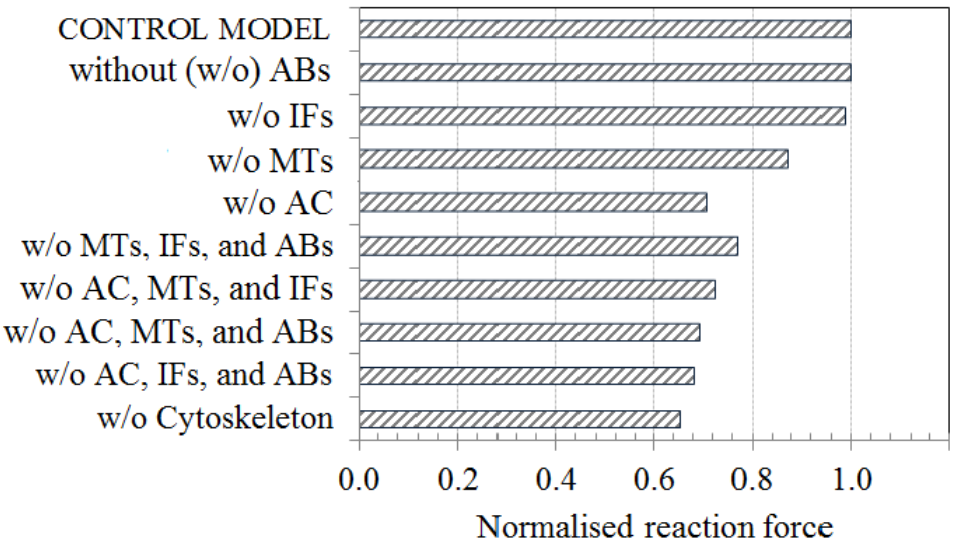

Fig. 4.6: Contribution of the cytoskeletal components individually and in mutual combination to cell's response to indentation (at a receptor), highlighting their synergistic effect. The reaction force of different cell models is normalized with respect to that from the control model.

The maximum reaction force of the cell model without cytoskeletal components was 1.52 times lower than the control model, highlighting their contribution in resisting the mechanical perturbations from exterior. For model without ABs and model without IFs, the reaction force was comparable to that of the control model, indicating their minimal effect on cell rigidity during indentation. When AC was removed the model showed approximately twofold decrease in the reaction force compared to the model with MTs being removed. Thus, AC and MTs play critical role in maintaining cell rigidity during indentation, analogous to that observed experimentally by Barreto et al. (2013). Furthermore, the cell models created without two or more cytoskeletal components were also incompetent to withstand cell forces, emphasizing their mutual interdependence during indentation.

4.3.4. Parametric variation of material properties

Figure 4.7 shows the results of parametric studies investigating the effect of varying modulus of elasticity of individual cellular components on the cell's response to indentation. During these studies, the prestress in ABs was retained constant. The variation of Young's modulus of AC considerably affected the reaction force of adherent cell model, probably due to constraining the volume of cytoplasm. Parametric analysis revealed that the adherent cell model was highly sensitive to the variations in elastic modulus of cytoplasm, whereas its sensitivity to the changes in elastic

modulus of nucleus was very limited that can be associated to their different volumes.

