Mechanical force promotes the proliferation and extracellular matrix synthesis of human gingival fibroblasts cultured on 3D PLGA scaffolds via TGF-β expression

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Abstract. Human gingival fibroblasts (HGFs) are responsible for connective tissue repair and scarring, and are exposed to mechanical forces under physiological and pathological conditions. The exact mechanisms underlying gingival tissue reconstruction under mechanical forces remain unclear. The present study aimfed to investigate the effects of mechanical forces on the proliferation and extracellular matrix synthesis in HGFs by establishing a 3-dimensional (3D) HGF culture model using poly(lactide-co-glycolide) (PLGA) scaffolds. HGFs were cultured in 3D PLGA scaffolds and a mechanical force of 0, 5, 15, 25 or 35 g/cm^2 was applied to HGFs for 24 h. A mechanical force of 25 g/cm^2 induced the highest proliferation rate, and thus was selected for subsequent experiments. Cell viability was determined using the MTT assay at 0, 24, 48 and 72 h. The expression levels of type I collagen (COL-1) and matrix metallopeptidase (MMP)-1 were examined by reverse transcription-quantitative polymerase chain reaction and ELISA, and transforming growth factor (TGF)-β expression was evaluated by ELISA. The application of mechanical force on HGFs cultured on the 3D PLGA scaffolds resulted in a significant increase in cell proliferation and COL-1 expression, as well as a decrease in MMP-1 expression. A TGF-β1 inhibitor was also applied, which attenuated the effects of mechanical force on HGF proliferation, and COL-1 and MMP-1 expression, thus suggesting that TGF-β signaling pathways may mediate the mechanical force-induced alterations observed in HGFs. In conclusion, these findings helped to clarify the mechanisms underlying mechanical force-induced HGF proliferation and ECM synthesis, which may promote the development of targeted therapeutics to treat various diseases, including gingival atrophy caused by orthodontic treatment.

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Abbreviations: ECM, extracellular matrix; HGFs, human gingival fibroblasts; MMP-1, matrix metallopeptidase 1; OD, optical density; PGA, polyglycolic acid; PLA, polylactic acid; PLGA, poly(lactide-co-glycolide); RT-qPCR, reverse transcription-quantitative polymerase chain reaction; TGF-β, transforming growth factor-β

Key words: mechanical force, human gingival fibroblasts, 3D culture, PLGA, TGF-β

Introduction

Orthodontic treatment alters the location of abnormally positioned teeth by applying mechanical force, which in turn affects the attachment apparatus, including the periodontal ligament, alveolar bone, cementum and gingiva (1). Tooth movement is achieved following alveolar bone remodeling and response of the periodontal ligament to mechanical force (2). In recent decades, alveolar bone and periodontal membrane remodeling under mechanical force have been widely studied (3). However, the mechanisms underlying gingival tissue reconstruction remain largely unexplored.

Gingival tissues are constantly exposed to the effect of physical forces (4). Mechanical stimuli are regulators of connective tissue homeostasis, and sustained mechanical stimulation may lead to modifications in cell activity and extracellular matrix (ECM) composition (5). Human gingival fibroblasts (HGFs) are the major cell type that constitutes human gingival connective tissue (6), which are responsible for the synthesis and degradation of the ECM and bone resorption, as well as the secretion of proteolytic enzymes (7-9). Accordingly, they are crucial for regulating the homeostasis of periodontal tissues under healthy and diseased states (10). Nevertheless, strategies to improve correction efficiency and promote HGFs to maintain the stability of adaptive remodeling following orthodontic treatment remain to be elucidated.

Transforming growth factor (TGF)-β is an important regulator of gingival tissue regeneration (11-13). Guo *et al* (14) demonstrated that mechanical shear force promotes the proliferation of gingival fibroblasts through activation of the TGF-β signaling pathway. In addition, Jeon *et al* (15) revealed that mechanical force induces the synthesis of type I collagen (COL-1) and osteopontin in HGFs through TGF-β signaling. Therefore, it was hypothesized that TGF-β may have an important role in converting mechanical force into

biochemical signals in HGFs, thus promoting cell proliferation and extracellular ECM synthesis.

Since other cells and ECM surround almost all cells in the *in vivo* environment in a 3-dimensional (3D) fashion, 2-dimensional (2D) cell culture does not adequately take into account the natural 3D environment of cells (16). The development of biological scaffold material provides a structural basis for 3D cell culture (17). Cells cultured in a 3D cell-based system more realistically mimic *in vivo* cell behaviors and provide more predictable results for subsequent *in vivo* experiments. Biodegradable poly(lactide-co-glycolide) (PLGA) is a biocompatible material that is widely used in clinical settings (18). In the present study, a 3D HGFs culture model was established, using PLGA scaffolds, in order to investigate the effects of mechanical force on the proliferation of HGFs, and to explore the functions of TGF-β in HGF proliferation, as well as COL-1 and matrix metallopeptidase (MMP)-1 expression. The results may provide a theoretical basis for the understanding of gingival remodeling under mechanical force.

