J. of Applied Tissue Engineering
2018; 5(1): 37-43

The evaluation and comparison of the elastic modulus of mechanically motivated and pure rabbit mesenchymal stem cell with mature chondrocytes

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Received: 15 May 2018/ Accepted: 8 June 2018

Abstract:

Background: Different cells are located in different anatomical location of the body and consequently they are exposed to different mechanical forces. Chondrocytes, which form the cartilage tissue, are located in the articular joints like knee and they are daily influenced by intermittent hydrostatic pressure of thousands of times. Mechanical stimulations of the cells have vital contributions on acquisition of their functional characteristics, particularly, elasticity of the cells, which is defined by elastic modulus is one the most imperative mechanical properties. The arrangement and organization of actin fibers in the cytoskeleton can determine the elasticity of the cells, and it has been illustrated that it plays a pivotal role in some important cellular activities such as motility or cell- cell interaction.

Methods and Materials: In this research, the stem cells and mature chondrocytes were extracted from rabbit adipose and cartilage tissue, respectively.A unique bioreactor was used to apply intermittent hydrostatic pressure (0-1 MPa, 0.5 Hz) to Rabbit Mesenchymal Stem Cells (RMSCs). After applying the forces, the elastic modulus of different groups of cells were assessed and compared by atomic force microscopy.

Results: The results demonstrated that applying the hydrostatic pressure can modify the elastic modulus of stem cells up to 90% and make them more resemble to the mature chondrocytes.

Conclusion: The result of this paper can be important for applications of cartilage tissue engineering.

Keyword: Stem cell, chondrocytes, Hydrostatic pressure, Elastic modulus, AFM

Introduction

Cells are living in an active environment and they have continuous interaction with their environment[1, 2]. Mechanical loads are one of the primary stimuli that exert to the different cells [3, 4]. There are numerous studies, which illustrate that these forces play crucial roles in vital characteristics of the cells [5, 6].Chondrocytes, which are located in the articular joints of the body like ankle, knee and vertebrae , are exposed fundamentally to hydrostatic pressure. This type of mechanical stimulation can regulate the essential traits of the chondrocytes and

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also can be considered as a motivating factor for differentiation of stem cells [7, 8].

Stem cells are undifferentiated cells that have the ability to transform to various phenotypes [9]. They can differentiate in the response to the mechanical and chemical factors [6, 10]. For example, the existence of TGF-B growth factor with certain concentration can incite chondrogenic differentiation [8, 11]. In addition, the applying of hydrostatic pressure to the stem cells can elevate the suggestive specific chondrocyte genes [6]

Cells membrane mechanical properties is one the most important criteria in analysis and investigation of different cells behavior. Mechanical properties of the membrane has direct influence on some indispensable cells traits such as migration [12, 13], adhesion [14, 15] and intracellular interactions [16]. Cells apparent mechanical properties can be of importance in diagnosis [17] and even treatment of some diseases, such as cancer and atherosclerosis [18]. There are several methods for investigation of the mechanical properties of cells. Elastic and viscoelastic properties of the cells can be evaluated by micropipette [19], magnetic approaches [20], optical methods [21] and atomic force microscopy (AFM) [22]. AFM approach is one the best methods for analysis of the cells' mechanical properties that has one the least intervention duo to a delicate cantilever indentation in ordinary physical and biological processes within the cells in comparison to other approaches such as micro pipette approach [23-25].

In this paper, by means of a specific bioreactor, which can mimic the in-vivo condition of chondrocytes culture via applying intermittent hydrostatic pressure to rabbit mesenchymal adipose stem cells (RMSCs), cells membrane elastic modulus before and after applying the force was assessed by AFM. In addition, chondrocytes were extracted and their mean elastic modulus was measured by same the procedure. By statistically comparison of the data, it is possible to claim that mechanically motivated RMSCs are more mechanically resemble to the differentiated adult chondrocytes.

Materials and methods

RMSCs extraction

Norwegian rabbit was obtained from Pasteur Institute of Iran and their adipose tissue extracted according to standard ethical codes (WMA Statement on Animal Use in Biomedical Research). Adipose tissue was digested in collagenase solution (0.1%, Sigma, USA) for 30 minutes in 37ºC. DMEM culture medium (Sigma, USA) with 10% FBS (Sigma, USA) added and the solution filtered and centrifuged (1000 RPM, 5 min). Digested fat tissue accumulated on the top and it was removed careful pipette and the remaining solution was mixed and centrifuged again. The above liquid was removed and the deposit dissolved in fresh culture medium supplemented with 10% FBS. The suspension transferred to 6 well cell culture plate and the red blood cells were removed after 2 over nights. The RMSCs attached to the substrate were cultured and proliferated till passage 3 that was favored for our analysis.