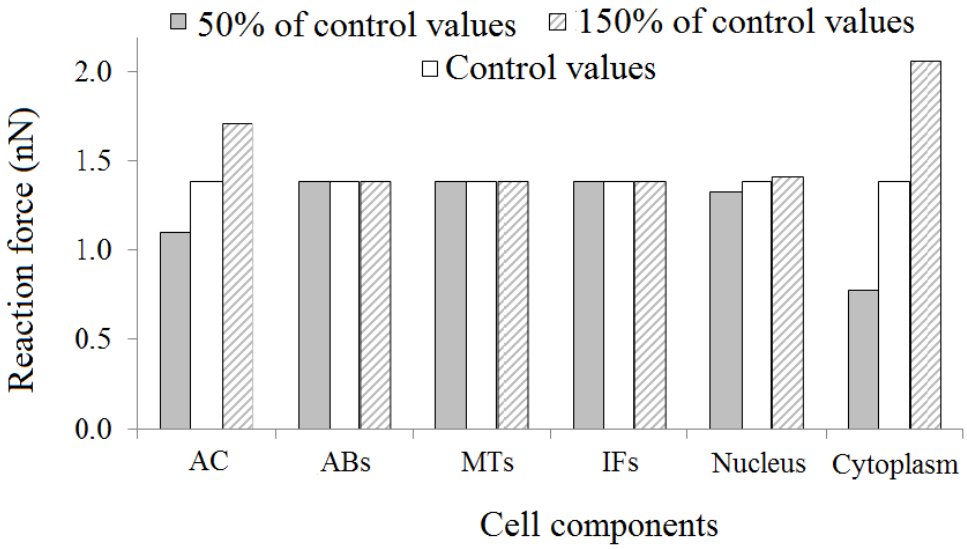

Fig. 4.7: The effect of varying elastic modulus of individual cell components from the control values (Table 3.1 and 3.2) on the overall cell reaction force during indentation.

On the contrary, changing the Young's modulus of ABs, MTs, and IFs to 50% or 150% of their control values did not affect the overall cell reaction force. Although not presented in Fig. 4.7, simulation results have demonstrated that inclusion of cytoplasm compressibility affect the overall cell reaction force approximately by only 2%.

4.3.5. Effect of increase in the density of cytoskeletal components

The structural arrangement of cytoskeletal components in this model is a simplified representation of their complexity in real cell. Thus, it is of high interest to study the effect of increase in their density on the cell's response to indentation.

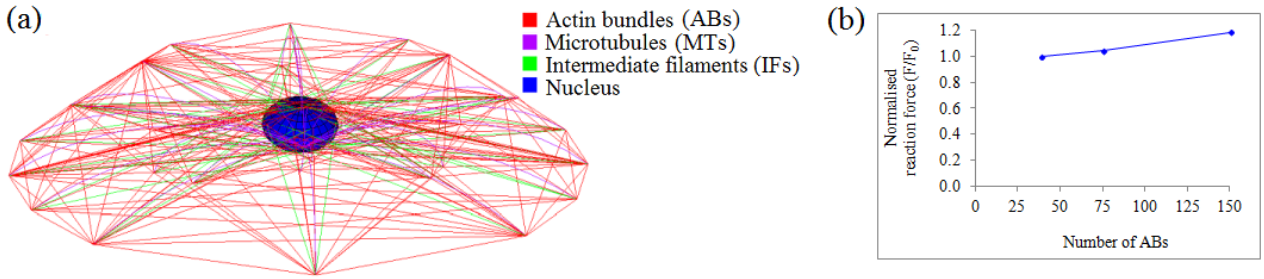

Fig. 4.8: (a) Additional ABs created in the model with new orientation for parametric studies and (b) their effect on the overall cell reaction force during indentation.

Figure 4.8a exhibits additional ABs created at the cell periphery and interior. An increase in overall cell reaction force was observed for increased number of ABs (Fig. 4.8b), which might be attributed to the more number of ABs resisting local mechanical load due to their new configuration. Increase in the density of elements representing MTs and IFs did not show much variation in the cell reaction force, whereas variation of AC thickness significantly changed the cell's response to indentation.

4.4. Summary of adherent cell model

The proposed bendo-tensegrity model of adherent cell describes the response of cytoskeletal components and cell as a whole to AFM indentation, thus satisfying the initial hypothesis. This

model describes the mechanical role of individual cytoskeletal components including stress/strain distribution within them. It provides quantitative characterization of nucleus deformation for distinct indenting sites on cell surface.

The simulation results exhibited that indentation site dominates the cell behavior where AC and MTs were essential for cell rigidity. Thus, the proposed model identifies the cytoskeletal components that influence the local mechanical response of cell. Parametric studies of material properties exhibited that changes in elastic modulus of AC and cytoplasm highly affected the cell's response to indentation. Further, parametric studies of increase in the density of cytoskeletal components revealed that the cell response was sensitive to increase in the density of ABs specifically in the cell interior and variations of the thickness of AC.