Materials and methods

3D culture of HGFs. HGFs were 3D cultured as previously reported (19). Gingival tissues were collected from healthy teeth extracted from 17 males and 19 females (age range, 10-14 years) during orthodontic extraction or gingival resection. Subsequently, the tissues were dissociated using 0.25% trypsin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) containing 100 ng/ml DNase (Roche Diagnostics, Basel, Switzerland) at 37˚C for 35 min. The digestion was terminated by adding Dulbecco's modified Eagle's medium (GE Healthcare Life Sciences, Logan, UT, USA) with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The dissociated HGFs were cultured in Eagle's minimum essential medium (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), 2 mM L-glutamine and 100 U/ml antibiotics (Gibco; Thermo Fisher Scientific, Inc.) in plastic culture flasks. Cells were maintained at 37˚C with 5% CO_2. The PLGA scaffold (size, 2 cm x 2 cm x 300 μm; porosity, 85%; average pore size, 80-120 μm) was synthesized using the solvent casting/particulate leaching technique (20). After 4-6 passages, $1x10^5$ HGFs were seeded onto a single sheet of PLGA scaffold. The present study was approved by the Hospital Institutional Review Board (approval no. 20150304-22) of Guangxi Medical University (Nanning, China). All donors and their guardians signed an informed consent form.

Application of mechanical force. Prior to the application of mechanical force, HGFs were allowed to stabilize for 48 h. HGFs were continuously compressed using the uniform compression method, as presented in Fig. 1. Briefly, the PLGA sheet was placed into the well with a wire stool to prevent it from floating. A HGF suspension was dripped into the well. After 24 h of incubation at 37˚C, the PLGA/HGF construct was moved to another well. After cell implantation for 6 h, glass bottles containing lead granules were placed on top of the 3D models. The bottles provided compressive stress of four magnitudes (5, 15, 25 and 35 g/cm^2) for 6, 24 and 72 h. Control cells (without application of mechanical force) were cultured under the same 3D conditions. Cells were also cultured at 37˚C in the presence of SB431542 (cat. no. HY-10431; MedChemExpress Monmouth Junction, NJ, USA) for 24 h, a TGF-β inhibitor, at 20 μM.

Immunocytochemistry. HGFs from the PLGA scaffold were plated on coverslips and incubated for 6 h at 37˚C in an atmosphere containing 5% CO_2, in order to allow cells to adhere and proliferate. Cells were harvested and fixed with 4% paraformaldehyde for 30 min at room temperature. Peroxidase activity was blocked using 3% hydrogen peroxide for 30 min at 37˚C. After blocking in normal rabbit serum (Santa Cruz Biotechnology, Inc., Dallas, TX, USA), the cells were incubated with monoclonal antibodies against vimentin (cat. no. MAB3404; 1:100) and cytokeratin (cat. no. MAB3400; 1:100) (both from Sigma-Aldrich; Merck KGaA) at 4˚C overnight. Following three washes with PBS for 5 min at room temperature, sheep anti-rat immunoglobulin G secondary antibody (1:5,000; cat. no. 5647; Abcam, Cambridge, UK) was added and incubated at 37˚C for 1 h. Subsequently, the membrane was analyzed using the UltraSensitive™ S-P Immunohistochemistry kit (cat. no. 40398a; Fuzhou Maixin Biotech. Co., Ltd., Fuzhou, China) according to the manufacturer's protocol. Finally, cells were counterstained with 3% hematoxylin for 60 sec at room temperature and examined using a fluorescence microscope (Olympus Corporation, Tokyo, Japan).

Immunofluorescence. Sections of the scaffolds (300 μm-thick) containing the HGFs were deparaffinized with xylene. Following rehydration with descending ethanol series, the samples were incubated for antigen retrieval in a microwave oven with EDTA buffer at pH 8.0 for 30 min at 95˚C, followed by fixation with 4% paraformaldehyde for 30 min at room temperature. Immunofluorescence analysis was performed to detect fibrillar actin (F-actin) and nuclei. Structures of F-actin were detected using tetramethylrhodamine-phalloidin (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China) staining solution at a concentration of 100 nM and incubated for 30 min at room temperature. Nuclei were stained with DAPI at 10 μg/ml for 30 sec at room temperature (Sigma-Aldrich; Merck KGaA), according to the manufacturer's protocol. After washing with PBS, samples were examined by confocal microscopy (fv-500; Olympus Corporation).

Cell viability. Following the application of mechanical force, HGFs-PLGA constructs were incubated with MTT reagent (Sigma-Aldrich; Merck KGaA) for 4 h at 37˚C, at a concentration of 20 μM. Subsequently, 500 μl DMSO (Sigma-Aldrich; Merck KGaA) was added into each well to dissolve the formazan and the optical density (OD) values were determined at 570 nm.

