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Regulation of Cyclic Longitudinal Mechanical Stretch on Proliferation of Human Bone Marrow Mesenchymal Stem Cells

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Abstract: Mechanical stimulation is critical to both physiological and pathological states of living cells. Although a great deal of research has been done on biological and biochemical regulation of the behavior of bone marrow mesenchymal stem cells (MSCs), the influence of biomechanical factors on their behavior is still not fully documented. In this study, we investigated the modulation of mechanical stretch magnitude, frequency, and duration on the human marrow mesenchymal stem cells (hMSCs) proliferation by an in vitro model system using a mechanical stretch loading apparatus, and optimized the stretch regime for the proliferation of hMSCs. We applied 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyl tetrasodium bromide (MTT) assay to estimate the overall proliferative effects of the stretch on hMSCs. We found that fibronectin coating increased adhesion to silicone chamber surface, however, it did not show significant effect on proliferation of hMSCs. A frequency of 1 Hz was more effective in stimulating hMSCs proliferation. At 1 Hz, 5% strain for 15, 30, 60 min, the significant increase of hMSCs proliferation was observed. Proliferation was enhanced at 1 Hz, 10% strain for 15, 30 min, while decreased for 60 min. At 1 Hz, 15% strain, 15 min stretch resulted in the decrease of proliferation, and 30 min and 60 min stretch showed an increased proliferation. Long time (12 and 24 h) strain application blocked the proliferation. These results indicate that mechanical stretch plays an important role in hMSCs growth and proliferation; an appropriate mechanical stretch regime could be a novel approach to promoting proliferation of hMSCs in vitro.

Keyword: Bone, Mesenchymal stem cells, Mechanical strain, Proliferation, Biomechanics

1 Introduction

Tissues and cells in the body are continuously exposed to a complex mechanical microenvironment. Mechanical stimulation including gravity, tension, compression, and shear stress are critical to the growth and function of living cell (1). It has been reported that skeletal unloading inhibits the in vitro proliferation of rat osteoprogenitor cells (2), while mechanical stretch (5% strain, 15 min/h, 1 Hz) induces rat fetal lung fibroblasts and epithelial cells proliferation with enhanced DNA synthesis (3). Moreover, 15 or 60 min uniaxial stretch (5% strain and 1 Hz) increase the incorporation of BrdU into the human tendon fibroblasts in vitro, but 30 min stretch at the same magnitude and frequency doesn't increase, on the contrary, decrease the proliferation of tendon fibroblasts (4). Interestingly, human bone osteoblasts proliferation depends on the number of applied load cycles, but not significantly depends on the frequency. 15 min stimulation with 400ε enough increases the growth of osteoblast-like cells while after 72 h stimulation proliferation is decreased (5). These research results strongly

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suggest that optimal mechanical stimulation can induce and promote the cell proliferation.

Stem cells are primal cells found in all multicellular organisms that retain the ability to renew them through mitotic cell division and can differentiate into a wide range of specialized cell types. Bone marrow is one of the most abundant sources for adult stem cells. Bone marrow mesenchymal stem cells (MSCs) are the pluripotent stromal cells derived from bone marrow capable of differentiating along multiple mesenchymal lineages (e.g. bone, adipose tissue, cartilage, muscle tissue) (6, 7). Moreover, the transplantation of ex vivo-expanded allogeneic MSCs has shown little immunogenic responses in vivo (8). Thus, it can serve as a basis for tissue engineering of autologous implants without concerns on transplant rejection and has been identified as an attractive cell source for a wide variety of tissue engineering strategies. However, isolation of marrow aspirates in great volume causes damage and pain, and it is difficult to isolate from the bone marrow 10^7-10^8 MSCs that are required for regeneration of large injured tissues (9). Therefore, expansion of MSCs in vitro is a prerequisite for the clinical application.

