

# Atomic Force Microscopy-Based Single-Cell Indentation: Experimentation and Finite Element Simulation

Hamid Ladjal, Jean-Luc Hanus, Anand Pillarisetti, Carol Keefer, Antoine Ferreira and Jaydev P. Desai

**Abstract**—In order to understand and characterize the mechanical property and response of the mouse embryonic stem cells (mESC), we used an atomic force microscope (AFM) combined with a PHANToM haptic feedback device. Atomic force microscopy has rapidly become a valuable tool for quantifying the biophysical properties of single cells or a collection of cells through force measurements. We report herein the mechanical characterization of single mESC using indentation-relaxation measurements with micro-sphere AFM probes for fixed and live undifferentiated mESC. During cell indentation for both live and fixed undifferentiated cells, we provided force feedback to the user in real-time through the PHANToM haptic feedback device as the AFM tip was deforming the cell. The force was amplified for the human operator to perceive the change in force during cell indentation by the AFM cantilever. This information can be used as a mechanical marker to characterize state of the cell (live and fixed). As the interpretation of atomic force microscopy-based indentation tests is highly dependent on the use of an appropriate theoretical model of the testing configuration, various contact models are presented to predict the mechanical behavior of an individual mouse embryonic stem cells (mESC) in different states. A comparison study with finite element simulations (FEM) of spherical tip indentation demonstrates the effectiveness of our computational model to predict the mESC deformation during indentation and relaxation nanomanipulation tasks.

## I. INTRODUCTION

The realization of promising potential of stem cells for engineering bone, cartilage, muscle and other connective tissues requires a proper characterization of their unique biological, biochemical, proteomic, and biomechanical properties that are yet to be fully elucidated. The mechanical properties such as elasticity, membrane tension, cell shape, and adhesion strength may play an important role in the cell fate and differentiation [1],[2],[3]. The mechanical properties of biological cells have been studied with different techniques [4], the most popular are optical tweezers [5], magnetic beads [6], and micropipette aspiration [7]. However, those methods cannot compete with the precision that can be attained with atomic force microscopy (AFM) method [8]. AFM has been widely used in the study of micro- and nanostructures including living cells.

Modern AFM techniques allow solving a number of problems of cell biomechanics by simultaneous evaluation of the local mechanical properties and the topography of the living

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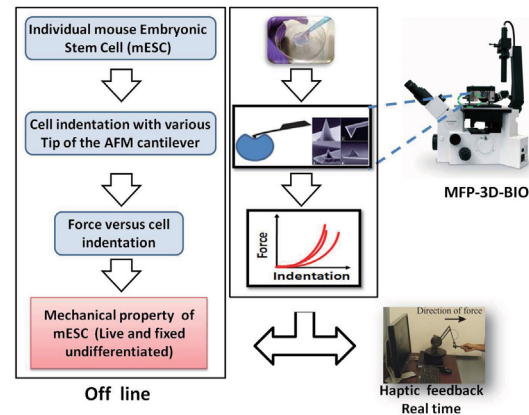


Fig. 1. Schematic showing the haptics-enabled atomic force microscopy: The AFM is used to obtain force and indentation data.

cells, at a high spatial resolution and force sensitivity [9]. In these experiments AFM cantilever serves as a microindenter to probe the cell, and further analysis of force-indentation data yields the local Young's modulus. In addition, AFM indentation technique can be used to characterize the viscoelastic behavior of the cell cytoskeleton [10], including viscosity [11], loss and storage moduli [12], and stress relaxation times. Fig.1 presents the AFM-based indentation techniques with force feedback that have been applied to characterize single-cell mouse embryonic stem cells (mESCs) [13]. The proposed indentation tests use microsphere-modified AFM probes in order to estimate global elastic modulus for the cell to reflect the true global response of a mESCs [14], [15]. Distribution of the indenting load over several  $\mu\text{m}^2$  area, averages the contribution of multiple cytoskeleton fibers and makes the contact analysis more accurate. Indeed, the use of 5  $\mu\text{m}$  diameter spherical indenter allows to reduce experimental elastic data dispersion several-fold compared to regular pyramidal sharp SiNi AFM tips [16], [17]. As the interpretation of AFM-based indentation tests is highly dependent on the use of an appropriate theoretical model of the testing configuration, our results demonstrate the applicability of the Hertz contact model as the most appropriate model compared to the capsule model or the JKR and DMT models. We have used the Hertzian contact model for the spherical tip indenter to compute the Young's modulus of the cells (live and fixed). We demonstrated that two dimensional mechanical models of mESCs are suitable for analytical studies and give a valuable understanding of the principles governing mechanical interactions within a cell. A numerical validation study of the Hertzian contact model has been

