

Effect of Mechanical Stimulation on Differentiation of Human Mesenchymal Stem Cells to Different Cell Lines: A Systematic Review

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Abstract

Background and Aim: Stem cells due to their great potential can help in establishing tissue engineering as a new treatment modality. Numerous studies have evaluated the effect of various chemical and mechanical stimuli on these cells. In this respect, the role of mechanical loads is undeniable. This systematic review evaluated studies on the effects of mechanical loads on differentiation of mesenchymal stem cells to different cell lineages published in the past 12 years.

Materials and Methods: In this systematic review, PUBMED database was used to search key words namely “human mesenchymal stem cell”, “strain,”, “mechanical loading,” and “differentiation”, in the literature published from 2000 to July 2012. The inclusion criteria were the publication year, language of articles, type of cells and study objectives.

Results: In total, 46 articles were evaluated qualitatively. In most studies, applied mechanical loads led to the anticipated differentiation. Studies showed that the combination of two forces increased differentiation. The amount of applied strain also influenced the type of differentiation.

Conclusion: This review indicated that advances made on the effects of mechanical loads on stem cells can be used for improving tissue engineering treatments.

Key Words: Human Mesenchymal stem cells, Strain, Mechanical loading, Differentiation

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Introduction

Tissue engineering has shown that instead of using artificial materials, we may induce regeneration of tissues [1]. Dental researchers hope to regenerate temporomandibular joint, alveolar bone, periodontal ligament, enamel, dentin and even a whole tooth [1].

Langer et al. defined tissue engineering as an interdisciplinary science using both engineering principles and biology for the development of biological organs that need to be repaired or healed [2]. Tissue engineering is based on 3 components

of mature stem cells, growth factors and extracellular matrix scaffold [3].

Adult stem cells are widely used as a cell source for tissue engineering and regenerative medicine because they can be procured from the autologous sources and can be isolated from bone marrow, dental pulp and adipose tissue and proliferate in the laboratory. Moreover, under in-vitro conditions, these cells are capable of differentiating into different cell lines namely osteoblasts, chondroblasts, myoblasts, adipocytes and ligament cells [4]. Induction of differentiation in these cells at the mo

lecular level requires a specific process with strong programming. Thus, studies have provided various environmental conditions to affect differentiation of human mesenchymal stem cells (such as involvement of different hormones, various load applications, cytokines, growth factors, insulin, steroids, BMPs and etc.) [5].

It has been confirmed that cells and tissues in their innate environment are exposed to mechanical loads such as compressive, tensile and shear forces influencing their development and natural function [6]. Mechanical loads are involved in regulation of tissue homeostasis and in development, function and repair of the main components of the musculoskeletal system namely bones, tendons, ligaments, teeth and cartilages [7]. Numerous studies have shown that mechanical loads stimulate the synthesis of extracellular matrix and may even improve the mechanical properties of the formed tissues [8]. In contrast, it has been demonstrated that absence of load can lead to tissue atrophy and bone loss [9]. Mechanical loads regulate fetal growth and development. Studies on completely paralyzed fetus of birds have detected specific developmental defects in the mandible and large bones indicating the impact of muscle contraction and consequent loads on bone development and stated that loads are necessary for correct morphogenesis of tissues [10]. As the result, tissue engineering currently uses mechanical loads as a tool for the formation of cartilage, ligament, muscle, cardiac muscle and bone under in-vitro conditions [11-16].

Some loads are related to the physical conditions of the understudy cells. For instance, cyclic uniaxial loads are effective for activation of mechano-transduction cascades and induction of differentiation of mesenchymal stem cells into smooth muscle cells. Some loads regulate the expression of matrix molecules without changing the expression of cartilage and bone differentiation markers such as type II collagen and alkaline phosphatase (ALP) [17]. To date, no review study evaluated the effect of various mechanical loads on differentiation of stem cells. This study aims to introduce various mechanical loads used in studies and review the effects of these loads on differentiation of human mesenchymal stem cells.