Mechanical stimulation has been increasingly recognized to play an important role in the regulation of stem cells function, including the modification of growth, proliferation and differentiation. Application of equiaxial cyclic strain (3%, 0.25 Hz) to human mesenchymal stem cell (hMSCs) inhibited proliferation and stimulated a 2.3-fold increase in matrix mineralization over unstrained cells (10). Kearney et al. explored effects of 0.17 Hz, 2.5% and 10% mechanical strain on proliferation of adult marrow stromal cells, and found that proliferation was not significantly altered after 1 day 2.5% strain, but 2 and 3 days of strain significantly reduced proliferation. Strain of 10% evoked a time dependent reduction in proliferation (11). Recently, it was reported that cyclic strain of 1 Hz, 10% inhibited proliferation of adipose-derived stem cell, and caused alignment of the cells and of the F-actin cytoskeleton perpendicular to the direction of strain (12). It seemed from these findings mechanical strain yields an inhibitory effect in stem cells proliferation, but results from other findings are inconsistent with these investigations. van Griensven et al. described 15 min strain of 1 Hz, 5% resulted in increased proliferation of hMSCs, 60 min of the same stain showed hardly any effect (13). Interestingly, the effects of strain depend on the orientation of cells. 1 Hz, 5% uniaxial strain increased MSCs proliferation. However, when micropatterning was used to align cells perpendicularly to the axis of mechanical strain, the changes of MSCs proliferation was not affected (14). Moreover, the response of MSCs to uniaxial strain and equiaxial strain was compared, and uniaxial strain was demonstrated to be a better fashion for MSCs proliferation (15, 16). Koike et al.. found 10-15% high magnitudes of strain applied to marrow stromal ST2 cells resulted in significant increase in proliferation and type I collagen mRNA level (17). Taboas et al.. uncovered the antithetic responses of MSCs vs. chondrocytes to physiologic levels of cytokines, growth factors, and mechanical forces (18). These studies show that a mechanical strain is crucial to proliferation of stem cells, but the fashion of stretch is extremely important, different strain loading may induce totally different effect. The different proliferation responses of stem cells to mechanical strain may be dependent on the cell types and mechanical regime as well as loading parameters like strain duration, frequencies and intensity in regulating cell physiology.

Despite a great deal of research has been done on proliferation regulation of stem cells exposure to mechanical strain, the combined effects of these loading parameters of mechanical strain, including magnitude, duration and frequency, applied in vitro to MSCs have not been fully addressed. In this study, using a specially developed cell stretch loading system, cyclic longitudinal uniaxial mechanical stretch was applied to hMSCs cultured in flexible silicone chambers, we sought to determine the effects of mechanical stretch and focused on the modulation of different mechanical stretch parameters on the proliferation of hMSCs.

2 Materials and Methods

2.1 Cell culture

Human bone marrow mesenchymal stem cells (hMSCs, JCRB 1136) were purchased from the cell bank, the Japan Health Sciences Foundation (Osaka, Japan). The cells were plated in 25-cm^2 culture flasks (Becton Dickinson Labware, USA) at 2×10^3 cells/cm^2 with the hMSCs special expansion medium (B3001, TAKARA BIO INC., Japan) to allow for cell proliferation without differentiation, and incubated at 37°C in a humidified atmosphere of 5% carbon dioxide and 95% air. The culture medium was changed two-three times per week. After reaching confluence (usually about 5 to 7 days), the cells were released with 0.25% trypsin/1mM EDTA (B3232, TAKARA BIO INC., Japan) and subcultured in 25-cm^2 cell culture flasks.

2.2 Cyclic longitudinal strain apparatus

Mechanical cell strain instrument (Model ST-140, STREX Co., LTD, Osaka, Japan) consisted of a control unit, a strain unit and rectangular, elastic silicone chambers in which the whole chamber, not only the cell culture surface, was deformable (Fig. 1 A). During stretch experiments, only strain unit was put into an incubator. The chambers were designed for use in the strain unit driven by an eccentric motor that allowed variation in magnitude (5-20%) and frequency (0.01-1.5 Hz) of the applied strain. The chambers were molded of a two-component silicone elastomer containing Silicosehl RTV270 and crosslinker A 47 (Rhône-Poulenc, Lübeck, Germany) at a ratio of 10:1. There are two types of chambers, named ST-CH-04 and ST-CH-10, respectively. ST-CH-04 chambers were 40 mm long, 25 mm wide, 10 mm high, and the wells had a 20 mm $\times$ 20 mm cell culture surface. ST-CH-10 chambers were 51 mm long, 35 mm wide, 10 mm high, and the wells had a 32 mm $\times$ 32 mm cell culture surface (Fig. 1 B and C). This automated instrument was designed to hold 4 ST-CH-04 chambers or 5 ST-CH-04 strain chambers with precise uniaxial mechanical strain synchronously (as shown in Fig. 1 A). The stretching over the entire cell culture surface is uniformity.

