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Interplay Between Cytokine-Induced and Cyclic Equibiaxial Deformation-Induced Nitric Oxide Production and Metalloproteases Expression in Human Alveolar Epithelial Cells

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Abstract

Ventilator-induced lung overdistension has been a growing concern in the management of mechanically ventilated patients. Mechanical ventilation triggers or enhances the net inflammatory and tissue remodeling activities. Although it has been shown that proinflammatory and tissue remodeling factors play important roles during airway remodeling, the interplay between them is not well understood. Thus, our objective was to study and characterize the molecular mechanism of cyclic equibiaxial deformation-induced airway inflammation and remodeling either in the presence or absence of a pre-existing inflammatory condition. This study was done using an *in vitro* dynamic model, which can simulate different mechanical ventilative conditions. Type II alveolar epithelial cell (A549) monolayers were exposed to the different levels of mechanical ventilative conditions using the Flexcell® Tension Plus™ 4000T system, which generated the different levels of cyclic equibiaxial deformation (5, 10, 15, and 20%) at 0.2 Hz deformation frequency. The production of nitric oxide (NO), the expression of metalloprotease-2 (MMP-2)/tissue inhibitor metalloprotease-2 (TIMP-2), and the activation of MMP-2 were measured under the different levels of cyclic equibiaxial deformation either in the presence or absence of TNF- α . Our study indicated that cyclic equibiaxial deformation-induced production of NO and MMP-2/TIMP-2. Higher levels of cyclic equibiaxial deformation increased the expression of the active form of MMP-2. In particular, in the presence of TNF- α , the more active form of MMP-2 was detected during both cyclic equibiaxial deformation and remodeling periods.

Keywords

Inflammatory cytokine; Mechanical deformation; Cyclic strain; Metalloproteases; Airway remodeling

INTRODUCTION

Ventilator-induced lung overdistension has been a growing concern in the management of mechanically ventilated patients. In clinical practice, mechanical ventilation is used as an effective life-saving strategy, but on the dark side it initiates injury and leaves the injured lung susceptible to rapid remodeling. Mechanical ventilation causes injury to the lung not only by the mechanical stress caused by a complex set of forces, but also by the inflammation that follows after. Typically acute respiratory distress syndrome and acute lung injury are the most commonly observed side effects of ventilator-induced lung injury.¹⁴

It is believed that mechanical ventilation triggers or enhances the net inflammatory activity between proinflammatory and anti-inflammatory mediators. The mechanisms underlying the sensing and conversion of inappropriate mechanical stretching into cytotoxicity, net inflammatory activity, and extracellular matrix remodeling have not been well characterized.

A negative effect of lung overdistension on net inflammatory activity in different localized cell types inside the lung is the most common phenomenon during a ventilative condition. Preliminary studies, including animal studies, have already shown injurious effects of mechanical ventilation on the lung by induction of inflammation following increased positive pressure.^{1,23} Current knowledge allows us to understand the chronological order of events, which take place under mechanical ventilative conditions: surfactant dysfunction, alveolar damage, proinflammatory mediator production, structural changes in the alveolar capillary, and changes in gene transcription.^{5,17} These events are highly interconnected and orchestrate a chain reaction. Thus, understanding of each individual event enables us to better model this process.

Understanding the effects of mechanical forces on altering production of surfactants and proinflammatory mediators has been a focal point of many studies. Animal studies have clearly shown the rapid increase of proinflammatory mediators like IL-8, TNF- α , and IL-6 in response to various ventilative conditions.^{1,23} The findings of animal studies showed the outcome of the entire process, but failed to show the individual contributors such as the process initiators and mediators. To better understand the process and reduce the complexity of the outcome, *in vitro* models with stretching⁷ or compressing²⁵ capabilities have been used. These *in vitro* strategies have provided information about the cell-cell interactions, the responses of individual cell types, and the originator and mediators of the entire process. Because A549 cells form a protective barrier at the surface of lung tissue, these cells have been the most commonly studied cell types in *in vitro* stretching or compressing systems. Due to exposure of mechanical forces, A549 cells have been shown to produce proinflammatory mediators such as IL-8, nitric oxide (NO), and IL-6.^{7,16} These proinflammatory mediators are known to play a crucial role in attracting immune cells and altering gene transcription by activating multiple pathways including the NF- κ B pathway and amplifying the inflammation.^{12,15} Along with proinflammatory mediators, anti-inflammatory mediators and remodeling factors are also produced by A549 cells, but the level of production is highly dependent on the surrounding conditions inside the lung.^{22,27} The remodeling factors like matrix metalloproteases (MMP) and tissue inhibitor metalloproteases (TIMP) are also known to play an important role during inflammatory conditions by promoting the tissue remodeling activities.^{8,18} Mechanical forces not only increase the inflammatory response, but also increase the remodeling activity in A549 cells by increasing MMP-2 and MMP-9 production.² Although both proinflammatory and remodeling factors play a crucial role during lung overdistension, the interplay between them has not been well characterized.

