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Effects of 17β-Estradiol combined with cyclical compressive stress on the proliferation and differentiation of mandibular condylar chondrocytes

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ABSTRACT

Objective: The aim of this study is to investigate the effects of 17β-Estradiol (E2) at different concentrations combined with cyclical compressive stress on the proliferation and differentiation of mandibular condylar chondrocytes (MCCs).

Design: MCCs, isolated from female Sprague-Dawley rats, were exposed to E2 at different concentration, cyclical compressive stress or the combination, effects of which on MCCs proliferation and differentiation were detected.

Results: E2 at physiological concentration (10^{-9} mol/L) has lower proliferative effects on MCCs, compared with non-physiological concentration (10^{-12} mol/L or 10^{-6} mol/L). For MCCs differentiation, effects of E2 at different concentration are totally opposite: E2 at 10^{-9} mol/L promotes MCCs differentiation, but at 10^{-12} mol/L or 10^{-6} mol/L, it inhibits MCCs differentiation. When combined with E2 at 10^{-9} mol/L, cyclical compressive stress shows synergistic effect on proliferation and differentiation. However, when combined with E2 at 10^{-12} mol/L or 10^{-6} mol/L cyclical compressive stress reverses the inhibition in MCCs differentiation provoked by E2 at 10^{-12} mol/L or 10^{-6} mol/L.

Conclusion: Effects of E2 combined with cyclical compressive stress on MCCs proliferation and differentiation are different, which suggests that orthodontist should take fully consideration of the levels of E2 and adopt comprehensive strategies, so as to achieve better orthodontic effect.

1. Introduction

The temporomandibular joint, unlike other joints in our body, always retains its strong life-long remodeling capabilities. The fibrocartilage that covers the surface of the temporomandibular joint is the basis for this ability (Tabeian et al., 2019). Physiological stimulation can lead to adaptive remodeling of the condylar cartilage and a wide range of biological effects, which are favorable for orthodontic treatment. However, abnormal stimulation leads to degeneration of the condylar cartilage (Shen & Darendeliler, 2005; Sriram, Jones, Alatli-Burt, & Darendeliler, 2009). Therefore, understanding the factors causing the condylar cartilage remodeling and its possible mechanisms are crucial for orthodontics. Previous studies have shown that the temporomandibular joint disorders and even progressive absorption often occur at puberty and menopause (Paulsen, 1997; Tashiro, Nishida, & Bereiter, 2015). The incidence in females is higher than that in males, which suggests that there may be a link between changes in endogenous estrogen and the onset of the temporomandibular joint disease (Bellinger et al., 2007; Lee, Kim, Kang, & Kim, 2006). Further studies have reported that the concentrations of E2 in the serum were significantly higher in patients with the temporomandibular joint disorder (including disc displacement, arthralgia, osteoarthritis, and joint absorption) than in healthy controls (Landi et al., 2005).

On the other hand, the condylar cartilage that carries and transfers the loads from the joints are often stimulated by direct or indirect mechanical stress, such as twin-block, flat bite plate, chincup and other devices (Sun et al., 2017). Some clinical studies reveal that if the applied stress is in the physiological range, it induces adaptive

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reconstruction of the condylar cartilage. In contrast, if it exceeds the physiological range, it will cause the degeneration of the condylar cartilage (Ghislanzoni, Toll, Defraia, Baccetti, & Franchi, 2011). Elder et al demonstrated that intermittent hydrodynamic pressures of a 7–10 MPa application increased the synthesis of the extracellular matrix of MCCs and the expression of collagen II and proteoglycans. However, the effect of the continuous application of this force is reversed (Elder & Athanasiou, 2009).

The above results indicate that the level of estrogen or compressive stress have important effects on MCCs proliferation and differentiation, but the effects of the combination of both on MCCs are rarely reported. In this study, we investigated the effects of estrogen combined with cyclical compressive stress on the proliferation and differentiation of MCCs in order to confirm whether they have a biological integration effect on cartilage tissue.

2. Materials and methods

2.1. MCCs isolation and culture

Female Sprague-Dawley rats, 4–6 days after birth, were sacrificed by neck dislocation and were soaked in 75% ethanol for 10 min. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki with the approval of the Institutional Medical Ethics Committee of the Third Affiliated Hospital of Sun Yat-sen University (Guangzhou, China). Cartilage from bilateral mandibular condyles was separated and cut into 1 mm × 1 mm × 1 mm pieces. We then digested the cartilage using 0.25% trypsin (Thermo Fisher Scientific, Inc., Waltham, MA, USA) at 37 °C for 30 min followed by centrifugation for 5 min at 1000 r/min (the rotor radius is 16.1 cm). Then, 0.2% type II collagenase (Thermo Fisher Scientific, Inc.) was used for digestion at 37℃ for 4 h followed by centrifugation for 3 min at 1000 r/min (the rotor radius is 16.1 cm). After washing with phosphate-buffered saline (Thermo Fisher Scientific, Inc.) 3 times, the cells were seeded in the culture dishes with Dulbecco's Modified Eagle's Medium (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) containing 10% fetal bovine serum (Thermo Fisher Scientific, Inc.), 100 IU/mL penicillin and 100 μg/mL streptomycin, and then, they were cultured at 37 °C in a humid atmosphere containing 5% CO_2 and 95% air. MCCs at passage 2 were used for further experiments.