Thus, the proposed adherent cell model based on the bendo-tensegrity concept opens new perspectives in studying the interdependence of cellular mechanical properties, mechanical contribution of individual cytoskeletal components, and the nucleus deformation.

5. DISCUSSION

The presented models aim at realistic simulation of the deformation of cell, primarily focusing on mimicking its structure to obtain realistic nucleus deformation. It is well established that cells respond to mechanical stimuli in a variety of ways that range from changes in cell morphology to activation of biochemical responses, which affect the cell's phenotype. The nucleus plays a central role in defining cell responses thus the current study provides an insight into its deformation by means of FE modeling of distinct single cell mechanical tests. The nucleus deformation of 8% (maximum first principal strain) was observed for cell stretching of 6.3 μm (20% global deformation), whereas the maximum first principal strain in nucleus was about 20% for cell compression of 16.132 μm (50% global deformation). Moreover, the maximum first principal strain in nucleus was approximately 4% under indentation (local deformation) of 2.5 μm at cell apex, suggesting that the nucleus deformation depends on the nature of biophysical stimulus [Prendergast (2007)]. Further, it is observed that the same maximum first principal strain of 4% in nucleus can be induced either by cell indentation of 2.5 μm at the apex or by cell stretching of 2.081 μm or by cell compression of 3.871 μm. This quantitative characterization of nucleus deformation during distinct mechanical tests of single-cell could be hypothetically decisive for initiating the mechanotransduction. It means that, from the point of view of biochemical responses, indentation of 2.5 μm could be equivalent to stretching by 2.081 μm or to compression by 3.871 μm.

Although it was intended to simulate various mechanical tests using the same model, the distinctness in the form and organization of actin protein for cell in suspended and adherent states served as a motivation for creating two distinct models of cell. In numerous experimental studies, for cell in suspended state a dense network of thin AFs is observed at cell periphery localized beneath the CM [Ofek et al. (2010), Li et al. (2006), Nagayama et al. (2006), Guck et al. (2005)], whereas for cell in adherent state a thin layer of actin-gel referred as AC is observed at the cell periphery along with thick ABs running almost uniformly in the longitudinal direction [Barreto et al. (2013), Matsumoto et al. (2012), Li et al. (2006), Nagayama et al. (2006), Deguchi et al. (2005)].

The tensile test simulation results demonstrated that the bended MTs were highly compliant with stresses below 1 MPa, whereas the straight ones (being in tension only) showed much higher stresses (Fig. 3.5b), highlighting the significant contribution of MTs arranged in loading direction to the cell stiffness during stretching. When stretched by approximately 100 % the suspended cell model gave the reaction force of 1.8 μN that evaluated the cell stiffness of 2.4 kPa, which is in good agreement with normalized stiffness of 2.6±0.5 kPa measured for cultured SMC [Nagayama et al. (2006)]. Although the simulated response showed higher non-linearity (strain stiffening) than the experiments (Fig. 3.3), this tendency is in accordance with other experimental results [Miyazaki et al. (2002)]. The contribution studies of the cytoskeletal components in tension and compression test simulations (Figs. 3.7a and 3.7b) demonstrated that removal of AFs reduced the cell stiffness by approximately 20%, which is less than the value observed experimentally [Nagayama et al. (2006), Ofek et al. (2009), Ujihara et al. (2012)]. This difference in stiffness might be attributed to the treatment with cytochalasin D that resulted in disruption of not only the deep actin fibers but also an actin meshwork beneath the CM [Ujihara et al. (2012)]. As a result, the cell stiffness was reduced substantially during experiments in contrast to the simulations.

The structural arrangement of cytoskeletal components in adherent cell model creates an elastic network that can sense and transmit mechanical stimuli and explains the different published results obtained with local experimental techniques, such as AFM. The adherent cell model shows that local application of load is preferably borne by AC and bulk components of the cell while the cytoskeleton is not much active, with the exception of MTs (Fig. 4.6) and this could be attributed to the longitudinal configuration/orientation of the ABs. When these were removed from the model, the overall cell reaction force was about the same as the control model (Fig. 4.6). At the same time, when present in the model they sustain high deformation to resist indentation (Fig. 4.4c), which is related to the fact that these elements were pre-stressed. Therefore, the prestress in ABs is identified as a requirement for force generation in the model and the AC to maintain its local surface rigidity, supported by MTs at receptors.