To enhance our understanding of molecular mechanisms for cyclic equibiaxial deformation-induced airway remodeling, we used an *in vitro* model, which can simulate different mechanical ventilation conditions. We hypothesized that cyclic equibiaxial deformation either in the presence or absence of a pre-existing inflammatory condition would affect the net inflammatory activity and airway remodeling. The Flexcell[®] Tension Plus[™] 4000T system was used to mimic ventilator-induced overdistension. Human type II alveolar epithelial cells (A549) were used as a model cell line for characterization of cellular response under cyclic equibiaxial deformation. Percentage area change generated by cyclic equibiaxial deformation was considered analogous to surface area change resulting from lung inflation, during mechanical ventilation. Different levels of cyclic equibiaxial deformation (5, 10, 15, and 20%) at a frequency of 0.2 Hz was applied to A549 cell

monolayers either in the presence or absence of inflammatory cytokine (TNF- α). Under these conditions, the expression of proinflammatory (NO) and remodeling factors (MMP-2/TIMP-2) were investigated.

MATERIALS AND METHODS

Cell Culture

Type II alveolar epithelial cells of human origin (A549) were purchased from ATCC (Manassas, VA). A549 is epithelial-like in morphology and originates from a human lung carcinoma patient. The cells were seeded at 3×10^5 cells/well onto six well BioFlex plates (Flexcell International, PA) containing 2 mL of F-12k culture medium, which was supplemented with 1% penicillin streptomycin (Invitrogen, CA) and 10% fetal bovine serum (Thermo Fisher Scientific, UT). Cells reached confluency in 48 h after seeding. After reaching confluency, cells were exposed either to cyclic equibiaxial deformation or to inflammatory cytokine (TNF- α) followed by cyclic equibiaxial deformation.

Exposure of A549 Monolayers to TNF- α

After reaching confluency, cells were incubated in serum-free media for 24 h before exposure to TNF- α . The cells were then exposed to TNF- α (10 ng/mL) in serum-free media for 24 h. Following TNF- α exposure, serum was returned to the culture media. Cells were then either grown in static condition (no cyclic equibiaxial deformation) as controls or exposed to cyclic equibiaxial deformation (Fig. 1b).

Cyclic Equibiaxial Deformation

The airway wall exists in a mechanically dynamic environment, where different amounts of circumferential and longitudinal expansion and contraction occurred during breathing movements or ventilation therapy. We used a physiologically relevant range of cyclic equibiaxial deformation 5, 10, 15, and 20%, which corresponds to 45, 60, 70, and 80% of the total lung capacity, respectively.²⁴ Flexcell® Tension Plus™ 4000T system (Flexcell International, PA) was used to equibiaxially elongate the monolayers of cells on silicone rubber bottoms of a BioFlex plate. The cell monolayers were exposed to the different levels of cyclic equibiaxial deformation for 24 h at frequency of 0.2 Hz and then grown in static condition for another 48 h. The 0 h time refers to the starting point of cyclic equibiaxial deformation (Fig. 1a).

Nitrite Measurement

The Griess Reagent system (Promega Corporation, WI) was used to measure the nitrite level in the media samples collected from all experiments. NO is highly unstable in the presence of oxygen, and is rapidly converted into NO₂⁻ (Nitrite) and NO₃⁻ (Nitrate) in liquid media. Thus, the level of nitrite measured in all media samples provided only the partial concentration of NO produced from the cells under different conditions.

Total MMP-2 Expression

All media samples were analyzed using Quantikine® Human/Mouse/Rat MMP-2 (total) Immunoassay (R&D Systems, MN) to detect total MMP-2 production. This assay detected both active and pro-active forms of the MMP-2.