conducted through a realistic two-dimensional finite element modeling (FEM). Finally, the FEM simulations validate the mESC stress relaxation properties (hysteresis was about 15-20 %) indicating that energy dissipation due to the material viscosity contribution is low at this probe velocity and force measurements are dominated by the cell elastic behavior. The proposed FEM model constitutes a basis for simulation of micro/nano-injection tasks using virtual haptic feedback model in tissue engineering and cancer research [18]. The paper consists of five sections. In Section II, we present the materials and methods used in our work. In Section III, we present the results of mouse embryonic stem cells characterization. In Section IV, we make a comparative study between the different analytical and numerical models. Finally, in Section V, we give some concluding remarks and the directions for future work.

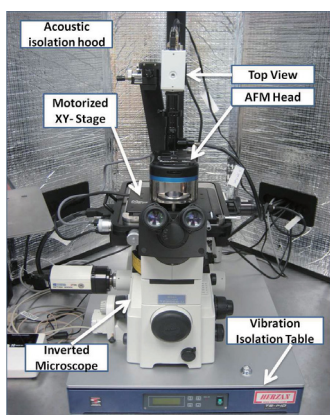


Fig. 2. Atomic force microscopy: The experimental set up for mESC indentation studies.

## II. EXPERIMENTAL SETUP

### A. Description

The nanoindentation of the cells was performed using the atomic force microscope (Model: MFP-3D-BIO, Asylum Research, Santa Barbara, CA) combined with PHANToM haptic feedback device (Sensable Technologies, Inc.). The AFM is integrated with a top view module and mounted on a active vibration isolation table manufactured by Herzan (Laguna Hills, CA) as shown in Fig.2. The top view module enables viewing of cells and easy alignment of the laser beam on the AFM cantilever. The XY stage (manual) allows the user to position the cell beneath the cantilever tip of AFM. The entire AFM set up is enclosed in a acoustic isolation chamber to prevent acoustic noise from interfering with the AFM measurements. The scan head range is  $90\mu\text{m}$  in  $x$ - and  $y$ - axes and  $40\mu\text{m}$  in  $z$ -axis. The Atomic force microscopy (AFM) system is used to obtain force and cell deformation data from biological samples. The cantilever is moved by the piezoelectric scanner in the  $z$  direction towards the cell. The stiffness depends not only on Young's modulus but also on the geometry of the tip-surface contact. Therefore, the geometry and spring constant of the cantilever are calibrated

in the same way for the live and fixed cells (see Fig.3). In the following experiments, we used a spherical tip (sphere diameter:  $5\mu\text{m}$ ) with spring constants  $0.006\text{N/m}$  and  $1.75\text{N/m}$  for the live and fixed cells respectively ( *we found in our experience that the fixed cells were stiffer compared to the live cells*).

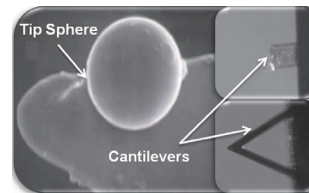


Fig. 3. View of the spherical tip used for the live and fixed (mESC): attached on different cantilevers ( $5\mu\text{m}$  diameter).