2.2. E2 and cyclical compressive stress application

The second passaged MCCs were seeded on the force-applying plate (Chengdu MIRACLE Technology, China) and were cultured in Dulbecco's modification of Eagle's medium Dulbecco containing E2 at different concentrations (including 10^{-12} mol/L, 10^{-9} mol/L or 10^{-6} mol/L) for 24 h. Then, 0.5 Hz 2000 μstrain cyclical compressive stress was applied to the cells for 2 h as described.

2.3. Cell counting Kit-8 assay

Cell proliferation was tested using cell counting Kit-8 (DOJINDO, Japan) according to the manufacturer's instructions. In brief, cells were digested by trypsin and seeded on 96-well plates at a density of 5000 cells per well. After they were incubated in an incubator containing 5% CO_2 at 37℃ for 24 h, the former medium was replaced by 100 μL serum-free Dulbecco's modification of Eagle's medium Dulbecco and 10 μL cell counting Kit-8 solution. The cells were incubated for 2 h followed by determination of absorbance at 450 nm (BioTek Instruments, Inc. Winooski, VT, USA).

2.4. Flow cytometry

Cell cycles were tested using a Cell Cycle Analysis Kit (Cat. KGA511, Keygen Biotech, China). After treatment, the cells were trypsinized and collected followed by centrifugation at 1000 r/min for 5 min (the rotor radius is 16.1 cm). Following phosphate-buffered saline washes, the cells were fixed using 70% ethanol at 4℃ overnight. After centrifugation and discarding the ethanol, the cells were stained with staining solution containing 100 μL of RNase A and 400 μL of Propidium Iodide at 4℃ for 30 min followed by an analysis by flow cytometry (Becton, Dickinson and Company, Franklin Lakes, NJ, USA).

2.5. Alkaline phosphatase activity assay

After the different treatments, the cells were washed with phosphate-buffered saline three times. The cells were treated with 70 μL of cell lysis buffer and were then collected followed by centrifugation at 14,000 r/min at 4℃ for 10 min (the rotor radius is 9.5 cm). A Bicinchoninic Acid protein assay kit (Thermo Fisher Scientific, Inc.) was used to detect the protein samples from each sample according to the manufacturer's instructions, and after that, the protein concentration was normalized using protein lysis buffer. Finally, cellular alkaline phosphatase activity was measured using an Alkaline Phosphatase Assay kit (Abcam, Cambridge, UK). The absorbance, at 405 nm, was determined by the Spectrum (Bio-Rad, Hercules, CA, USA).

2.6. Quantitative real-time PCR

Total RNA was extracted using 1 mL TRIzol, followed by RNA quality and quantity detection using a nanodrop spectrophotometer (ND-1000, Nanodrop Technologies, CA, USA). The RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA) was used to reverse transcribe the total RNA into cDNA according to the manufacturer's instructions. The RT-PCR was performed using Platinum™ SYBR™ Green qPCR SuperMix-UDG (Invitrogen, USA) according to the manufacturer's instructions. All the primers were designed and verified by our group, which are listed as following: Sox9 (F: 5'-CCAGAGAACGCACATCAAGA-3'; R: 5'-TCTGGTGGTCGGTGTAGTCA-3'), Col2a1 (F: 5'-CTCAAGTCGC TGAACAACCA-3'; R: 5'-GTCTCCGCTCTTCCACTCTG-3'), Runx2 (F: 5'-GCCGGGAATGATGAGAACTA-3'; R: 5'-TGGGGAGGATTTGTGAAGAC-3'), Col10a1 (F: 5'-GTCCAAGAGGTGAACCTGGA-3'; R: 5'-TGTTACCCC CTGGTTAGCAC-3'), GAPDH (F: 5'-ATGACTCTACCCACGGCAAG-3'; R: 5'-TACTCAGCACCAGCATCACC-3'). All the RNA levels were normalized to the level of GAPDH.