Zymography for MMP-2 Activity Measurement

Gelatin-based Zymography was performed on all media supernatant samples in a 12% polyacrylamide resolving gel under nonreducing condition. Granular gelatin, dissolved in deionized water, was copolymerized in the polyacrylamide resolving gel with a final

concentration of 1 mg/mL. A 6% polyacrylamide stacking gel was used. Prior to performing zymography measurement, all samples were incubated at 37 °C with 2× sample buffer (62.5 mM Tris-HCL, pH 6.8, 5% SDS, 40% Glycerol and 0.1% Bromophenol blue) in 1:1 ratio for 30 min. All samples mixed with sample buffer were run at 200 V for 45 min under constant voltage mode. After electrophoresis, gels were washed four times at 15 min intervals each in renaturing buffer (2.5% Triton X-100 in 50 mM Tris pH 7.4, 5 mM CaCl₂ and 1 μM ZnCl₂) on a rotating shaker. After renaturing MMPs, the gels were incubated at 37 °C in developing solution (50 mM Tris pH 7.4, 5 mM CaCl₂ and 1 μM ZnCl₂) overnight. Gels were stained with 0.5% Coomassie Blue R-250 in 40% ethanol and 10% Acetic acid for 1 h. Prior to imaging, the gels were briefly destained in 40% ethanol and 10% acetic acid solution for 5 min. The G-Box gel imaging system (Syngene, MD) was used to analyze active and inactive forms of MMP-2. In active and active forms of MMP-2 were detected at 72 and 62 kD region, respectively.

Total TIMP-2 Expression

The TIMP-2 ELISA kit (EMD-Calbiochem, CA) was used to measure the level of TIMP-2 expression in media supernatant samples.

Total Protein Measurement

Total protein from cell lysate of all samples was measured using the BCA total protein assay (Pierce, IL).

Statistical Analysis

Statistical analyses were carried out using two-way analyses of variance (ANOVA) followed by Dunnett's multiple comparison tests to determine where significance exists ($p < 0.05$). All graphs were prepared by plotting mean data (sample size, $n = 3$) with corresponding standard error.

RESULTS

Effect of TNF- α on NO Production and Cell Growth in A549 Cells Under a Static Condition (Without Cyclic Equibiaxial Deformation)

Exposure of TNF- α resulted in a significant increase of NO production in A549 cells (0% cyclic equibiaxial deformation, Fig. 3a). While A549 cell monolayers were exposed to TNF- α in a serum-free media for 24 h, NO production significantly increased due to inflammatory stimuli. NO production peaked at 48 h after TNF- α exposure ($p < 0.05$) and remained significantly higher than the level of NO concentration (0% cyclic equibiaxial deformation, Fig. 3a). Total protein concentration in cell lysate samples collected from the same experiments showed the normal cell growth up to 48 h, followed by decrease for 72 h (0% cyclic equibiaxial deformation, Fig. 3b). The decreased total protein concentration in the presence of TNF- α could be due to cytotoxicity of TNF- α to A549 cells in cultures. The increased levels of cell necrosis might influence the level of inflammation and remodeling measured at the longer time points (i.e., >48 h).^{10,19}

Effect of Cyclic Equibiaxial Deformation on Inflammatory Response of A549 Cells

Nitrite concentration was measured from media supernatant of A549 cultures following different levels of cyclic equibiaxial deformation (5, 10, 15, and 20%). Cyclic equibiaxial deformation increased nitrite concentration during the period of cyclic equibiaxial deformation and remodeling (Fig. 2a). Nitrite concentration at 72 h was significantly higher than the control (at 0 h). Total protein concentration was significantly decreased during cyclic equibiaxial deformation between 0 and 24 h, but it returned to control level during

remodeling period between 24 and 72 h (Fig. 2b). Nitrite concentration and total protein concentration were not significantly changed in 0% cyclic equibiaxial deformation (static control) during the entire period of experiments (Fig. 2a, b).

Effect of Cyclic Equibiaxial Deformation on Inflammatory Response of A549 Cells in the Presence of TNF- α

Nitrite concentration was measured in media supernatant of A549 cultures following different levels of cyclic equibiaxial deformation (5, 10, 15, and 20%) in the presence of TNF- α . Nitrite concentrations were significantly increased immediately following exposure to cyclic equibiaxial deformation, and remained higher than the control (Fig. 3a). However, in the presence of TNF- α , cyclic equibiaxial deformation did not cause significant decrease in total protein concentration. In fact total protein concentration was higher than the control during the remodeling period (Fig. 3b). This indicates a different cell growth response as compared to the response of those that underwent cyclic equibiaxial deformation without the exposure of TNF- α (Fig. 3b).

