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Finite Element Modeling of 3D Human Mesenchymal Stem Cell-Seeded Collagen Matrices Exposed to Tensile Strain

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Abstract

The use of human mesenchymal stem cells (hMSCs) in tissue engineering is attractive due to their ability to extensively self-replicate and differentiate into a multitude of cell lineages. It has been experimentally established that hMSCs are influenced by chemical and mechanical signals. However, the combined chemical and mechanical *in vitro* culture conditions that lead to functional tissue require greater understanding. In this study, finite element models were created to evaluate the local loading conditions on bone marrow derived hMSCs seeded in three dimensional collagen matrices exposed to cyclic tensile strain. Mechanical property and geometry data used in the models were obtained experimentally from a previous study in our laboratory and from mechanical testing. Eight finite element models were created to simulate three-dimensional hMSC-seeded collagen matrices exposed to different levels of cyclic tensile strain (10% and 12%), culture media (complete growth and osteogenic differentiating), and durations of culture (7 and 14 days). Through finite element analysis, it was determined that globally applied uniaxial tensile strains of 10% and 12% resulted in local strains up to 18.3% and 21.8%, respectively. Model results were also compared to experimental studies in an attempt to explain observed differences between hMSC response to 10% and 12% cyclic tensile strain.

Keywords

Uniaxial Tensile Strain; hMSCs; Collagen; Bioreactor; Finite Element Modeling

Introduction

Functional bone tissue engineering for large critical defects remains an elusive goal in restorative medicine. Key to *in vitro* generation of nascent tissue with appropriate material properties for withstanding *in vivo* loading are the combined mechanical and chemical stimuli used to culture cells. Previous studies have shown that mechanical loading induces differentiation of human mesenchymal stem cells (hMSCs) and mesenchymal tissue into tissues such as bone, fibrous tissue, cartilage, and smooth muscle cells (Ignatius 2004, Altman 2002, Campbell 2006, Carter 1998, Kelly 2005, Park 2004). We have previously shown that cyclic tensile strain, even in the absence of osteogenic differentiating medium (i.e. hMSCs maintained in growth medium alone), promotes osteogenic differentiation of

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bone marrow-derived hMSCs when seeded in 3D type I collagen matrices (Sumanasinghe 2006). The cyclic tensile strains that were applied to hMSCs *in vitro* in that study (10% and 12%), however, were much greater than the strains bone typically experiences under physiological conditions (0.09% to 0.21%) (Miki 1995, Hillam 1996, Burr 1996).

This study focused on engineered three dimensional hMSC-seeded collagen constructs cultured in TissueTrain (Flexcell Int. Hillsborough, NC) culture plates. The TissueTrain system has been used in previous studies to impart mechanical stimuli to a variety of cells in three-dimensional culture (Garvin 2003, Qi 2006), and to investigate the effects of cyclic tensile strain on osteogenic differentiation of hMSCs. In brief, the system works by drawing a vacuum against a rubber membrane, which in turn pulls two anchors in tension. Between the anchors, a linear cell-seeded collagen matrix is created so that cyclic uniaxial tension may be applied (Fig. 1).

A previous study in our laboratory examined bone morphogenetic protein-2 (BMP-2) expression in 10% and 12% cyclically strained 3D hMSC-seeded collagen matrices at 7 and 14 days as compared to unstrained controls using the Tissue Train system. The results of that study showed that matrices exposed to cyclic tensile strain increased BMP-2 expression in the absence of osteogenic growth supplements; however differences existed between apparently similar 10% and 12% strain levels with 10% cyclic tensile strain leading to a greater increase in BMP-2 expression than 12% cyclic tensile strain (Sumanasinghe 2006 A).

As a biomaterial and engineered tissue scaffold, collagen has been modeled in numerous finite element analysis (FEA) studies. Two-dimensional FEA has been used to quantify forces exerted by corneal fibroblasts on a surrounding collagen matrix (Roy 1999). FEA has also been used to predict differentiation and repair of cartilage surrounding injuries (Cullinane 2003). Two-dimensional models have been created to simulate extension of prealigned collagen gels. (Chandran 2007). Collagen fiber alignment has been predicted during compaction in cell-seeded tissue equivalents (Ohsumi 2008). To our knowledge, no previous study has examined the local stress and strains within cell-seeded collagen gel matrices cultured under cyclic tensile strain using three dimensional FEA.

