Finite Element Simulation of Contact Mechanics of Cancer Cells in Manipulation Based on Atomic Force Microscopy

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Abstract:
The theory of contact mechanics deals with stresses and deformations which arise when the surfaces of two solid bodies are brought into contact. In elastic deformation contact occurs over a finite area. A regular method for determining the dimensions of this area is Hertz Contact Model. Appearance of atomic force microscope results in introduction of Contact Mechanics into biology. Low elasticity modulus of biologic particles, causes large deformation against foreign forces, therefore to understand them, studying their behavior is essential. Here, in studying these particles we have used finite element which is a new tool in biology. In this paper indentation of three prostate cancer cells CL_{-1}, CL_{-2} and LNCaP which have low elasticity modulus and are considered ductile materials was conducted using Hertz contact mechanics model. For modeling, in this section the contact equations of two spheres were used and simulated by using finite element method (FEM). The results of these two steps were compared with available experimental data on these cells to verify simulations and results. These results include force-displacement diagram which shows particles behavior against foreign load. In this presentation we tried to study the behavior of these cells through different methods and make a comparison. Using finite element approach in studying characteristics of these particles was new.
Keywords: Nano-manipulation, Hertz contact mechanics model, Cancer cell, FE.

1. INTRODUCTION

Nano-manipulators have been made to apply an external force in both contact and non-contact types. Atomic force microscopy and scanning tunneling microscopy are the manipulators that are widely used [1]. One method for measuring mechanical properties of molecules is atomic force microscopy. Atomic force microscope is able to provide three dimensional images of biological samples in physiological environments. Thus, this tool has many applications in manipulation of molecules [2]. Atomic force microscope is a powerful tool for measuring molecule and cell forces and displacement [3, 4]. Atomic force microscope is a powerful tool for measuring molecule and cell forces and displacements. The structure of this tool has been shown in Figure 1. Manipulation can be interpreted as particle transferor in nano-scales and with nano-scale precision. This movement can be initially considered as pushing and in more complex manipulations as pulling, lifting or pointing.

Korayem and Zakeri modeled manipulation of micro and nano-scale particles on rough surfaces [5, 6]. Korayem et al. presented a theoretical analysis on manipulation of submerged nanoparticles in liquid medium based on AFM [7]. In line with
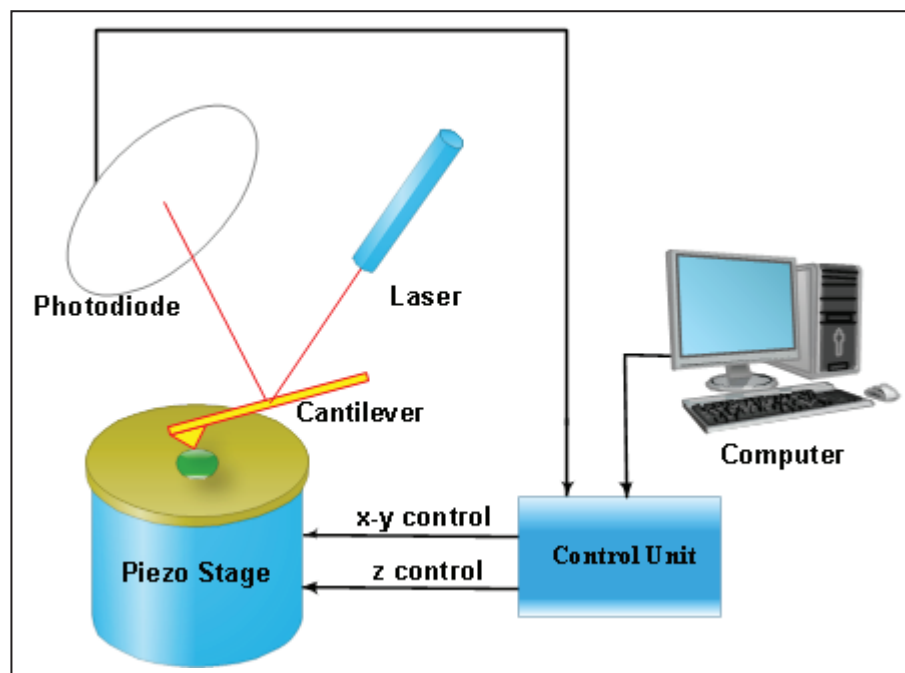
Figure 1: *Schematic of the AFM instrument*

manipulation in view of molecular dynamics Korayem, Sadeghzadeh *et al*. have conducted some studies such as dynamic nano-manipulation modeling of polystyrene nano-rods with an atomic force nano microscope. In their proposed model three nano-major forces were considered. This model consisted of a nine-step strategy to drive nano-rods [8].

In the recent decades, study of biological molecules and estimation and calculation of their mechanical properties has been considered by scientists. The activities performed by Butt and his colleagues cover a wide field of the application of atomic technology in atomic force microscopes for spectroscopy of molecular forces [9]. Molecule force spectroscopy and cell mechanics were described well by Evans [10]. Living cells as physical bodies have some physical and structural properties that enable these cells withstand the physiological environments such as mechanical stimuli occurring within and outside the body. Any deviation from these properties not only destroys the integrity of the cells, but also impairs their biological functions. In their revision, Lim *et al*. tested some of the mechanical models which are used for determining the mechanical responses of living cells created by their exposure to both transient and dynamic loads [11].