Materials and Methods

For this review study, we searched PubMed database using "human stem cells", "differentiation", "strain" and "mechanical loading" key words. The inclusion criteria were year of publication from 2000 to June 2012, English and French articles, studies on mature human mesenchymal stem cells and use of mechanical loads to assess cell differentiation. Studies on animal models or evaluating other effects of mechanical loads on cells were excluded.

Results

Our search yielded 848 articles out of which 805 were excluded after evaluation of title and abstracts. The excluded articles were studies on animal stem cells, those outside the selected time period, articles in languages other than English and French and those evaluating the effect of mechanical loads on cell proliferation. The remaining 48 articles were thoroughly studied out of which 2 were excluded due to inappropriate study design and lack of control group. A total of 46 articles were eventually evaluated and categorized based on the type of load used.

Loads used in studies:

The load can be applied from any angulation or direction. Usually, several loads are combined causing complex stresses in a structure. Stresses used in different studies can be divided into three main groups of tensile, compressive and shear. On the other hand, in terms of being constant or variable, loads can be divided into two groups of static and dynamic. In terms of duration of load application, they can be categorized into 3 groups of interrupted, intermittent and continuous. Static load is exerted constantly for a specific time period and does not change during this time; whereas, dynamic loads change. This change can be interrupted. For example, load can be applied to cells once and does not repeat. Dynamic load may be intermittent and applied to cells at specific time intervals. Dynamic load may also be applied continuously at a specific frequency to cells [18].

Tensile load:

Tension is the result of two loads applied along a straight line but at completely opposite directions; or when an object is fixed at one side and is stretched in a direction opposite to the fixed side

resulting in increased length of the specimen [19]. Tensile forces may be uniaxial or equibiaxial. In uniaxial tensile forces, the scaffold is stretched at one direction; while in equibiaxial load application, the scaffold is stretched at all directions [18]. Of 46 articles evaluated qualitatively, 26 used tensile loads for differentiation (Table 1). Of these 26 studies, one study used tensile load along with magnetic force and another one compared cyclic tensile load with continuous load. Only in 2 studies load application did not lead to the desired differentiation and in one study load application prevented the differentiation of cells. Two studies showed that the amount of applied strain affected the type of differentiation of stem cells. One study confirmed that equibiaxial tensile force prevented myogenic differentiation; whereas, uniaxial load stimulated it.

Compressive load:

Compression results from two loads at the same direction along a straight line or when an object is fixed at one side and is compressed at the opposite side towards the fixed side [19]. Of all the evaluated articles, 13 had used compressive forces (Table 2). Two articles used a combination of compressive and shear loads for differentiation. Combination of compressive and shear loads significantly increased the differentiation of chondrogenic markers. In all articles in this group, load application led to the desired differentiation.

Shear load:

Shear results from two parallel loads that are not applied along a straight line [19]. Of 46 studies evaluated, 7 used shear loads (Table 3). In this group, application of mechanical load at different frequencies led to the desired differentiation. In one study, interrupted and continuous shear loads were compared and interrupted load was found to be more effective.

Discussion

Overall, chemical induction is the most common method for differentiation of stem cells. However, it has been understood that tissue engineering in tissues under load application requires mechanical stimulation [61, 62]. Comparison of reviewed articles revealed that mechanical loads applied were mainly dynamic and uniaxial and only two articles used static equibiaxial loads. Although several stu-

dies have shown positive effects of static loads [63], dynamic loads can better simulate in-vivo conditions. Review of these 46 articles showed that the efficacy of dynamic loads for cell differentiation was greater than that of static loads. In the majority of studies, the effect of mechanical loads caused the expected differentiation.