The goal of the present study was to use finite element analysis to simulate the hMSC-seeded collagen matrices under the mechanical loading conditions in which they were grown in culture. We hypothesized that local strains experienced by cells within the matrices would differ from the global strains applied by the bioreactor, and these local strains would change with time in culture. To investigate this hypothesis, we created finite element models of the three-dimensional hMSC-seeded collagen gels in an attempt to answer the following questions: 1. What are the local stress and strain fields experienced by cells within the three-dimensional collagen gels? 2. How do the local stress and strain fields vary at different time points in culture? 3. What may be the difference between 10% and 12% applied global cyclic tensile strain that causes a significant difference in expression of BMP-2?

Methods

Mechanical Testing

Eleven nonwoven Nylon mesh specimens (Cerex Advanced Fabrics Cantonment, FL) were tested in tension to determine the elastic modulus of the Cerex anchor of the TissueTrain (Flexcell Int., Hillsborough, NC) device (Fig. 1B). The mesh material consists of spunbond Nylon 0.09 mm thick with a basis weight of 0.60 ounce per square yard which was stamp cut to dog bone shaped test specimens. The cross sectional area of each sample was determined by averaging measured width at three locations and multiplying it by the

manufacturer-provided thickness. Previously unstrained specimens were soaked in phosphate buffered saline (PBS) for one hour then tested in tension using an EnduraTEC ELF 3200 (Bose, Minnetonka, MN) under ramp displacement control at 0.05 mm/sec (0.2% strain/sec) to 150% of maximum strain experienced by constructs when strained within the TissueTrain bioreactor. A 500 gram load cell was used to record the resulting forces. Elastic modulus was calculated by averaging the stiffness data from the linear portions of the stress-strain histories for each specimen.

Linear elastic moduli data of unstrained, 10%, and 12% axially strained hMSC-seeded collagen matrices cultured in either complete growth or osteogenic medium were previously determined experimentally after 7 or 14 days through similar mechanical testing (Sumanasinghe 2007). In brief, Young's moduli and failure stresses of the hMSC-seeded collagen constructs were determined one day after completing culture strain regimen by applying a 0.5% per second constant strain rate to a 10 mm central section of each matrix until failure using a 500 gram load cell with a force resolution of 0.03 g.

Actin Staining

At 14 days in culture, actin morphology and alignment was visualized by staining the hMSC-seeded collagen matrices with Alexa 594 phalloidin (Molecular Probes, Eugene, OR). Matrices were rinsed twice in PBS and fixed for 30 minutes with 10% formalin. After fixing, matrices were rinsed twice in PBS then incubated in buffer consisting of 0.2% Triton X-100 with 0.5% bovine serum albumin in PBS for 30 minutes. Matrices were then stained with 1 μ g/ml phalloidin in buffer for 20 minutes while protected from light. Following staining, matrices were rinsed twice with PBS and imaged at 10X magnification using fluorescent microscopy (Leica Microsystems Inc., Bannockburn, IL).

Finite Element Modeling

Previous studies have shown that mesenchymal tissue and type I collagen gel scaffolds initially have a linear elastic response under physiological loading frequencies (Loba 2004, Roeder 2002). Therefore, the matrices were modeled as isotropic, linear elastic materials based on these studies and previous mechanical testing results from our laboratory (Sumanasinghe 2007).

In the present study, finite element models were designed and solved using ABAQUS CAE version 6.4-3 (ABAQUS, INC. Providence, RI). A total of eight axisymmetric models, each representing one-half of the hMSC-seeded collagen gel at different time points, strain levels, and in either osteogenic or growth medium (Fig. 2), were constructed using geometry and material property data obtained experimentally (Table 1) and a Poisson's ratio of 0.49. Other studies have modeled type I collagen and hMSCs with Poisson's ratios near 0.49 or 0.50 (Simha 1999, Zhang 2005, Darling 2008). Models in the present study simulated hMSC-seeded collagen gels cultured in either osteogenic differentiation or complete growth medium, and exposed to 10% or 12% cyclic tensile axial strain, after 7 or 14 days time in culture. The shaft of each matrix was approximated with an elliptical cross section using width and thickness data taken in a previous study (Table 1) (Sumanasinghe 2008). In that study, the width and thickness data were taken by imaging the constructs in two planes and using Scanflex image analysis software (Flexcell, Hillsborough, NC), and mechanical property data were determined using the same technique described above for nonwoven Nylon anchors. Average widths of the elliptical sections ranged from 0.386 mm to 0.850 mm and average thicknesses ranged from 0.221 mm to 0.442 mm. The anchor-attachment zone of the hMSC-seeded collagen gel was modeled to follow the anchor geometry with a small surrounding thickness (Fig. 3). The shaft was joined to the anchor attachment zone by lofting. One anchor was created for each model using geometry measurements taken from a

TissueTrain plate (Flexcell Int.). An embedding constraint was used to attach the anchor into the matrix. The model was loaded by constraining axial motion at the midsection and applying a 10% or 12% tensile deformation to the end of the anchor.