Mechanical properties such as elasticity, membrane tension, and cell shape and adhesion resistance may play an important role in reproduction. Mechanical properties of biological cells have been studied by various methods. The most common of these methods are optical tweezers, magnetic beads and micropipette respiration. However, these methods cannot present the accuracy that is obtained by atomic force microscope [12].

2. METHODS AND MODELING

2.1. Prostate cancer

Prostate cancer is the second most common form of cancer in U.S. male population. Although the primary tumor originates in the prostate, the prostate cancer cells frequently spread to other organs, particularly the bones and the lymph nodes [13].

Metastasis is the movement or spreading of cancer cells from one organ or tissue to another. Despite advances in the early diagnosis and therapeutic strategy, metastasis often defeats the possible curing strategy and leads to a lethal condition. Metastasis involves multiple processes such as invasion, migration, intra/extra-vasation, and changes in cell adhesion [14].

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During these processes, cancer cells interact with the surrounding environment and undergo mechanical deformations. Thus, the enhanced mechanical compliance of cancer cells is suggested to play a critical role in the metastatic progression. The enhanced mechanical compliances were widely observed from cancer cells compared with benign cells. Recently, Atomic Force Microscope (AFM) based nano-mechanical study using the pleural fluids of patients demonstrated the possible clinical use of mechanical properties for cancer detection. Nevertheless, the multi-faceted aspects of metastasis impose difficulties in evaluating the relationship between mechanical compliances and metastatic potential [14].

Increasing evidence suggests that the altered mechanical compliances of cancer cells might be generated by the alterations in the dynamic rearrangements of the Focal Adhesions (FAs). In addition, the intracellular calcium ($[Ca^{2+}]$) in dynamics is recognized as an important messenger mediating the changes in FAs to the long-range changes in the mechanical compliances. Extensive efforts have been made to identify the molecular signaling pathways regulating the unique mechano-biology in cancer cells. Several pathways such as Rho GTPase and focal adhesion kinase have been revealed to contribute to the mechanical signature of cancer cells during cancer progression. Nevertheless, it is unclear if the combined mechanical signatures such as mechanical compliances, cell-to-substrate adhesions, and calcium dynamics can indicate the metastatic progression of cancer cells [14].

Bastatas *et al*. investigated this relationship in 2012. Bastatas *et al*. used the lowly (LNCaP) and highly (CL_{-1}, CL_{-2}) metastatic human prostate cancer cells. The AFM-based nanomechanics were performed to determine the elastic moduli and the cell-to-substrate adhesion. The elastic moduli, the calcium dynamics, and the migratory ability are greater in CL_{-1} and CL_{-2} than LNCaP. CL_{-1} and CL_{-2} also display a significantly larger area of cell-to substrate adhesions while the LNCaP displays a limited adhesion. These properties were slightly reduced in CL_{-2} compared with CL_{-1} cells. Ca^{2+} plays a prominent role in the regulation of several cellular functions relevant for the acquisition of a metastatic phenotype .They found that $[Ca^{2+}]$ in dynamics is greater in highly than in lowly metastatic prostate cancer cells with the ATP stimulation. A high

migratory ability is required for the metastatic progression. Bastatas *et al*.'s data suggest that highly metastatic cells (CL_{-1} and CL_{-2}) migrate at faster rates than lowly metastatic cells (LNCaP). Thus, it can be concluded that the enhanced elastic moduli, the cell-to-substrate adhesion, the Ca^{2+} dynamics, and the migratory ability are consistently correlated with highly metastatic signatures of prostate cancer cells [14].

2.2. Hertz contact model

As shown in Figure 2, while the two spheres have contact with each other, a shared surface whose geometry is the basis of our discussion in contact mechanics is created. The start of contact mechanics was 1882 when an old article on the contact of elastic solids was published by Heinrich Hertz.
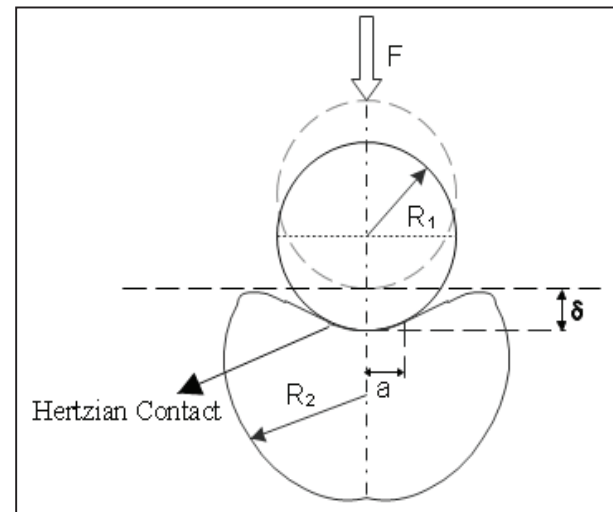

Figure 2: Contact of two elastic spheres

His assumptions were based on the following statements:

1. Compared to body size, the dimensions of the contact area are small.
2. The two contact surfaces are smooth and frictionless.
3. The distance between two surfaces whose shapes are not changed, is considered in $h = Ax^2 + By^2$ form.
4. The deformation is elastic and can be calculated by considering each object as a semi-infinite elastic body [15].

$$F_{(adh)Hertz} = 0 \tag{1}$$

$$\delta = \frac{a^2}{R} \tag{2}$$

$$a^3 = \frac{R}{k}F \tag{3}$$

The effective radius of the two surfaces with radius of R_1 and R_2 is $R = \dfrac{R_1 R_2}{R_1 + R_2}$ and *k is the reduced elastic module that can be obtained from the following equation:*

$$\frac{1}{k} = \frac{m}{2}\left(\frac{1-v_1^2}{E_1} + \frac{1-v_2^2}{E_2}\right) \tag{4}$$

m is a constant and dependent parameter on the geometry of the tip (*m = 1* for a cylindrical geometry, *m =1.5* for spherical geometry and *m =2* for conical forms; E_1 and E_1 are elastic modulus; v_1 and v_2 are the Poisson ratio) [16].

Because the Hertz formalism is based on the theory of linear elasticity, it must be possible to define measures of stress and strain that satisfy a Hookean relationship.

In modeling AFM contact tip and cells, the geometry of these particles was considered spherical and two sphere equations were used in simulations.

Table 1: *Geometry details of cells*

Cancer Cell	$h_0(\mu m)$	$D_0(\mu m)$
CL$_{-1}$	8	20
CL$_{-2}$	7	20
LNCaP	7.5	20

2.3. Finite element

Finite element method has been grown from the need for solving the issues related to the structural analysis and complex elasticity. The Hrennikoff and

Table 2: *Specification of modeling*

cancer cell	Form of Elements	Elements No.
CL$_{-1}$	Triangular	7632
CL$_{-2}$	Triangular	6684
LNCaP	Triangular	7312

Courant's study is an extension of this method that can be referred to [17]. Currently, with the advent of finite element softwares, this method has been widely used in biological fields. These applications have been developed from the analysis of the loads on the muscles and body organs to in vivo and in vitro cells. For example, uniaxial stretching of the cells was investigated by Gladilin *et al.* using three-dimensional finite element analysis [18]. Limited sampling performed by White revealed that this method can be used for calculating the optical hooking efficiency of electric objects with indeterminate shapes [19]. Hamid Ladjal *et al.* modeled cell indentation with finite element software and compared the results with those of the Hertz contact model [20].

Finite element simulation was performed using ABAQUS 6.10-1 software. Accordingly, the following assumptions were considered in the finite element modeling:

1. The problem is about symmetric axis.
2. The behavior of AFM tip is elastic.
3. The cell is homogeneous and incompressible.

Figure 3 shows a schematic view of cell geometry in simulation. Table 2 determines each cell dimensions for modeling. The selective geometry for each cell is chosen according to their dimensions.

Finite element method (FEM) is based on mesh which takes up the most time, cost and effort. Meshing somehow determines the type of numerical solution. In meshing two dimensional bodies, two types of quad and tri elements can be used. Meshing technique in this model has structure. Two dimensional areas which are meshed via this method should have two
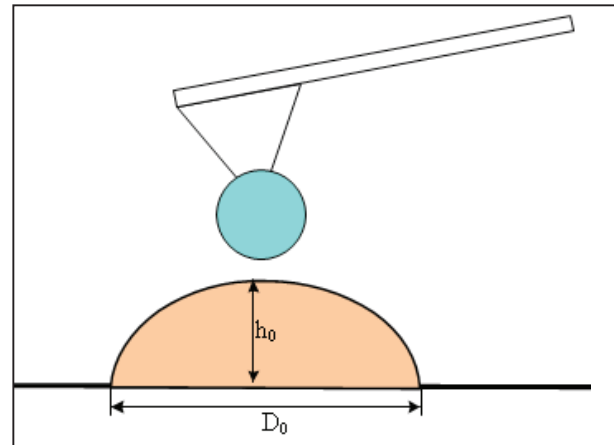
Figure 3: *Geometry used for indentation in Abaqus*
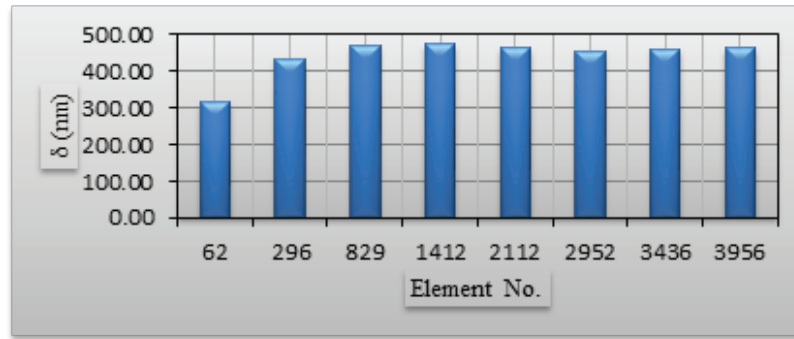
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Figure 4: Changes of indentation depth versus elements No. in the 1 nN force