## III. MOUSE EMBRYONIC STEM CELLS CHARACTERIZATION

### A. Cell culture preparation

It is recommended to use specially coated dishes or slides to facilitate adhesion. Such Petri dishes or slides are available commercially. Cells should be attached to some rigid substrate, usually either a slide or the bottom of a Petri dish. In our study, the mouse embryonic stem cells (mESC) R1 (SCRC-1011, American Type Culture Collection (ATCC), Manassas, VA) were grown on 0.1 % gelatin coated plates in the absence of feeder cells. The ES medium consisted of 1000 U/ml leukemia inhibitory factor (LIF, ESGRO, Chemicon, Temecula, CA), 15 % fetal bovine serum (FBS)(Invitrogen), and basic medium that included Knockout Dulbecco's modified Eagle's medium (Invitrogen), 2 mM L-glutamine, 1x non-essential amino acids, and 0.1 mM mercaptoethanol. Differentiation was induced by removal of LIF from the medium. Prior to experiments, cells were dispersed using trypsin to obtain single cells and were plated on 60 mm tissue culture petri dish. Fixed mouse ES cells were obtained by treating the live mouse ES cells with 4 minutes and were stored in phosphate buffered saline (PBS).

### B. Geometry of the cell

The cells were  $10$  and  $15\mu\text{m}$  in diameter. The phase contrast module enabled imaging low contrast, transparent cells in fluid via an inverted microscope as shown in Fig.4(a). The cells were stained with Hoescht 33342 and were visualized by fluorescence microscopy as shown in Fig.4(b). The three-dimensional geometry of mESC colony is visualized via the AFM contact mode imaging (Fig.5). The mESC colony size can be estimated through image analysis (ImagePro software from Asylum). To determine the cells height, two force indentation measurements are necessary. First, initial height The cell's height  $h = h_0 - h_1$  is is measured by bringing the AFM tip in contact with the hard substrate  $h_0$  firstly and then bringing the AFM tip on the cell surface gives  $h_1$  .

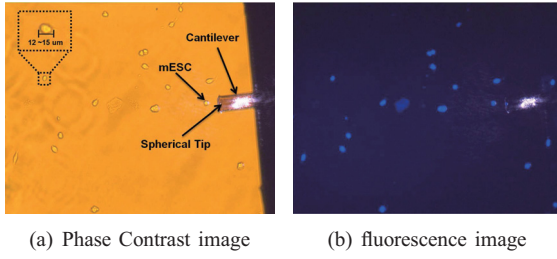


Fig. 4. Phase contrast image and fluorescence image of the mESC using the spherical cantilever tip.

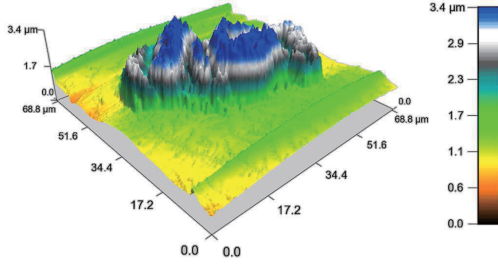


Fig. 5. Contact mode AFM (mESC colony).

#### IV. MECHANICAL CHARACTERIZATION OF MESC: REVIEW OF ANALYTICAL MODELS

To estimate the mechanical properties of biological cells using the AFM, various analytical models can be used to estimate Young's modulus of mESC in live as well as fixed cells.

##### A. The Hertz contact

The Hertz contact model has been used extensively by the AFM community to quantify the mechanical property of biological samples using AFM [8], [9]. The Hertz contact model describes the simple case of elastic deformation of two perfectly homogeneous smooth surfaces touching under load. The forces measured are dominated by the elastic properties. In our work, the geometry of the tip used for all experiments is spherical. The mechanical interaction between the spherical tip and mESC can be described by the Hertz contact model of two elastic bodies [20]. The model assumes that:

- The material properties of the tip and the cell are *isotropic and homogeneous*.
- The normal contact of the two bodies is *adhesionless and frictionless*.
- The contact geometry is assumed to be *axisymmetric, smooth and continuous*.

