

Cadherin-11 modulates cell morphology and collagen synthesis in periodontal ligament cells under mechanical stress

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ABSTRACT

Objective: To examine the role of cadherin-11, an integral membrane adhesion molecule, in periodontal ligament cells (PDLs) under mechanical stimulation.

Materials and Methods: Human PDLs were cultured and subjected to mechanical stress. Cadherin-11 expression and cell morphology of PDLs were investigated via immunofluorescence staining. The mRNA and protein expressions of cadherin-11 and type I collagen (Col-I) of PDLs were evaluated by quantitative real-time polymerase chain reaction and Western blot, respectively. Small interfering RNA was used to knock down cadherin-11 expression in PDLs. The collagen matrix of PDLs was examined using toluidine blue staining.

Results: Cadherin-11 was expressed in PDLs. Mechanical stress suppressed cadherin-11 expression in PDLs with prolonged force treatment time and increased force intensity, accompanied by suppressed β -catenin expression. Simultaneously, mechanical stress altered cell morphology and repressed Col-I expression in a time- and dose-dependent manner in PDLs. Moreover, knockdown of cadherin-11 with suppressed β -catenin expression resulted in altered PDL morphology and repressed collagen expression, which were consistent with the changes observed under mechanical stress.

Conclusions: Results of this study suggest that cadherin-11 is expressed in PDLs and modulates PDL morphology and collagen synthesis in response to mechanical stress, which may play an important role in the homeostasis and remodeling of the PDL under mechanical stimulation. (*Angle Orthod.* 2017;87:193–199)

KEY WORDS: Cadherin-11; β -catenin; Mechanical stress; Periodontal ligament cells; Type I collagen

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INTRODUCTION

The periodontal ligament (PDL) is a specialized connective tissue that attaches a tooth to the alveolar bone. Periodontal ligament cells (PDLs) are the predominant cell type in the PDL and play an important role in PDL homeostasis and remodeling.¹ PDLs are subjected to mechanical stimulation generated by mastication, speech, and orthodontic treatment and can sense and respond to mechanical stress.² Although mechanical stimulation alters biological activities in PDLs,^{3,4} how PDLs respond to mechanical stimulation has not been fully elucidated.

Cadherins mediate calcium-dependent homophilic cell-cell interaction and play critical roles in tissue formation and cell sorting during development.⁵ Cadherin-11 (encoded by the CDH11 gene) is a type II classic cadherin that mediates intercellular adhesion between cells of the same type, which is crucial for tissue morphogenesis and architecture.⁶ Cadherin-11 is mostly expressed in mesenchymal-type cells such as fibroblasts and osteoblasts,^{7,8} which suggests that

cadherin-11 may also be expressed in PDLCs. Mechanical stimulation can regulate cadherin-11 expression in synovial fibroblasts.⁹ However, the response and role of cadherin-11 in PDLCs under mechanical stimulation remain unknown.

Collagen is the main component of the extracellular matrix (ECM) in the PDL.¹⁰ PDLCs secrete ECM components, such as collagen, building up the PDL collagen fibers and having significant function in the remodeling of PDL collagen.¹¹ Collagen expression in cultured PDLCs can be regulated by mechanical stimulation.^{12,13} During orthodontic tooth movement, PDL collagen is degraded in the compression region, with altered expression of type I collagen (Col-I) and cytoskeleton of cells.^{14,15} Hence, the present study aimed to test the hypothesis that cadherin-11 is expressed in PDLCs and modulates cell morphology and collagen synthesis in PDLCs in response to mechanical stress.

MATERIALS AND METHODS

Cell Culture

The protocol used to obtain human tissue samples in this study was approved by the Ethical Guidelines of Peking University (PKUSSIRB-201311103) and was performed with appropriate informed consent. Human PDLCs were isolated from PDLs of normal, orthodontically extracted bicuspid as previously described.¹⁶ The harvested PDLCs were cultured with alpha modification of Eagle's medium containing 15% fetal calf serum (GIBCO, Carlsbad, Calif), 100 U/mL penicillin, and 100 g/mL streptomycin (Biofluids, Rockville, Md) and were used at passage 4. The alignment of cultured PDLCs was observed using a light inverted microscope.