Studies showed that the combination of both loads increased differentiation. Also, the percentage of applied strain can affect the type of differentiation. The drawback of these studies was that the period of application of mechanical strain on cells was widely variable. For instance, for evaluation of osteogenic differentiation of stem cells one study used 3% continuous tensile strain for 2 weeks. In another study, tensile strain for a few hours or dynamic load was applied. Other studies used shear or compressive strains at different magnitudes and time intervals. Thus, comparison of results is not feasible and we cannot definitely state that what type of mechanical load at what frequency and for how long will lead to the desired differentiation.

The most commonly used stem cells in the mentioned 46 studies were bone marrow cells used in 36 articles. Dental pulp stem cells were used in 5, adipocyte stem cells in 4 and umbilical cord and endometrial stem cells were used each in one article. These cells were cultured on various natural and synthetic polymer and ceramic 3D scaffolds, composite scaffolds, hydrogels and demineralized bone. However, in the majority of studies (17 articles) cell carrier was a silicon membrane or a flexible plate that was not 3D and cells were grown on it in a single layer. Considering the composite structure and 3D nature of bone this issue can be considered as a limitation of these studies. Another important issue is that the osteogenic and odontogenic effects of dentin or demineralized bone have been well confirmed [64]. In some other studies a scaffold with osteogenic property was used [50] and the cumulative effect of scaffold and the applied force was considered altogether. Since the first interaction between the cells and scaffold occurs through cell adhesion, surface characteristics of the substrate are the main key in success of tissue engineering [65]. Cell adhesion leads to the attachment of cells to the substrate and provides

Table 1. Studies evaluating the effect of tensile loads on differentiation of stem cells

Authors	Year of publication	Stem cell source	Applied load	Scaffold or carrier	Surface treatment of scaffold or carrier	Type of differentiation	Results
Leong et al, (20)	2012	Bone marrow	Uniaxial, cyclic, tensile	Modified polycaprolactone (PCL)	-	Neurogenic	Cyclic stretching of cells was done at 0.5%, 2% or 3.5% amplitudes and 0.5, 1 or 1.5Hz frequencies for 8 h. Significant upregulation of neurogenic genes' expression occurred at 0.5% strain amplitude and 0.5 Hz frequency.Rac1 but not RhoA was activated at this load.
Tabatabaei et al, (21)	2012	Dental pulp and endometrium	Uniaxial, equibiaxial, tensile	Silicon	Collagen	Osteogenic	Application of 3% static tensile load for 2 weeks discontinued the expression of CD90 marker (expressed in stem cells) in the stretched cells.
Kreja et al, (22)	2012	Bone marrow and fibroblasts	Uniaxial, cyclic-interrupted, tensile	Polylactic acid (PLA)	-	Ligament	Application of mechanical load had no effect on the expression of genes for ligament markers on undifferentiated mesenchymal stem cells but increased the expression of type I and II collagen, fibronectin and tenascinC (ligament matrix markers) in fibroblast cells derived from anterior cruciate ligament
Haghghi-pour et al, (23)	2012	Bone marrow	Uniaxial, cyclic, tensile	Silicone with/without IGF-1 (insulin-like growth factor 1)	Collagen	Myogenic	After application of 10% cyclic uniaxial strain at 1 Hz, different levels of MyoG mRNA and MyoD between groups indicated initiation of myogenic differentiation due to mechanical strain. Comparison of levels of Myf5, MyoD, MyoG and Myf6 mRNA among test groups revealed that a combination of mechanical loads and growth factors leads to the highest expression of myogenic genes
Zhang et al, (24)	2012	Bone marrow	Continuous, cyclic	-	-	Osteogenic	Application of 10% continuous mechanical strain at 1 Hz decreased the proliferation, induced the osteogenic differentiation of cells through activation of Runx2 and increased alkaline phosphatase activity and expression of ALP, collagen type I and osteocalcin mRNA genes. Level of phosphorylation of ERK ½ increased at the onset of loading.
Glossop et al, (25)	2010	-	Tensile, uniaxial + magnetic force	-	-	-	Application of two mechanical loads for an hour during a 24h period had no effect on expression of IL-1B or MAP3K.
Cai et al, (26)	2010	Dental pulp	Uniaxial, cyclic, tensile	Polyethylene	-	Osteogenic, odontogenic	Load application inhibits the gene expression of osteogenic markers and proteins namely BMP2, osteocalcin, and alkaline phosphatase. Also, gene expression of odontogenic markers like DSPP, DSP and BSP was inhibited
Friedl et al, (27)	2009	Bone marrow	Uniaxial, cyclic, tensile	Silastic dishes	Fibronectin	Osteogenic	Application of 3000 mustrainstrain at 1Hz frequency 6 times a day for 72h significantly increased the expression of osteogenic marker genes and ALP activity. Linear correlation analysis showed a correlation between the phenotypic strain response and do-