Flow cytometry. Trypsin was used to detach and collect the cells from the PLGA scaffold, and cells were washed twice with PBS at 4˚C. The cells were collected by centrifugation at 300 x g for 5 min and were fixed in 1 ml cold 70% ethanol for 30 min at 4˚C. Cells were resuspended in 1 ml propidium iodide staining solution (cat. no. CCS012; Multisciences Lianke Biotech Co., Ltd., Hangzhou, China) and incubated for 30 min at room temperature. The stained cells were analyzed

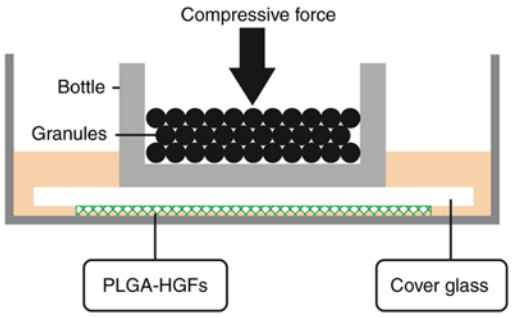

Figure 1. Schematic diagram of the uniform compression method of mechanical force loading. PLGA-HGFs, poly(lactide-co-glycolide)-human gingival fibroblasts.

for DNA content by flow cytometry (BD Accuri™ C6; BD Biosciences, Franklin Lakes, NJ, USA). The number of cells in the S and G2/M phases was divided by the number of cells in the G0/G1 phase, in order to calculate the proliferation index (ModFit LT software; version 4.0; Verity Software House, Inc., Topsham, ME, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from HGFs on the PLGA scaffold using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. An additional round of purification was performed with deoxyribonuclease I (ribonuclease-free; Takara Bio, Inc., Otsu, Japan) to remove genomic DNA. RNA quality was assessed using RNA 6000 Nano-Chips on an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). All samples showed intact 18S/28S bands. Total RNA (1 μg) underwent cDNA synthesis using an ExScript RT reagent kit (Takara Bio, Inc.), according to the manufacturer's protocol. RT-qPCR was performed using a T100™ Thermal Cycler system (Bio-Rad Laboratories, Inc., Hercules, CA, USA), according to the manufacturer's protocol. The following primers were used: TGF-β1, forward, 5'-CGCATCCTAGACCCTTTCTCCTC-3' and reverse, 5'-GGTGTCTCAGTATCCCACGGAAAT-3'; COL-1, forward, 5'-GAGGGCAACAGCAGGTTCACTTA-3' and reverse, 5'-TCAGCACCACCGATGTCCA-3'; MMP-1, forward, 5'-ACAACTGCCAAATGGGCTTGA-3' and reverse, 5'-CTGTCCCTGAACAGCCCAGTACTTA-3'; and GAPDH, forward, 5'-GCACCGTCAAGGCTGAGAAC-3' and reverse, 5'-TGGTGAAGACGCCAGTGGA-3'. The RT-qPCR reactions were performed on an ABI 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.) with SYBR Premix Ex Taq (Takara Biotechnology Co., Ltd., Dalian, China) at 95˚C for 30 sec, followed by 40 cycles at 95˚C for 5 sec and 60˚C for 31 sec, after which a melt curve analysis was performed at 95˚C for 15 sec, 60˚C for 1 min and 95˚C for 15 sec. All reactions were performed in triplicate and average of $2^{-\Delta\Delta Ct}$ (21). GAPDH was used as an internal control. Each experiment was repeated at least three times.

ELISA. ELISA kits were used to quantify TGF-β1 (cat. no. JL10706), COL-1 (cat. no. CSB-E08082h) and MMP-1 (cat. no. CSB-E08082h) levels (Quantikine; R&D Systems, Inc., Minneapolis, MI, USA) according to the manufacturer's protocol. A standard curve was created with the serially diluted TGF-β1 standard provided in the kit. Samples were measured at 450 nm using a microplate reader (Model 3550; Thermo Fisher Scientific, Inc.). Data were linearized by plotting the log of the TGF-β1 concentrations vs. the log of the OD; TGF-β1 concentrations were determined by linear regression. COL-1 and MMP-1 protein levels were detected using the same method. Each sample was assessed in triplicate.

Statistical analysis. All data are presented as the means ± standard deviation of three independent experiments. One-way analysis of variance (ANOVA) with Dunnett's post hoc test or two-way ANOVA with Bonferroni post hoc test was used for multiple comparisons. Statistical analyses were performed using SPSS 21.0 (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Mechanical force induces HGF proliferation. As presented in Fig. 2A, HGFs had a long, fusiform shape, with radial and swirling growth. In addition, HGFs were positive for vimentin staining (Fig. 2B) and negative for keratin staining (Fig. 2C). Based on the sample origin combined with the mesodermal nature of the cells (rather than epithelial), the cells were considered to be HGFs. Histological sections revealed 2-3 layers of HGFs overlying the surface of the 3D scaffold. Throughout the scaffold, the cells exhibited an elongated shape and were arranged in multiple layers (Fig. 2D). In addition, microfilaments, stained in red, were arranged along the longitudinal axis of the HGFs, as detected by F-actin staining (Fig. 2E).

Forces of 5, 15, 25 and 35 g/cm^2 were applied in the present study. Compared with in the control group (no mechanical force), HGF proliferation increased under the action of 5 and 15 g/cm^2 for 24 h, and cell proliferation activity reached its peak in response to 25 g/cm^2 (Fig. 3A). At 35 g/cm^2, HGF proliferation was inhibited and cell death increased, as measured by flow cytometry (Fig. 3A).