The relationship between the indentation  $\delta$ , and the loading force  $F$  is given by:

$$F = \frac{4}{3} E^* R^{\frac{1}{2}} \delta^{\frac{3}{2}} \quad (1)$$

where  $E^*$ ,  $R$  are the combined modulus and the relative curvature of the tip and the cell respectively

$$\frac{1}{E^*} = \frac{1 - \nu_1^2}{E_1} + \frac{1 - \nu_2^2}{E_2} \quad (2)$$

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2} \quad (3)$$

where  $(E_1, \nu_1, R_1)$  and  $(E_2, \nu_2, R_2)$  represents the elastic modulus, Poisson's ratio, and the radius of the cell and spherical indenter respectively. The elastic modulus of the silicon nitride cantilever and silicon cantilever were 222.22 GPa and 168.17 GPa used for the live and fixed cells respectively. The elastic modulus of the cells is in range of KPa [11]. Hence, our assumption that the tip used for probing is infinitely stiff compared to the cell and Hertz contact model is valid. Thus, Eq. 1 can be rewritten as:

$$F = \frac{4E}{3(1 - \nu^2)} R^{\frac{1}{2}} \delta^{\frac{3}{2}} \quad (4)$$

##### B. The Capsule contact

The second model considers the biological cell to be composed of a cell membrane and cytoplasm. It's the capsule model [23]. The model assumes that the cell membrane is a thin film and that the inner cytoplasm provides a uniform hydrostatic pressure on the membrane. The model assumes the following:

- The cell membrane is linearly elastic.
- The deformation of the cell membrane is caused by stretching and the bending is neglected.
- The cell is free of initial membrane stress or residual stress.
- The cell volume is constant.

The force ( $F$ ), indentation ( $\delta$ ) relationship is given :

$$F = \frac{2\pi h R_0 [1 + R_0 / (2R_s)]^2 E}{(1 - \nu)(1 + R_0 / R_s)^4} \varepsilon^3 + \frac{\pi}{2\sqrt{2}} E h^2 \sqrt{\varepsilon} \quad (5)$$

where  $E$ ,  $\nu$ , and  $h$  represent the elastic modulus, Poisson's ration and the thickness of the capsule membrane respectively.  $R_0, R_s$  is the radius of the capsule and the spherical indenter respectively.  $\varepsilon$  is the relative deformation of the capsule given by  $\varepsilon = 1 - \frac{H}{2R_0}$  ( $H$  is the height of the capsule after indentation). The present model considers stretching as well as bending of the cell membrane, in our experiments the bending deformation term can be neglected for  $\varepsilon > 0.15$  [23].

##### C. JKR and DMT contact

The JKR and DMT contact models modify the Hertz theory by taken into account the adhesion between two elastic spheres. The model assumes that the adhesion force operates over a short distance within the contact region. According to this theory [25], contact radius  $a$ , indentation  $\delta$  and pull-off force  $P_{off}$  are represented by :

$$\begin{cases} a^3 = \frac{3R}{4E^*} (F + 3\pi R w + \sqrt{6\pi R w F + (3\pi R w)^2}) \\ \delta = \frac{a^2}{R} - \sqrt{\frac{2\pi w a}{E^*}} \\ P_{off} = \frac{3}{2} \pi R w \end{cases} \quad (6)$$

The DMT theory assumes the adhesion force operates just outside the contact zone where the surfaces are small distance apart. According to the DMT model, contact radius  $a$ , indentation  $\delta$  and pull-off force  $P_{off}$  are represented by the following equations:

$$\begin{cases} a^3 = \frac{3R}{4E^*} (F + 3\pi R w + 2\pi R w) \\ \delta = \frac{a^2}{R} \\ P_{off} = 2\pi R w \end{cases} \quad (7)$$

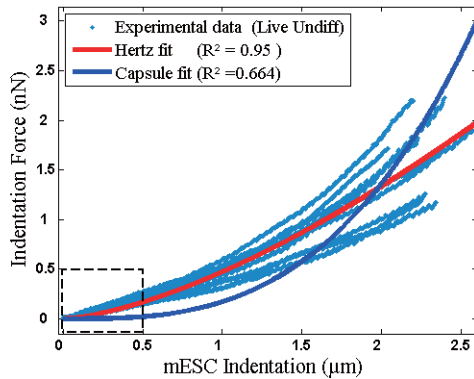


Fig. 6. Force versus indentation for live undiff mESC.