							nor's body mass index ($r=-0.91$, $P<0.001$)
Huang et al, (28)	2009	Bone marrow	Cyclic, tensile	Flexcell tension system	Type I collagen, vitronectin, fibronectin, laminin	Osteogenic	Application of 3% load at 0.1 Hz activated the focal adhesion kinase (FAK) phosphorylation, upregulated the transcription and phosphorylation of core-binding factor alpha-1, and increased ALP activity and deposition of mineralized matrix. Among the ECM proteins, fibronectin and laminin had the highest supporting effects on osteogenic differentiation induced by stretching compared to type I collagen and vitronectin
Ghazanfari et al, (17)	2009	Bone marrow	Cyclic, tensile	Silicon	Type I collagen	Myogenic	Application of 5% and 10% stretch showed that proliferation of cells increased by increasing the amplitude of load. Strain application regulated smooth muscle alpha-actin, re-oriented actin fibers and caused differentiation of cells to smooth muscle cells without the need for growth factors
Diederichs et al, (4)	2010	Adipose tissue	Strain, cyclic	Collagen silicone	-	Osteogenic	Application of 5% strain at 1Hz for 15min increased the activity of ALP, osteocalcin, osteopontin and BMP 2/4 indicating osteogenic differentiation. Long-term strain application (after the first 15 min, load was repetitively applied for more than 8h) decreased these effects.
Hanson et al, (29)	2009	Adipose tissue	Cyclic, continuous, interrupted, tensile	Type I collagen	Osteogenic medium	Osteogenic	Two cell lines after 14 days of culture in osteogenic medium were subjected to tensile strain with one cell line depositing approximately nine times as much calcium as the other. One group received 10% strain at 1 Hz and the other 10% strain, 1 Hz, 10s rest after each cycle. Similar results were obtained in both groups although cyclic tensile strain had stronger osteogenic effect on cells with high calcium deposition.
Chen et al, (7)	2008	Bone marrow	Cyclic, tensile	Flexible bottomed plates	Type I collagen	Osteogenic ligament	Application of 3%, 6% and 10% strain at 1Hz frequency for 8 and 48h yielded the following results: significant reduction in expression of cell proteins, increased expression of matrix metalloproteinase 3 regardless of the magnitude of load, increased osteoblastic markers (Cbfα1, alkaline phosphatase and osteocalcin) in response to 3% strain but increased ligament markers (type I and type III collagen and tenascin-C) in response to 6% strain. Cbfα1 and ALP increased in the first 8h but decreased afterwards and remained unchanged. Type I and type III collagen mRNA and tenascin-C significantly increased in 10% strain after the first 48h and remained unchanged in the 48h rest.
Han et al, (30)	2008	Dental pulp	Cyclic, tensile	Silicon	Atelocollagen (ethanol + collagen)	Myogenic, osteogenic	In the first 4 days, proliferation in response to 5% and 8% strain was equal. RT-PCR analysis showed that strain application increased the expression of collagen and osteopontin