Mechanical stress was applied on cultured PDLCs using the uniform method.^{17,18} In brief, a glass cover and additional metal weights on top were placed over an 80% confluent cell layer in six-well plates. The PDLCs were subjected to different intensities of mechanical stress ranging from 0 to 2 g/cm² for 24 hours or at 1 g/cm² for different time ranging from 0 to 24 hours.

Small Interfering RNA Transfection

Cadherin-11 small interfering RNA (siRNA) and negative control siRNA were purchased from GenePharma (Suzhou, China). The siRNA sequences of CDH11 were as follows: siCDH11 number 1: 5'-AGGAAGUAGGAAGAGUGAAAGCUAA-3'; siCDH11 number 2: 5'-CAUCGUCAUUCUCCUGGUCAUUGUA-3'. The PDLCs were transfected with siRNA by

lipofectamine RNAiMax reagent (Invitrogen, Carlsbad, Calif) according to the manufacturer's protocol.

Immunofluorescence Staining

Immunofluorescence staining was performed as previously described.¹⁹ Briefly, cultured PDLCs were washed with phosphate-buffered saline, fixed using 4% paraformaldehyde fixative, and incubated with cadherin-11 (1:200; Cell Signaling Technology, Danvers, Mass), β -catenin (1:200; Cell Signaling Technology), and phalloidin (1:200; Sigma, Danvers, Mass). The nuclei were counterstained with 4', 6-diamidino-2-phenylindole. Confocal microscopic images were acquired using a Zeiss laser scanning microscope (LSM 510; Jena, Germany), and the images were processed using LSM 5 Release 4.2 software.

Quantitative Real-Time Polymerase Chain Reaction (PCR)

Total RNA was isolated from cells with Trizol reagent (Invitrogen). Reverse transcription and real-time PCR were performed as previously described.¹⁹ The primers designed by Primer Premier 5.0 software and commercially synthesized were as follows: human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) sense/antisense, 5'-ATGGGGAAGGTGAAGGTCG-3'/5'-GGGGTCATTGATGGCAACAATA-3'; human cadherin-11 sense/antisense, 5'-AGAGGTCCAATGTGGGAACG-3'/5'-GGTTGTCCTTCGAGGATACTGT-3'; and human Col-I sense/antisense, 5'-CCAGAA-GAAGTGGTACATCAGCAA-3'/5'-CGCCATACTCGAAGTGAATC-3'. The efficiency of the newly designed primers was confirmed by sequencing. Relative quantification of gene expression was assessed with the comparative cycle threshold method, using GAPDH as internal control.

Western Blot

Western blot was performed as previously described in detail.¹⁹ Antibodies of cadherin-11 (1:1000; Cell Signaling Technology), β -catenin (1:1000; Cell Signaling Technology), Col-I (1:1000; Proteintech, Wuhan, China), and GAPDH (1:1000; Santa Cruz Biotechnology Inc, Santa Cruz, Calif) were used. Each experiment was repeated three times to obtain comparable results.

Toluidine Blue Staining

Cells were fixed in 4% paraformaldehyde and toluidine blue (3%) stain, and were incubated overnight at 4°C. Ethyl alcohol (95%) was subsequently added, excess dye was washed out, then the cells in plates were observed by optical microscope.

Statistical Analysis

Statistical analysis was performed with SPSS 13.0 (IBM Corp, Armonk, NY). Comparisons among groups were statistically analyzed by one-way analysis of variance, followed by the least significant, difference multiple-comparison test. All data were presented as mean \pm SD. Statistical significance was considered at $P < .05$.

RESULTS

Cadherin-11 and β -Catenin Expressions in PDLCs Were Suppressed Under Mechanical Stress

Cadherin-11 plays an important role in sensing and transmitting force into the cell interior and interacts with β -catenin to regulate cell functions.²⁰ Immunofluorescence showed that cadherin-11 was expressed in PDLCs and β -catenin located in both cytoplasm and nucleus. Under mechanical stress (1 g/cm^2) for 24 hours, the expressions of cadherin-11 and β -catenin were suppressed, and β -catenin activity in the nucleus was prominently reduced (Figure 1A).

