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Experimental extraction of modulus of elasticity of gastric cancer tissue using cylindrical developed contact mechanics models

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Abstract

The physical and chemical properties of living tissues change with the change in their physiological conditions during disease. AFM can perform surface imaging and ultrastructural observation of living tissues at nanometer resolution under near-physiological conditions and collect force spectroscopic information, which enables the study of tissue mechanical properties. In this research, AFM was used to measure the elasticity modulus of stomach cancer tissue. For this purpose, in order to bring the theoretical results closer to the experimental reality, three-dimensional modeling of the developed contact theories has been done. Since in most of the past research, the shape of spherical target particles has been assumed, as an important innovation, in this research, cylindrical contact models including Hertz, Dawson, Nikpour, Hoeprich, and Lundberg have been modeled, and the simulation of each of these models has been done using MATLAB software. The simulation results of contact models have been compared with the results of experimental work. From the results obtained from this comparison, the modulus of elasticity in kilopascals at the most appropriate penetration depth of the AFM needle for biological tissue has been extracted. The results have shown that the Hoeprich model is a suitable model for theoretical simulation and is closest to the experimental results. By comparing the obtained results and the previous results, the difference percentage of the results for gastric cancer tissue is between 3 and 20% at the end, and the validity of the results was checked.

Keywords: Nanomanipulation; Young module extraction; T4A Cancer cell; Cylindrical contact models.

1. Introduction

Stomach cancer is a disease in which malignant

digestive system that processes the nutrients (vitamins, minerals, carbohydrates, fats, proteins, body. The stomach wall consists of 5 layers of tissue. The layers of the stomach wall are: mucosa, submucosa, muscle, subserosa (connective tissue) and serosa. Stomach cancer starts in the mucosa and spreads by growing through the outer layers. Age, diet and stomach disease can affect the risk of stomach cancer.

Atomic force microscope has made it possible to examine cells and molecules on a nanometer scale.

Figure 2 graphically shows how the atomic force microscope works. During the movement of the

(cancerous) tissues, as seen in Figure 1, form in the lining of the stomach. The stomach is the part of the

and water) in the food we eat and helps eliminate waste from the

tip of the pole on the surface of the sample, the force between the tip of the pole and the surface is measured by the amount of deviation (in contact mode) or oscillation (non-contact mode). In modeling the shape of tissues using atomic force microscope, one of the important points is to use a suitable and accurate contact model.

One of the methods used in the past to extract the modulus of elasticity and Poisson's ratio of biological particles is the use of a tensile test machine to extract the stress-strain diagram of soft tissues. The elastic modulus is the result of dividing the stress by the strain in the linear region of the stress-strain diagram. But this is not possible at the micro and nano level.

Investigating the effect of the mechanical properties of the surfaces in nanoparticles is related to the mechanics and dynamics of contact during the manipulation process, in other words, in the pre-contact stage, the amount of force is equal to the displacement frequency of the tip of the atomic force microscope, and in the post-contact stage, the indentation depth and geometry. It depends on biological samples. This requires accurate knowledge of existing surface forces and their effect on the mechanical analysis of tissue and soft materials.

Calzado et al [1] studied the relationship between cytoskeleton structure and stiffness of three types of breast cancer cells with different degrees of malignancy including healthy (MCF-10A), non-invasive tumorigenic (MCF-7) and invasive tumorigenic (MDA-MB-231) have done.

Li et al [2] have determined the mechanical properties of living single cells that are closely related to the health and function of the human body. In this study, atomic force microscope indentation using a small spherical needle has been performed to describe the tensile properties of healthy (MCF-10A) and cancerous (MCF-7) human breast cells.

Rebelo et al [3] compared the viscoelastic properties of different kidney cancer phenotype cells with atomic force microscopy. Comparison of the three cell lines shows that non-tumor cells are less deformable and more viscous than cancer cells, and cancer cell lines have distinct viscoelastic properties.

Kawano et al [4] have investigated the elasticity of colon cancer tissue. Generally, cancerous tissue is hard to the touch. However, the elastic nature of cancer tissue is not well understood. The aim of this study was to evaluate the clinical efficacy of measuring the modulus of elasticity in colon cancer tissue.

Liu et al [5] have investigated the effects of tumor necrosis factor on the mechanical properties of human colon cancer cells (HCT116) by atomic force microscopy. In this study, atomic force microscopy was used to investigate the morphology and mechanical properties of EMT in HCT116 human colon cancer cells.