							and decreased expression of smooth muscle alpha actin. FACS analysis found no change in CD105 expression but CD90 expression decreased in response to 8% strain.
McMahon et al, (31)	2008	Bone marrow	Cyclic, tensile	Collagen+ glycosaminoglycan	-	Chondrogenic	10% strain at 1Hz frequency for 7 days increased the synthesis of glycosaminoglycans
Friedl et al, (27)	2007	Bone marrow	Cyclic, tensile	Silastic	Fibronectin	Osteogenic, chondrogenic	Strain application significantly increased the expression of genes related to primary chondrogenic and osteogenic markers (ALPL, SPPT, SPARC, Runx2, DCN, LUM and Sox9) and increased the activity of ALP ($P<0.051$ standard error of mean: $+38\%\pm12$)
Ward et al, (32)	2007	Bone marrow	Tensile	Type I collagen	-	Osteogenic	Strain application increased mineralized matrix and activated ERK1/2. Addition of MEK inhibitor decreased the activity of ERK leading to decreased expression of osteogenic genes, decreased production of mineralized matrix and blocking of the effect of applied loadon decreasing the expression of non-osteogenic markers.
Sumana-singhe et al, (33)	2006	Bone marrow	Uniaxial, cyclic, tensile	Collagen gel	-	Osteogenic	A significant increase occurred in expression of BMP2 in 8% strain at days 7 and 14 compared to the control group. Increased BMP2 was seen in 12% strain; which was significant at day 14.
Wiesmann et al, (34)	2006	Bone marrow	Uniaxial, cyclic, tensile	Polycarbonate	Osteoconductive medium	Osteogenic	14 days of 2000 mustrain, 200 cycles per day at 1 Hz led to expression of type I collagen and osteocalcin in response to mechanical stimulation. Calcium content increased in both test and control groups but at day 21, calcium content of the test group was more than control group.
Lee et al, (35)	2007	Adipose tissue	Uniaxial, cyclic, tensile	Flexible plate	Type I collagen	Myogenic	Application of 10% strain at 1Hz inhibited the proliferation of cells and led to orientation of cells and F actin cytoskeleton perpendicular to the direction of strain. Application of strain in absence of TGF B1 decreased the expression of primary markers of smooth muscle cells (a SMA and h1-calponin)
Kang et al, (36)	2012	Umbilical cord	Uniaxial, cyclic	Flexible plate	Osteogenic medium	Osteogenic differentiation	0%, 5% and 10% strains were applied. By increasing the strain, CD73, CD90 and CD105 surface antigens decreased. Groups under strain produced more elastin and sulfatyl glycosaminoglycan than the control group. RT-PCR analysis showed that mechanical stimulation increased the expression of mRNA for markers of osteoblastic differentiation.
Kimelman-Bleich et al, (37)	2011	Bone marrow	Dynamic	Hydrogel	-	Osteogenic differentiation	Load application increased cell metabolism by 6.8 times, ALP activity by 12.5 times, BMP-2 secretion by 18.2 times and formation of mineralized tissue by 1.72 times in the hydrogel environment compared to the control

							group.
Haasper et al, (38)	2008	Bone mar-row	Cyclic, longitudinal	Flexible silicon plates	-	Osteogenic differentiation	Application of 2% and 8% strain for 2h three times a day for 3 days and evaluation at days 4 and 7 yielded the following results: Cbfal level was significantly different at various time points. At day 4, differentiation in the group subjected to strain was more than the dexamethasone group.
Simmons et al, (39)	2003	Bone mar-row	Cyclic, equibiaxial	Silicon	Type I collagen	Osteogenic	Strain inhibited the proliferation and increased the mineralized matrix by 2.3 times compared to the control group. Mechanical stimulation activated ERK1/2 and P38 mitogen activated protein kinase but had no effect on the activity or C-Jan N-terminal phosphorylation.
Sen et al, (40)	2008	Bone mar-row	Strain, biaxial, cyclic	Bioflex plate	Collagen	Osteogenic	2% strain for 5 days (6h/day) despite the placement of cells in adipogenic differentiation medium prevented adipocyte differentiation and despite being in this medium cells showed markers of osteoblastic differentiation.
Park et al, (41)	2004	Bone mar-row	Cyclic, uniaxial, equibiaxial	Silicone membrane	Type I collagen/ gelatin	Myogenic	Application of equibiaxialstrain reduced smooth muscle a-actin and smooth muscle 22a after one day and decreased alpha actin in stress fibers. Application of uniaxial load increased the expression of smooth muscle a-actin and smooth muscle 22a after one day and caused transient increase of type I collagen. Thus, uniaxial load was more effective for myogenic differentiation.