When applying 25 g/cm^2, no significant difference in cell proliferation was observed after 6 h of stimulation, whereas cell proliferation was significantly increased after 24 (peak) and 48 h ($P<0.05$; Fig. 3B). However, with the gradual increase of application time, the number of cells gradually decreased, and cell proliferation was inhibited after 72 h (Fig. 3B).

COL-1 and MMP-1 mRNA and protein expression is altered in response to mechanical force in HGFs. Under 25 g/cm^2, the mRNA and protein expression levels of COL-1 were upregulated compared with in the control group; the highest expression was detected at 24 h, whereas the expression level was decreased at 48 and 72 h (Fig. 4A and B). Conversely, the mRNA and protein expression levels of MMP-1 were lowest at 24 h, and were consequently increased with time, although they remained lower than the control group (Fig. 4C and D).

Mechanical force increases TGF-β1 expression in HGFs. The results of RT-qPCR analysis demonstrated that the mRNA expression levels of TGF-β1 were upregulated in response to mechanical force; the highest expression was detected at 24 h,

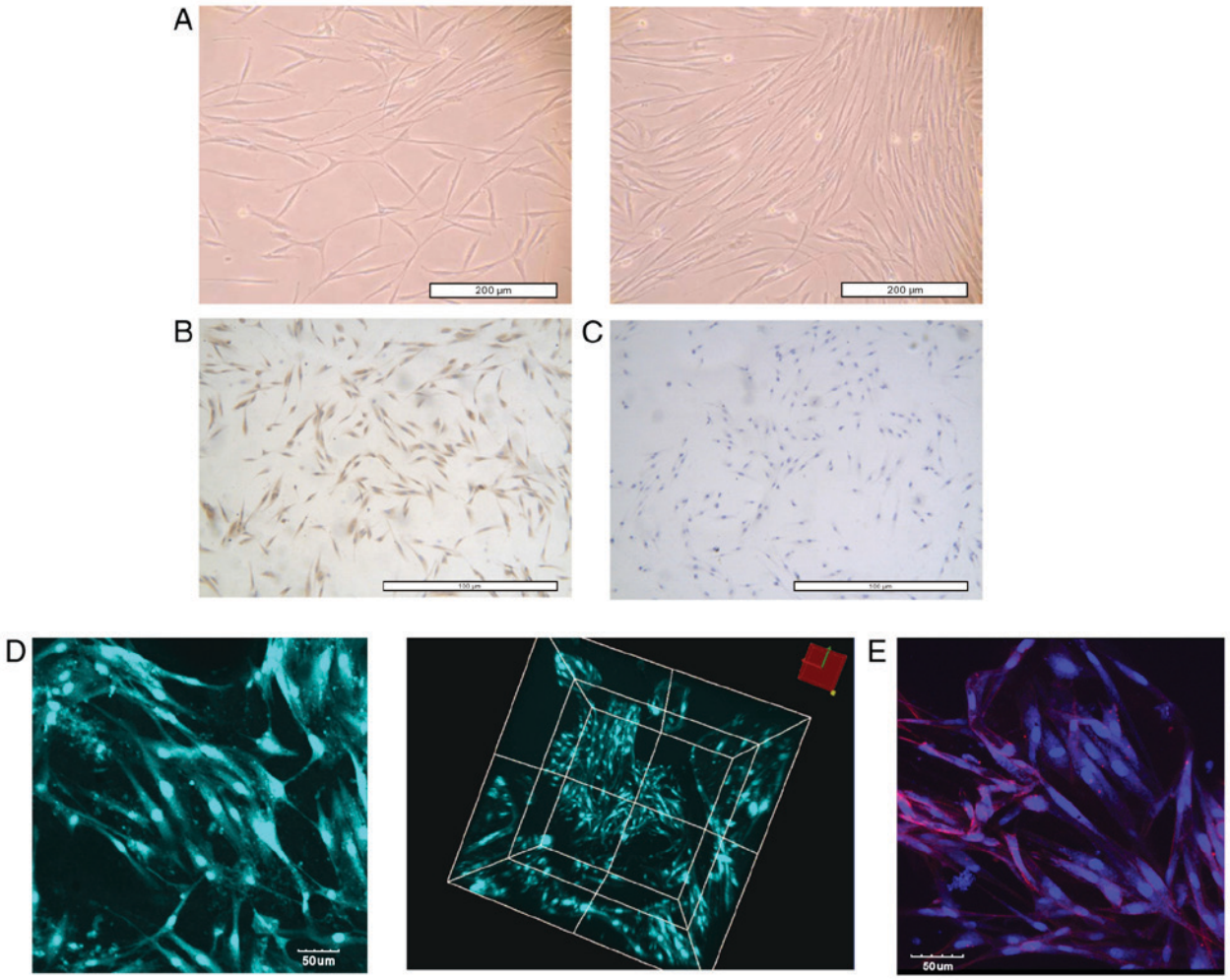

Figure 2. Identification and morphology of HGFs on the 3D PLGA culture model. (A) Representative micrographs of HGFs under an optical microscope. Immunohistochemical staining of (B) vimentin and (C) cytokeratin. (D) Growth mode of HGFs cultured on the 3D PLGA scaffold. (E) F-actin in HGFs was stained with tetramethylrhodamine-phalloidin. HGFs, human gingival fibroblasts; PLGA, poly(lactide-co-glycolide).