2.3 Coating of strain chamber and microplate

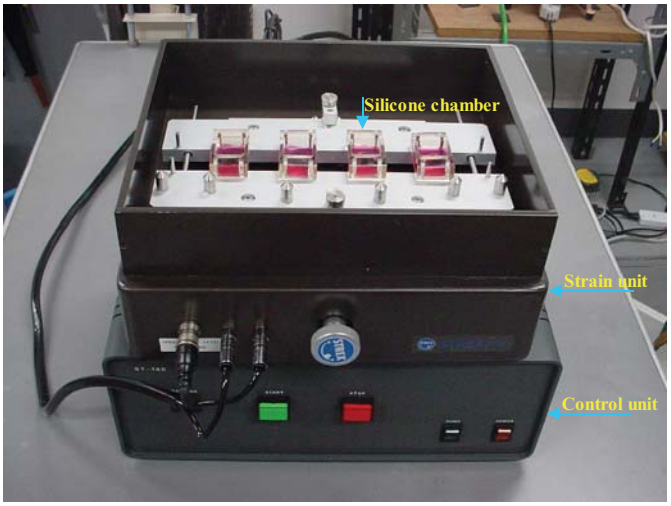
Coating of strain chamber was performed in the following procedures. New chambers were cleaned and autoclaved at 121°C for 20 min. Concentrated stocks of human fibronectin (COSMO BIO CO., LTD. Tokyo, Japan) were diluted to 5μg/ml concentration in phosphate buffered saline (PBS, pH 7.4), and coated strain chamber by adding appropriate amount of diluted fibronection to culture surface for 4 hours in incubator at 37°C. Aspirate remaining fibronection solution after incubation and rinse the chamber carefully with sterile distilled water, avoid scraping the culture bottom surface. The coated chambers are ready for seeding cells.

To assess the effects of fibronectin coating on proliferation of hMSCs, 96 well microplates were coated with fibronectin by the same method. The coated microplate are ready for cell culture and proliferation assay.

2.4 Cell stretching experiments

hMSCs were harvested, counted and plated in the chamber precoated with fibronectin at a density of 10^4 cells/cm^2. After 12 h of culture, the concentration of fetal bovine serum was reduced to 0 for 24 h in order to align most cells into G0 phase of the cell cycle. Subsequently, the chambers were mounted on strain unit of the strain apparatus by 4 holes for hooks, one end of the chamber was firmly attached to a fixed frame, while the other end was held on a movable frame. The movable frame was connected to a motor driven shaft. The cells in the silicone chambers were exposed to a stretch treatment at amplitude of 5% to 15% and frequency of 0.01 Hz to 1.5 Hz. Short time strain was applied for 15, 30 and 60 min, the observation periods after cessation of strain were 6 and 24 h. Long time strain was applied for 12 and 24 h. As a control, static cells were grown on strain chamber at the same conditions, but did not receive any strain.

(A)



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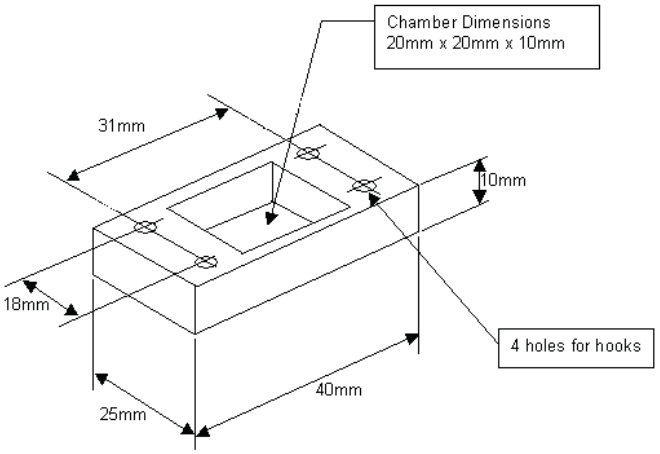

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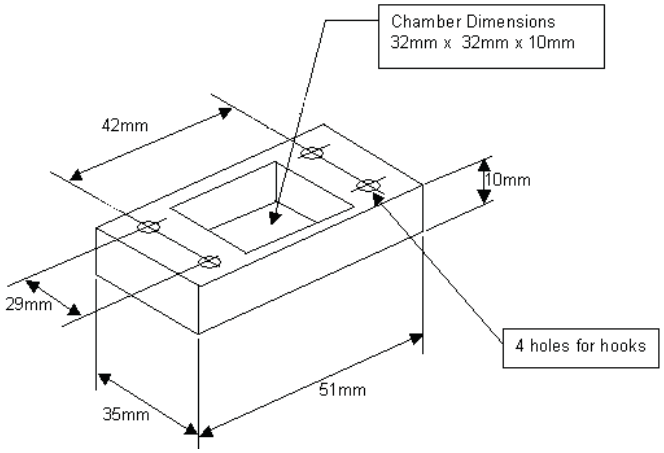

Figure 1: Cell cyclical mechanical stretch device. (A) Photograph of the mechanical strain instrument (Model ST-140, STREX Co., LTD, Osaka, Japan). It consisted of a control unit, a strain unit and elastic silicone chambers (as marked by arrowheads, respectively) and could provide stretch of variation in magnitude (5-20%) and frequency (0.01-1.5 Hz). (B) Schematic diagram of silicone chamber ST-140-04. (C) Schematic diagram of silicone chamber ST-140-10 (from http://www.b-bridge.com/eng/products/strex/st-ch.htm).