Each completed model consisted of approximately 15,000 hexahedral elements. A linear static solution was conducted and stress and strain fields were recorded for each model. The reaction forces on the anchors necessary to achieve each strain were also recorded.

Results

Mechanical testing

Eleven nonwoven nylon mesh specimens had an average stiffness of 16.3 MPa with a standard deviation of 3.6 MPa. All specimens exhibited linear stress-strain relationships to the applied displacements (Fig. 4). The data from one specimen were not included due to inadequate nylon fiber mesh density, which led to immediate rupture.

Cytoskeleton Staining

Representative images taken near the center of the hMSC-seeded collagen gels appeared to show increased number of actin fibers and more consistent actin organization in matrices globally strained at 10% (Fig. 5A, 5C) compared to 12% (Fig. 5B, 5D). Fiber alignment in the applied strain direction (horizontal) also appeared to be more consistent in matrices globally strained at 10%.

Finite element modeling

Axial strain—The strain magnitudes were highest within the central contracted portion of the matrices, gradually decreasing with the increase in cross section to the anchor, and lowest in the portion overlapping the anchors (Fig. 6). All models indicated local strains higher than the applied global strains within the central portions of the matrices. Depending on the specimen type being modeled, the increase in local strain over global strain varied from 6.4% to 9.8%. The greatest increase of 9.8% over applied strain was found in the 14 day matrix strained globally at 12% (local strain = 21.8%) while cultured in complete growth media (Fig. 6A). The lowest increase of 6.4% over applied strain was found in the 7 day matrix globally strained at 10% (local strain = 16.4%) cultured in osteogenic differentiation media (Fig 6B).

Axial Stress—Stress fields followed a similar distribution to the strain fields described above (Fig. 7). The stress experienced by the central portion of the matrices varied from 83.2 KPa in the model representing a 7 day matrix globally strained at 10% cultured in complete growth medium (Fig. 7A) to 430 KPa as indicated in the model of a 14 day matrix globally strained at 10% cultured in complete growth medium (Fig. 7A).

Axial displacement—Axial displacements were linear through the central contracted portions of the matrices (Fig. 8). As the model stiffness increased through the greater cross section near the anchor, the change in displacement decreased. The portions of each model where the collagen gel overlapped the anchors exhibited much stiffer behavior than the rest of the model.

Effect of culture duration—Axial strain increased only marginally within the central section of all models from 7-day to 14-day hMSC-seeded collagen matrices (Table 2). Axial stress within the central contracted portion increased in models of matrices cultured in complete growth medium from day 7 to day 14 (Table 2). The greatest increase was within the 10% globally strained matrices cultured in complete growth media. The lowest increase

in stress was indicated by the models of the 12% globally strained matrices cultured in osteogenic differentiation media.

Discussion

In the present study, we created 3D finite element models to determine the local stress and strain fields experienced by hMSCs cultured in 3D collagen matrices and exposed to global cyclic tensile strains of 10% and 12%. We have previously shown that these apparently similar magnitudes of cyclic tensile strain result in distinctly different responses by hMSCs in 3D collagen gels with respect to BMP-2 mRNA expression, matrix contraction, nuclear morphology and orientation, and tensile properties of hMSC-seeded collagen gels (Sumanasinghe 2006, 2007 2008.). In the present study, the local strains varied with axial location in all models. The greater stiffness of the nylon nonwoven anchors caused stress shielding of the surrounding portion of the more compliant hMSC-seeded collagen matrix, resulting in the portion between the anchor tips experiencing an increased strain.

The 14 day models yielded higher axial stresses than 7 day models representing equivalent global strains and medium types, although the change was less for specimens cultured in osteogenic differentiating media as compared to complete growth media. The increases in stress from 7 day models to 14 day models were due in large part to decreases in cross sectional area. The 14 day specimens cultured in complete growth media had smaller cross sectional areas than the 14 day specimens cultured in osteogenic differentiating media. The smaller matrix area contributed to the increased stress. The actin fibrillar network appeared to be more diffuse and have more densely concentrated aggregates in the 12% global strain matrices (Fig. 5B, 5D) compared to the 10% global strain matrices (Fig. 5A, 5C), which appear to have greater fibrillar density and alignment in the global strain direction. This difference may be due to actin cytoskeleton damage that appears to occur in the 12% global strain matrices (Fig. 5B, 5D). It is possible that local strains resulting from the 12% global strain may be above a threshold that the hMSCs can tolerate without damage, in light of the encouraging experimental results from the 10% global applied strain matrices, which exhibited a significantly higher fold change in expression of BMP-2 over unstrained controls (Sumanasinghe 2006). A connection between mechanical stretch and rat MSC F-actin filament arrangement has been previously reported (Qi 2005). Work by other investigators suggests that actin filament bundles affect overall cell mechanical properties (Deguchi 2006). Such a strain threshold would need to be confirmed with experimental analyses quantitating F-actin content and morphology.