Effect of Cyclic Equibiaxial Deformation on MMP-2 Expression in A549 Cell Monolayers Either in the Presence or Absence of TNF- α

The total MMP-2 (*inactive* and *active*) expression was observed in cells both exposed to cyclic equibiaxial deformation (Fig. 4a) and cyclic equibiaxial deformation in presence of TNF- α (Fig. 4b). During the first 24-h period of cyclic equibiaxial deformation, the level of MMP-2 expression was lower than the basal level of MMP-2 expression. However, during the period of remodeling following cyclic equibiaxial deformation, the level of MMP-2 expression began to increase and continued increasing up to 72 h following cyclic equibiaxial deformation. Cells grown in a static condition (0% cyclic equibiaxial deformation, Fig. 4a) did not show significant change in MMP-2 expression. But cells grown in a static condition with TNF- α (0% cyclic equibiaxial deformation, Fig. 4b) showed significant increase in the total MMP-2 expression level for 48 and 72 h.

Effect of Cyclic Equibiaxial Deformation on MMP-2 Activation in A549 Cell Monolayers Either in the Presence or Absence of TNF- α

Without any cyclic equibiaxial deformation, only the inactive form of MMP-2 expression was detected at the 48- and 72-h intervals (Table 1 and Fig. 5a, 0% cyclic equibiaxial deformation). The active form of MMP-2 expression appeared immediately following exposure to cyclic equibiaxial deformation. Under the 10% of cyclic equibiaxial deformation, both active and pro-active forms of MMP-2 expression were observed at 24 and 48 h (Table 1). At 72 h after cyclic equibiaxial deformation, the pro-active form of MMP-2 expression prevailed under the lower levels of cyclic equibiaxial deformation (5 and 10%). Under the higher levels of cyclic equibiaxial deformation (15 and 20%), all MMP-2 expression was detected as active forms (Table 1 and Fig. 5a).

In the presence of TNF- α without cyclic equibiaxial deformation, the active form of MMP-2 was observed at both 0 and 24 h. Thereafter, all MMP-2 expression was detected as pro-active forms (Table 2 and Fig. 5b, 0% cyclic equibiaxial deformation). Exposure to TNF- α alone induced the activation of MMP-2 up to 24 h. Following the exposure of cyclic equibiaxial deformation in the presence of TNF- α , all MMP-2 expression was observed as active forms during both cyclic equibiaxial deformation and the remodeling period (Table 2 and Fig. 5b). These results were significantly different from those observed in A549 cell monolayers exposed to cyclic equibiaxial deformation alone (Table 1 and Fig. 5a).

Expression of TIMP-2

Cyclic equibiaxial deformation did not affect TIMP-2 expression until 24 h, but TIMP-2 expression increased significantly at 48 h following cyclic equibiaxial deformation and remained higher for 72 h (Fig. 6a). The level of TIMP-2 expression increased in a similar pattern to that observed in the expression of MMP-2 (Fig. 4a).

Cyclic equibiaxial deformation-induced expression of TIMP-2 in the presence of TNF- α dramatically increased at 24 h following cyclic equibiaxial deformation and remained higher up to 72 h ($p < 0.05$) (Fig. 6b). The expression of TIMP-2 significantly increased at 24 h following stretching although the expression of MMP-2 decreased at the same time interval, especially in the presence of TNF- α (Fig. 4b).

DISCUSSION

We used human type II alveolar epithelial cell monolayers with cyclic equibiaxial deformation, simulating normal breathing and ventilator conditions to study the mechanisms of ventilation-induced lung injury. In this study, we showed the effect of different levels of cyclic equibiaxial deformation on the net inflammatory response (e.g., NO production) and the net tissue remodeling activity (e.g., expression of MMP-2 and TIMP-2), either in the presence or absence of a pre-existing condition of inflammation (e.g., exposure to TNF- α). It was observed that different levels of cyclic equibiaxial deformation on A549 cell monolayers induced the different levels of inflammatory activity and the net tissue remodeling activities, either in the presence or absence of pre-existing inflammatory stimulus, both during cyclic equibiaxial deformation (first 24 h) and postcyclic equibiaxial deformation (next 48 h).