2.5 Proliferation assay

To determine the proliferation of hMSCs, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrasodium bromide) kit (Chemicon International, Inc. USA), a cell proliferation ELISA system, was used. All the MTT assay operations were performed according to the manufacturer's instructions. Briefly, appropriate MTT solutions were added to strain chambers at desired time points and incubated at 37°C for 4 h. The intense purple colored formazan derivative formed during active cell metabolism was eluted and dissolved in 0.04 M HCl (in 95% isopropanol). 100μl of each solution was transferred to a 96-well microtiter plate. The optical density (OD) of each probe was measured in an ELISA plate reader (Model 680, BIO-RAD, USA) with a test wavelength of 570 nm and a reference wavelength of 630 nm. OD values obtained at a wavelength of 570 nm were subtracted from the values obtained at 630 nm to standardize the different measurements. Relative proliferation rates were determined by comparing strained cells with static control cells.

2.6 Statistical analysis

Results are expressed as mean values $\pm$ standard deviation. Statistical analysis to compare results between two groups was carried out by unpaired Student's t-test and a value of $p < 0.05$ was considered to be statistically significant.

3 Results

3.1 Cell morphology in silicone chamber

When hMSCs were seeded in silicone chamber without coating, it could not adhere and grow due to the hydrophobic property of silicone membrane surface, died cells were observed at the second day. We selected some adhesive substrates to coat the culture surface of silicone chamber and found that fibronectin is a suitable coating for our experiments. Fig. 2 shows morphology of hMSCs growing in silicone chamber surface coated with 5μg/ml human fibronectin in quiescent condition, the majority of hMSCs maintained their elongated fusiform shape resembling fibroblasts and grew well in fibronectin coated silicone chamber.

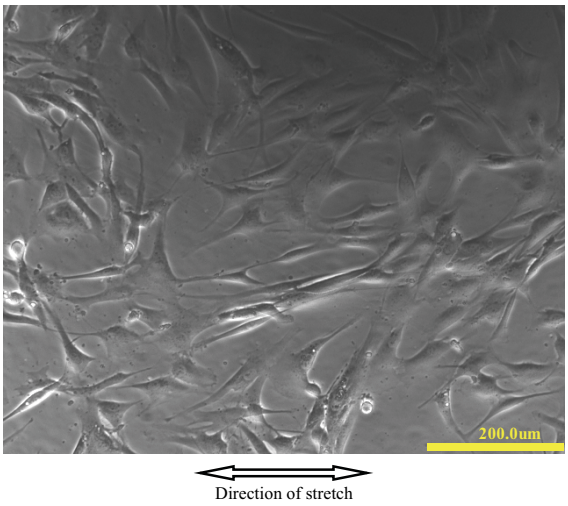

Figure 2: Photomicrograph of hMSCs in silicone chamber coated with 5μg/ml fibronectin. Cells exhibited an elongated fusiform shape resembling fibroblasts. Arrowhead showed the direction of stretch in strain experiments.

3.2 Effects of fibronectin on proliferation of hMSCs

To determine whether the fibronectin coating treatment affects hMSCs proliferation, we measured proliferation of hMSCs cultured in coated 96 well microplate using MTT assay. Fig. 3 A and B indicate the OD values of hMSCs, with or without fibronectin treatments. Compared to control, no significant difference was detected in fibronectin treatments. However, fibronectin coating significantly promoted adhesion of hMSCs to silicone surface (Fig. 2).

3.3 Effects of strain frequency on hMSCs proliferation

To explore effect of strain frequency on proliferation of hMSCs, the cells cultured in silicone chamber were exposed to 10% stretch for 15 min at different frequency from 0.01-1.5 Hz. After strain cessation, the cells were continuously cultured for 24 h and the proliferation assay by MTT was performed. As shown in Fig. 4, we found that in the case of 10% stretch for 15 min at 0.01 Hz, proliferation rate of hMSCs compared to control cells did not have any change (1.01$\pm$0.01 times,