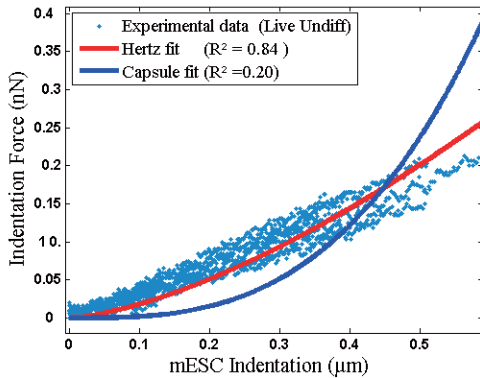


Fig. 7. Force versus indentation for live undiff mESC (small indentation).

#### D. Comparison between experimental data and analytical models

We evaluate and compare the different analytical models to determine whether it appropriately predicts the force-indentation relationship of mESC. The figures (Fig.6, Fig.7, Fig.8, Fig.9) show the force versus cell indentation for live and fixed undifferentiated mESC using the capsule and the Hertz model respectively for small and rather larger indentation range. Based on the experimental data, we performed a least square fit on the data set and the corresponding  $R^2$  value was found to be 0.91 and 0.98 for the fixed and live undifferentiated mESC respectively. The figure (Fig.10) present the force (loading and unloading) versus time of

fixed and live undifferentiated mESC. In our experiments, we observed that the adhesion force exists for only one of live undifferentiated but does not exist for the fixed cells, this could be due to the wear of the spherical tip [25]. *There are no adhesion forces, there is no reason to use the JKR or DMT models.*

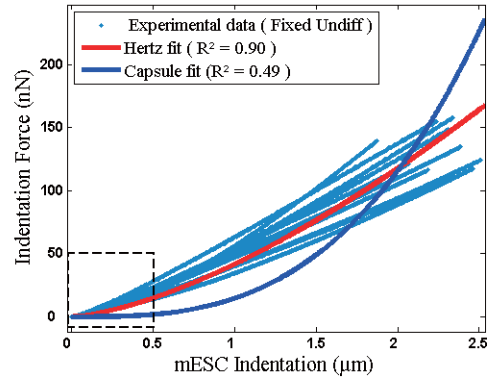


Fig. 8. Force versus indentation for fixed undiff mESC.

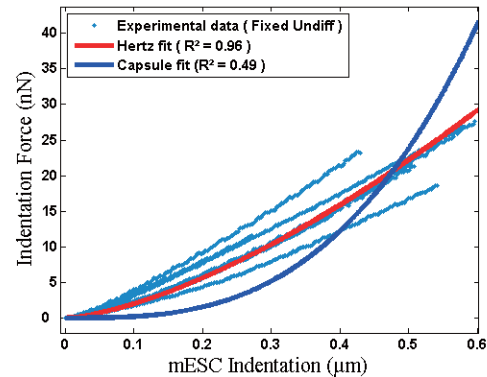


Fig. 9. Force versus indentation for fixed undiff mESC (small indentation).

The equation (4) was used to determine the global elastic modulus of the mESC. The average elastic modulus was  $17.87KPa$  and  $0.217KPa$  for the fixed and live undiff respectively (see Fig.11). The standard deviation for the fixed and live undiff cells was  $3.37KPa$  and  $0.05KPa$ . Finally, from our experiments, we infer that Hertz model appropriately describes the mechanical behavior of the live and fixed undifferentiated mESC.

FEM simulations may help us to answer the unsolved question: *in which indentation range can we use the Hertz model to determine the cell global equivalent Young modulus since the Hertz theory was shown to be valid at small deformations* ?

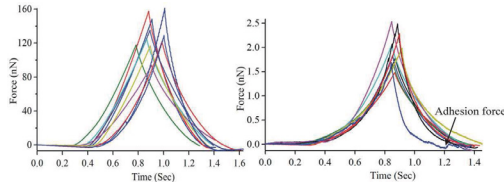


Fig. 10. Load-unload versus time for fixed and live undiff.

## V. FINITE ELEMENT MODEL (FEM) SIMULATION OF CELL INDENTATION RESULTS

### A. Finite Element Simulations of spherical tip indentation for live and fixed mESC

The finite element simulations are performed using an industrial research and development finite element code Cast3M<sup>1</sup>. The FE model of the mESC is based on the following hypothesis:

- The mechanical problem is *axisymmetric*.
- The spherical tip is *undeformable*.
- the cell is supposed to have *homogeneous, isotropic (visco)hyperelastic and nearly incompressible material properties*.