properties: 1. No holes, separate edges and separate dots are allowed on the surface. 2. The area should be limited with 3 to 5 lines. To determine accuracy in finite element, the indentation for a specific force was calculated so that adequate number of elements for simulating was determined. For instance, the indentation for a 1nN force in a CL_{-1} cell is drawn for different numbers of elements. Details can be viewed in Figure 4.
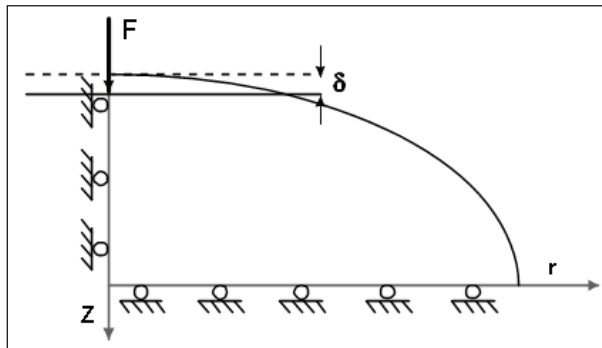
Figure 5: Schematic model of the contact problem

According to the posed assumptions, the scheme of the

problem will be like what is provided in Figure 5. The simulation profile for each cell is brought in Table 2. In Figure 6 the simulated shape is shown before and after stimulation. This Figure describes the change in the shape of the cell. According to this Figure, going away from the area where the force is applied, decreases the amount of stress.

3. SIMULATION AND RESULTS

3.1. Hertz's simulation results

Empirical data were used for mechanical properties in Hertz modeling. Highest load was contributed to CL_{-1} due to its higher modulus of elasticity comparing to the other two. Using equations 3 and 4, force-indentation and contact radius-indentation diagrams can be drawn. Contact radius indicates maximum contact surface during loading. LNCaP has the greatest deformation due to its low modulus of elasticity. Generally it can be inferred that, when same load is exerted, the particle with lower modulus of elasticity shows lower resistance. Figure 7 confirms this claim.
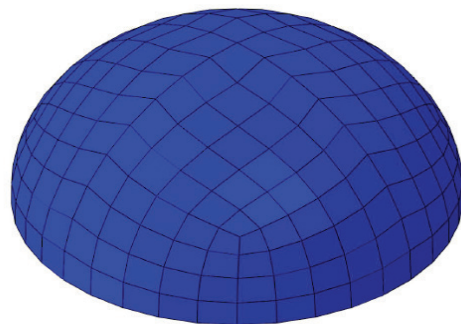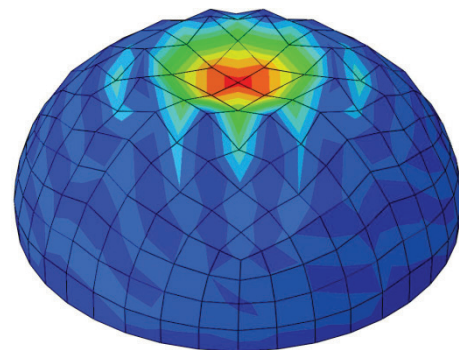

Figure 6.a: Cell geometry before loading
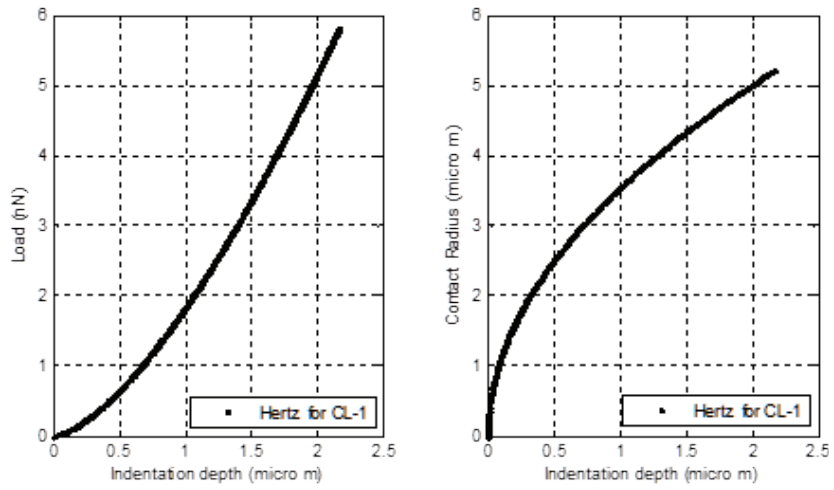