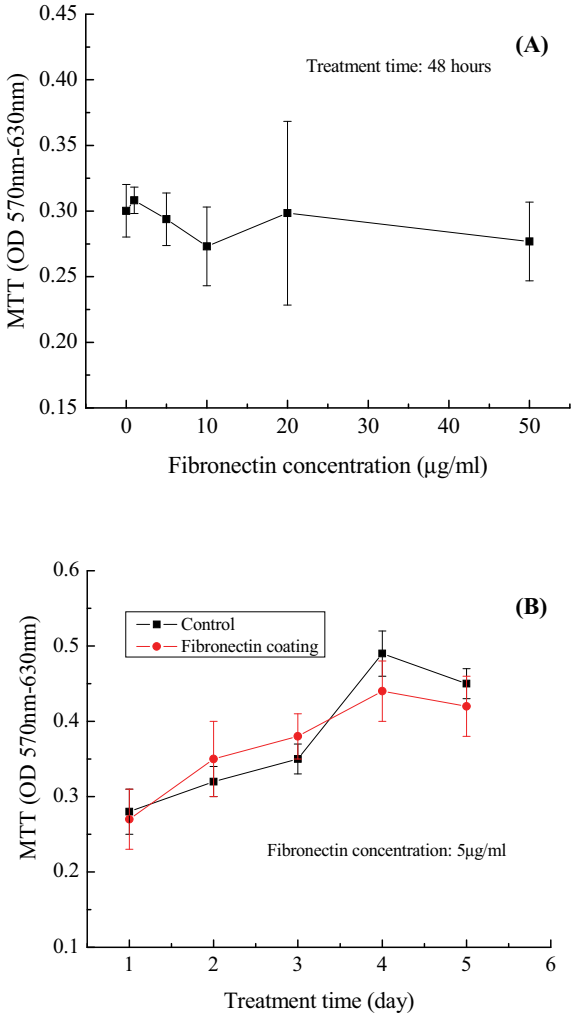

Figure 3: Effects of fibronectin coating on the proliferation of hMSCs. The cells were cultured in 96 well microplate coated with fibronectin and MTT assay was used to examine the proliferation of hMSCs. OD values were obtained using a reading plate at 570 nm and 630 nm with a 96-well micro test spectrophotometer. Data represent means ± standard deviation of five separate samples. (A) Effects of fibronectin concentration on the proliferation (treatment time: 48 h). (B) Effects of treatment time on the proliferation (5μg/ml fibronectin).

$p > 0.05$). However, the same stretch at 0.1 Hz resulted in a lower proliferation rate (0.83±0.07 times, $p < 0.05$). Subsequently, a higher proliferation rate was observed at 0.5Hz (1.12±0.03 times, $p < 0.05$) and the highest proliferation rate was found at 1.0Hz (1.70±0.04 times, $p < 0.01$). The proliferation rate at 1.0Hz was also remarkably higher than that of 0.5Hz. However, application of high frequency strain at 1.5Hz resulted in an evident decrease of proliferation rate compared to control cells (0.77±0.01 times, $p < 0.01$). Therefore, the stretch frequency in subsequent experiments was designed at 1 Hz.

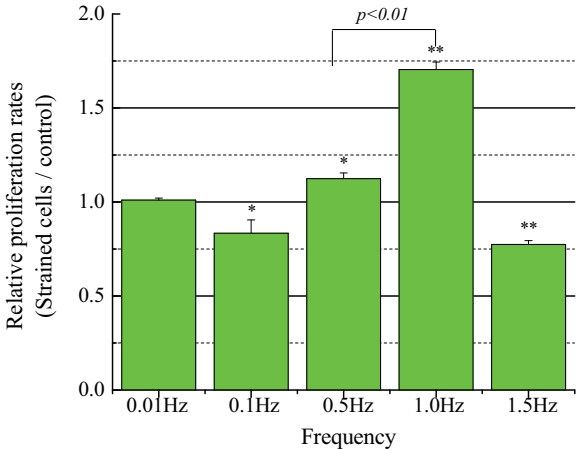

Figure 4: Cell proliferations at 24 h and different frequency after 15 min of 10% stretch. Data represent relative proliferation rates of strained cells vs. control cells depicted at line 1. Proliferation was measured using MTT assay. *p <0.05, **p <0.01.

3.4 Effects of short time strain on hMSCs proliferation

In 1 Hz, 5% strain stretching group, 6 h after 15, 30 and 60 min of mechanical stretch, relative proliferation rates of hMSCs were significantly increased to 1.35±0.15, 1.12±0.06 and 1.32±0.37 times respectively, compared to control cells ($p <$ 0.05). Then the proliferation rates returned to levels as seen in controls at 24 h; no significant differences were observed in comparison to control cells (Fig. 5).