The cellular tensegrity models envision AFs as tension supporting cables and MTs as compression supporting struts [Ingber (1993)]. Although these models successfully explain several observations in cell mechanics, the excessive compression stiffness of the struts introduces non-realistic artifacts in tension test simulations, as shown by Bursa et al. (2012, 2006). The previous tensegrity-based models neither take into account the influence of flexural behavior of MTs nor predict the mechanics of individual cytoskeletal components during cytoskeleton disruption studies. The proposed bendo-tensegrity models modify this structural concept by taking into account both flexural (buckling) as well as tensional behavior of MTs and having no restriction on the spatial distribution of fibers.

Implementing the hybrid modeling approach, the interaction of discrete and continuous elements is ensured at the nodes representing FAs and thus, the external forces transmitted through FAs are sensed by the entire discrete structure as well as all the continuum parts. These models defined the structural adaptability for cytoskeleton components, where they can move independently of each other and can be completely removed individually or in combination without disintegrating the cell structure, as opposite to the tensegrity theory. In both proposed models, the suggested form and organization of individual cytoskeletal components allow exploration of the force transmission pathways for mechanical stimuli acting on the cell surface to propagate to the nucleus, ultimately resulting in mechanotransduction. The quantitative information on forces transmitted by individual cytoskeletal components in distinct cell types can be obtained by varying the mechanical properties of individual cytoskeletal components in them. The simulation results show capability of the proposed models in describing the response of not only the cell as a whole but also nucleus and individual cytoskeletal components to distinct mechanical stimuli.

Although the proposed models have some advantages over the previous models, they have certain limitations as well. The structural arrangement of cytoskeletal components does not capture their true complexity and dynamic behavior as observed in living cells. These models also do not take into consideration the viscoelastic nature of cell. Due to their passive nature, they are not able to capture the active responses of cell such as remodeling of AFs and MTs exhibited under mechanical loading.

Taken together, the proposed models describe the short term response of intracellular components and cell as a whole to distinct external mechanical stimuli not only qualitatively but also quantitatively, thus contribute to better understanding of structure-function paradigm of living cells. An attempt is made to develop these models as computational tools that may serve as a novel way to investigate the cell mechanical behavior, which, when combined with *in vitro* observations could be more effective.

6. CONCLUSIONS

6.1. Concluding remarks

The present thesis was aimed towards a realistic computational modeling of cytoskeleton and cell as a whole. Two FE bendo-tensegrity models focusing on cytoskeletal mechanics of cell in different states were proposed to study passive cell behavior: a suspended cell model and an adherent cell model. In this study, three mechanical tests of single cell were simulated and the results obtained were compared with corresponding published experimental data. Tensile test with micropipettes and compression test with microplates were simulated by means of the suspended cell model to elucidate the global cell response, while AFM indentation test was simulated by means of the adherent cell model to explicate the local cell response. The findings of this thesis predict and explain distinct cellular behaviors that emerge from mutual interaction between specific cytoskeletal components observed under different experimental conditions. The main findings of this thesis can be summarized as follows:

- The proposed models provide simulation of the cell mechanical responses during tension, compression, and indentation tests, aid to illustrate the mechanical role of individual cytoskeletal components including stress/strain distribution within them, and offer quantitative information on the nucleus deformation hypothetically decisive for mechanotransduction.
- Analogous to cellular tensegrity concept, the proposed models incorporate the preexisting tensile stress and interaction among cytoskeletal components. Further, this concept is modified by incorporating both flexural (buckling) as well as tensional behavior of MTs, waviness of IFs, and with no restriction on the spatial distribution of cytoskeletal components, where they can move independently of each other. Therefore, they predict relations among cellular mechanical properties and stress/strain distributions within the specific cytoskeletal components, under different mechanical loading conditions.
- Depending on external mechanical stimuli, the models predict role of specific cytoskeletal components in force transmission through the cell. Parametric studies of the mechanical role of the components of cytoskeleton exhibited that AFs and MTs play a crucial role in cell stiffness including its increase with stretching. It was observed that, even though AFs are tension bearing elements, they contribute significantly to the compressive properties of cell. On the other hand, AC and MTs were essential for cell rigidity during indentation and the indentation site dominated the cell behavior.
- Parametric studies of material properties demonstrated that the dependence of reaction force on deformation of the suspended cell model during both tensile and compression tests was affected by changes in elastic modulus of AFs, CM, and cytoplasm, whereas during tensile test the same was also affected by changes in MTs modulus of elasticity. On the other hand, the reaction force of adherent cell model during indentation test was affected by changes in elastic modulus of AC and cytoplasm. Furthermore, inclusion of cytoplasm compressibility affected the reaction force of suspended cell model during compression only.
- Parametric studies of increase in the density of cytoskeletal components revealed that the suspended cell model during tensile test was sensitive to the increase in the density of both AFs and MTs, whereas the same during compression test was sensitive to the increase in the density of AFs only. On the other end, the adherent cell model during indentation test was sensitive only

to the increase in the density of ABs. The models were sensitive to the changes in thickness of the surface layer, created of shell elements representing CM in the suspended cell model or AC in the adherent cell model.

- These parametric studies identify the biological parameters in cells that influence tissue mechanics the most and provide valuable guidelines about the structure of individual cytoskeletal component for future cell-phenotype modeling.
- The proposed cell models take into account the distinctness in form and organization of actin protein for cell in different states (suspended and adherent) and highlight its influence on the interpretation of force-deformation measurements.

The proposed models may thus contribute to a better understanding of various cellular mechanical processes such as mechanotransduction or cytoskeleton remodeling.

6.2. Future works

Some of the previous limitations of the proposed models serve as a basis for their optimization. The followings are recommended to be incorporated:

- By applying appropriate boundary conditions, multiple other single-cell mechanical tests (MTC, MA, etc.) can be also simulated. Further these models can be incorporated into multi-scale models, which may provide a key link between the responses of tissues and cells to distinct mechanical stimuli, that is, to explain the relations between macroscopic mechanical loads and the mechano-biological response at the molecular level.
- Most of the *in vitro* studies measure the cell deformation over time or frequency. Under external mechanical loads, cells deform exhibiting both solid-like elastic and fluid-like viscous behaviors. Therefore, cells and their components can be better described as viscoelastic materials and the specific mechanical properties measured will depend on the time scale [Hoffman and Crocker (2009)]. In the proposed models, viscoelastic properties can be defined for the continuous components using a standard linear solid model.
- Following the approach put forward by Maurin et al. (2008) using granular media, inclusion of the centrosome in the proposed models might explain the link between nucleus and MTs, however, little is known about the mechanics of centrosome. To achieve this, computational simulations with or without the centrosome should be performed which can help in better understanding of its contribution to cell mechanics under different loading conditions.
- Active cell responses could be incorporated in the proposed models using a cytoskeletal remodeling description proposed by Deshpande et al. (2006) following the approach of Dowling et al. (2013). The remodeling process is based on three coupled phenomena: an activation signal that triggers actin polymerization and myosin phosphorylation, the tension-dependent assembly of the actin and myosin into stress fibers (SFs), and the cross-bridge mechanics between the actin and myosin filaments that generates the tension.

Implementing the bendo-tensegrity concept with hybrid modeling approach, the proposed models present cell geometries in different states with more realistic morphological representations of the cytoskeletal proteins that can predict reliable cell mechanical responses, which aids in research areas of drug development, tissue engineering, investigation of cancer cells, or regenerative medicine therapies.

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[6] Bansod Y.D. and Burša J. *Tensegrity models of mechanical behaviour of living cell.* Computational Mechanics 2013 conference, Spicak - Czechia, 4-6 November, 2013.

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