Bioreactor characteristics

The bioreactor, which has been used for applying of hydrostatic pressure, was described in previous researches [8]. Briefly, a controllable hydraulic pump of 5 lit/min capacity equipped by an expansion valve has been used. The user can control the hydraulic

valves by means of a computer. Fig. 1 shows the schematic view of the bioreactor.

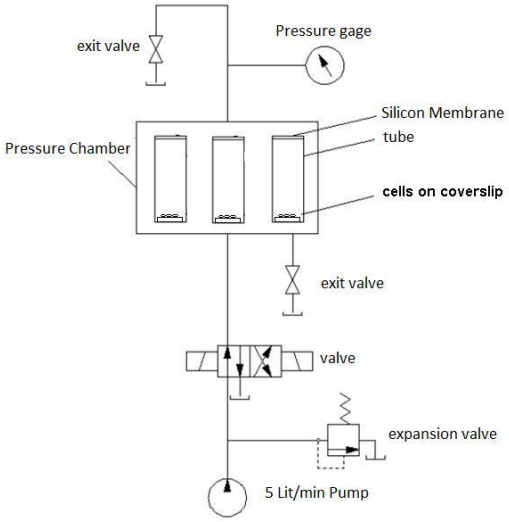


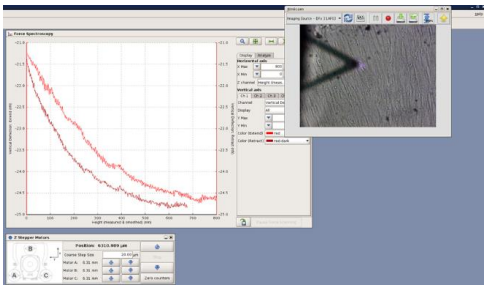
Fig. 1. Schematic view of the bioreactor. Cells in passage three are cultured on a coverslip and positioned in the tube. The tubes were set in the pressure chamber and intermittent hydrostatic pressure was applied.

Applying of hydrostatic pressure

Passage 3 RMSCs were cultured on a coverslip (SPL Life Science, Korea) and after 12 hours they were transferred to the tube. The end of the tubes were covered by a silicon membrane, and as a result, the hydrostatic pressure could be transferred prior to locating the samples into the bioreactor. Cells were exposed to the intermittent hydrostatic pressure between 0 and 1 MPa gage pressure with the frequency of 0.5 Hz for 2 hours. After applyingof the hydrostatic pressure, the cells were prepared for AFM test.Three groups of cells have been analyzed in this research. The examined groupsincluded RMSCs without any special treatment, mechanically motivated RMSCs and chondrocytes. Chondrocyteswere taken from the national cell bank of the Pasteur Institute of Iran.

AFM Analysis

Cells membrane elastic modulus was determine by AFMNanoWizard 2(JPK Germany). A soft (spring constant of 0.04 N/m)V shape cantilever (HYDRA6V, Appnano) of 0.04 N/m force constant was used. The force of 3 nN and the approach velocity of 1.6µm/s and indentation depth of less than 0.5 microns were set in contact mode of spectroscopyand the elastic modulus of the cell membrane was assessed. Fig.2a shows cells under the AFM microscope and Fig.2b illustrates the force diagram obtained by the device.



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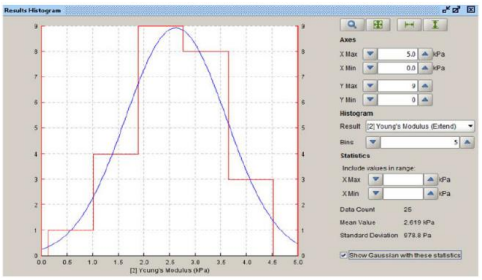

B

Fig. 2. A) View of cells under the AFM microscope, about 20 random points were selected on the cells and their elastic modulus have been investigated. B) A typical graph of elastic modulus.

Statistical analysis

All tests were independentlyperformed three times. In AFM Analysis more than 20 cells were selected and the elastic modulus of 10 points were measured on each cell. All the data provided in mean±SD and t-test paired statistical analysis was performed by SPSS software. The criterion for significant difference was set as P value less than 0.05.