In 10% strain stretching group, significant increase of proliferation rates were detected both at 6 h and 24 h after 15 and 30 min of mechanical stretch, compared with control cells ($p <$ 0.05). The values are as follows: 6 h, 1.45±0.05,

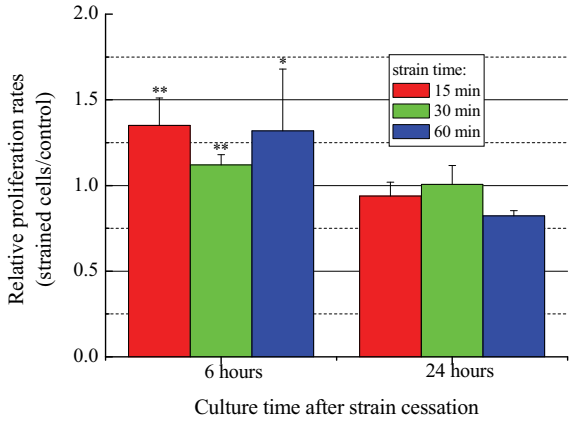

Figure 5: Effects of 5% mechanical stretch on the proliferation of hMSCs. Data represent relative proliferation rates of strained cells vs. control cells depicted at line 1. Proliferation was measured using MTT assay. Strain was applied with a frequency of 1 Hz and for the duration indicated. $*p < 0.05$, $**p < 0.01$.

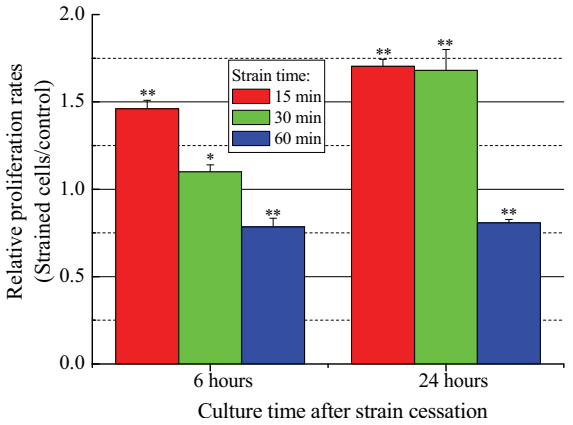

Figure 6: Effects of 10% mechanical stretch on the proliferation of hMSCs. Data represent relative proliferation rates of strained cells vs. control cells depicted at line 1. Proliferation was measured using MTT assay. Strain was applied with a frequency of 1 Hz and for the duration indicated. $*p < 0.05$, $**p < 0.01$.

1.09±0.04; 24 h, 1.70±0.04, 1.68±0.12. In contrast with control level, 60 min of mechanical stretch shows an obvious inhibitory effect on hMSCs proliferation ($p < 0.01$). The relative proliferation rates at 6 and 24 h are 0.78±0.05 and 0.80±0.02 (Fig. 6).

In 15% strain stretching group, no significant difference was found after 15 min of mechanical stretch at 6 h (1.01±0.07 times, $p > 0.05$), however, the relative proliferation rate markedly decreased at 24 h (0.52±0.02 times, $p < 0.01$). Interestingly, the proliferation rates of 30 min of mechanical stretch firstly represented a clearly decrease at 6 h (0.69±0.03 times, $p < 0.01$), and then an evident increase of proliferation rate was detected at 24 h (1.23±0.14 times, $p < 0.01$). After 60 min of mechanical stretch, slight increase of proliferation rate was observed at 6 h (1.05±0.04 times, $p > 0.05$), and significant increase of proliferation rate was found at 24 h (1.71±0.03 times, $p < 0.01$), as shown in Fig.7.

3.5 *Effects of long time strain on hMSCs proliferation*

When the cells were subjected to 1 Hz, 5% elongation for different treatment time, we evaluated

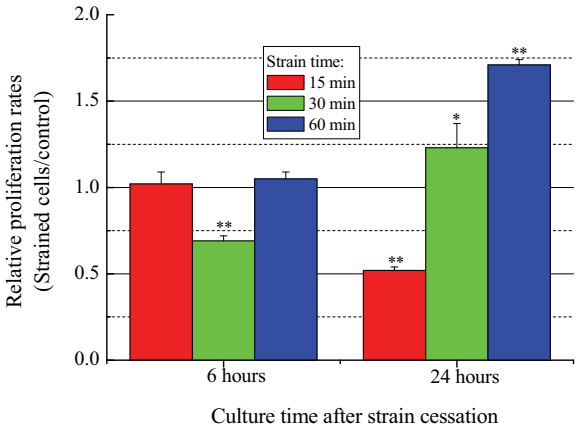

Figure 7: Effects of 15% mechanical stretch on the proliferation of hMSCs. Data represent relative proliferation rates of strained cells vs. control cells depicted at line 1. Proliferation was measured using MTT assay. Strain was applied with a frequency of 1 Hz and for the duration indicated. $*p < 0.05$, $**p < 0.01$.

the effects of duration of stretch on hMSCs proliferation. 6 h after application of short time strain (15 and 60 min), cell proliferation rates was significantly increased compared with control cells. In contrast, proliferation of long time strained hMSCs significantly decreased after 12 and 24 h

stretch (12 h: 0.74±0.02 times, $p < 0.01$; 24 h: 0.45±0.01 times, $p < 0.01$. Fig. 8).