The mRNA and protein expressions of cadherin-11 in PDLCs were suppressed with prolonged stress treatment time (4, 8, 12, and 24 hours) at an intensity of 1 g/cm^2 , accompanied by suppressed β -catenin protein expression (Figure 1B,C). In addition, the suppressed mRNA and protein expressions of cadherin-11 expression were observed at an intensity of 0.5 g/cm^2 and further decreased at intensities of 1, 1.5, and 2 g/cm^2 of mechanical stress, accompanied by suppressed β -catenin protein expression (Figure 1D,E). These results suggest that cadherin-11 is expressed in PDLCs and that PDLCs respond to mechanical stress with a suppressed cadherin-11/ β -catenin pathway.

Mechanical Stress Altered PDLC Morphology and Repressed Col-I Expression

To investigate the function of mechanical stress-suppressed cadherin-11 on PDLCs, we observed the morphology and cytoskeleton organization of PDLCs under mechanical stress. Under mechanical stress (1 g/cm^2) for 24 hours, PDLCs displayed less aligned and loose cell-cell conjunction (Figure 2A) and appeared significantly elongated morphologically with denser F-actin distribution (Figure 2B), compared with PDLCs from the control group.

Apart from morphological changes, application of mechanical stimulation also affected a variety of cellular processes in PDLCs.²¹ Col-I is the major component of the PDL.²² We found that mRNA and protein expressions of Col-I in PDLCs were repressed by mechanical stress with prolonged stress treatment time (4, 8, 12, and 24 hours) and various stress

intensities (0.5 g/cm^2 , 1 g/cm^2 , 1.5 g/cm^2 , and 2 g/cm^2) (Figure 3), which were consistent with the suppressed expression of cadherin-11 in response to mechanical stress. These data indicate that mechanical stress-suppressed cadherin-11 could alter cell morphology and repress collagen synthesis in PDLCs.

Knockdown of CDH11 Altered Cell Morphology and Repressed Col-I Expression in PDLCs

To confirm whether the suppressed cadherin-11 contributed to the altered PDL morphology and repressed collagen expression under mechanical stress, cadherin-11 expression in PDLCs was knocked down using CDH11-targeting siRNA. Two siCDH11 sequences were used to confirm the efficacy of siCDH11 transfection. Compared with negative control, PDLCs transfected with siCDH11 sequences resulted in significant suppression of cadherin-11 at both the mRNA (Figure 4A) and protein levels (Figure 4B). β -Catenin protein expression was also suppressed (Figure 4B).

Knockdown of cadherin-11 expression in PDLCs resulted in elongated cell morphology and denser F-actin distribution, which were consistent with the changes produced by mechanical stress (Figure 4C). Furthermore, Col-I expression in PDLCs was repressed after depletion of cadherin-11 at the mRNA (Figure 4D) and protein levels (Figure 4E) and at the extracellular collagen matrix (Figure 4F), which were also consistent with the effect of mechanical stress. These data suggest that cadherin-11 could modulate cell morphology and collagen synthesis in PDLCs.

DISCUSSION

In the present study, we were the first to demonstrate that cadherin-11 modulates cell morphology and collagen synthesis of PDLCs in response to mechanical stress. First, cadherin-11 was expressed in PDLCs, and the expressions of cadherin-11 and β -catenin were suppressed by mechanical stress with prolonged stress treatment time and increased stress intensity. Second, mechanical stress altered PDL morphology and repressed collagen expression in time- and dose-dependent manners. Moreover, knockdown of cadherin-11 expression in PDLCs resulted in altered cell morphology and repressed collagen expression, which were consistent with the changes produced by mechanical stress in PDLCs. These data indicate that mechanical stress suppresses the cadherin-11/ β -catenin pathway in PDLCs, which may contribute to altered PDL morphology and repressed collagen synthesis.