2.7. Statistical analysis

All the experiments were performed at least three times independently. All the data are presented as the mean ± standard error. All statistical analyses were conducted by Statistical Product and Service Solutions (SPSS, version 18.0) using one-way analysis of variance or two-way analysis of variance with Bonferroni's posttests. $P < 0.05$ was considered a statistically significant difference.

3. Results

3.1. Effects of different concentrations of E2 combined with cyclical compressive stress on MCCs proliferation

Effects of different concentration of E2 on MCCs proliferation are different, especially when it is outside the range of physiological concentration (M. C. Ng, Harper, Le, & Wong, 1999). Fig. 1A shows that 10^{-9} mol/L E2 (the physiological concentration is 10^{-10}-10^{-8} mol/L E2) has lower effects on MCCs proliferation, compared with the concentration of 10^{-12} mol/L or 10^{-6} mol/L (outside of the physiological concentration). When combined with cyclical compressive stress, the effects of E2 on MCCs proliferation between 10^{-9} mol/L and 10^{-12} mol/L or 10^{-6} mol/L were different. Although, cyclical compressive stress could promote MCCs proliferation obviously, it could not further elevate MCCs proliferation when combined with E2 at 10^{-12} mol/L or 10^{-6}

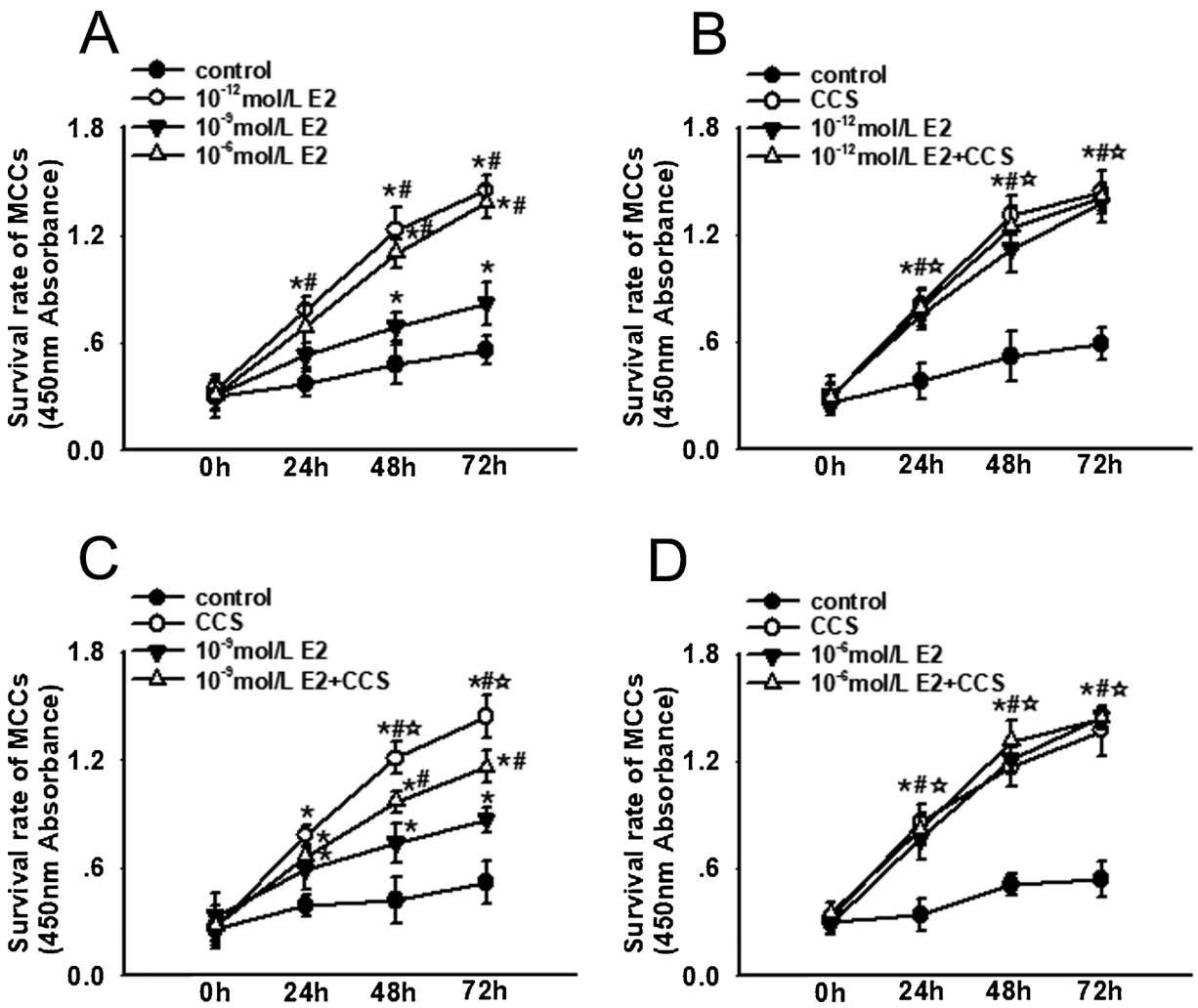


Fig. 1. Effects of different concentrations of E2 combined with cyclical compressive stress on MCCs proliferation.
(A) Effects of different concentrations of E2 on MCCs proliferation at 0 h, 24 h, 48 h and 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-9} mol/L E2 group. (B) Effects of 10^{-12} mol/L E2 combined with CCS on MCCs proliferation at 0 h, 24 h, 48 h and 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ (CCS group *vs* control group); $\#P < 0.05$ (10^{-12} mol/L E2 group *vs* control group); $\star P < 0.05$ (10^{-12} mol/L E2 + CCS group *vs* control group). (C) Effects of 10^{-9} mol/L E2 combined with CCS on MCCs proliferation at 0 h, 24 h, 48 h and 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-9} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-9} mol/L E2 + CCS group. (D) Effects of 10^{-6} mol/L E2 combined with CCS on MCCs proliferation at 0 h, 24 h, 48 h and 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ (CCS group *vs* control group); $\#P < 0.05$ (10^{-6} mol/L E2 group *vs* control group); $\star P < 0.05$ (10^{-6} mol/L E2 + CCS group *vs* control group). MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol.

mol/L (Fig. 1B and D). In contrast, the combination of 10^{-9} mol/L E2 and cyclical compressive stress could further increase MCCs proliferation, compared with E2 group, but diminish effects of cyclical compressive stress on MCCs proliferation (Fig. 1C).