Tissue remodeling activities that occurred by cyclic equibiaxial deformation were positively regulated by the increased expression and activation of MMP-2 and TIMP-2. Total MMP-2 expression was decreased during the period of cyclic equibiaxial deformation for 24 h, and significantly increased for 48 and 72 h for all levels of cyclic equibiaxial deformation either in the presence or absence of TNF- α (Fig. 4). TIMP-2 expression was steadily increased both during cyclic equibiaxial deformation and postcyclic equibiaxial deformation either in the presence or absence of TNF- α (Fig. 6). The decrease in total MMP-2 expression at 24 h may be due to the decrease in the total number of cells (Fig. 3b) during the period of cyclic equibiaxial deformation. Following cyclic equibiaxial deformation, as cell growth was restored, MMP-2 and TIMP-2 expression increased significantly to a level higher than the control. The expression level of MMP-2 shown in Fig. 5 includes both active and pro-active forms of MMP-2. To identify active (62 kDa) and pro-active (72 kDa) forms of MMP-2 from the total expression of MMP-2, zymography was used to detect the level of the active form of MMP-2 under different levels of cyclic equibiaxial deformation either in the presence or absence of TNF- α (Fig. 5).

Without the exposure of TNF- α and cyclic equibiaxial deformation, no detectable level of MMP-2 expression was observed with zymography. Only the inactive form of MMP-2 was detected during the remodeling period (at 48 and 72 h) (Table 1 and Fig. 5a, 0% cyclic equibiaxial deformation). All MMP-2 expression was observed to have active form dominant during cyclic equibiaxial deformation (at 24 h) and during the remodeling period (at 48 and 72 h). Under 10% cyclic equibiaxial deformation, both active and pro-active forms of MMP-2 were detected during the whole period following cyclic equibiaxial deformation. At 72 h after cyclic equibiaxial deformation, the inactive form of MMP-2 started to appear in cultures that underwent the lower levels (5 and 10%) of cyclic equibiaxial deformation (Table 1 and Fig. 5a). As shown in Fig. 6a, the level of TIMP-2 expression increased during the postcyclic mechanical strain, which might have played a

role in the inactivation of MMP-2. An increased level of TIMP-2 expression has also been known to stimulate cell growth through the mediation and activation of NF- κ B, which is also responsible for the expression of proinflammatory proteins, including inducible nitric oxide synthase (iNOS), which increases the level of NO production.¹³ In this study, we cannot rule out the possibility that A549 cells produced TNF- α in response to cyclic equibiaxial deformation and were stimulated to produce NO by secreted TNF- α .

To investigate the effects of cyclic equibiaxial deformation on cell inflammation and tissue remodeling activity under a pre-existing inflammatory condition, A549 cell monolayers were exposed to TNF- α before starting cyclic equibiaxial deformation. The pattern of production of NO, the expression of MMP-2, and TIMP-2, induced by cyclic equibiaxial deformation in absence of TNF- α (Figs. 2a, 4a, and 6a), were different than those induced in the presence of TNF- α (Figs. 3a, 4b, and 6b).

TNF- α induced NO production without cyclic equibiaxial deformation reached peak production at 48 h and remained significantly higher than control for the entire 72 h duration (0% cyclic equibiaxial deformation, Fig. 3a). There were significant differences in cyclic deformation induced NO production based on the presence or absence of TNF- α (Figs. 3a and 2a). Cyclic equibiaxial deformation-induced NO production was peaked at 48 h in the presence of TNF- α (Fig. 3a), whereas it was shown to continuously increase up to 72 h in the absence of TNF- α (Fig. 2a). Cyclic equibiaxial deformation induced NO production in the presence of TNF- α was similar to that induced by TNF- α alone, but it exhibited a significantly higher level ($p < 0.05$).