Cadherins are a large superfamily of calcium-dependent adhesion proteins mediating cell-cell cohe-

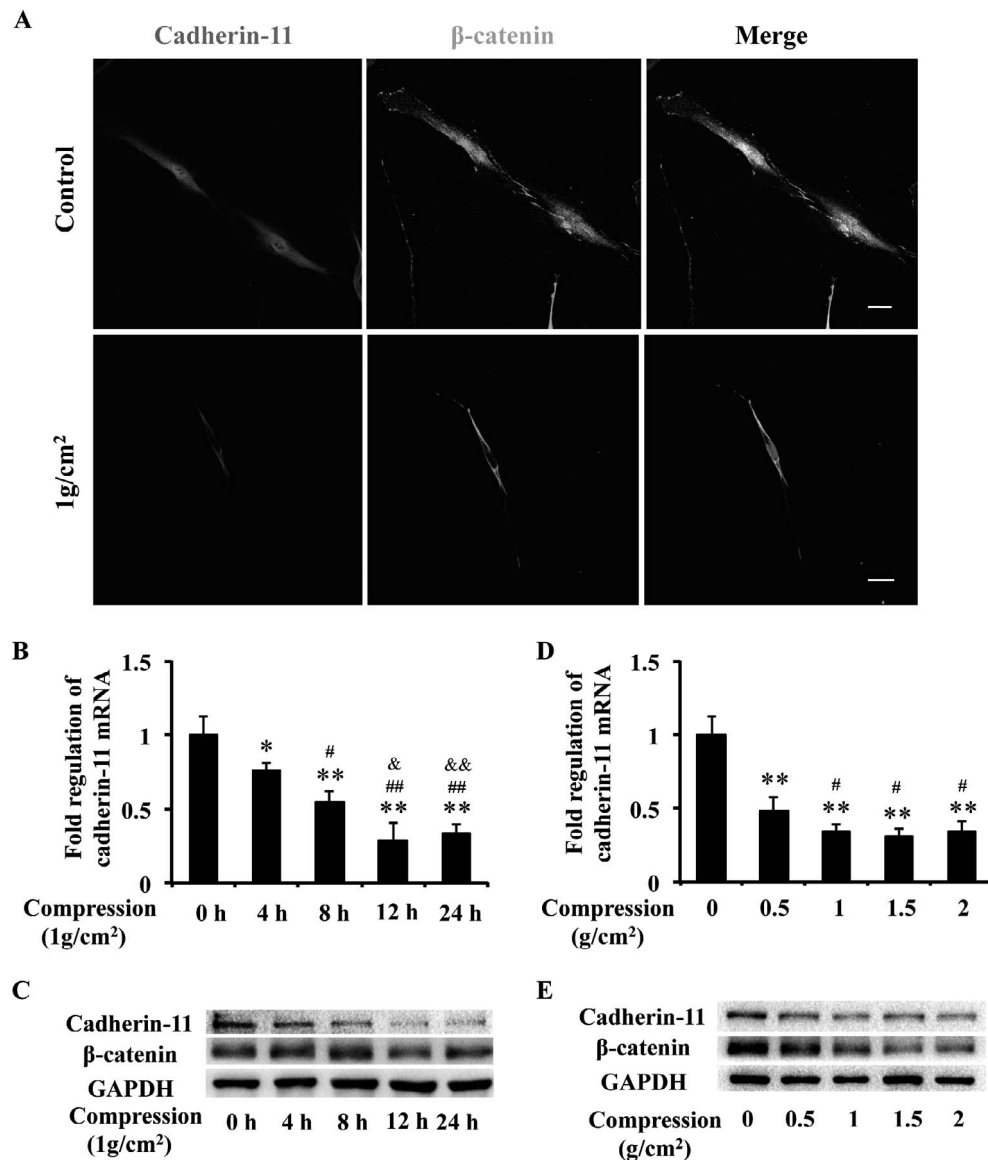


Figure 1. Cadherin-11 and β -catenin expressions in PDLCs were suppressed under mechanical stress. (A) Immunofluorescence staining showed that cadherin-11 (red) was expressed in PDLCs and β -catenin (green) located in both cytoplasm and nucleus. Under mechanical stress, the expression of cadherin-11 (red) and β -catenin (green) was suppressed, and β -catenin (green) activity in the nucleus prominently reduced. Scale bars: 20 μ m. (B, C) The mRNA and protein expressions of cadherin-11 and protein expression of β -catenin in PDLCs were suppressed with prolonged stress treatment time, examined by real-time PCR (B) and Western blot (C). * $P = .05$; ** $P = .01$ versus 0-hour group; # $P = .05$ ## $P = .01$ vs 4-hour group; & $P = .05$, && $P = .01$ vs 8-hour group. (D, E) The mRNA and protein expressions of cadherin-11 and protein expression of β -catenin in PDLCs were suppressed under mechanical stress with variable intensities, examined by real-time PCR (D) and Western blot (E). ** $P = .01$ vs 0 g group; * $P = .05$ vs 0.5-g group.