I(strain); 2D (Two-dimensional); 3D (Three-dimensional); COL(Collagen); GAG (Glycosaminoglycan); GDF-5 (Growth and differentiation factor 5); PCL (Poly(Caprolactone)); PLGA (poly(L -Lactide/glycolide) acid); VN (Vitronectin); FN (Fibronectin); LN (Laminin); PLLA (Poly(L -Lactic) acid); DEX (Dexamethasone); ALP (Alkaline phosphatase); OPN (Osteopontin); BMP (Bone morphogenic protein); LOX (Lysyl oxidase); BSP (Bone sialoprotein); OCN (Osteocalcin); RUNX2 (Runt-related transcription factor 2); PPAR g (Peroxisome proliferator-activated receptor-gamma).

Table 2. Studies evaluating the effect of tensile loads on differentiation of stem cells

Authors	Year of publication	Stem cell source	Applied load	Scaffold	Scaffold surface treatment	Type of differentiation	Results
Michalopoulos et al, (42)	2012	Bone marrow	Cyclic, compressive	Collagen, alginate	-	Osteogenic	Osteogenic differentiation was observed in 10% strain (increased Cbfa 1 at days 7 and 21) and chondrogenic differentiation occurred in 15% strain (increased expression of Cbfa 1, Sox9 and Aggrecan)
Bian et al, (43)	2012	Bone marrow	Dynamic, compressive	Hyaluronic acid, hydrogel	-	Chondrogenic	Load application increased the glycosaminoglycan and collagen of the medium. This increase at high cellular density medium (60 million cells/ml) was greater than in the low density medium (20 million cells/ml). Load application uniformed spatial distribution of cells in chondrogenic matrix in both densities and significantly decreased hypertrophic markers and degree of calcification.
Schatti et al, (44)	2011	Bone marrow	Dynamic, shear/ compressive	Fibrin, polyurethane, composite	-	Chondrogenic	Shear and compressive loads were capable of inducing chondrogenic induction independently but when combined significantly increased the expression of chondrogenic