Figure 6.b: Cell geometry after loading

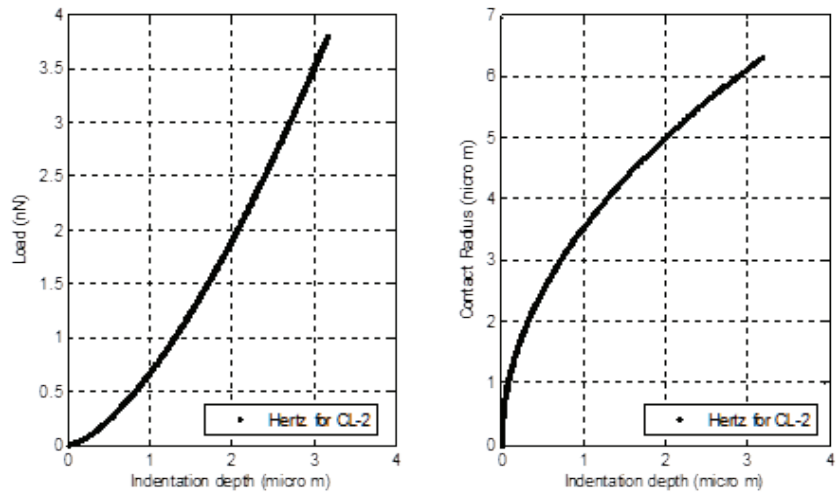**Figure 7.a:** *Force and contact radius curves obtained on CL_{-1}*

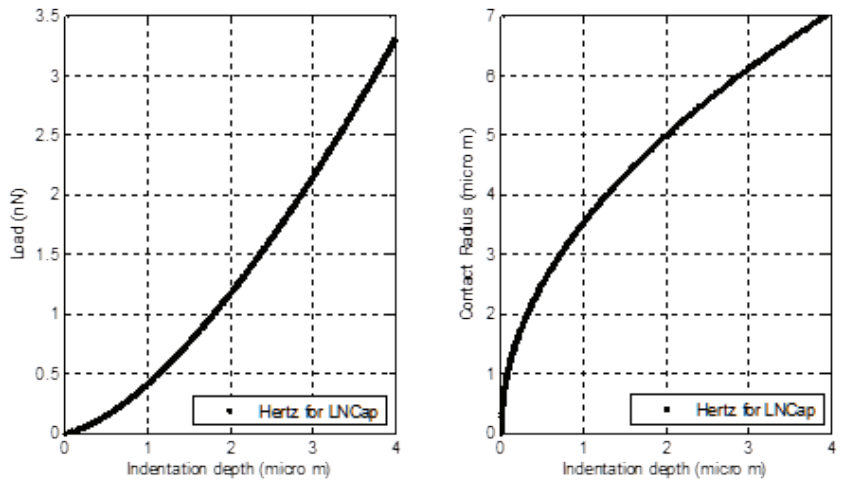**Figure 7.b:** *Force and contact radius curves obtained on CL_{-2}*

Figure 7.c: *Force and contact radius curves obtained on LNCaP*
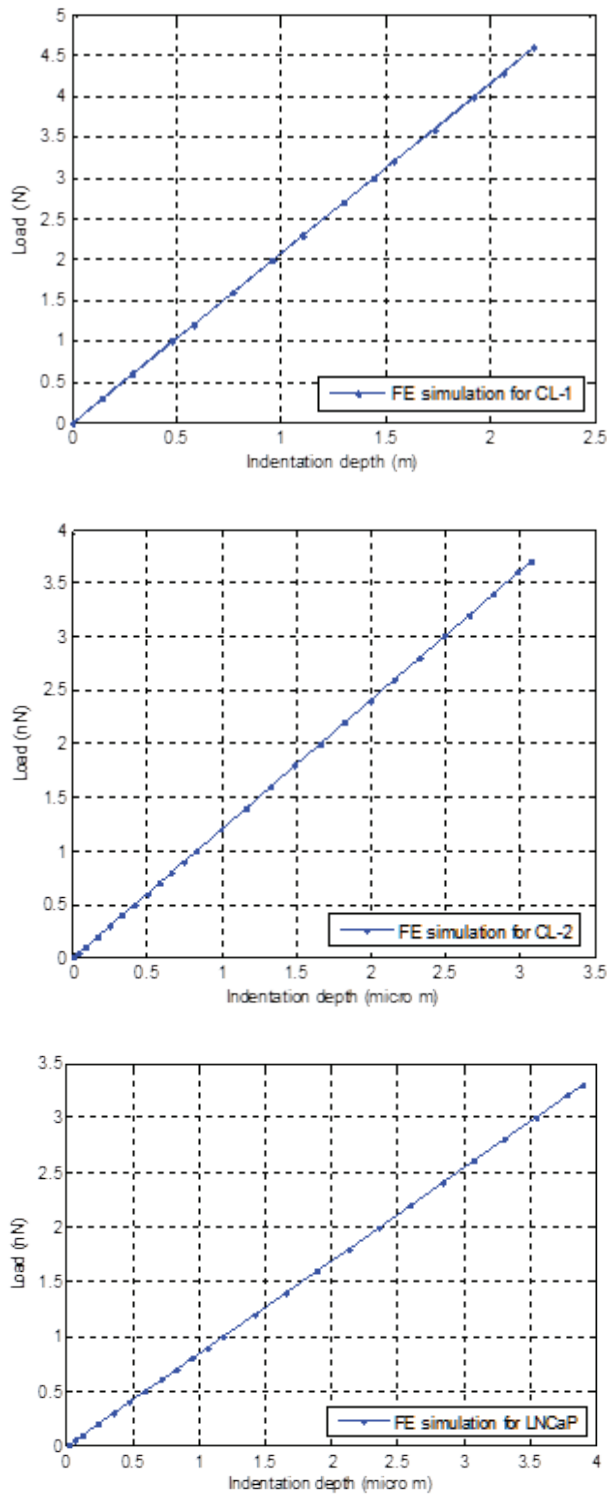
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Figure 8: *Force versus indentation for*
a. CL_{-1}
b. CL_{-2}
c. LNCaP