sion in soft tissues.⁵ Moreover, cadherin complexes are force sensors that transduce fluctuations in intercellular tension into intracellular signals to regulate tissue remodeling.²⁰ A previous study has shown that hydrostatic pressure increased cadherin-11 expression in synovial fibroblasts.⁹ In the present study, the PDLCs were subjected to mechanical stress, and cadherin-11 expression was significantly suppressed, accompanied by suppressed β -catenin expression. β -Catenin is recruited directly to the cadherin cytoplasmic

tail, functioning as a signal transducer in the Wnt signaling pathway.^{23,24} Wnt/ β -catenin signaling is required for mechanotransduction in bone.²⁵ Mechanical strain and fluid shear stress induce nuclear translocation of β -catenin in calvarial osteoblasts and osteocytes.^{26,27} Our findings, for the first time, reported that cadherin-11 was expressed in PDLCs and suggest that cadherin-11/ β -catenin axis might play an important role in the signal transduction of mechanical stimulation to biological function in PDLCs.

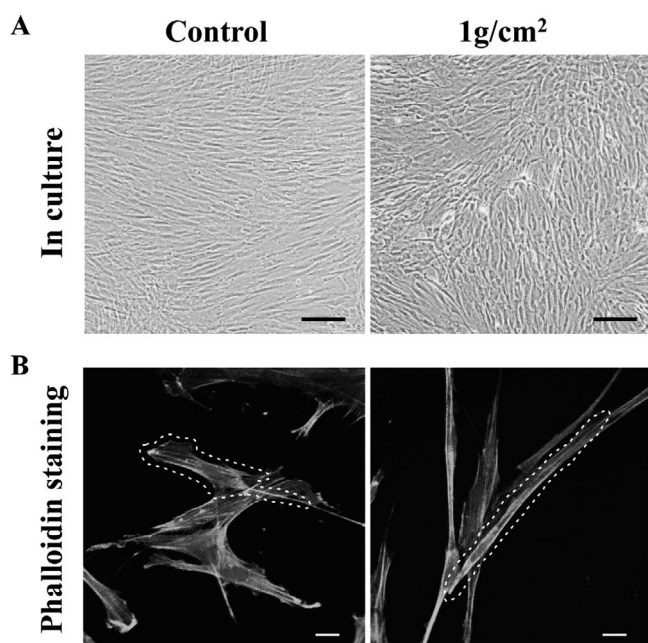


Figure 2. Mechanical stress altered cell alignment (A) and morphology (B) in PDLCs. (A) Scale bars: 200 μm . (B) Scale bars: 20 μm .

Cadherin-11 regulated PDLC morphology under mechanical stress. Actin cytoskeleton can be coupled to sites of adherens junctions, and they produce force to generate changes in cell shape and organization.⁵ In this study, mechanical stress resulted in loose cell-cell conjunctions, elongated morphology, and denser F-actin distribution in PDLCs, accompanied by the suppressed expressions of cadherin-11 and β -catenin. A previous study has shown that compressive force reduced the F-actin level, leading to PDLC spreading,²⁸ which is

consistent with our study. The cadherin-catenin complex plays important roles in regulating the rearrangement of actin cytoskeleton and controlling tissue morphogenesis.²⁹ Our results further showed that knockdown of cadherin-11 expression resulted in altered PDLC morphology, which was consistent with the changes produced by the application of mechanical stress. These findings suggest that the suppressed cadherin-11/ β -catenin pathway under mechanical stress is not only a concomitant phenomenon but also may be the cause of altered morphology of the PDLCs, and indicate that cadherin-11 might be involved in the architecture of PDL by regulating the morphology of PDLCs.