The assumption of homogeneity, which may seem to be wrong, implies that we study a *global equivalent elastic model of the cell*. Furthermore, as already mentioned, the microsphere AFM-indentation test averages the contribution of multiple cytoskeleton fibres and smoothes the area contact geometry as needed for the use of the Hertz contact model. We used the Saint-Venant Kirchhoff hyperelastic model for which the elastic potential is given by:

$$W(\mathbf{E}) = \frac{\lambda}{2}(\text{tr } \mathbf{E})^2 + \mu (\text{tr } \mathbf{E}^2) \quad (8)$$

Since the strain energy function  $W$  is quadratic in terms

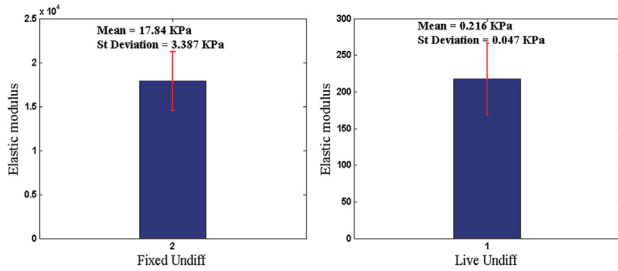


Fig. 11. Elastic modulus for live and fixed undifferentiated mESC calculated from the Hertz contact model for the small deformation (range  $0.5\mu\text{m}$ ) with the error bar.

of strain invariants, we obtain a linear relation between the second Piola-Kirchhoff stress tensor ( $\mathbf{S}$ ) and the Green-Lagrange strain tensor ( $\mathbf{E}$ ):

$$\begin{aligned} \mathbf{S} &= \lambda (\text{tr } \mathbf{E}) \mathbf{I} + 2 \mu \mathbf{E} \\ \text{or } S_{ij} &= \lambda E_{kk} \delta_{ij} + 2 \mu E_{ij} \end{aligned} \quad (9)$$

<sup>1</sup>The FE code Cast3M is developed by the Department of Mechanics and Technology (DMT) of the French Atomic Energy Agency (CEA - DEN/DM2S/SEMT)

The Saint-Venant Kirchhoff law extends the Hooke's law for large deformations. The main difference is that the stress field, which was a *linear* function of the gradient of the displacement field  $\text{grad } \underline{U}$ , is now a *quadratic* function of  $\text{grad } \underline{U}$ . Since a rate sensitivity effect and hysteresis may be observed in the force vs. indentation loading-unloading curves, a first attempt was made to describe this viscoelastic dissipative behavior with a classical Kelvin-Voigt viscoelastic model. The relation between the second Piola-Kirchhoff stress tensor ( $\mathbf{S}$ ), the Green-Lagrange strain tensor ( $\mathbf{E}$ ) and the Green-Lagrange strain rate tensor ( $\dot{\mathbf{E}}$ ):

$$\begin{aligned} \mathbf{S} &= (\lambda (\text{tr } \mathbf{E}) \mathbf{I} + 2 \mu \mathbf{E}) + \eta \left( \lambda (\text{tr } \dot{\mathbf{E}}) \mathbf{I} + 2 \mu \dot{\mathbf{E}} \right) \\ S_{ij} &= (\lambda E_{kk} \delta_{ij} + 2 \mu E_{ij}) + \eta \left( \lambda \dot{E}_{kk} \delta_{ij} + 2 \mu \dot{E}_{ij} \right) \end{aligned} \quad (10)$$

TABLE I  
MECHANICAL AND GEOMETRICAL PROPERTIES USED IN THE FEM

	Fixed Undiff	Live Undiff
Diameter of the cell( $\mu\text{m}$ )	12	10
height of the cell( $\mu\text{m}$ )	8	7.2
Elastic modulus (KPa)	23.5	26.7
Indentation range ( $\mu\text{m}$ )	2	0.5
Poisson ratio	0.49	0.49
Diameter of the Tip( $\mu\text{m}$ )	5	5