The exposure of TNF- α before cyclic equibiaxial deformation had a positive effect on tissue remodeling activities, especially on the expression of MMP-2 (Fig. 4b). Cyclic equibiaxial deformation in the presence of TNF- α affected not only the expression of MMP-2, but also the activation of MMP-2 (Figs. 4a, 5b, and Table 2). The exposure to TNF- α might have increased proteolytic activity by increasing MMP-2 and MMP-9 activity.^{8,11} Both MMP and TIMP expression was increased by TNF- α .¹¹ TNF- α exposure alone induced an increase in the MMP-2 expression at 48 and 72 h (0% cyclic equibiaxial deformation, Fig. 4b). Exposure to cyclic equibiaxial deformation also induced an increase in the MMP-2 expression at 48 and 72 h (Fig. 4a). In the presence of TNF- α without cyclic equibiaxial deformation, the active form of MMP-2 was observed at both 0 and 24 h. Thereafter, all MMP-2 expression was detected as pro-active forms (Table 2 and Fig. 5b, 0% cyclic equibiaxial deformation). Exposure of TNF- α alone induced the activation of MMP-2 up to 24 h. Following the exposure of cyclic equibiaxial deformation in the presence of TNF- α , all MMP-2 expression was observed as the active form during both cyclic equibiaxial deformation and the remodeling period (Table 2 and Fig. 5b). This suggests that TNF- α exposure prior to cyclic equibiaxial deformation-induced prolonged activation of MMP-2.

Cyclic equibiaxial deformation or TNF- α exposure induced the release of inflammatory and tissue remodeling mediators through the different interconnected pathways. Mechanical deformation increases the cytoplasmic Ca²⁺ concentration through various pathways and mechanisms, which include an increase in intracellular inositol 1,4,5-trisphosphate (IP3) concentration; the activation of stretch activated calcium channels, and the repair of stretch activated plasma membrane damage.^{3,26} The increased concentration of cytoplasmic Ca²⁺ boosts the Calmodulin (CaM) activity and amplifies the basal level of NO by increasing the activity of constitutive nitric oxide synthase (cNOS).²⁰ On the other hand, TNF- α induced the activation of NF- κ B, resulting in the induction and activation of iNOS and the release of a large amount of NO.⁶ Increased NO production following cyclic equibiaxial deformation in the presence of TNF- α (Fig. 3a) could be explained by the combined effect of cNOS and iNOS.^{7,9} Mechanical ventilation also increased tissue remodeling activities through the

increased activity of metalloproteases in airway epithelial cells.⁴ Haseneen *et al.* have shown that mechanical stretch induced the expression of MMP (MT1-MMP) in the presence of the inducer, EMMPRIN.⁸ Little is known about how MMP-2 and TIMP-2 are up-regulated in airway epithelial cells during and after cyclic mechanical strain in the presence of TNF- α . TNF- α may have increased the expression of MMP-2 and TIMP-2 through the activation of the NF- κ B pathway^{13,21} or possibly through interaction with MMPs, which underwent mechanical ventilation-induced activation⁴ as shown in Figs. 4 and 6.

In conclusion, our study supports the idea that exposure to cyclic equibiaxial deformation, either in the presence or absence of pre-existing inflammation, positively regulates the inflammatory and net tissue remodeling activities in A549 cells. Cyclic equibiaxial deformation-induced NO production and MMP-2/TIMP-2 expression. Higher levels of cyclic equibiaxial deformation increased the active form of MMP-2 in both instances. However, in the presence of TNF- α more of the active form of MMP-2 was detected during both cyclic equibiaxial deformation and remodeling periods.

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FIGURE 1.

(a) Experimental scheme for cyclic equibiaxial deformation of A549 cells. Following confluency, A549 cell monolayers were incubated in serum-free media for 24 h. The cell monolayers were then either exposed to the different levels of cyclic equibiaxial deformation for 24 h and grown in static condition for another 48 h or grown in static condition. Time 0 h marks the end of serum-free media incubation and refers to the starting point of cyclic equibiaxial deformation or static condition growth. (b) Experimental scheme for cyclic equibiaxial deformation of A549 cells in presence of TNF- α . Following confluency, A549 cell monolayers were incubated in serum-free media for 24 h before TNF- α exposure. Cell layers were exposed to TNF- α for 24 h and then grown in static condition or under cyclic equibiaxial deformation. The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h marks the end of TNF- α exposure and refers to the starting point of cyclic equibiaxial deformation.

FIGURE 2.

(a) Effect of cyclic equibiaxial deformation on inflammatory response of A549 cells. Nitrite concentration was measured from media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%). The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to the starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. *

Significantly higher than the control ($p < 0.05$). (b) Effect of cyclic equibiaxial deformation on A549 cell proliferation. Total protein concentration was measured in cell lysate of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%). The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of

cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$).

FIGURE 3.