Changes in the cellular environment can affect adhesion complexes, modify the actin organization, and then initiate downstream biosynthetic responses. The primary function of PDLCs is elaboration of collagen-predominant ECM. Our study showed that mechanical stress suppressed cadherin-11 and Col-I expression in PDLCs. Moreover, knockdown of cadherin-11 expression resulted in repressed PDLC collagen expression. A previous study has shown that cadherin-11 was necessary for cell-cell adhesion and matrix production of the synovial lining.³⁰ Additionally, knockdown of cadherin-11 in tendons results in cell separation and misalignment of the collagen fibrils in the tendon ECM.³¹ Our results are consistent with these previous studies. A previous study has shown that PDL undergoes degradation and PDLCs lose their regular arrangement on the compression side under orthodontic force.³² Our study might reveal a novel mechanism by which cadherin-11 modulates PDLC morphology and collagen synthesis under mechanical stimulation.

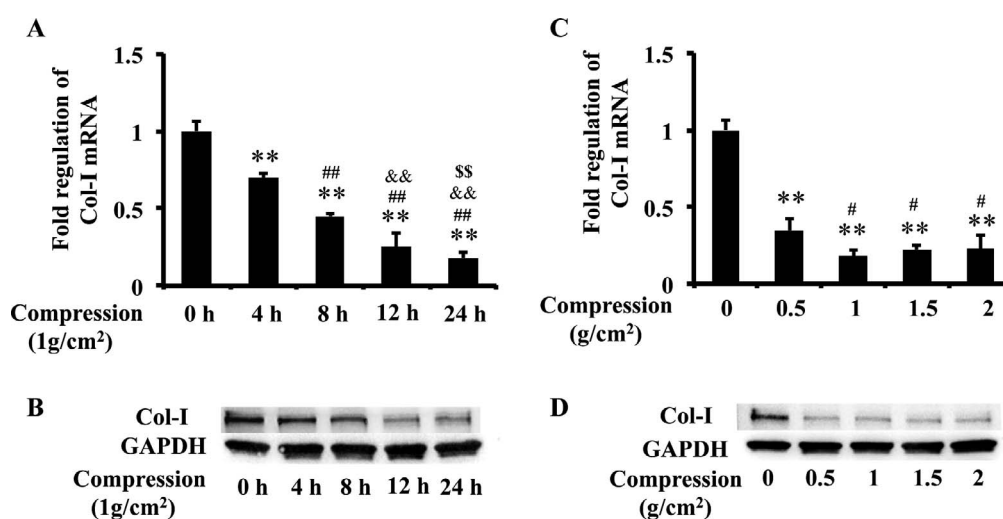


Figure 3. Mechanical stress repressed Col-I expression in PDLCs. (A, B) The mRNA and protein expressions of Col-I in PDLCs were repressed with prolonged stress treatment time, examined by real-time PCR (A) and Western blot (B). ** $P = .01$ vs 0-hour group; ## $P = .01$ vs 4-hour group; && $P = .01$ vs 8-hour group; \$\$ $P = .01$ vs 12-hour group. (C, D) The mRNA and protein expressions of Col-I in PDLCs were repressed under mechanical stress with variable intensities, examined by real-time PCR (C) and western blot (D). ** $P = .01$ vs 0-g group; # $P = .05$ vs 0.5-g group.