Another large component affecting the local stress and strain fields was the effect the hMSCs had on cross section of the collagen matrix as a result of matrix contraction by the cells. As cells exert traction forces on their environment, the substrate or 3D matrix surrounding them is contracted (Harris 1987, Tranquillo 1992, Choquet 1997, Sumanasinghe 2008). With longer times in culture, the hMSCs continued to contract the collagen matrix, causing the volume, thickness, and width to decrease. In three of the eight models, the predicted axial stress experienced by matrices in culture was greater than the failure stress levels reported previously during mechanical testing (specifically 430, 157, and 365 kPa model stress as compared to 171, 124, and 195 kPa failure stress for cell seeded collagen gels cultured in complete growth medium subjected to 10% strain for 7 days, and 12% axial for 7 and 14 days respectively) (Sumanasinghe 2007). This was likely due to variations in methods by which the failure stress was calculated in the previous experimental studies and this study. The previous experimental studies measured cross sectional area of the hMSC-seeded collagen matrices in an unstrained condition. When mechanical testing was conducted, the engineering failure stress was calculated based on the area of the initial unstrained cross section. The finite element models created in this study decreased in cross

section as strain was applied due to Poisson effects, and the axial stress reported was based on the changing geometry.

As illustrated by the difference between 7 day and 14 day time points (Table 2), the global applied strains of 10% and 12% did not create consistent stresses within hMSC-seeded collagen matrices throughout the culture duration. It is possible that the hMSCs deposited collagen or calcium within the matrix that might have caused the stiffness of the matrix to increase. Some studies have also shown mechanical strain increases stiffness of cell-seeded collagen gels (Shearn 2007, Sumanasinghe 2007) while another found no significant increase (Nirmalanandhan 2007). Mineralization was not verified in this study. In a bioreactor system without feedback such as the one investigated here, this straining method could lead to a decrease in the local strain experienced by cells. Another study found effective increases of 10% to 20% over prescribed strains on two-dimensional, biaxial BioFlex (Flexcell, Int) culture plates which use similar rubber membrane substrates (Thompson 2007).

A limitation of this study is that the mechanical property and geometry data used in the models were taken from bone marrow-derived hMSCs from a single donor. It is possible that other cell lines may exhibit different results, as the amount of mineral or collagen deposited by hMSCs within the matrices may vary. It should also be noted that this study modeled hMSC-seeded collagen matrices in the linear elastic range of displacement, due to the applied cyclic strain frequency of 1 Hz. Another study used much longer loading durations and found viscoelastic properties of both cell-seeded and acellular type I collagen gels matrices when held in tension for one hour (Karamichos 2006). This difference in material response of collagen gels is expected, due to the significant difference between loading frequencies used in the two studies.

In this study, we simulated 3D hMSC-seeded collagen gel matrices through 3D finite element modeling. We applied geometry and material property data previously determined experimentally to create models of matrices at 7 and 14 days cultured in complete growth medium or osteogenic medium and subjected to 10% or 12% global tensile strain. These models were used to determine the local stresses and strains within the matrices at 7 and 14 days. We also suggest that damage to the actin fibrillar network may occur at strains above 10% which may inhibit expression hMSC of BMP-2 mRNA.

This study emphasizes the importance of understanding the relationship between global strains applied by a bioreactor and local strains experienced by cells in culture. Transitioning from one bioreactor system to another or between different cell types requires an understanding of this strain relationship in order to achieve desired results. As the use of mechanical stimulation becomes increasingly common to a multitude of cell types in culture, a more complete understanding of the effective loading applied to cells in culture should enhance experimental results.