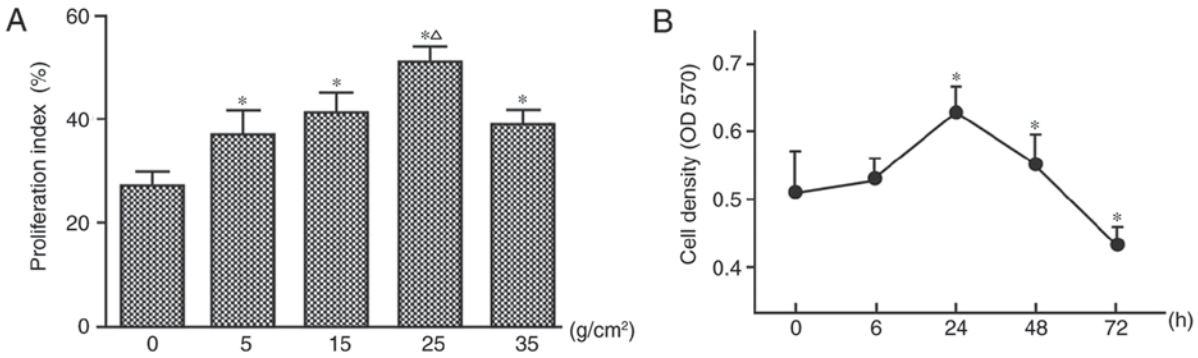

Figure 3. Mechanical force induces the proliferation of HGFs. (A) Proliferation index was calculated by flow cytometry following the application of four different force magnitudes (0, 5, 15, 25 and 35 g/cm^2) for 24 h. (B) HGF proliferation under 25 g/cm^2 for 0, 6, 24, 48 and 72 h, as measured by MTT assay. *P<0.05 vs. the control group; $^\Delta$P<0.05 vs. 5, 15 and 35 g/cm^2. One-way analysis of variance followed by Dunnett's post hoc test was used to analyze data. HGFs, human gingival fibroblasts; OD, optical density.

whereas the expression level was decreased at 48 and 72 h (P<0.05; Fig. 5A). In addition, compared with in the control group, no significant alterations in TGF-β1 protein expression were observed at 6 h; however, protein expression was significantly increased after 24 h, and was decreased over time after this point.

TGF-β1 inhibitor reverses the effects of mechanical force on cell proliferation, and COL-1 and MMP-1 expression in HGFs. To determine whether TGF-β1 mediated the effects of mechanical force on HGF proliferation, as well as COL-1 and MMP-1 expression, cells were treated with the TGF-β1 inhibitor SB431542 at 20 μM. The results indicated that mechanical