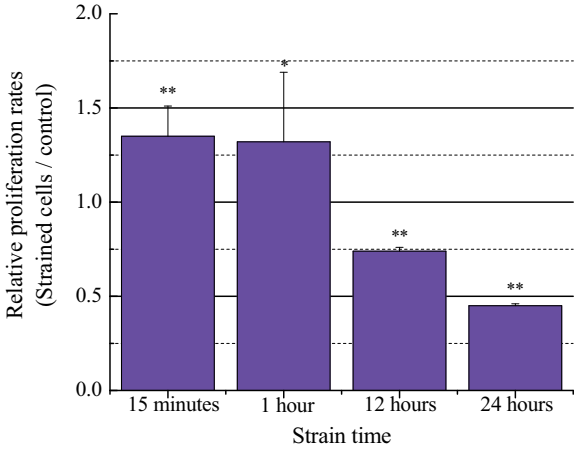

Figure 8: Effects of the duration of stretch on the proliferation of hMSCs. Data represent relative proliferation rates of strained cells vs. control cells depicted at line 1. Proliferation was measured at 6 h after strain application using MTT assay. Strain was applied with a frequency of 1 Hz, amplitude of 5% and for the duration indicated. $*p < 0.05$, $**p < 0.01$.

No detached cells from the substrate were observed during all the experiments.

4 Discussion

In the present studies we used a cyclic longitudinal strain device and assessed the regulation of mechanical uniaxial stretch on proliferation of hMSCs. Our results demonstrated that a short time (15-60 min) stretch of 1 Hz, 5% strain causes an increase in hMSCs proliferation at 6 h and returns to normal at 24 h during the time points under investigation (6 h and 24 h). These results are consistent with van Griensven et al..'s findings (13), and suggest that small magnitudes of strain (5%) result in increase in proliferation of hMSCs, but the sustaining time of cell proliferation response is short (Fig. 5). With the increase of magnitude of stretch, 1 Hz, 10% strain for 15 min and 30 min, increased proliferation was observed at all the time measurements, indicating that the sustaining time of proliferation response is extended. 60 min 10% strain, however, resulted in a decrease in cellular proliferation, indicating the cessation of self-renewal of hMSCs in this duration (Fig. 6). When the cells were exposed to 1 Hz, 15% strain, 15 min stretch did not yield a difference in proliferation at 6 h, but a decrease proliferation was detected at 24 h. 30 min stretch represented a lower proliferation at 6 h, unexpectedly, it gave rise to a significant increase of the proliferation at 24 h. 60 min stretch caused an increase proliferation after 6 and 24 h (Fig. 7). These results suggest that higher strain (15%) for shorter strain time causes a decrease in hMSCs proliferation. Subsequently, a longer strain time (30-60 min) maybe activates protective processes and leads to an increase in cellular proliferation. Koike et al.. reported that high magnitudes of 15% strain increase the proliferation of marrow stromal ST2 cells and low magnitudes of 0.8% or 5% strain lead to osteoblastic differentiation (17). Their results are inconsistent with our results and other findings. This difference of proliferation in response to the mechanical strain maybe is due to the different cell type used in the experiment.

This study also demonstrates that both of low frequency (0.1 Hz) and high frequency (1.5 Hz) are detrimental; only an appropriate frequency (1 Hz) is optimal to hMSCs proliferation (Fig. 4). Our findings are in good agreement with those of Kaspar et al.. (19) and Kearney et al.. (11), who demonstrated that 0.17 Hz strain have the effect of reducing the proliferative capacity of rat mesenchymal stem cells. Our study also demonstrates that long time strained hMSCs showed an obvious reduction of proliferation (Fig. 8). This is in agreement with a recent study by van Griensven et al.. (13), who also demonstrated that application of repetitive long time ($3\times$ 8 h) cyclic longitudinal strain (1 Hz, 5% strain) resulted in lower proliferation rates of hMSCs. Our results together with these findings suggest that short time mechanical strain may be beneficial to the proliferation of hMSCs.