The geometrical characteristics were obtained from experimental data, namely the diameter and height of the cell (Table.I). The main difficulty of identifying material properties from the non linear force-displacement curve is that this geometrical non-linearity can come from both large strains and contact non-linearities. Indeed, the cells are indented  $\approx 2\mu\text{m}$  which is not infinitesimal compared to the height of the cells  $\approx 8\mu\text{m}$ . If the non-linearity is attributed only to the contact geometry, the Young's modulus values deduced from the classical Hertz equations may be unreliable [26]. A classical solution, *computationally very expensive*, is to perform an inverse finite element solution to determine the optimum values of (visco) hyperelastic material properties. We chose a different strategy, computationally more efficient: we limited the fitting to data points that do not exceed  $0.5\mu\text{m}$  ( $\approx 5\%$  of relative nominal strain). The viscosity coefficient was roughly estimated by successive simulations. Numerical simulations used two structure meshing to verify FEM approximation convergence; a first, coarse mesh, is composed of 398 nodes (10 contact nodes) and 724 triangles and a second one, fine mesh, is composed of 1628 nodes (26 contact nodes) and 3108 triangles (Fig.13). The results of mESC deformations are visualized in (Fig.14) and (Fig.15) for a fixed mESC. The FEM analysis shows that moderate to large deformations are observed around the contact region ( $\approx 20\%$  for  $0.34\mu\text{m}$  and  $\approx 40\%$  for  $1\mu\text{m}$ ). As a validation, the Hertz contact model and FEM simulations have been compared with force-displacement curves obtained from AFM indentation experiments on both undifferentiated fixed (Fig.17) and live undifferentiated (Fig.16) single mESC.

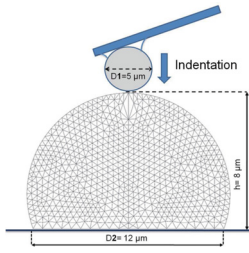


Fig. 12. Mesh geometry of the cell.

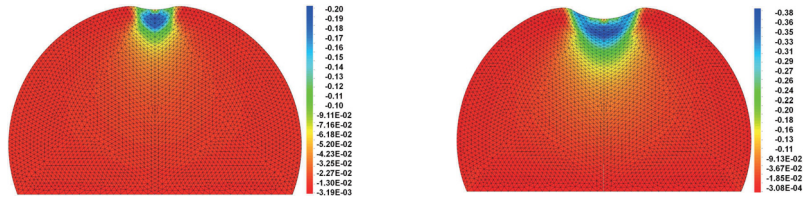


Fig. 13. (b): Small indentation range ( $0.34 \mu\text{m}$ ). Fig. 14. (a): Large indentation range ( $1 \mu\text{m}$ ).

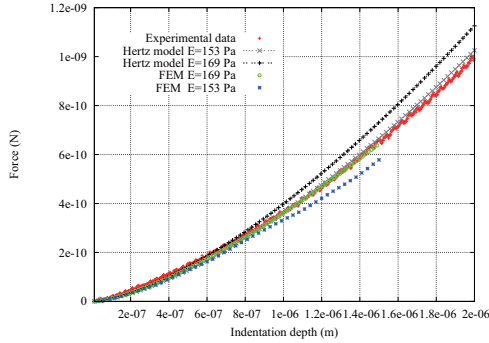


Fig. 15. Comparison between the FEM simulation (large and small indentation range) and experimental data indentation for live undiff cells: .

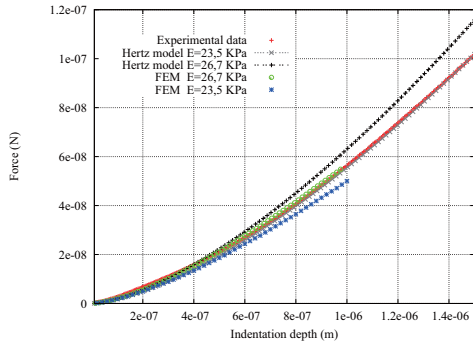


Fig. 16. Comparison between the FEM simulation (large and small indentation range) and experimental data for fixed undiff cells.