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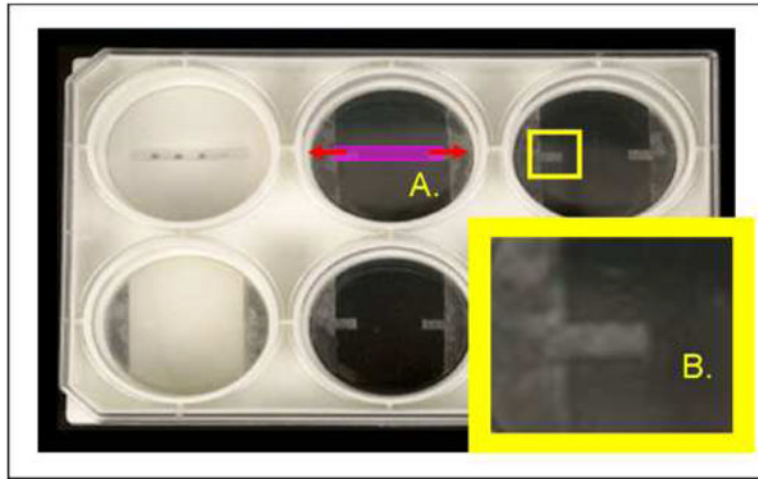


Figure 1. TissueTrain culture plate: A. Illustration of MSC-seeded collagen gel set between anchors and direction of uniaxial tensile strain. B. Detail of nylon nonwoven anchor.

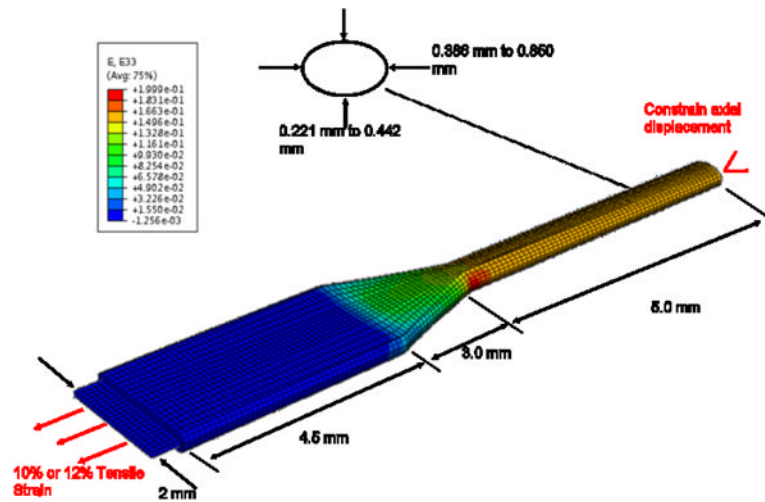


Figure 2. Representative example of matrix geometry with embedded anchor and boundary conditions. Detail shows shaft cross section. Shading illustrates strain resulting from matrix loading. Model shown is globally strained at 10% at 7 days in culture with osteogenic differentiating medium.

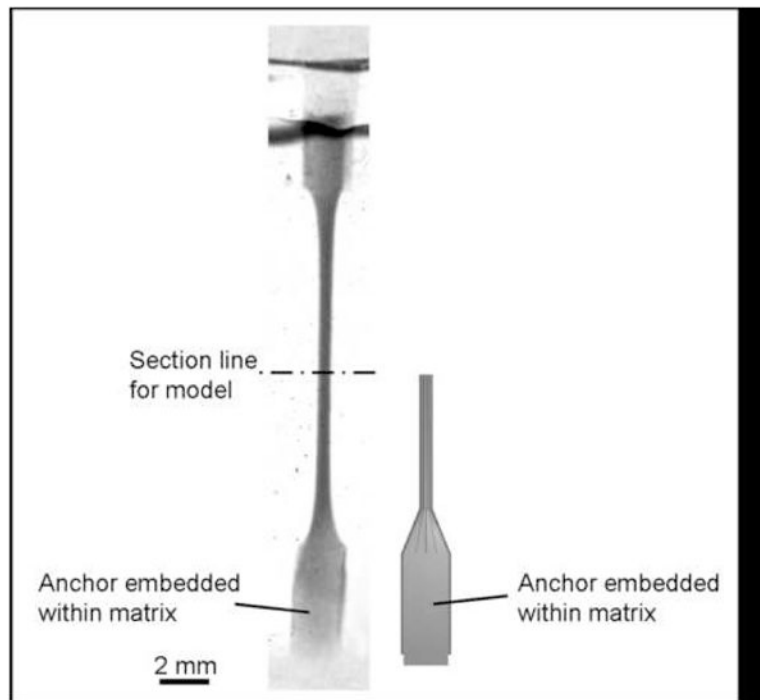


Figure 3. Comparison of representative hMSC-seeded collagen matrix on left with axisymmetric finite element model on right.