3.2. Finite element results

After simulating using finite element method (FEM), f-δ diagram was drawn for each particle. These diagrams show elastic behavior of these particles against foreign exerted forces. Figure 8 includes these diagrams. In this simulation only elastic properties of materials were used. The slope of each diagram is equal to resistance of that cell against exerted load meaning modulus of elasticity. CL_{-1} has the highest slope and LNCaP has the lowest which confirms what we claimed above.

3.3. Experimental results

During the study of Lyndon Bastatas *et al.* on CL_{-1}, CL_{-2} and LNCaP as three prostate cancer cells using atomic force microscope, the amount of δ was calculated by subtracting the displacement of cantilever from scanner and applied force displacements by multiplying the spring constant k in displacement of cantilever. The elastic modulus values were calculated for these values by graphing the force versus penetration depth curves for these cells [14]. These values are shown in Table 3.

To verify the simulation results, they have been brought along with empirical data. These Figures indicate the differences between methods for each cell. (Figures 9.a, 9.b and 9.c)

Based on Figures 9.a and 9.c, Hertz contact model provides a good stimulation of the behaviors of CL_{-1} and LNCaP cells against the applied force. Further, the curve obtained from Hertz contact model fits the experimental data. Figure 9.b shows that this theory is to some extent far from the true results. The main reason for such difference is the assumed spherical geometry of the CL_{-2} which is against its real shape. The results obtained from finite element are somehow different from those of the other two data sets. The reasons will be discussed in the section related to sources of error.

It is expected that modeling via finite element method (FEM) based on elastic property, shows linear behavior. Diagrams number 8 and 9 show proof to this claim. In CL_{-2} and LNCaP cells, for equal forces, FEM method shows more rigidity comparing to Hertz model and less indentation.

In Figure 10 the error value for each cell is given. As you can see in modeling using Hertz

Table 3: Mechanical properties of Cells

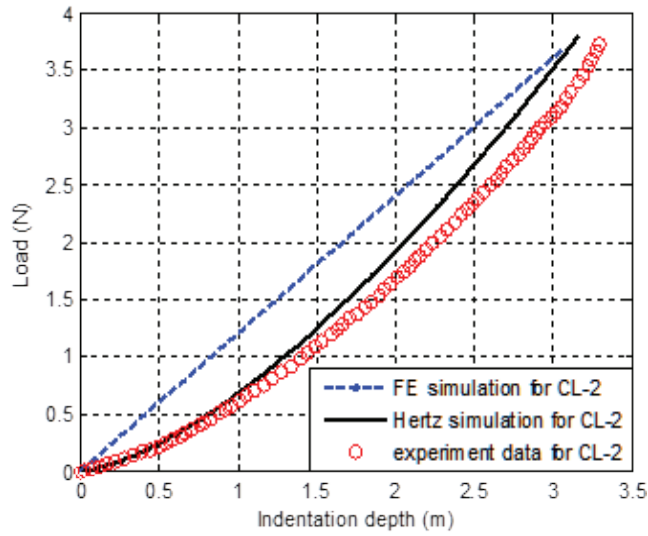| cancer cell	Modulus of elasticity [Pa]	Poisson's ratio
CL-1	391±236	0.5
CL-2	259±89	0.5
LNCaP	196±89	0.5

Figure 9.a: Force-Indentation (f–δ) curves obtained from Hertz model, Experiment [14] and Finite Element of CL_{-1} cell
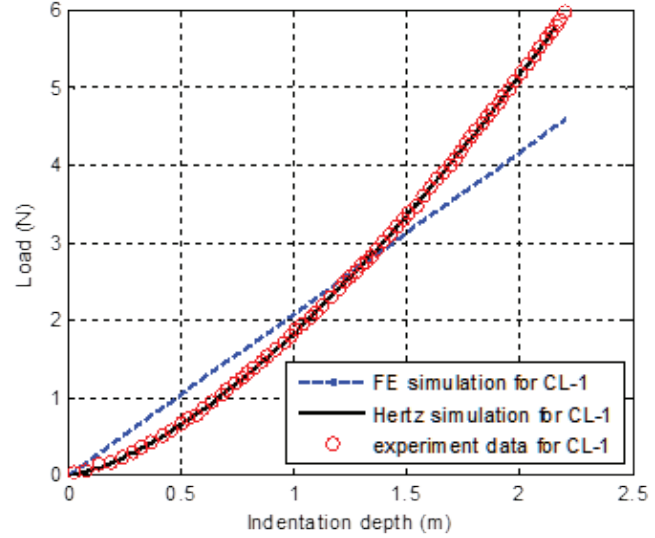