### B. PHANToM Haptic Device

In order to provide force feedback to the user during the cell contact, the AFM is integrated with the PHANToM device. The deflection channel of the AFM controller was connected to a data acquisition board (model:DS1103, dSPACE Inc, Wixom, MI). The force exerted on the cell and hence transmitted to the haptic feedback device is given by:

$$F = Kd \quad (11)$$

Where  $K$  is the spring constant of the cantilever and  $d$  the cantilever deflection. Thus, our haptics-enabled AFM system obtains the relationship between the force exerted on the cell and the corresponding deformation. This tool can be used as a mechanical marker to characterize the biological state of the cell. The haptic feedback was rendered in real time to the

operator during cell indentation by the AFM tip (the delay of the feedback is neglected). The operator was able to feel the change in the stiffness during cell deformation between the fixed and live cells..

### C. Analysis and Discussion

Despite variations between cells of the same population, the experimental data show that fixed cells are more stiffer than live cells ( $10^2$  to  $10^3$  magnitude of order). Nevertheless, fixed and live mESC present common mechanical non-linear responses. The finite element simulations show good agreement with both Hertz contact model and experimental indentation for low to moderate deformations (around 10% of relative nominal strain (see Fig.15, Fig.16). Furthermore, as expected, the finite element simulations show perfect adequation with Hertz contact model for low deformation. The differences between, on the one hand, experimental data and FEM simulations and, on the other hand, analytical Hertz contact results for large deformations is explained by the fact that the Hertz contact model is only valid for the low deformations [21], [22]. These comparisons show clearly that:

- if the Young modulus is deduced from the full datasets, the finite element simulation show good agreement to experimental results but only until moderate deformations ( $< 10\%$ ).
- if the Young modulus is deduced from a truncated dataset, the finite element simulation show good agreement to experimental results for the whole simulation and differ significantly from Hertz contact model for large deformations ( $> 10\%$ ).

The hysteresis was quantified experimentally and numerically by subtracting the area under indentation and retraction curves, which represents the viscous dissipation of energy into the cell. The FEM simulations and experimental data are shown in (Fig. 17). Calculated in this manner, hysteresis was about 15-20 %, indicating that energy dissipation due to the material viscosity contribution is low at this probe velocity and force measurements are dominated by the cell elastic behavior. A first attempt has been made to approach observed experimental hysteresis with a simple Kelvin-Voigt model. Preliminary simulations show quantitatively good agreement with experimental data.

## VI. CONCLUSIONS AND FUTURE WORK

Atomic force microscopy has rapidly become a valuable tool for quantifying the biophysical properties of single

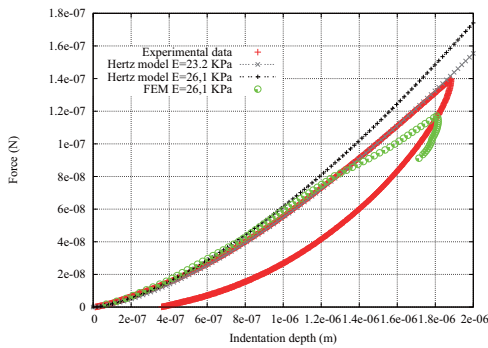


Fig. 17. Experimental viscoelastic hysteresis and finite element simulation.

cells. We presented the mechanical characterization of single mESC indentation-relaxation studies using microsphere-modified AFM probes for different fixed and live undifferentiated mESCs. As the interpretation of atomic force microscopy-based indentation tests is highly dependent on the use of an appropriate contact model, we used Hertz contact model based on our recent work on analyzing the various contact models appropriate for the AFM tip and mESC interaction. Finite element simulations (FEM) of spherical tip indentation proves the effectiveness of computational model to predict the mESC deformation behavior during mESC indentation and relaxation tests. As a perspective, the proposed FEM model constitutes a basis for simulation of nano-injection tasks using virtual haptic feedback model for tissue engineering applications and development of new therapies for regenerative medicine. In our ongoing and future work, we are developing a real-time force-feedback simulator dedicated to AFM indentation for various living cells. To achieve these goals, the proposed finite element model (FEM) takes into account the nonlinear elasticity cell model, the geometry and properties of AFM-tip indenters (spherical and pyramidal probes).

## VII. ACKNOWLEDGMENTS

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