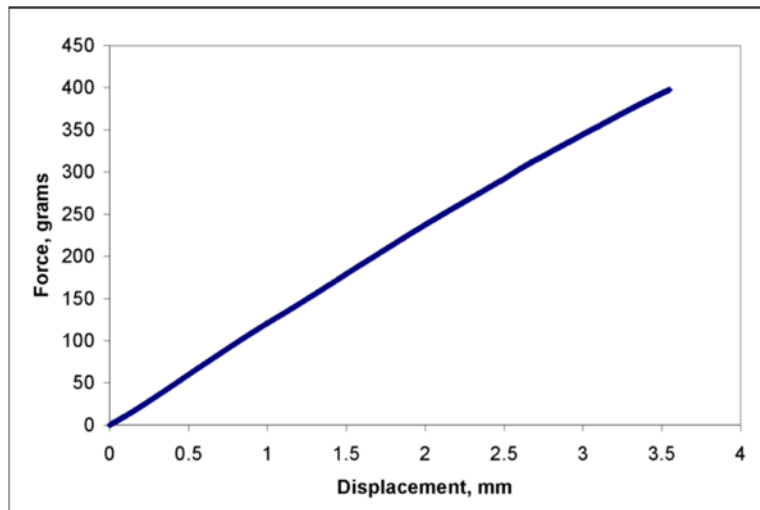


Figure 4. Representative example of linear response to loading during mechanical testing of nylon nonwoven mesh anchors.

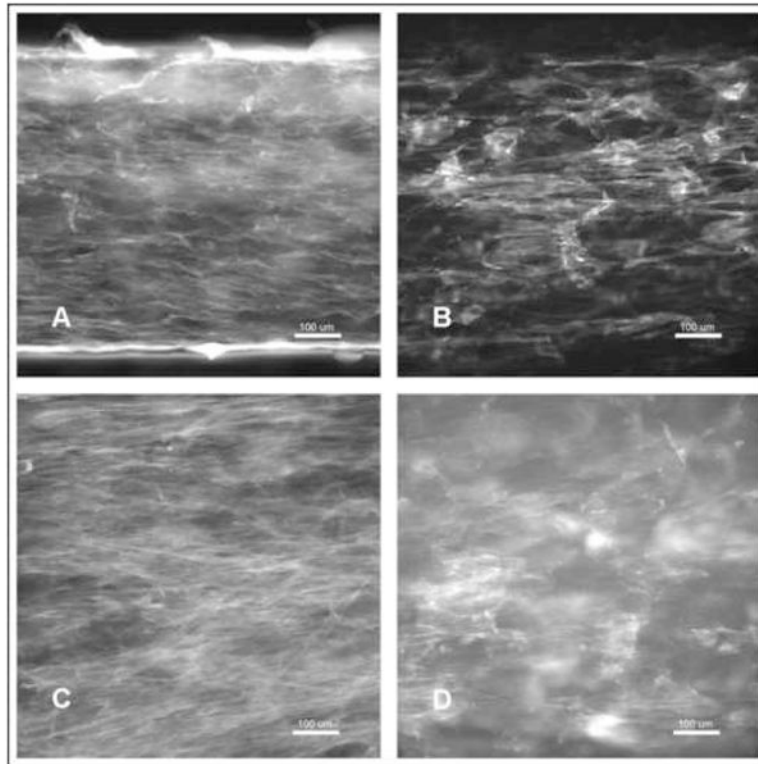


Figure 5. Actin filaments within matrices stained with Alexa 594 phalloidin. Matrices globally strained at 10% (A and C) and 12% (B and D). Matrices cultured in complete growth medium (A and B) and osteogenic differentiation medium (C and D).