The review of the researches and studies that have been carried out shows that in most researches, biological particles are assumed to be spheres, while it can be said that considering the actual shape of cellular tissues, the assumption that all tissues are spherical is not correct. Therefore, according to the experimental work carried out in this article and the images obtained from stomach cancer tissue using atomic force microscope, it has been observed that the shape of the studied cancer tissue was closer to cylindrical geometry; Therefore, it can be considered as the main and practical innovation of the current research in the examination of cylindrical geometry for the examination of gastric cancer tissue. In this research, in the modeling section, Lundberg, Dawson, Nikpour, Hoeprich and Hertz contact models of contact modes have been examined to investigate the contact between the base plate and the cell tissue of gastric cancer (T4a), then to extract The modulus of elasticity of gastric cancer cell tissue (T4a) has been studied. In order to simulate and extract data from the contact models, according to the images obtained from the atomic force microscope, a cylindrical nanoparticle with a length of 70 nm and a diameter of 150 nm has been used.

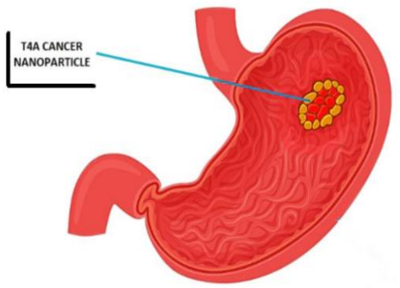

Figure 1. Gastric cancerous tissue (T4A)

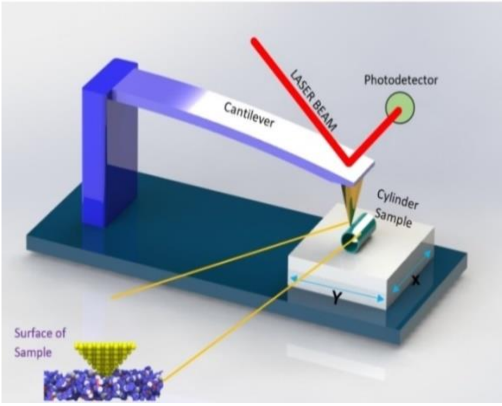

Figure 2. Schematic of atomic force microscope operation

2. Cylindrical contact theory models for soft biological materials

In this section, cylindrical contact models for application in soft biological materials have been investigated. For this purpose, first the Hertz contact model as one of the first and most important contact models and then newer models such as Lundberg, Dawson, Nikpour and Hoeprich have been investigated and developed for use in this research.

2-1- Hertz contact model

One of the important models of cylindrical contact was presented by Hertz [6]. The most important flaw of this model is not paying attention to adhesion force. Therefore, if there are surface forces, this model will not work. For this reason, the use of this model will be useful in the simulation of nanomanipulation, when the amount of external force is not greater than the amount of surface force.

2-2- Lundberg contact model

Lundberg's contact model [7] has focused more on the force distribution between plane/cylinder.

2-3- Dowson contact model

In the field of plane/cylinder contact mechanics, Dawson [8] is another person who studied in this field.

2-4- Nikpur contact model

In the field of contact mechanics for plane/cylinder, many models have been presented, and the contact model of Nikpur [9] is one of them.

2-5- Hoeprich contact model

The contact model of Hoeprich [10] is a contact model, using a cylindrical roller that is placed between two flat planes in a compressed form. The basis of this model is for plane/cylinder contact mechanics, as presented by Hoeprich.

3. Experimental results of examining cancerous tissue with atomic force microscopy

At this stage, stomach cancer tissue is prepared for examination. The washing of these tissues was done after separation and after placing the stabilizer for 60 seconds, the desired tissue was washed with salt in three stages and finally, the tissue was dried. The prepared tissues were stored in an antibacterial solution with 4% formalin at a temperature below 10 degrees Celsius for 2 days and then transferred to the laboratory. In the laboratory, due to the limitation of the height of the existing atomic force microscope, for taking pictures, the height of the slide containing the tissue has been reduced and is ready for testing. Then the machine operator calibrates and ensures the operation of the machine and the most suitable needle is selected for testing. For this purpose, the images taken from the sample using an atomic force microscope have been examined first with dimensions of 20 micrometers in order to identify the location of the tissue and then with dimensions of 1 micrometer in order to identify the geometry, shape and real dimensions of the tissue.