Results and Discussion

Fig. 3 shows the elastic modulus of RMSCs as anegative control group (without any mechanical treatment), mechanically motivated RMSCs (intermittent hydrostatic pressure) and chondrocytes extracted from the knee articular joint. Results illustrated that the mechanical elasticity of chondrocytes are essentially higher than that of the stem cells, and also it was seen that the mechanically motivated stem cells have an elasticity modulus in between. It has been illustrate that mechanical forces have essential influences on vital characteristics of different type of cells. Mechanical stimuli can regulate the migration, proliferation, differentiation and mechanical properties of the cells [26, 27]. One the major mechanical properties of the cells that have influences on some significant cells' biological traits is elastic properties of the cell membrane. It has been assessed that the elastic properties of the cells are dependent on the arrangement and organization of actin fibers and intracellular biological processes[15].

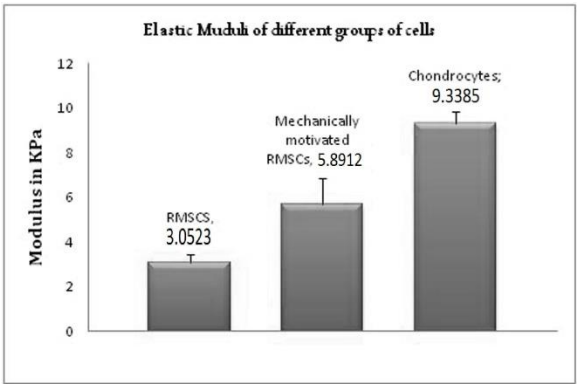

Fig. 3. The comparison of elastic moduli of RMSCs, mechanically motivated RMSCs and chondrocytes. Error bars representstandard deviations.

In this research the mechanical elastic properties of stem cells and mechanically motivated stem cells have been assessed and their results were compared to that of differentiated mature chondrocytes. The chondrocytes are located in a very especial mechanical environment with unique combination of forces. For example, during each cycle of regular walking, hydrostatic pressure of several MPa applied to the chondrocytes. This continuous special mechanical stimulation can alter some of the most significant biological specifications of the chondrocytes and the resulted mechanical properties of mature chondrocytes have essential dedications on essential functionalities of chondrocytes.

Atomic force microscopy (AFM) is one of the recent scientific approaches that has opened its ways to the field of biomedical engineering. Determination of the cells elastic and viscoelastic properties, calculation of adhesive forces between cells and their substrates [28] and delivering special macromolecules in to the cells are among the most crucial applications of AFM.

Among the several methods for determination of the cells' mechanical properties, such as micropipette

aspiration approach [29] or magnetic field application [20], atomic force microscopy provides the most precise results with minimum amount of interventions, and therefore can be categorized as one of the best approaches for evaluation of the mechanical properties in the cellular level[30].

Our results demonstrated that the mechanical stimulation of RMSCs by hydrostatic pressure can enhance and elevate elastic properties of these cells and make them more mechanically resemble to the differentiated mature chondrocytes. Previous researches have explained that many imperative criteria associated with cells' functionality such as their interaction with substrate and adjacent cells or their motility abilities are determined by the arrangement and organization of actin fibers and the structure of the cytoskeleton [31, 32]. These features also dictate the apparent mechanical properties of cells and therefore the elasticity of cells as one the most important mechanical properties can be one the determinant factors in functional evaluation of the cells.

Differentiation of stem cells in the response to the mechanical motivation by different type of forces or chemical agent like growth factors has been widely assessed and there are numerous studies in this field. But few of These studies have regarded the mechanical properties of final obtained cells as one the essential factors in complete and functional differentiation and the major intensity of these type of research is just on expression of genes in RNA and protein level [33]. While the differentiation of stem cells has been assessed broadly based on gene expression in different levels, this research focused on the comparison of the elasticity between different cell groups. Although there is a great difference between the mechanical properties of RMSCs and rabbit chondrocytes, our results showed that this difference can shrink and after applying of hydrostatic pressure the RMSCs have more resemble elasticity to the mature chondrocytes. It seems that the arrangement and organization of actin fibers in cytoskeleton undergoes alteration in the response to the mechanical stimulation and become more similar to that of the mature chondrocytes in comparison to RMSCs without any special treatment and the result is that the mechanically motivated RMSCs have higher elastic modulus.

The results of this paper can well demonstrate the pivotal role of mechanical stimuli in acquisition of chondrocytes characteristics in stem cells. Especially in comparison with other researches [33] it has been well proven that application of mechanical forces for short interval of times also can induce chondrocytes vital characteristics in stem cells. Membrane mechanical traits can be considered as one of the primary factors in appropriate functioning of cartilage cells and also it can characterize and distinguish different cells. The result of this study can be important in application of cartilage tissue engineering.

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