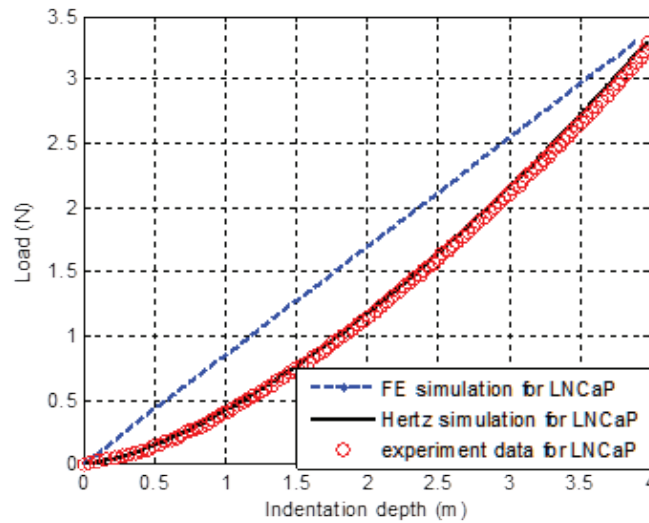*Figure 9.b:* Force-Indentation (f–δ) curves obtained from Hertz model, Experiment [14] and Finite Element of CL_{-2} cell

Figure 9.c: Force-Indentation (f–δ) curves obtained from Hertz model, Experiment [14] and Finite Element of LNCaP cell

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method, maximum value of error belongs to CL_{-2} and CL_{-1} in the minimum value of error group. The first point that should be noticed here is the spherical geometry assumption of the three cells which caused error development and proximity in results. Changes in cell geometry cause change in contact area. Considering contact to be elastic is another cause of error development which can be eliminated through changing current method. Ignoring adhesion is another problem of Hertz theory which causes error development. While using Hertz method it should be noted that loading must be in elastic area of particles. Error distribution is uniform during loading and will not change much.

For all three particles, the value of error in finite element method (FEM) is maximum value at the beginning of the contact while in Hertz theory error distribution is not uniform and changes. Due to intersection of this diagram with empirical diagram, in CL_{-1}, error values reduce force up to 2.7 nN and then rise. For other two particles the error value of this method decreases constantly as the force increases. As it can be observed in Table 1 the value of modulus of elasticity for these materials has a wide range while in simulation via this method, it is considered an average and constant value. This and low modulus of elasticity are among main reasons for error development. Accuracy in meshing is one of the factors that can affect developed error.

4 . CONCLUSION

Because of its ability to move nano-scale objects and measure reaction forces in the movements of biological samples, atomic force microscope is very useful.

Atomic force microscope (AFM) is becoming a key technique in biology for studying properties. Progress in methods of preparing samples, instrumentation and recording conditions for live cells still needs improvement in accuracy and force measurements Repeatability.

Identification of contact area and the applied force is very important in stimulating manipulation. Using such manipulation, the behaviors of particles-

especially biological particles that because of low elasticity module are very mutable can be predicted. Using more accurate contact mechanics models will guide us towards better anticipation of behavior of these materials. Using elastoplastic models which are capable of showing multiple behaviors of materials can increase accuracy. Hertz model is efficient when surface forces can be neglected; using more recent models can solve this problem.

In this study, the attempt is to use contact mechanic equations and finite element modeling in order to accede the experimental results. The findings of such studies can be used in laboratory projects and in bio environments such as the mediums. In Hertz's contact model, two surfaces are assumed to be in sphere contact and the results were to an acceptable extent near the experimental data. Thus, it is possible to approach the experimental data using more exact contact theories.

The new action which has been conducted in this paper was the finite element approach in micro/Nano dimension for studying the behavior of a species of live cells. Progress and development of these kinds of activities can be suitable for solving analytical problems which solving them is hard or impossible. Through increasing calculation capacity which is one of the prerequisites of finite element methods (FEM), we can be hopeful for a better future in this respect.

One of the causes of error in Hertz's model is elasticity of the contact between two bodies in collision, regardless of the adhesion energy and by considering the spherical cell geometry. Of course, we know that Hertz' theory acts well when the amount of the applied external force is more than that of the surface force. In addition to the items listed, it is possible to refer to truncation error and simplification of the physical model for the finite element model. In fact, we should not ignore the low elasticity modules of cancer cells, because such elasticity causes severe changes in the lengths. Similarly, the existence of elastic modulus variation is another factor in error production.