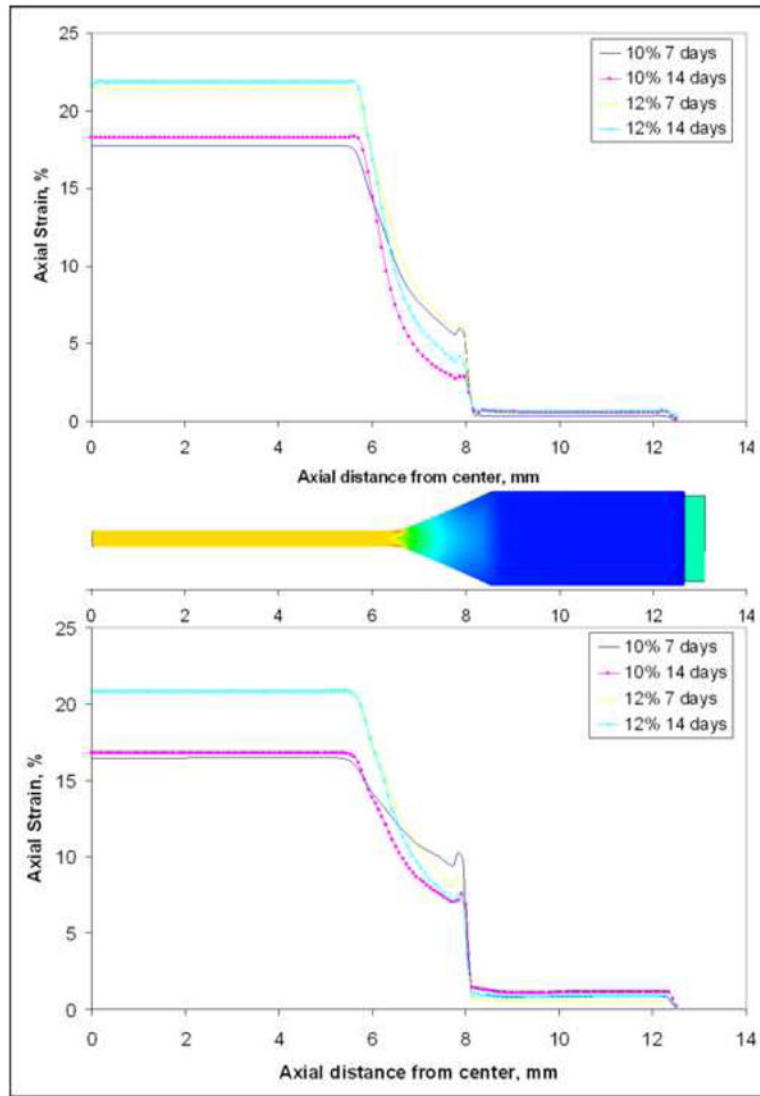


Figure 6. Axial strain as a function of distance from center of matrices cultured in A) complete growth medium and B) osteogenic differentiating medium.

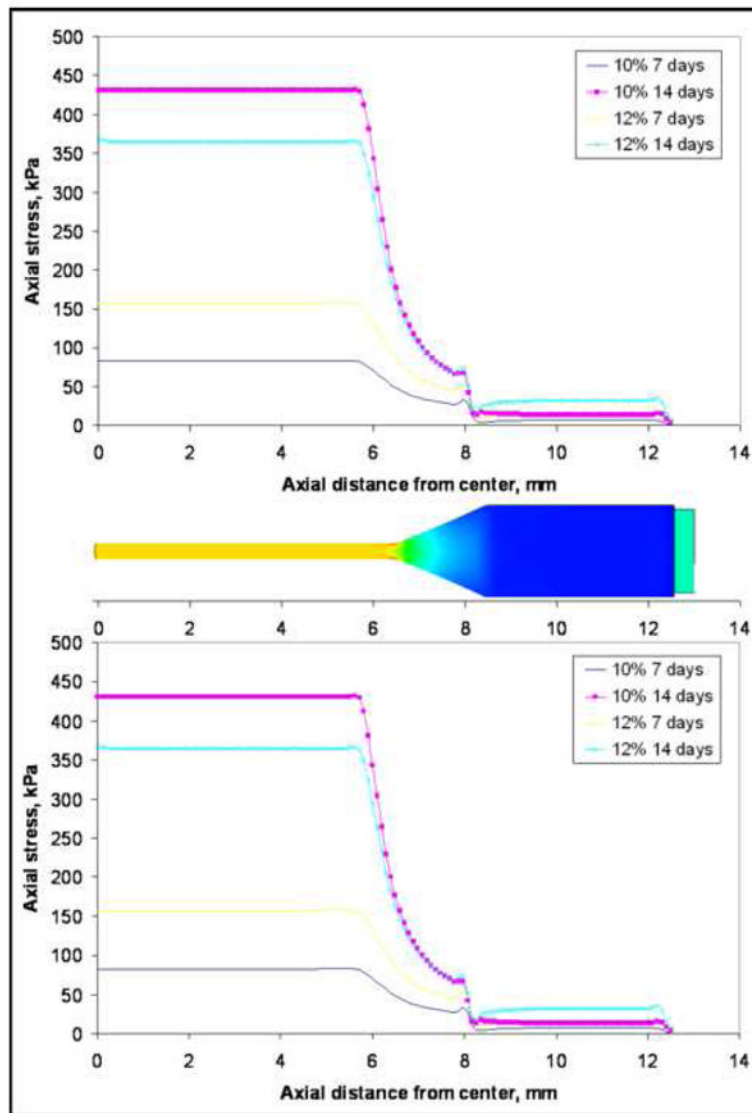


Figure 7. Axial stress as a function of distance from center of matrices cultured in A) complete growth medium and B) osteogenic differentiating medium.