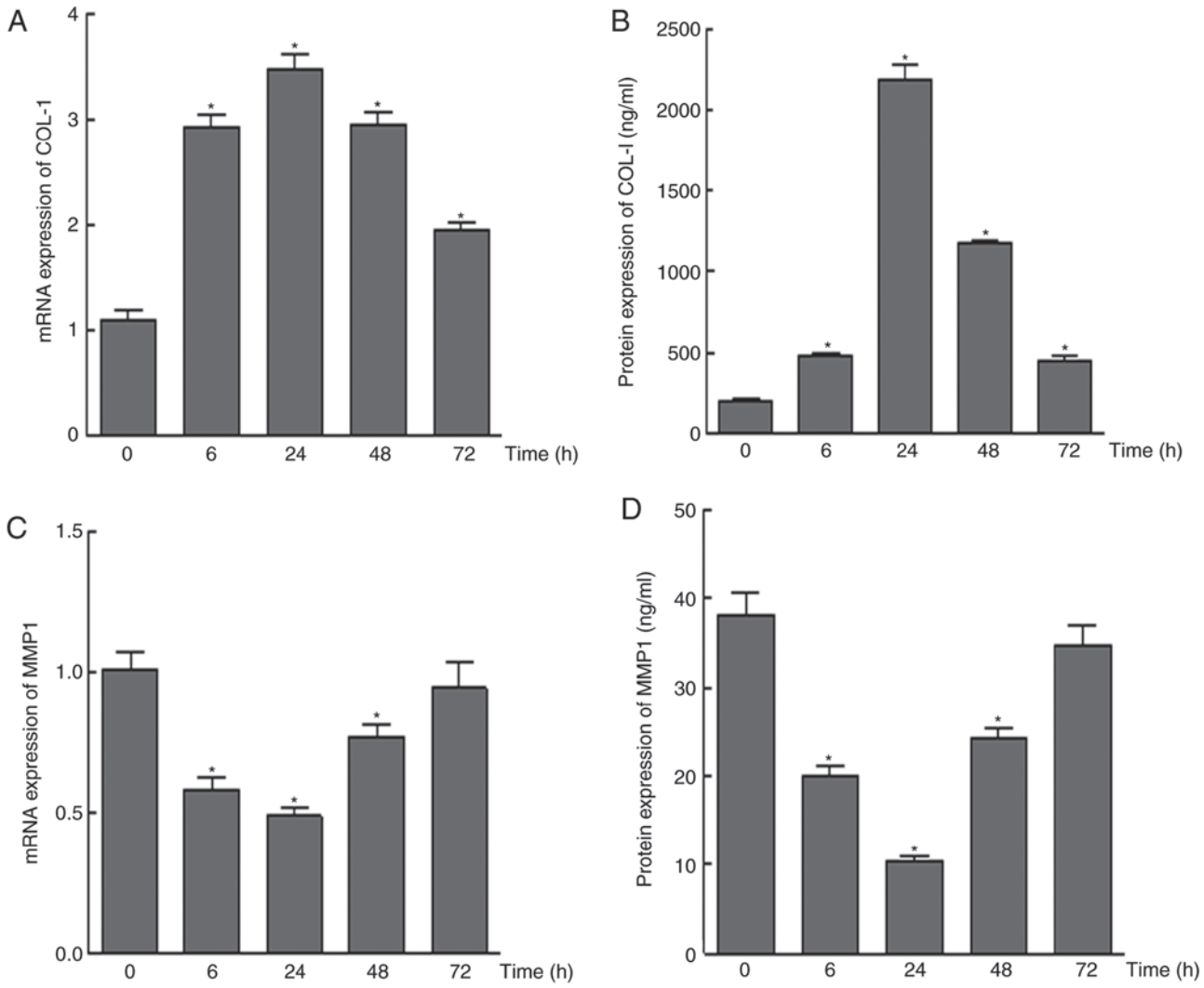

Figure 4. COL-1 and MMP-1 mRNA and protein expression is altered in response to mechanical force in human gingival fibroblasts. mRNA and protein expression of (A and B) COL-1 and (C and D) MMP-1 following the application of mechanical force. *P<0.05 vs. the control group. One-way analysis of variance followed by Dunnett's post hoc test was used to analyze data. COL-1, type I collagen; MMP-1, matrix metallopeptidase.

force-induced upregulation of COL-1 expression and downregulation of MMP-1 expression were reversed in the presence of SB431542 (Fig. 6). These data suggested that TGF-β1 may be a key regulator in the of HGF biological function induced by mechanical force. As shown in Fig. 7, presents the effects of the TGF-β1 inhibitor SB431542 were also determined on cell proliferation. Compared with in the control group, cells only treated with SB431542 for 24 h did not exhibit a significant decrease in cell proliferation (P>0.05). However, continuous stimulation with SB431542 for 24 h significantly decreased mechanical stress-induced cell proliferation compared with in the mechanical stress group (P<0.05).

Discussion

The present study strongly indicated that the application of mechanical force on HGFs cultured on 3D PLGA scaffolds led to a significant increase in cell proliferation and in COL-1 gene expression, as well as a decrease in MMP-1 expression. Furthermore, these effects were mediated, at least partially, via the TGF-β signaling pathway. These findings further elucidated the mechanisms underlying mechanical force-induced HGF proliferation and ECM synthesis, and may aid in the development of targeted therapeutics, in a pre-clinical and clinical setting.

A growing body of evidence has suggested that 3D cell culture systems, in contrast to 2D culture systems, more accurately represent the microenvironment in which cells reside in tissues (16). Therefore, the behavior of 3D-cultured cells is more reflective of the actual *in vivo* cellular responses. Kang *et al* (22) attempted to use collagen gel to establish a 3D culture model of periodontal ligament cells; however, the porosity and pressure resistance, as well as other characteristics of the periodontal ligament are significantly different to those of collagen gel (22). The PLGA used in the present study is a copolymer of polyglycolic acid (PGA) and polylactic acid (PLA), which has the advantages of PGA in terms of cell adhesion, and PLA in terms of mechanical strength (18,23). In addition, it is more consistent with periodontal ligament tissue, regarding its physical characteristics. Furthermore, hypoxia must be considered. In previous studies, four-point bending and Flexcell loading models were used to expose the cells to