The values of error in terms of foreign load are given for all three cell types. Error value of Hertz
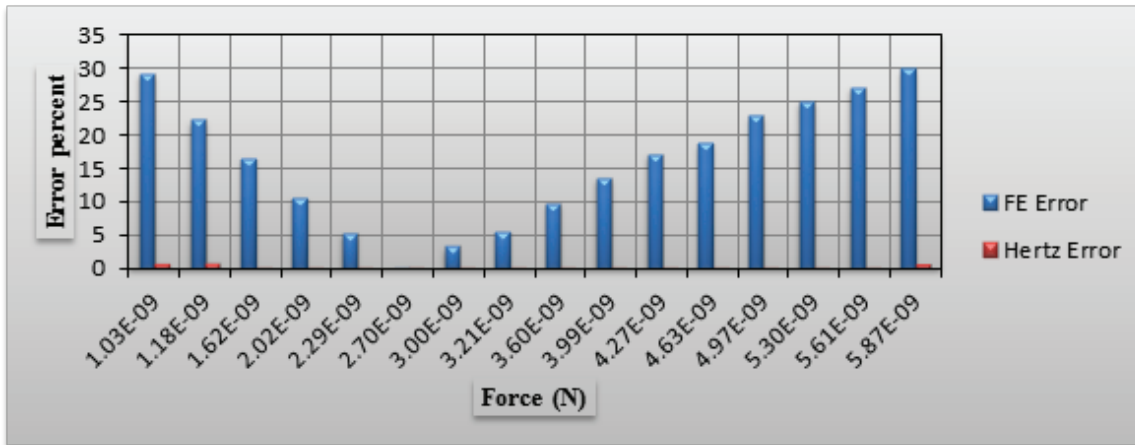
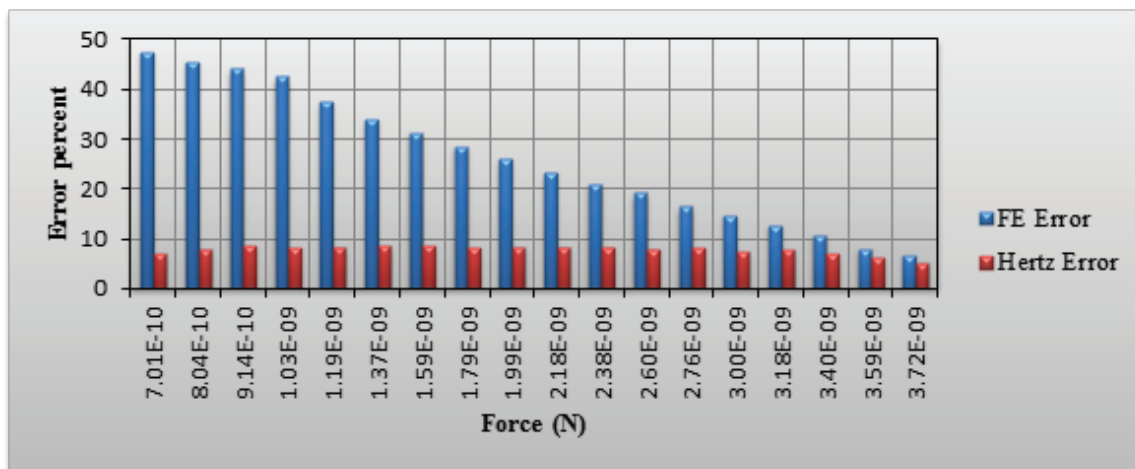*Figure 10.a:* *Changes of error versus force in the Hertz contact model and finite element method for CL$_{-1}$*

Figure 10.b: *Changes of error versus force in the Hertz contact model and finite element method for CL$_{-2}$*
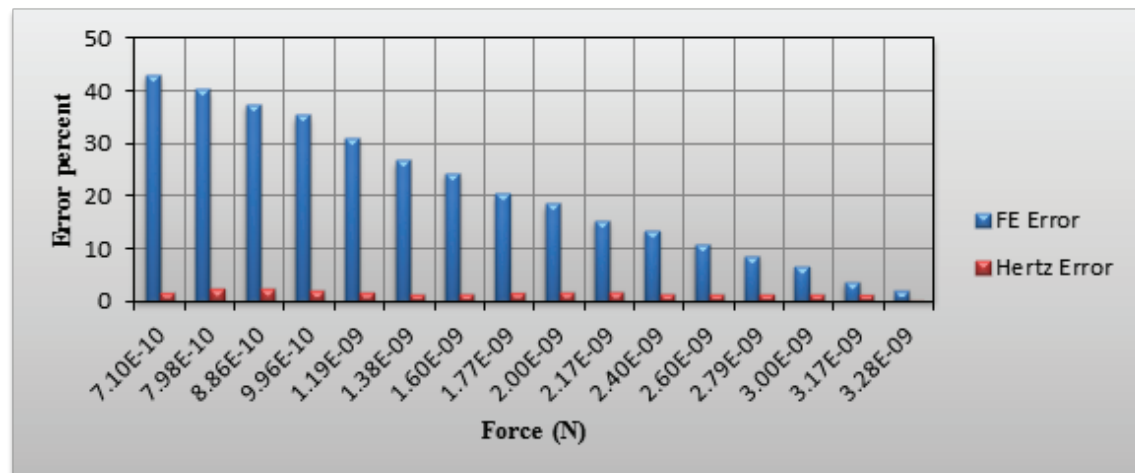

Figure 10.c: *Changes of error versus force in the Hertz contact model and finite element method for LNCaP*

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model for CL_{-1} is under 0.9 percent. This value for CL_{-2} and LNCaP are less than 8.6 and 2.4 percent, respectively. Error values in finite element modeling are higher due to the cases mentioned above. These values for CL_{-1}, CL_{-2} and LNCaP are less than 29.9, 47.4 and 43.1 percent, respectively.

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