3.2. Effects of different concentrations of E2 combined with cyclical compressive stress on the cell cycle of MCCs

We noticed that from the time point of 48 h, effects of E2 at 10^{-9} mol/L and 10^{-12} mol/L or 10^{-6} mol/L combined with cyclical compressive stress on MCCs proliferation were different. In order to confirm their effects on MCCs proliferation, we tested the cell cycles of MCCs. Table 1 showed that the changes of cell cycles of MCCs were coincident with the results of MCCs proliferation (Fig. 1). Table 1 showed that E2 (at 10^{-9} mol/L and 10^{-12} mol/L or 10^{-6} mol/L) could prompt MCCs enter into the S or G2/M phase, manifested as the proportion of MCCs in the G0/G1 phase was reduced, but that in S and G2/M phase was increased. The proliferation promoting effect of 10^{-9} mol/L was less than 10^{-12} mol/L or 10^{-6} mol/L. cyclical compressive stress could enhance effects of E2 on MCCs proliferation when E2 was at 10^{-9} mol/L, but had no enhancement on MCCs proliferation when E2 was at 10^{-12} mol/L or 10^{-6} mol/L. The results of proliferation index were coincident with the changes of cell cycles (Table 1 and Fig. 2).

3.3. Effects of different concentrations of E2 combined with cyclical compressive stress on MCCs differentiation

Alkaline phosphatase activity is a sensitive indicator of early bone formation, which could reflect MCCs differentiation to some extent (O'Grady & Morgan, 2019). Fig. 3A showed that E2 at 10^{-9} mol/L could increase the alkaline phosphatase activity of MCCs obviously, but at 10^{-12} mol/L or 10^{-6} mol/L, it decreased the alkaline phosphatase activity of MCCs instead. When combined with cyclical compressive stress, inhibitory effects of E2 at 10^{-12} mol/L or 10^{-6} mol/L could be reversed (Fig. 3B and D). The combination of cyclical compressive stress and E2 at 10^{-9} mol/L manifested as synergy: alkaline phosphatase activity of MCCs was much higher than cyclical compressive stress or E2 at 10^{-9} mol/L respectively (Fig. 3C).

Table 1
Changes of cell cycles of MCCs.

Groups	G0/G1 phase	S phase	G2/M phase	PI
control	77.90 ± 0.09	11.88 ± 0.27	10.22 ± 0.21	22.10 ± 0.09
10^{-12} mol/L E2	72.85 ± 0.25*#	16.00 ± 0.21*#	11.15 ± 0.29*	27.15 ± 0.25*#
10^{-9} mol/L E2	76.44 ± 0.49*	12.26 ± 0.41*	11.3 ± 0.58*	23.56 ± 0.49*
10^{-6} mol/L E2	70.85 ± 0.13*#	16.82 ± 0.10*#	12.33 ± 0.14*#	29.15 ± 0.13*#
CCS	72.15 ± 0.27*	15.40 ± 0.14*	11.45 ± 0.23	27.85 ± 0.27*
10^{-6} mol/L E2 + CCS	72.33 ± 0.29*⋆	15.84 ± 0.40*	11.83 ± 0.33	27.67 ± 0.29*⋆
10^{-9} mol/L E2 + CCS	74.95 ± 0.04*	15.63 ± 0.09*	11.42 ± 0.11	25.05 ± 0.04*
10^{-12} mol/L E2 + CCS	72.78 ± 0.19*⋆	15.54 ± 0.16*	11.68 ± 0.17	27.22 ± 0.19*⋆