Using the two-dimensional and three-dimensional images obtained from the experimental test and with the help of MATLAB software, the images obtained from the experimental sample and the experimental diagrams of the force according to the depth of penetration, the range of the elastic modulus of the target tissue has been calculated using different models of the cylindrical contact theory.

4. Conclusions from the comparison of contact models and test results

In this section, using the obtained experimental results as well as the force diagrams according to the experimental penetration depth obtained using the atomic force microscope, which were repeated 5 times, the range of elasticity modulus of the desired tissue has been extracted.

For this purpose, by assuming the tissue to be cylindrical, according to the theories of the contact model developed in this research, as well as placing the dimensions and specifications obtained from the atomic force microscope images in the codes written in MATLAB, the diagrams of force theory according to the depth of penetration assuming different values of the modulus of elasticity. It is drawn and the results are compared with the average experimental results obtained and the approximate range of the modulus of elasticity is obtained using the theories of Hertz, Lundberg, Nikpour, Hoeprich and Dawson.

In Figure 3, assuming a modulus of elasticity of 2 MPa, force theory diagrams in terms of penetration depth, with assumptions obtained from atomic force microscope results, for five cylindrical contact models developed in this research, including the contact models of Hertz, Lundberg, Dawson, Nikpour and Hoeprich. , has been drawn. The theoretical results of these five models have been compared with the average experimental results of force in terms of penetration depth obtained from the atomic force microscope. The large difference between the results of the five theoretical models and the experimental results, considering that at the same force, the experimental results show a much greater depth of penetration than the theoretical results, indicates that the elasticity modulus of the desired tissue is much less than 2 MPa.

According to the results obtained from figure 3, in figure 4 the theoretical results of contact models of Hertz, Lundberg, Dawson, Nikpour and Hoeprich are drawn, assuming a modulus of elasticity of 100 kPa. The difference between the results of the five theoretical models and the experimental results, in this figure as well, considering that in the same force, the experimental results show a lower penetration depth than the theoretical results, indicates that the elasticity modulus of the target tissue is more than 100 kPa.

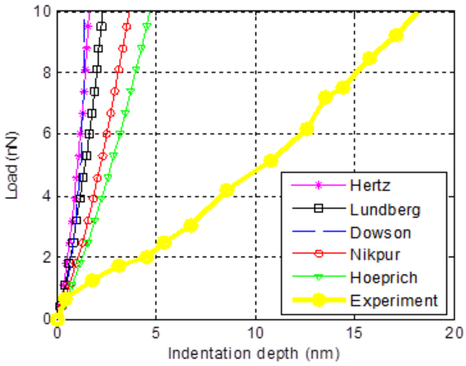

Figure 3. Loading diagram - depth of penetration assumption of E=2 Mpa

Finally, according to the results of Figure 3 and Figure 4, in Figures 5 and 6, the theoretical results are drawn assuming the modulus of elasticity of 250 kPa and 350 kPa, and the comparison of the theoretical and experimental results in these two figures, while verifying the Hoeparich model, according to Considering the effect of the equivalent radius as well as the available logarithmic relations, as a suitable contact model, the lower and upper range of the elastic modulus of the desired tissue shows 250 kPa and 350 kPa, respectively. The non-linear behavior of force and depth of penetration in the theoretical diagrams, especially in the lower elastic modulus, can be seen as the result of adhesion effects, which show themselves in a greater way, especially in the case of bio-particles with a lower elastic modulus.

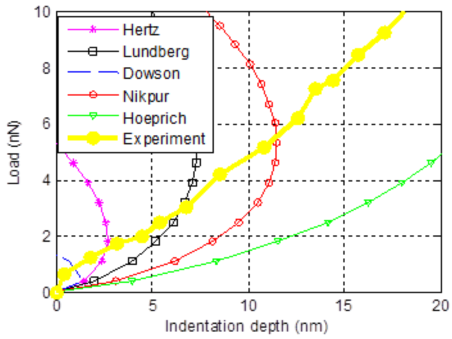

Figure 4. Load diagram - depth of penetration with assumption of E=100 Kpa

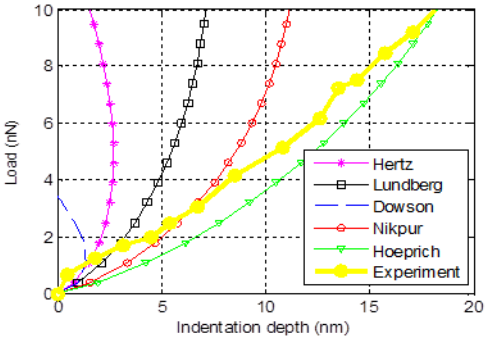

Figure 5. Loading diagram - depth of penetration with the assumption of E=250 Kpa