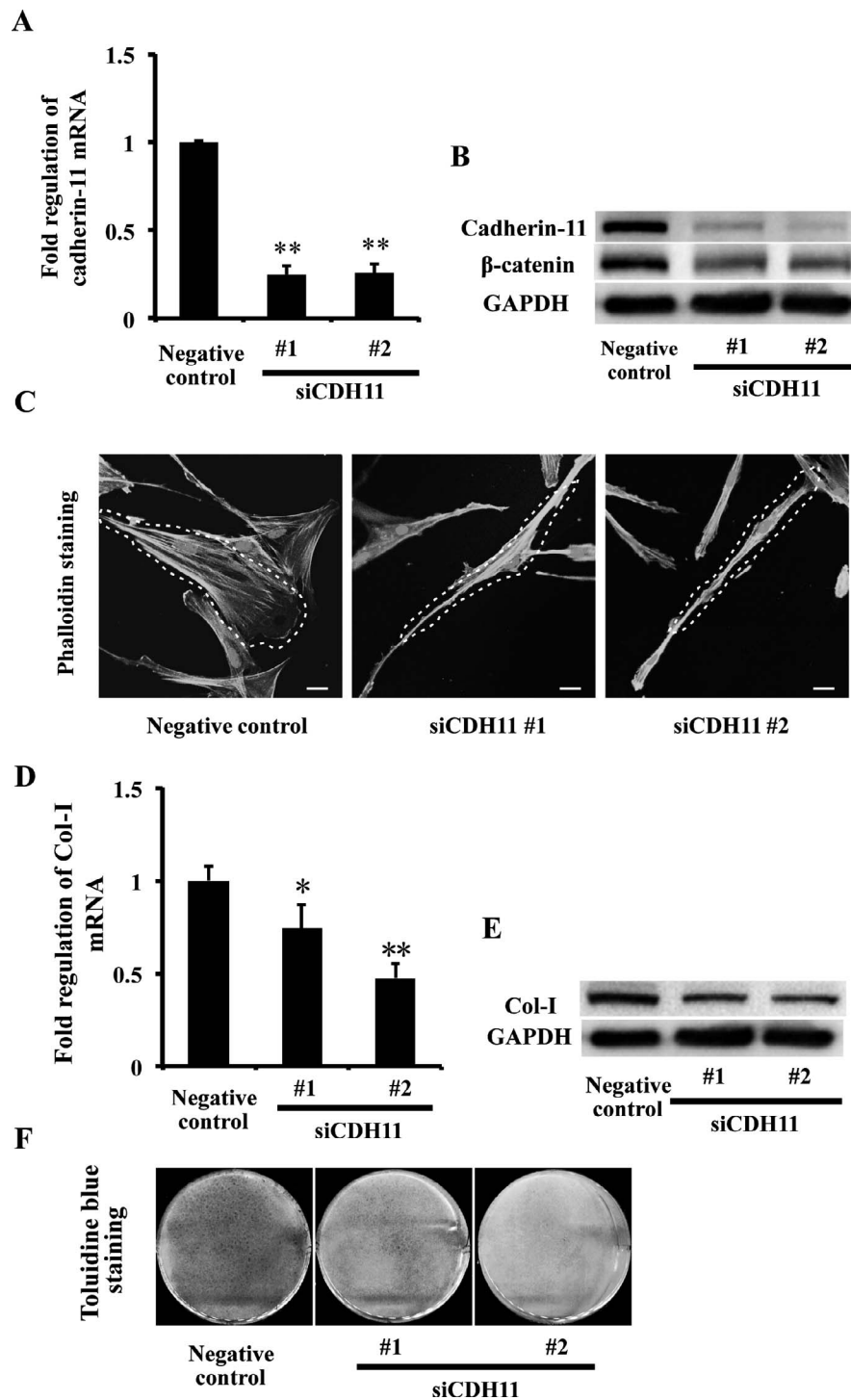


Figure 4. Knockdown of CDH11 altered PDLC morphology and repressed Col-I expression. (A, B) The mRNA and protein expressions of cadherin-11 and protein expression of β-catenin in PDLCs were suppressed in the siCDH11 No.1 and siCDH11 No. 2 groups, examined by real-time PCR (A) and Western blot (B). ** $P = .01$ vs negative control group. (C) Phalloidin staining showed morphology of PDLCs in negative control, siCDH11 No.1, and siCDH11 No. 2 groups. Scale bars: 20 μm. (D, E) The mRNA and protein expressions of Col-I in PDLCs were repressed in the siCDH11 No.1 and siCDH11 No. 2 groups, examined by real-time PCR (D) and Western blot (E). * $P = .05$, ** $P = .01$ vs negative control group. (F) Toluidine blue staining showed that collagen matrix of PDLCs was repressed in the siCDH11 No.1 and siCDH11 No. 2 groups compared with the negative control group.

CONCLUSIONS

- For the first time, our results demonstrate that cadherin-11 is expressed in PDL cells and modulates PDL cell morphology and collagen synthesis in response to mechanical stress, which provides a new understanding of how PDL cells respond to mechanical stimulation during physiological activity.

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