Effects of different concentrations of E2 on MCCs cell cycle at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$ *vs* 10^{-9} mol/L E2 group; ⋆$P < 0.05$ *vs* 10^{-9} mol/L E2 + CCS group. MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol.

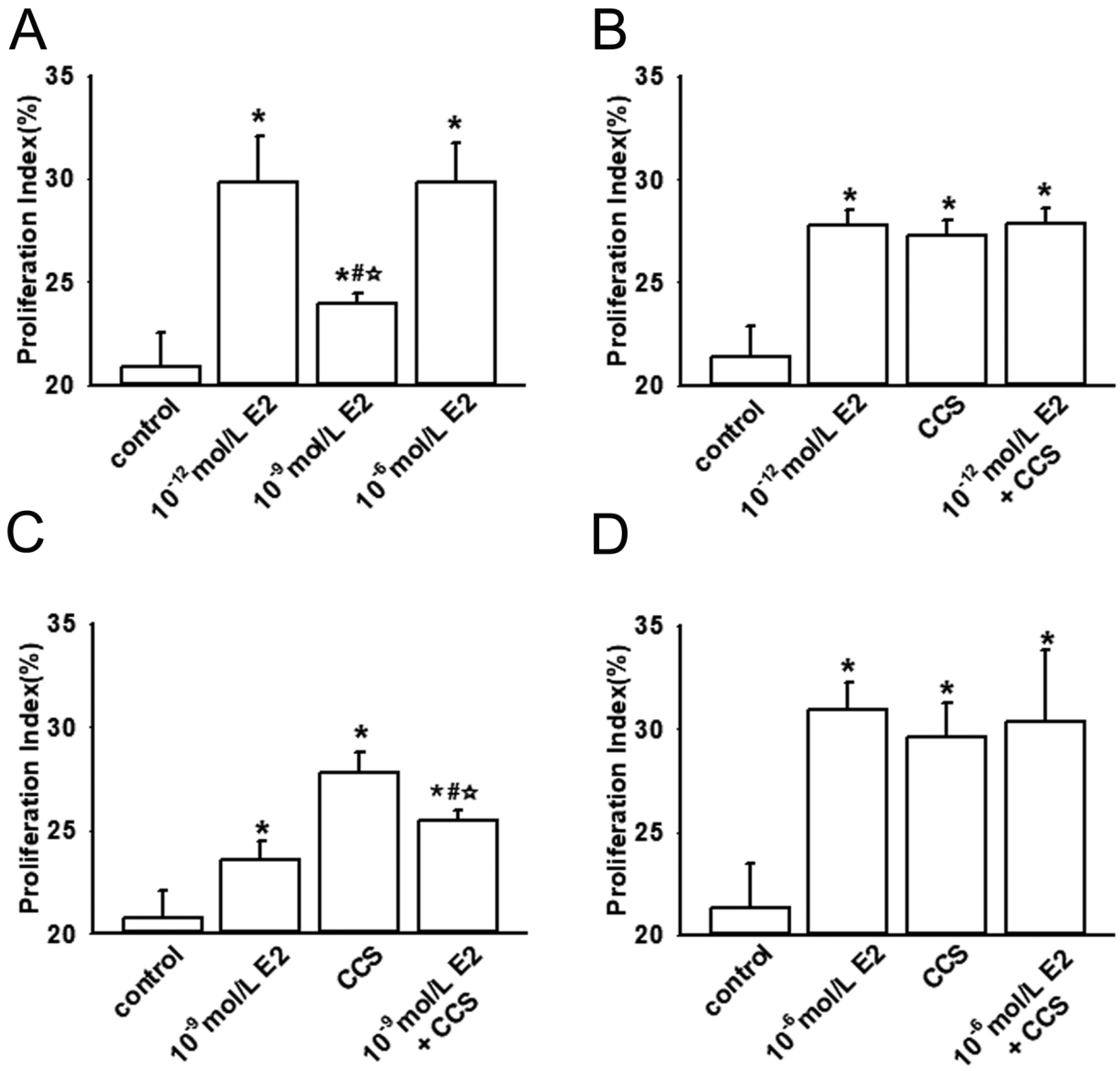


Fig. 2. Effects of different concentrations of E2 combined with CCS on the cell cycle of MCCs.
(A) Effects of different concentrations of E2 on MCCs cell cycle at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$ *vs* 10^{-12} mol/L E2 group; ⋆$P < 0.05$ *vs* 10^{-6} mol/L E2 group. (B) Effects of 10^{-12} mol/L E2 combined with CCS on MCCs cell cycle at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group. (C) Effects of 10^{-9} mol/L E2 combined with CCS on MCCs cell cycle at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$ *vs* 10^{-9} mol/L E2 group; n = 6, ⋆$P < 0.05$ *vs* CCS group. (D) Effects of 10^{-6} mol/L E2 combined with CCS on MCCs cell cycle at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group. MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol.

