Effects of Equiaxial and Uniaxial Tensile Strain Generated by Orthodontic Forces on Human Mesenchymal Stem Cells

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Abstract

Objective: The orthodontic force-induced tissue strain produces local alterations in cellular and extracellular matrix reorganization, leading to the transformation and differentiation of cells. The present study was designed to investigate the influence of mechanical force on the CD90 expression of human mesenchymal stem cells (endometrial stem cells and dental pulp stem cells).

Methods: The mesenchymal stem cells (MSC) from passage 3-5 were seeded onto silicone membrane and cultured in medium with or without static mechanical stimulation (equiaxial and uniaxial strains). After 2 weeks, cultured cells were analyzed for expression of CD90.

Results: After 14 days in culture, immunofluorescence staining of cultured MSC demonstrated that mechanical stimulation of MSCs compared with control group, resulted in decreased CD90 expression.

Conclusion: The decrease of CD90 expression in mechanically stimulated cultures compared to unstimulated control cultures suggests the possibility of differentiation of stem cells to other cells.

Key words: Uniaxial strain, equiaxial strain, dental pulp stem cells, endometrial stem cells

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Introduction:

Mechanical forces are one of the important biologic factors which influence the expression of surface markers, reproduction and differentiation of stem cells (1). Dental movement during orthodontia and induction of controlled mechanical forces results in biologic reactions in tissues surrounding the teeth which are accompanied by cell responses to adapt the system to the changed conditions. The simplest interpretation to understand cell behavior against applied forces can be formulated by explaining the behavior of the spring against forces. When a spring is stretched, a change in spring length (strain) happens and internal tension called stress is created in the spring. Studies of researchers have shown that application of orthodontic forces also results in stress of matrix and cells and that following changes in membrane potential and activation of ion canals, a change in cell shape and then differentiation of these cells to other cells happen (2). If we were aware of the accidental changes which occur in tissues around teeth due to their orthodontic movements, we could adapt our appliances in a way to only apply necessary changes and to prevent unnecessary ones. In addition, if the type of changes was known, the limitations ahead in the treatment would be better considered (3). Therefore, knowing the relation between the inducing mechanical strains and biological responses caused by them seems essential. Another of the potential applications of inducing strains on cells is in bone tissue engineering. In tissue engineering, 3 factors are used; stem cells, scaffolds and inducing morphogens. Morphogens are extra cellular signals governing
morphogenesis, which are supplied by adding growth factors and other special substances to culture media. But recent studies have shown that instead of this method it is possible to use mechanical signals which are obtain following application of forces on cells (4, 5).

Till now various studies evaluated the effects of mechanical forces on stem cells differentiation to different categories (1, 6-9). Cells used in these kinds of researches, to study osteogenesis, were mainly stem cells from bone marrow, which contains a heterogenic population of differentiated and undifferentiated cells. So it’s not completely clear that either the differentiated cells are bone precursor cells or undifferentiated stem cells, which responded to the mechanical signals.

The uterine endometrium has a well vascularized stroma, which in normal physiologic conditions, regenerates more than any other body tissue and usually during women menstrual hemorrhages its superior functional layers are detached and rebuild by the inferior basal layer (10). Today, the presence of stem cells in the endometrium has been proved by flow cytometry as well as the identification of different markers, particularly CD105 and CD90 (11). Also, the simultaneous presence of CD146 and PDGF Receptor β markers on endometrial stem cells which existed in high amounts implied that the origin of stem cells in the endometrium is the same as in dental pulp and bone marrow (12). Considering that endometrial stem cells can differentiate to other cells like odontoblasts, chondrocytes and osteoblasts (10, 13), it is possible to advance the following theory, that with the assessment of the effect of uniaxial mechanical strains on these cells which are from the mesenchymal cell category and similar to bone marrow, we can make an appropriate prediction regarding the behavior of stem cells surrounding the tooth under orthodontic forces.

Dental pulp stem cells have till now been the object of numerous studies, various markers such as CD90 were identified on their surface and their differentiation to different tissues has been studied (14, 15). Taking into account that several studies have investigated the effect of uniaxial tensile strains on these cells (1, 8), in this study, we assessed the effect of equiaxial forces on these cells.

Accordingly, the objective of this study was to evaluate two types of tensile strains, equiaxial and uniaxial, on the expression of CD90 (Thy-1) in stem cells of dental pulp and endometrium.