							genes. Type I collagen and glycosaminoglycan were present in the two groups with only one type of applied load (shear or compressive)
Sittichoke-chaiwut et al, (45)	2010	Bone marrow	Dynamic, compressive	Polyurethane with/without dexamethasone	-	Osteogenic	Collagen content was significantly higher in groups subjected to loads compared to the control group ($P<0.01$). No significant difference was found in ALP activity, collagen content and calcium production between groups without load and dexamethasone supplement and the group receiving load without dexamethasone.
Li et al, (46)	2010	Bone marrow	Cyclic, compressive	Fibrin-biodegradable polyurethane along with TGF-B1 at 2 concentrations of 0.1 ng/ml and 10ng/ml	-	Chondrogenic	Based on the concentration of TGF-B1 in the medium, mechanical load stimulated hMSCs chondrogenic differentiation. Lower concentration of TGF-B1 had greater effect on gene expression. In absence of TGF-B1, mechanical load stimulated gene transcription and synthesis of TGF-B 1&3 protein.
Kisiday et al, (47)	2009	Bone marrow	Dynamic, compressive	Agarose hydrogel	-	Chondrogenic	Application of load for 12h/day in absence of TGF-B1 significantly increased proteoglycan synthesis compared to the control group without TGF-B. Level of H-proline and S-sulfate in the test group was 2% and 14% higher, respectively than the control group with TGF-B but level of glycosaminoglycan was 67% higher.
Pelaez et al, (48)	2009	Bone marrow	Cyclic, compressive	Fibrin gel at 3 concentrations of 8, 40 and 60mg/ml	-	Chondrogenic	10% load was applied at 0.1, 0.5, and 1.0 Hz frequency and viability of cells was maintained at over 0.5 Hz frequency and 40 mg/ml fibrin concentration.
Campbell et al, (49)	2006	Bone marrow	Dynamic, compressive	Alginate+ 10ng/ml TGFB	-	Chondrogenic	Application of 15% interrupted load at 1Hz frequency in presence of 10ng/ml TGFB for 8 days increased the expression of chondrogenic markers (type I and II collagen, Sox9 and aggrecan) compared to the control group without TGFB and in absence of load.
Mauney et al, (50)	2004	Bone marrow	Compressive	Partially demineralized bone	-	Osteogenic	Application of 3% mechanical load at 5mm/min and 250 cycles/day for 16 days along with 10nM concentration of dexamethasone caused osteogenic differentiation with significant increase in ALP activity and mineralized matrix compared to the control group
Yu et al, (51)	2009	Dental pulp	Compressive, hydrostatic, dynamic	Glass lamella	Poly-L-lysine	Odontogenic	Application of different hydrostatic pressures at 0.5 Hz frequency for 1, 2, 3 or 4h decreased the number of cells and increased differentiation.
Angele et al, (52)	2004	Bone marrow	Cyclic compression	Composite, hyaluronan, gelatin	-	Chondrogenic	Application of load for 4h/day for 7 days increased the content of collagen and proteoglycan.
Wagner et al, (53)	2008	Bone marrow	Cyclic hydrostatic compression	Collagen sponge	-	Chondrogenic, osteogenic	Application of one MPa load at 1Hz frequency (4h/day) for 10 days increased chondrogenic markers but no change occurred in expression of RUNX2.
Ogawa (54)	2009	Bone marrow	Cyclic hydrostatic compression	Collagen scaffold	-	Chondrogenic	Application of 0.5 MPa load at 0.5 Hz frequency for 7 days increased chondrogenic markers after 4 weeks.
Finger et al, (55)	2007	Bone marrow	Cyclic hydrostatic compression	Agarose gel	-	Chondrogenic	Application of 7.5 MPa load at 1Hz frequency (4h/day) for 14 days increased chondrogenic markers after 2 weeks.

Table 3. Studies using shear loads for differentiation of stem cells

Authors	Year of publication	Stem cell source	Applied load	Scaffold or carrier	Surface treatment of scaffold or carrier	Type of differentiation	Results
Liu et al, (18)	2012	Bone marrow	Fluid shear stress, interrupted/continuous	PLGA and fibrin gel	-	Osteogenic	Application of interrupted load caused osteogenic differentiation of cells. Expression of osteogenic genes and ALP activity were higher in interrupted compared to continuous load application. Application of interrupted load increased ERK ½ and FAK activity.
Schatti et al, (44)	2011	Bone marrow	Dynamic, shear/compressive	Fibrin/ Polyurethane composite	-	Chondrogenic	Shear and compressive load alone were capable of inducing chondrogenic differentiation but combination of loads significantly increased the expression of chondrogenic genes. Histological analysis revealed that type I collagen and glycosaminoglycan were present in the two groups receiving only one type of load (shear or compressive).
Yourek et al, (56)	2010	Bone marrow	Shear	-	With and without osteogenic medium	Osteogenic	Immediately after load application ALP activity increased in the osteogenic medium under load application. At days 4 and 8, the mRNA expression related to BMP2 and osteopontin in the group receiving load was higher than the group without load.
Zhang et al, (57)	2009	Bone marrow	Shear	PET membrane	-	Myogenic	By application of shear load with 90dyn/cm ² intensity, cells expressed smooth muscle alpha actin after 24h and smooth muscle alpha actin and calponin after 48 and 72h. Smooth muscle myosin heavy chain was more prominent at 24 and less prominent at 72h.
Knippenber et al, (58)	2005	Adipose tissue	Pulsating fluid flow shear stress	-	-	Osteogenic	A bone cell-like response was shown by adipose tissue-derived mesenchymal stem cells to fluid shear stress after induction of osteogenic differentiation by 1,25-dihydroxyvitamin D3. Mechanical loading increased the production of nitric oxide and upregulated cyclooxygenase-2, but not cyclooxygenase-1.
Henrionnet et al, (59)	2012	Bone marrow	Calibrated agitation	3D-alginate	-	Chondrogenic	After 28 days of culture, mechanical load in the medium without TGF-B resulted in formation of types I and II collagen and increased the expression of chondrogenic markers like COMB and Sox9.
Li et al, (60)	2010	Bone marrow	Combination of cyclic compression and surface shear stress	Fibrin-polyurethane composite	-	Chondrogenic	After 7 days of loading (1h/day) chondrogenesis in the group receiving mechanical loads was significantly higher than in the control group.