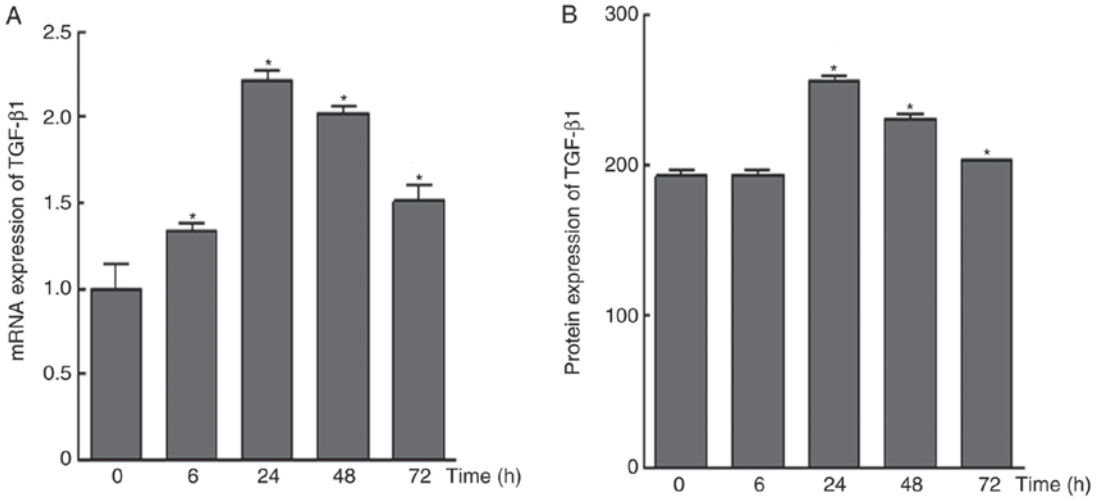

Figure 5. Mechanical force results in elevated TGF-β1 expression in human gingival fibroblasts. Expression levels of TGF-β1 (A) mRNA and (B) protein following the application of 25 g/cm^2 mechanical force for 0, 6, 24, 48 and 72 h. *P<0.05 vs. the control group. One-way analysis of variance followed by Dunnett's post hoc test was used to analyze data. TGF-β1, transforming growth factor-β1.

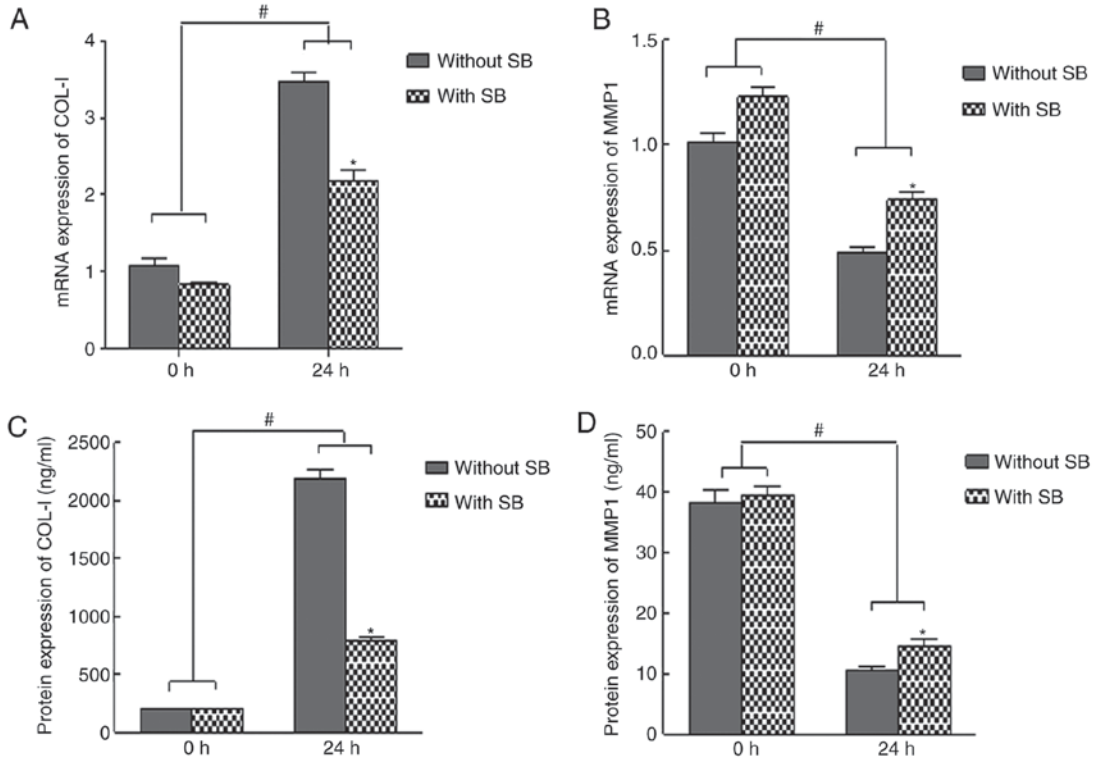

Figure 6. Inhibition of TGF-β prevents the effects of mechanical force on COL-1 and MMP-1 expression in human gingival fibroblasts. Effects of the TGF-β1 inhibitor SB (20 μM) on mechanical force-induced (A) COL-1 and (B) MMP-1 mRNA expression. Effects of SB on (C) COL-1 and (D) MMP-1 protein expression. *P<0.05 vs. cells without SB431542; #P<0.05 vs. the corresponding control group at 0 h. One-way analysis of variance followed by Dunnett's post hoc test was used to analyze data. COL-1, type I collagen; MMP-1, matrix metallopeptidase; SB, TGF-β1 inhibitor SB431542; TGF-β1, transforming growth factor-β1.

aerobic conditions (24). When applying mechanical forces to the teeth, due to vascular atresia, the periodontal ligament cells on the pressure side suffer simultaneously from mechanical and hypoxic stimuli, which interact and promote osteoclast formation, facilitating the absorption of alveolar bone on the pressure side. The combination of these stimuli were demonstrated to be beneficial to orthodontic tooth movement (25,26). In the present study, cells in the central part of the complex were under hypoxic and nutrient deprivation conditions due to limited diffusion following the application of mechanical force. Therefore, the PLGA 3D model simultaneously simulated the effects of mechanical force and the hypoxic environment, providing a solid foundation for subsequent experiments.