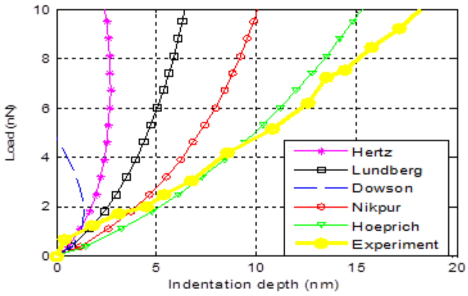

Figure 6. Loading diagram - depth of penetration with the assumption of E=350 Kpa

5. Verification of results

In order to check the validity, the comparison of the results obtained from this research with other reference sources and the results of this comparison are shown in Table 1. The results in this table show that for the lower limit of the modulus of elasticity, the minimum and maximum differences between the results of this research and previous researches are 8 and 20%, respectively, and for the upper limit of the modulus of elasticity, the minimum and maximum differences between the results of this research and previous researches are respectively 3 and It is 17 percent.

The upper range of the elastic modulus of the desired tissue using the developed cylindrical contact model of Hoepritch will be 350 kPa and the lower range of the elastic modulus of the desired tissue using the developed cylindrical contact model of Hoapritch will be 250 kPa.

Validation and comparison of the research results with other sources is shown in Figure 7.

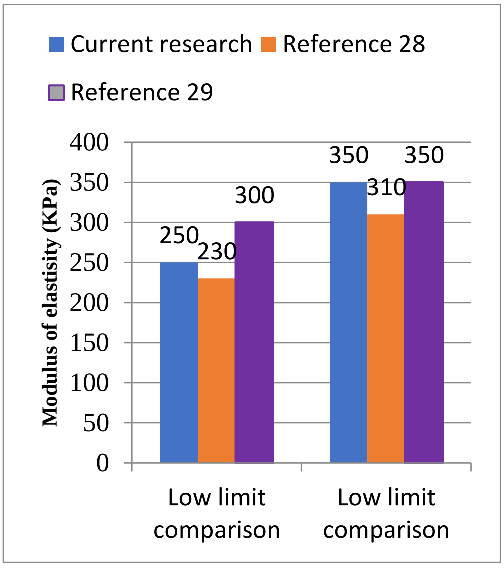

Figure 7. Comparison of the research results with the conducted researches

Table 1. Verification of the obtained results

Model	Reference [11]	Reference [12]	Research results
Lower limit of modulus of elasticity (KPa)	230	300	250
Difference percentage with reference (%)	8%	20%	-
Upper limit of modulus of elasticity (KPa)	310	350	300
Difference percentage with reference (%)	3%	17%	-

6. Results and Discussion

Analyzing and obtaining the mechanical properties of biological particles such as cancerous tissues in order to understand the behavior of these particles against force provides great help in creating bio-robots and modern treatment methods. One of the new and practical tools that has advantages such as not damaging healthy tissues and relatively high speed, is the atomic force microscope. Also, considering that the obtained results can be extended to other biological micro-robots with a single pole, this method is highly preferred over other methods.

In this research, the modulus of elasticity of gastric cancer tissue has been extracted using cylindrical contact models developed by Hertz, Lundberg, Dawson, Nikpour and Hoeprich. From the results obtained in this research, it can be generally concluded that in the range of the desired force and for the examined tissue, assuming the cylindrical shape of the tissue, the Hoeprich model is a suitable model for theoretical simulation, which is closest to the experimental results.

Hertz and Dawson models, due to the type of behavior they have and with the increase and then decrease of the depth of penetration due to the increase in force, which is probably due to the failure to consider the adhesion force in these two models, have a great difference with the experimental results, and therefore They are not recommended at all.

Lundberg and Nikpour models also showed behavior close to the experimental results in high values of the modulus of elasticity, but with the decrease of the modulus of elasticity, the behavior of these two models also changed compared to the experimental results and the use of these two models, especially for tissues and Particles with low modulus of elasticity are not recommended.

Also, due to the fact that the effect of equivalent radius is considered in them, the models of Nikpour and Hoeprich are more complete models from the scientific point of view, and the obtained theoretical results also confirm this issue. Also, it seems that the Hoeprich model had a more accurate prediction than the Nikpour model according to the existing logarithmic relationships.

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