the signals guiding cell differentiation [66]. Some authors have shown that covering the substrate surface with extracellular matrix molecules namely collagen, fibronectin or laminin improves effective seating and expansion of cells on the substrate [67]. The most commonly used material for surface coverage of the substrate was collagen used in 10 articles.

Lysine, gelatin, fibronectin, atelocollagen, laminin, vitronectin and fibronectin have also been used sometimes along with collagen. In 30 articles, substrate was not coated. One study evaluated the effect of several surface treatments on osteogenic differentiation and reported fibronectin and laminin to be more effective than others.

Of the 46 articles evaluated, 2 studies used a combination of loads and growth factors and showed that combination of loads with growth factors like IGF1 and TGF β increased the expression of markers.

Osteogenic, chondrogenic, adipogenic, myogenic, tenogenic and neurogenic differentiation of mesenchymal stem cells under different in-vitro conditions were evaluated. Osteogenic differentiation was the most common type with 25 articles. Since bone defects are very common causing a significant problem in treatment [3], studies have mostly focused on osteoblastic differentiation followed by chondrogenic differentiation in 14 cases. Odontogenic, tenogenic, myogenic, neurogenic and adipogenic differentiations were reported in 1-2 articles each.

Use of mechanical loads in tissue engineering requires targeted research to assess and compare the effects of dynamic tensile, shear and compressive loads with similar frequency and strain on one type of stem cells (cultured on a specific scaffold with similar surface treatment). By doing so, we can find out what force at what frequency and percentage of strain will cause the best regeneration of tissue. Selection of positive (cells in the desired differentiation medium) and negative (cells in a conventional medium) control groups in conditions completely similar to the treatment group (in conventional medium subjected to load) without the load application is very important. In most studies evaluated, only one control group was present. As long as using scaffolds, cells and protocols of different loads in studies, we cannot determine the optimal type of load for each differentiation [68]. This study had some limitations:

1. Only studies on human stem cells were evaluated
2. This review study evaluated articles by June 2012 and since then new articles have been added to the database.
3. Only PubMed database was searched and future studies are recommended to use several databases.

Conclusion

Based on the results of studies evaluated, mechanical loads-especially tensile- play an important role in differentiation of stem cells to different cell lines. Several studies have evaluated application of mechanical load to mesenchymal stem cells with

various degrees of success. However, due to the different protocols used, meta-analysis of these studies is usually problematic. Studies have differences in terms of human or animal stem cells, tissue origin of stem cells, type of scaffold used for load application, type of applied load and protocol of load application. Moreover, in some cases, mechanical loads have been used with growth factors or differentiation media. These factors are responsible for not obtaining comparable results. Simulating clinical conditions as much as possible can help making a decision regarding the use of mechanical loads for tissue engineering.

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