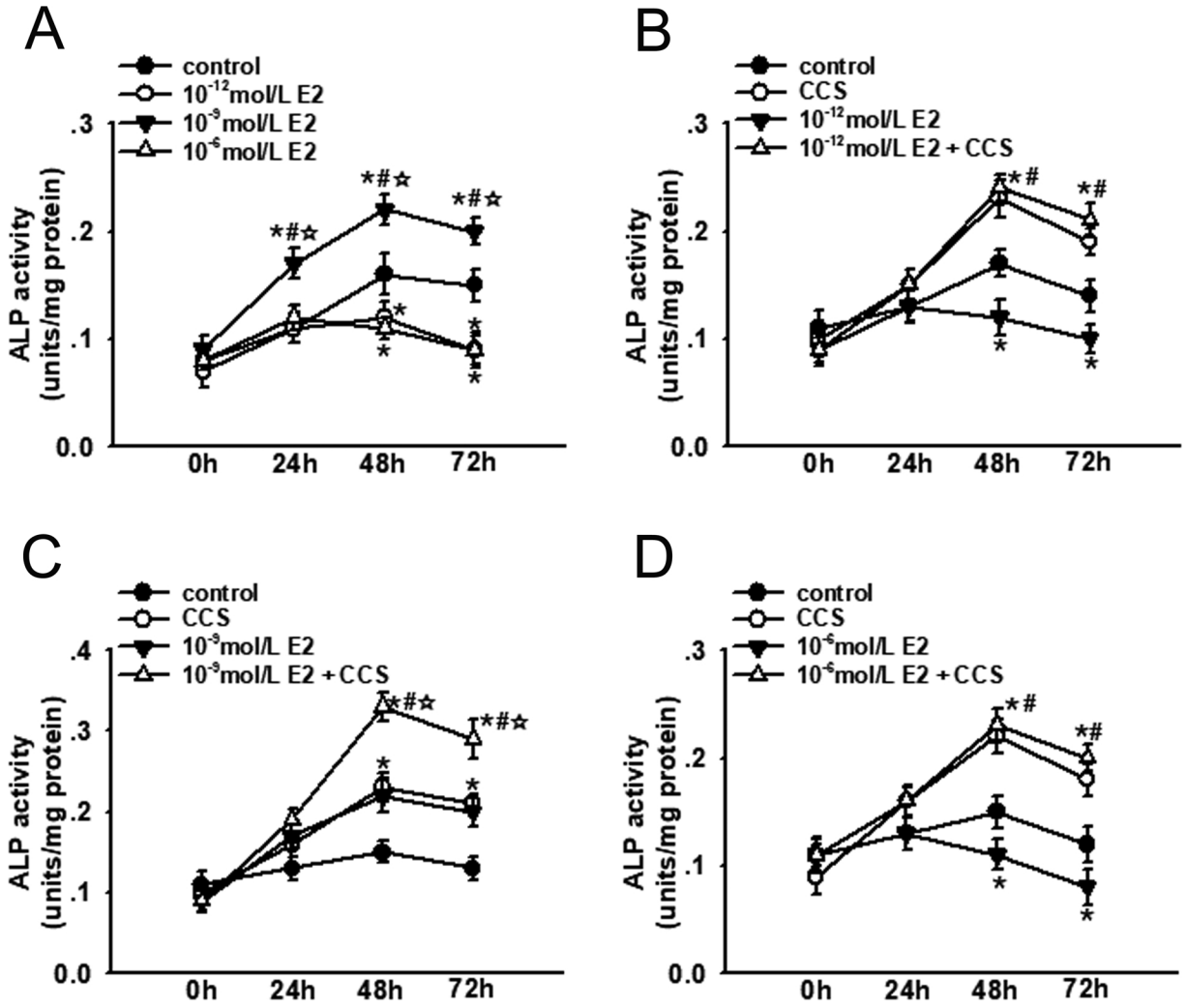


Fig. 3. Effects of different concentrations of E2 combined with CCS on the levels of alkaline phosphatase activity.
(A) Effects of different concentrations of E2 on MCCs alkaline phosphatase activity. Values represent the means ± standard errors, n = 6, $^{*}P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-6} mol/L E2 group. (B) Effects of 10^{-12} mol/L E2 combined with CCS on MCCs alkaline phosphatase activity. Values represent the means ± standard errors, n = 6, $^{*}P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group. (C) Effects of 10^{-9} mol/L E2 combined with CCS on MCCs alkaline phosphatase activity. Values represent the means ± standard errors, n = 6, $^{*}P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-9} mol/L E2 group; n = 6, $\star P < 0.05$ *vs* CCS group. (D) Effects of 10^{-6} mol/L E2 combined with CCS on MCCs alkaline phosphatase activity. Values represent the means ± standard errors, n = 6, $^{*}P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-6} mol/L E2 group. MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol; ALP: alkaline phosphatase.

3.4. Effects of different concentrations of E2 combined with cyclical compressive stress on differentiation-related gene mRNA expression

Besides alkaline phosphatase activity, we further detected the contents of Sox9, Col2a1, Runx2, Col10a1 mRNA, which were always considered to be the marker genes reflecting MCCs differentiation (Almatrafi et al., 2019; Cammarata-Scalisi et al., 2019; Janssen et al., 2019). The changes of these gene mRNA were the same as the results of alkaline phosphatase activity in Fig. 3. E2 at 10^{-12} mol/L or 10^{-6} mol/L reduced the mRNA