Methods:

1- Preparation of cell culture substrate silicone membrane
Cured Silicone Platinum Membrane with characteristics such as transparency is very appropriate for medical purposes. Transparency of the silicone membrane makes the observation and imaging of the cells by invert microscope possible. This membrane was prepared from Poly Dimethyl Siloxaen (PDMS) (Pasteur Institute, Iran). The prepared membrane was cut in bands with 60mm in length and 15mm in width in order to be placed in the uniaxial strain applying device. For the equiaxial device, this membrane was cut in circles with 5cm radius. In the middle part of both membranes, a range of 10x10mm was marked using color points, in order to evaluate the morphologic changes of cell categories before and after the experiment (Figure 1).

![Figure 1- silicone membrane for uniaxial strain applying device (a) and for equiaxial device (b)](image-url)
2- Correction of silicone surface properties with a collagen cover

In order to provide appropriate conditions for cell adhesion to silicone membranes, surface operations to improve the surface properties by covering the surface with an appropriate hydrophilic and biocompatible substance are necessary. In the present study, the silicone membrane covering was performed with collagen (Type A, Sigma, Germany). For this purpose, collagen diluted in phosphate buffer saline (PBS) was placed in the center of the membrane (Figure 2) and after drying and rinsing of the membrane with PBS (sigma), the membrane was conserved in refrigerator, till the time of transfer of the cells.

![Collagen on the silicone membrane before drying](image)

**Figure 2- Collagen on the silicone membrane before drying**

3- Transfer of the cells to the membranes

Dental pulp stem cells and endometrial stem cells were obtained from the Cellular and Molecular Biology Laboratory of the dental school of Shahid Beheshti University of Medical Sciences. These cells were identified via assessment of surface markers (using flow cytometry) and differentiation to various cell categories. For cell culture, DMEM (Dulbecco’s Modified Eagle Medium) (Gibco, Invitrogen, USA) culture media was used, containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 units/ml of penicillin and 100 mg/ml of streptomycin in an incubator with 5% CO2 and 37°C. In the fourth passage from every category of dental pulp and endometrial cells, 10000 cells were counted using the hemocytometer and were transferred to the membrane in the 1cm² marked area of the silicone membrane. In every category, one group of cells were assigned as control group in sterile petri dish and placed in the incubator. The scaffolds of the experimental groups, after 24 hours in the incubator were evaluated using the invert microscope (Nikon, Japan), and after assurance of appropriate adhesion of cells, they were prepared for loading.

![Transfer of dental pulp stem cells to silicone membrane for equiaxial strain applying device (a), and endometrial stem cells for uniaxial device (b)](image)

**Figure 3- Transfer of dental pulp stem cells to silicone membrane for equiaxial strain applying device (a), and endometrial stem cells for uniaxial device (b)**

4- Cells loading

In laboratory researches, loading and stretching of cells are usually performed via uniaxial or equiaxial stretching on an elastic bed. In this study, the equiaxial strain loading device designed by the Pasteur Institute was used for...
dental pulp stem cells loading and the uniaxial strain loading device designed by the Pasteur Institute was used for endometrial stem cells. The device was built with the capacity of static or periodic strain, various frequencies and percentages of strain. In this study, considering that orthodontic forces are static, the static tensile strain capacity of the device was used. The experimental groups’ silicone membranes were fixed in the device steel brackets and put under loading in normal culture environment. Taking into account that in orthodontic usual experiments, strain speed is 0.1-1mm per min (16), loading was determine to be of static type, and with an amount of strain of 3% for a period of 2 weeks (Figure 4).

Figure 4- Schematic of the strain loading device, equiaxial (a) and uniaxial (b)

5- Assessment of stem cells after loading

Two weeks after cells’ culture on membrane, cells of two groups, experimental and control were rinsed with PBS and fixed for 30mn in 4°C with paraformaldehyde (Merck, Germany). And after rinsing using 1/5% goat serum (Santa cruz, USA), the non-specific antibodies were blocked for one hour then incubation with CD90 primary antibodies (1:100; Santa cruz) was performed for over-night at 4°C. Following which incubation with secondary antibodies anti mouse IgG-FITC (1:100; Santa Cruz) was performed for 3 hours. In the final stage, after rinsing with PBS, cells were photographed using fluorescent microscope (Novel, China).

Results:

Cells cultured on collagen-coated silicone membranes, showed covered with collagen, showed good adhesion after 24h (Figure 5).

Figure 5- Image of endometrial stem cells (a) and dental pulp stem cells (b) after 24 hours of culture on silicone membrane
After the two weeks loading in the strain device, staining with specific markers (CD90) for mesenchymal stem cells (MSC) showed that while this marker was widely expressed in the control groups, in the experimental groups this marker was not expressed (Figure 6, a-b). This status was completely similar for both dental pulp and endometrial stem cells’ groups. In both groups, DAPI staining showed the existence of cells on both experimental and control scaffolds (Figure 6).