Mechanical stimulation has an important function in the improvement of orthodontic tooth movement. Kook *et al* (27,28) demonstrated that periodontal ligament fibroblasts secrete relatively higher levels of tumor necrosis factor-α in the compression side compared with the tension

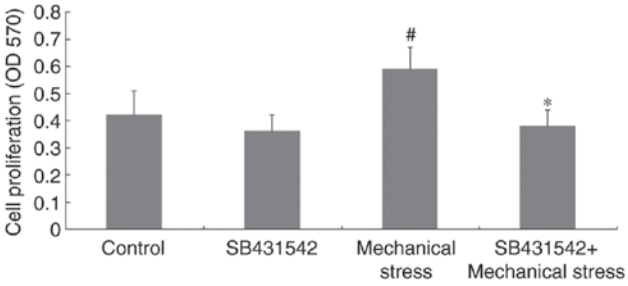

Figure 7. Effects of the TGF-β1 inhibitor SB431542 on cell proliferation *P<0.05 vs. the mechanical stress group; #P<0.05 vs. the control group. Two-way analysis of variance with Bonferroni post hoc test was used to analyze data.

side following application of mechanical stimuli, thereby facilitating bone resorption during orthodontic tooth movement (27,28). Improved simulation of orthodontic treatment *in vitro* is an important branch of cell biomechanics research. The concept of optimal orthodontic force in orthodontic treatment was first described in 1932 by Schwarz (29). The optimal orthodontic force was calculated by analyzing the capillary pressure at the periodontal ligament on the root surface. Notably, periodontal tissue may undergo ischemia and necrosis under high orthodontic force. In the majority of mammals and humans, the force value is 20-26 g/cm^2; therefore, in the present study, force magnitudes of 5, 15, 25 and 35 g/cm^2 were applied. It was revealed that when the force magnitude was 5 and 15 g/cm^2, HGF proliferation was enhanced, and cell proliferation was the highest at 25 g/cm^2. In addition, when the force magnitude reached 35 g/cm^2, HGF proliferation was inhibited. These results were consistent with those of a previous study (30). Thus, 25 g/cm^2 was selected for the subsequent experiments.

Mechanical forces of various magnitudes applied to HGFs affect cell proliferation, function and ECM metabolism through the autocrine or paracrine secretion of various cytokines (31). In the present study, HGF proliferation and COL-1 expression was increased after 6 h and peaked at 24 h under 25 g/cm^2. COL-1 expression began to decrease after 48 h, and cell proliferation activity began to decrease after 72 h. In addition, MMP-1 expression was decreased after 6 h of force application under 25 g/cm^2, and reached a nadir at 24 h. Its expression subsequently increased after 48 h. Previous studies have demonstrated that orthodontic force as an extrinsic mechanical stimulus not only moves the teeth, but also rebuilds equilibrium through evoking a biological cellular response within periodontal tissues (32). The alterations in HGF proliferation by different mechanical forces may have reflected the establishment of a new equilibrium.

TGF-β is expressed in several cell types, including HGFs, and is involved in the proliferation and differentiation of these cells (33,34). The functions of TGF-β are autocrine or paracrine, since they are involved in the regulation of HGFs during oral inflammation as well as wound healing processes at the site of injury (35). Previous studies have evaluated the effects of TGF-β on cell proliferation and collagen metabolism in primary human periodontal ligament cells *in vitro*. It was revealed that TGF-β significantly increases cell proliferation, as well as COL-1 collagen expression (36). Furthermore, Kimoto *et al* (37) focused on the effects of intermittent mechanical strain on the cytokine synthesis of HGFs *in vitro* in a 2D cell culture model, revealing that mechanical stretching induced TGF-β secretion. These findings suggest that HGFs synthesize and secrete TGF-β as autocrine or paracrine factors that affect bone remodeling and root resorption. The levels of these factors are altered in response to mechanical stress. Consistent with previous studies, the results of the present study determined that TGF-β levels were increased 6 h after force application under 25 g/cm^2, whereas TGF-β levels began to decrease after 48 h. The selective TGF-β inhibitor SB431642 prevented the effects of mechanical force on HGF proliferation, upregulation of COL-1 expression and downregulation of MMP-1, indicating that TGF-β served an important role in gingival tissue remodeling mediated by mechanical force. Nevertheless, the effects of TGF-β on HGFs should be further demonstrated using a more rigorous approach that includes genetic knockouts, small interfering RNAs and overexpression vectors. This will be performed in future experiments.

In conclusion, HGFs were cultured on 3D PLGA scaffolds and the results strongly suggested that inhibition or reduction of local TGF-β expression decreased HGF proliferation and COL-1 expression, and increased MMP-1 expression. These effects may lead to an increased degradation of collagen fibrils in the ECM and decreased gingival hyperplasia following orthodontic treatments. The modulation of TGF-β pathway may contribute to the development of novel therapeutic strategies to promote gingival tissue remodeling.

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Availability of data and materials

The datasets used and/or analyzed during the current study are included in this published article.

Authors' contributions

LN conceived and coordinated the study, designed, performed and analyzed the experiments, and wrote the paper. YZ, NL, SL, YW, ZC, LW, SZ and SM carried out data collection, data analysis and manuscript revisions. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the Hospital Institutional Review Board (approval no. 20150304-22) of Guangxi Medical University (Nanning, China). All donors and their guardians signed an informed consent form.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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