contents of Sox9, Col2a1, Runx2, Col10a1 in MCCs. In contrast, E2 at 10^{-9} mol/L increased the contents of these gene mRNAs (Fig. 4A to D). When MCCs were exposed to cyclical compressive stress, the mRNA contents of Sox9, Col2a1, Runx2, Col10a1 in MCCs were also increased. More importantly, when cyclical compressive stress combined with E2 at 10^{-12} mol/L or 10^{-6} mol/L, the inhibitory effects of E2 on the mRNA of Sox9, Col2a1, Runx2, Col10a1 in MCCs were reversed. Compared with cyclical compressive stress or E2 at 10^{-9} mol/L exposure, the combination of cyclical compressive stress and E2 could further reinforce the role in promoting differentiation (Fig. 5A to D).

4. Discussion

Effects of E2 combined with cyclical compressive stress on MCCs proliferation and differentiation are different. When combined with E2 at physiological concentration, cyclical compressive stress shows synergistic effect in proliferation and differentiation. However, when combined with E2 at non-physiological concentration cyclical compressive stress has no synergistic effect in MCCs proliferation, but reverses the inhibition in MCCs differentiation provoked by E2 at non-physiological concentration. Therefore, we believe that reasonable cyclical compressive stress could correct effects induced by abnormal E2 concentration on condylar cartilage proliferation and differentiation.

Previous investigations also showed that there might be a link between changes in endogenous estrogen and the onset of the temporomandibular joint disease (Bellinger et al., 2007; Okamoto, Hirata, Takeshita, & Bereiter, 2003; Tashiro et al., 2015). Our results show that abnormal E2 concentration has a great influence on cell proliferation