Up to the present, little is known about the mechanisms that link mechanical stretch stimulation and cellular proliferation response. One possibility is that a suitable stretch affects DNA syn-

thesis (20) and/or regulates growth factors (21) in cells and enhances the proliferative capacity. On the other hand, some stretch parameters are unfavorable to cell proliferation and result in the cessation of self-renewal in this condition, and maybe this regime of stretch induces cell damage or apoptosis (22). As for hMSCs, however, it has been proved that apoptosis and cell death rates do not have significant change after strain application (13). Stem cells are a kind of special cells possessing self-renewal and multi-directional differentiation potential, it has been shown that bioconjugated quantum dots are capable of labeling hMSCs during proliferation and differentiation (23). The cessation of self-renewal often marks the onset of lineage commitment in stem cells. Titushkin et al.. examined the biomechanical changes in hMSCs membrane and cytoskeleton in osteogenic differentiation, and also demonstrated the important role of external physical force to regulate stem cell fate (24). Therefore, it is possible that the decreased proliferation of hMSCs indicate these stretch conditions may trigger mechanisms involved in hMSCs differentiation to another cell type, which needs for further detailed studies.

In summary, although little is known about the credible mechanisms that link mechanical stretch and stem cells proliferation, there is consensus that applying mechanical stimulation to these cells produces dramatic effects. Our results proved that mechanical stretch plays an important role in regulating hMSCs growth and proliferation. Meanwhile, the fashion of stretch is extremely important, different stretch loading may induce totally different effect. Mechanical stretch of 1 Hz, 10% strain for 15 min looks more favorable to the proliferation of hMSCs. These results suggest that an appropriate mechanical stretch regime would be a novel approach to promoting proliferation of hMSCs in vitro.

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References

1. Ingber DE (1997) *Annu Rev Physiol* 59: 575-599.
2. Kostenuik PJ, Halloran BP, Morey-Holton ER, Bikle DD (1997) *Am J Physiol* 273: 1133-1139.
3. Xu J, Liu MY, Tanswell AK, Post M (1998) *Am J Physiol* 275: 545-550.
4. Zeichen J, van Griensven M, Bosch U (1999) *Am J Sports Med* 28: 888-892.
5. Kaspar D, Seidl W, Neidlinger-Wilke C, Beck A, Claes L, Ignatius A (2002) *J Biomech* 35: 873-880.
6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Morman MA, Simonetti DW, Craig S, Marshak DR (1999) *Science* 284: 143-147.
7. Jiang M, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, Reyes M, Lenvik T, Lund T, Blackstad M, Du J, Aldrich S, Lisberg A, Low WC, Largaespada DA, Verfaillie CM (2002) *Nature* 418: 41-49.
8. Koc ON, Lazarus HM (2001) *Bone Marrow Transpl* 27: 235-239.
9. Tsutsumi S, Shimazu A, Miyazaki K, Pan H, Koike C, Yoshida E, Takagishi K, Kato Y (2001) *Biochem Biophys Res Commun* 288: 413-419.
10. Simmons CA, Matlis S, Thornton AJ, Chen S, Wang CY, Mooney DJ (2003) *J Biomech* 36: 1087-1096.
11. Kearney EM, Prendergast PJ, Campbell VA (2006) *Bioengineering In Ireland Conference*, Galway: 90.
12. Lee W-CC, Maul TM, Vorp DA, Rubin JP, Marra KG (2007) *Biomechan Model Mechanobiol* 6: 265-273.

13. van Griensven M, Diederichs S, Kasper C (2005) in *Topics in tissue Engineering*, Eds Ashammakhi N, Reis RL (E-book), Vol 2: Chapter 12.

14. Kurpinski K, Chu J, Hashi C, Li S (2006) *Proc Natl Acad Sci U S A* 103: 16095-16100.

15. Park JS, Chu JS, Cheng C, Chen F, Chen D, Li S (2004) *Biotechnol Bioeng* 88: 359-368.

16. Kurpinski K, Park JS, Thakar RG, Li S (2006) *Mol Cell Biomech* 3: 21-34.

17. Koike M, Shimokawa H, Kanno Z, Ohya K, Soma K (2005) *J Bone Miner Metab* 23: 219-225.

18. Taboas JM, Tuan RS (2006) *Mol Cell Biomech* 3: 189-191.

19. Kaspar D, Seidl W, Neidlinger-Wilke C, Ignatius A, Claes L (2000) *J Biomech* 33: 45-51.

20. Galvin DJ, Watson RW, Gillespie JI, Brady H, Fitzpatrick JM (2002) *Am J Physiol Renal Physiol* 283: 1192-1199.

21. Liu M, Skinner SJ, Xu J, Han RN, Tanswell AK, Post M (1992) *Am J Physiol* 263: 376-383.

22. Hammerschmidt S, Kuhn H, Grasenack T, Gessner C, Wirtz H (2004) *Am J Respir Cell Mol Biol* 30: 396-402.

23. Moioli EK, Shah B, Clark PA, Stroscio M, Mao JJ (2006) *Mol Cell Biomech* 3: 153-155.

24. Titushkin IA, Cho M (2006) *Mol Cell Biomech* 3: 151-151.