(a) Effect of cyclic equibiaxial deformation on inflammatory response of A549 cells in the presence of TNF- α . Nitrite concentration was measured in media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%) in the presence of TNF- α (10 ng/mL). Cell monolayers were exposed to TNF- α for 24 h and then grown in either static condition or under cyclic equibiaxial deformation. The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$). # Significantly higher than other time points under the same condition ($p < 0.05$). (b) Effect of cyclic equibiaxial deformation on A549 cell proliferation in the presence of TNF- α . Total protein concentration was measured in cell lysate of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%) in the presence of TNF- α . Cell monolayers were exposed to TNF- α for 24 h and then grown in either static condition or under cyclic equibiaxial deformation. The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$).

FIGURE 4.

(a) Effect of cyclic equibiaxial deformation on MMP-2 expression in A549 cell monolayers. Total MMP-2 expression was measured in media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%). The cell monolayers were exposed to different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$). (b) Effect of cyclic equibiaxial deformation on MMP-2 expression in A549 cell monolayers in the presence of TNF- α . Total MMP expression was measured in media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%) in the presence of TNF- α . Cell monolayers were exposed to TNF- α for 24 h and then grown in either static condition or under cyclic equibiaxial deformation. The cell monolayers were exposed to the different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$).

FIGURE 5.

(a) Effect of cyclic equibiaxial deformation on MMP-2 activation in A549 cell monolayers. Active and pro-active forms of MMP-2 were analyzed by zymography following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%). Inverted view of zymography gels were presented for better visualization of the gelatinase activity. Pro-active and active forms of MMP-2 were detected at 72 and 62 kD region, respectively. One of three gel pictures was presented in this figure. All results were presented in Table 1. (b) Effect of cyclic equibiaxial deformation on MMP-2 activation in A549 cell monolayers in the presence of TNF- α . Active and pro-active forms of MMP-2 were analyzed by zymography following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%) in the presence of TNF- α . Inverted view of zymography gels was presented for better visualization of the gelatinase activity. One of three gel pictures was presented in this figure. All results were presented in Table 2.

FIGURE 6.

(a) Effect of cyclic equibiaxial deformation on TIMP-2 expression in A549 cell monolayers in the absence of TNF- α . Total TIMP-2 expression was measured in media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%). The cell monolayers were exposed to the different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective cyclic equibiaxial deformation level. * Significantly higher than the control ($p < 0.05$). (b) Effect of cyclic equibiaxial deformation on TIMP-2 expression in A549 cell monolayers in the presence of TNF- α . Total TIMP-2 expression was measured in media supernatant of A549 culture following different levels of cyclic equibiaxial deformation (0, 5, 10, 15, and 20%) in the presence of TNF- α . Cell monolayers were exposed to TNF- α for 24 h and then grown in either static condition or under cyclic equibiaxial deformation. The cell monolayers were exposed to the different levels of cyclic equibiaxial deformation for 24 h and then grown in static condition for another 48 h. Time 0 h refers to starting point of cyclic equibiaxial deformation and control for respective level of cyclic equibiaxial deformation. * Significantly higher than the control ($p < 0.05$).

TABLE 1

Qualitative analysis results from zymography of media samples from A549 cells exposed to cyclic equibiaxial deformation in the absence of TNF- α .

Cyclic equibiaxial elongation levels (%)	Time (h)			
	0	24	48	72
5	None	A	A	A
5	None	A	A	I
5	None	A	A	I
10	None	A/I	A/I	A/I
10	None	A/I	A/I	A/I
10	None	A/I	A/I	I
15	None	A	A	I
15	None	A	A	A
15	None	A	A	I
20	None	A	A	A
20	None	A	A	A
20	None	A	A	I
0 (Control)	None	None	I	I
0 (Control)	None	None	I	I
0 (Control)	None	None	I	I

A, Active; I, Pro-active.

TABLE 2

Qualitative analysis results from zymography of media samples from A549 cells exposed to cyclic equibiaxial deformation in the presence of TNF- α .

Cyclic equibiaxial elongation levels (%)	Time (h)			
	0	24	48	72
5	A/I	A	A	A
5	A/I	A	A	A
5	None	A	A	A
10	A/I	A	A	A
10	A/I	A	A	A
10	A	A	A	A
15	A/I	A	A	A
15	A/I	A	A	A
15	A/I	A	A	A
20	A/I	A	A	A
20	A/I	A	A	A
20	A/I	A	A	A
0 (Control)	A	A	I	I
0 (Control)	A/I	A	I	I
0 (Control)	A	A	I	I

A, Active; I, Pro-active.