**Figure 6- Immunofluorescence staining of cells with CD90, in control group (b) and group under mechanical stimulation (a), DAPI staining in control group (c) and group under mechanical stimulation (d)**

**Discussion:**

Studies have shown that osteogenesis which occurs during the orthodontic tooth movement is due to inducing molecules which are stimulated by tensile strain and have effect on stem cells or bone precursors surrounding periodontal ligament, which finally results in bone formation (17). In this study, dental pulp stem cells after culture on silicone membrane covered by collagen were put under static equiaxial tensile strain, while endometrial stem cells after culture on membrane were put under static uniaxial tensile strain. This mechanical stimulation was applied on cells for 2 weeks. Different laboratory studies reported various results in response to the application of mechanical forces on stem cells and some of these results were contradictory. While Lee et al revealed in their study that mechanical stresses led to differentiation of dental pulp stem cells to odontoblasts (18), some scientists stated that these forces have no influence on dental pulp stem cells (19). Cai et al after having put dental
pulp stem cells in an osteogenic media and applied uniaxial tensile strains on them reached the conclusion that application of strain is an obstacle to osteogenic differentiation of cells. In that study the strain applied was 2000 µ with 1Hz frequency and cells were placed under forces for 6 hours (1).

Ji-Yeon et al, in 2011, studied the combined effect of mechanical strain and the surface model of the scaffold on bone marrow stem cells differentiation. In that study, the culture media was completely free of osteogenic differentiating factors and the result of the study showed that 3% strain caused differentiation to osteoblasts and 10% strain led to differentiation to smooth muscle cells (20).

It seems that various mechanical stresses induce different effects on stem cells (21) and since the duration of force induction, type of force applied, frequency of stimulation and type of stem cells were different in the various studies, comparison of the results of these studies appear difficult (22, 23).

Researches have demonstrated that the expression of CD90 surface marker (Thy-1) decreases in the differentiation of stem cells toward osteoblasts (24). In a study performed by Wiesmann et al on the expression of CD90 surface marker after induction of tensile strain on bone marrow stem cells, absence of expression of this marker was reported (25). Also Han et al, in the evaluation of the effect of dynamic uniaxial tensile strain on dental pulp stem cells, recorded the reduction of expression of CD90 (8). In the present study, the influence of static tensile strain on the expression of CD90 surface marker (Thy-1) in MSCs was assessed. Surface marker CD90 (Thy-1) is usually used as a positive marker of MSCs (25). In this study, this marker was well expressed in cells of the control group, while in cells under mechanical strain, the amount of its expression considerably decreased. Till now, no study was performed regarding the effects of uniaxial mechanical strains on endometrial stem cells. Furthermore, the effect of equiaxial strains on dental pulp stem cells was evaluated for the first time. In most of the studies performed, the type of force induced was dynamic and periodic, but in this study, in order for the work platform to simulate the conditions of application of orthodontic forces, static forces were chosen. In addition, the minimal strain was chosen for the device in order to apply the least force.

One of the limitations of this study was the use of fixed geometric membranes. The reality is that in orthodontic appliances, from a biological point of view, the forces applied to the brackets are of no importance. But instead, what is important is the force applied on a unit of dental root surface which is transmitted to the periodontal ligament and bone, and for a determined amount of force applied to the brackets, with an increase in root surface, the amount of force applied on a unit of surface area. Therefore in subsequent studies, the device should be designed in order to make the use of scaffolds with various geometries possible.

Another limitation of this study was the use of silicone membranes, while tooth and surrounding bone are formed by the combination of organic and mineral substances. Thus use of composite scaffolds for studies will bring laboratory conditions as closer as possible to In vivo conditions. Finally, the qualitative nature of the results of the study didn’t make an accurate comparison between the two experimental groups which were put under two different types of strains possible and shows the need for quantitative studies like Real time RT-PCR.

Conclusion:

This study showed that uniaxial and equiaxial mechanical strains which are exerted when applying orthodontic forces result in decrease in expression of surface marker in mesenchymal stem cells compared to the control group. This
matter is indicative of the differentiation of the cells to other types of cells, with their identification constituting the second phase of this study. Although the exact reason of the influence of mechanical stimulation on reproduction and differentiation of cells is not determined, but it seems that the effect of mechanical signals on cell receptors and on transport of gases and necessary nutrients to the cell play a role in this matter. The first phase of this study did not show a difference in the response of the experimental groups’ cells to the type of strain (Uniaxial and equiaxial).

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