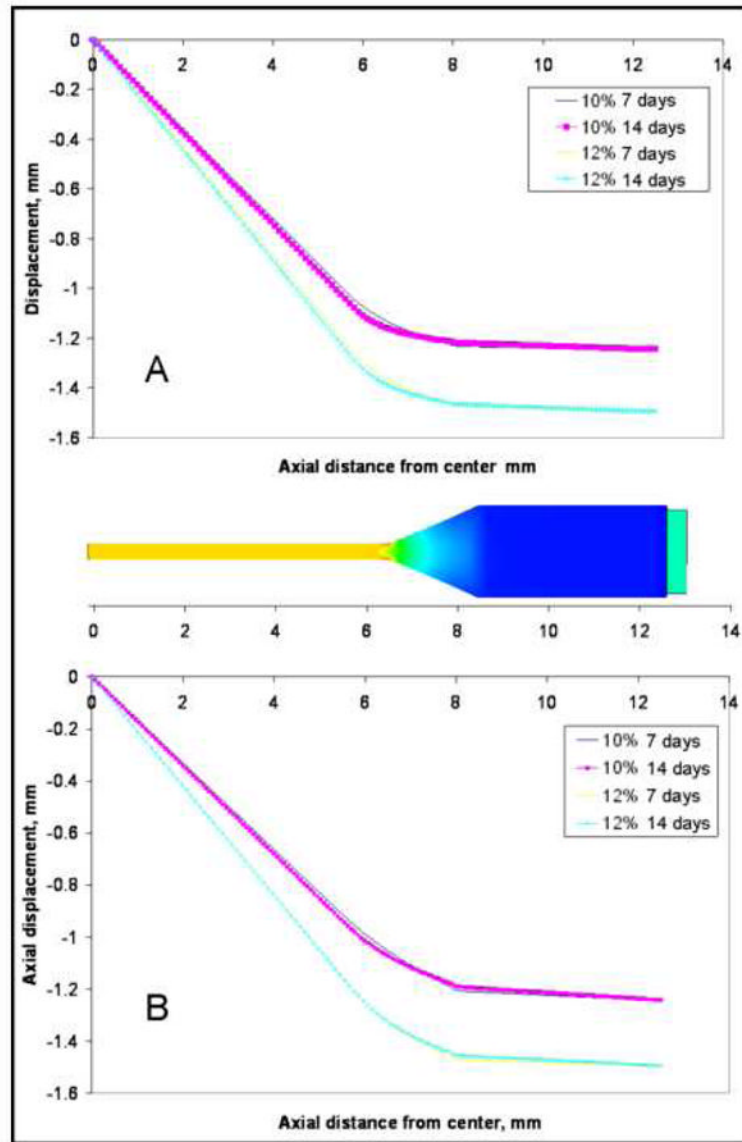


Figure 8. Axial displacement as a function of distance from center of matrices cultured in A) complete growth medium and B) osteogenic differentiating medium.

Table 1

Elastic Moduli and geometry of hMSC-seeded collagen gel matrices measured with respect to medium type (CGM: complete growth medium, ODM: osteogenic differentiating medium), time in culture and global cyclic tensile strain(Sumanasinghe 2007).

Medium	Time	Strain, %	E, kPa	Width, mm	Thickness, mm
CGM	7 days	10	469.24	0.6133	0.3205
		12	736.57	0.5724	0.2984
	14 days	10	2356.07	0.3756	0.2212
		12	1670.40	0.4118	0.2354
ODM	7 days	10	615.25	0.8498	0.4426
		12	493.04	0.7064	0.3456
	14 days	10	1339.20	0.6655	0.3897
		12	984.88	0.6310	0.3318

Table 2

Summary of finite element model results in center of cell-seeded construct by medium type, time in culture and global applied strain. CGM: Complete growth medium; ODM: Osteogenic differentiating medium.

Medium	Time	Global Applied Cyclic Tensile Strain, %	Average Local Axial Strain, % (St Dev)	Average Local Axial Stress, kPa, (St Dev)
CGM	7 days	10	17.7 (0.03)	83.2 (0.1)
		12	21.4 (0.01)	157.5 (0.3)
	14 days	10	18.3 (0.02)	430.4 (0.1)
		12	21.8 (0.04)	365.1 (0.5)
ODM	7 days	10	16.4 (0.08)	101.2 (0.3)
		12	20.8 (0.06)	224.8 (0.1)
	14 days	10	16.8 (0.0003)	102.6 (0.2)
		12	20.8 (0.04)	205.2 (0.2)