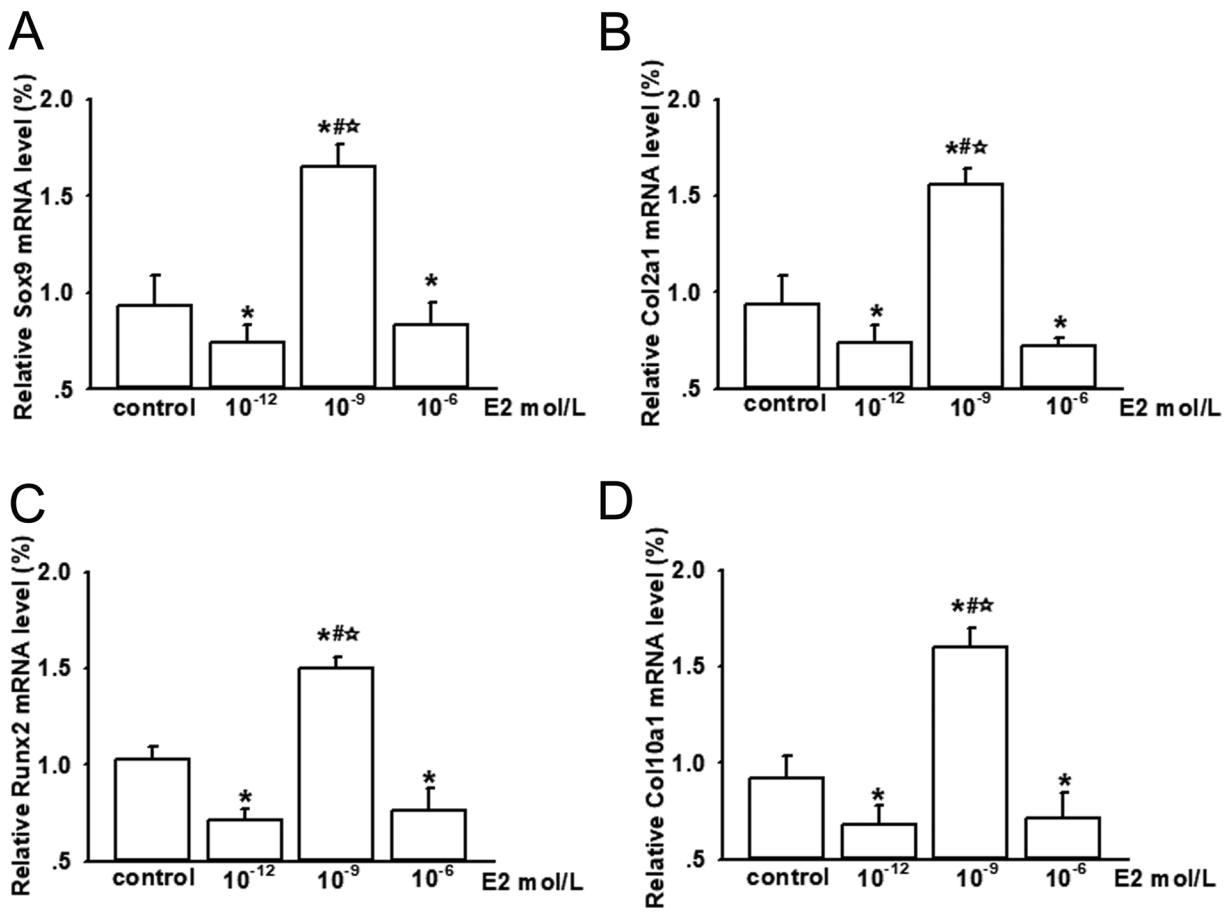


Fig. 4. Effects of different concentrations of E2 on differentiation-related gene mRNA expression at 72 h.
(A) Effects of different concentrations of E2 on Sox9 mRNA at 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-6} mol/L E2 group. (B) Effects of different concentrations of E2 on Col2a1 mRNA at 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-6} mol/L E2 group. (C) Effects of different concentrations of E2 on Runx2 mRNA at 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-6} mol/L E2 group. (D) Effects of different concentrations of E2 on Col10a1 mRNA at 72 h. Values represent the means ± standard errors, n = 6, $*P < 0.05$ *vs* control group; $\#P < 0.05$ *vs* 10^{-12} mol/L E2 group; $\star P < 0.05$ *vs* 10^{-6} mol/L E2 group. MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol.

and differentiation. It is detrimental to condylar cartilage remodeling and orthodontics. E2 could regulate the proliferation of condylar cartilage, so as to change the thickness of cartilage and the synthesis of proteoglycan. Effects of E2 on condylar cartilage differentiation are mainly manifested in regulating cell maturation, altering the expression of cytokines such as IL-β, IL-6 and IL-8, which always lead to the temporomandibular joint arthritis (Yun, Chae, & Lee, 2008). Effects of different concentration of E2 on MCCs proliferation or differentiation tend to be dose-dependent bidirectionality. It might be relative with that different concentrations of E2 have different effects on growth factors. For example, insulin-like growth factors-1, which participates in the proliferation of MCCs, regulates the secretion of type II collagen, and plays an important role in cartilage formation. E2 at physiological concentration can increase the expression of insulin-like growth factors-1, while non-physiological concentration of E2 can lead to a decrease in its expression (Tanko, Sondergaard, Oestergaard, Karsdal, & Christiansen, 2008). For Interleukin-1β, it plays an important role in cartilage destruction. Physiological concentration of E2 can reduce the degradation of extracellular matrix caused by Interleukin-1β and the re-expression of matrix metalloproteinases to protect articular chondrocyte, but E2 at non-physiological concentration can enhance the effect of Interleukin-1β, resulting in the damage of articular chondrocyte (Richette et al., 2004). E2 can activate the transcription of target genes by directly binding to E2 receptors, ERα and ERβ in the nucleus, leading to changes in the expression of growth factors, which is a classic signal transduction pathway of E2 receptor (Hall, McDonnell, & Korach, 2002). Recently, it has been found that E2 can also activate some intracellular signaling pathways, such as Wnt, Src/Ras/ERK, p38/MARK, or interact with other transcription factors, regulating growth factors expression (El-Tanani, Fernig, Barraclough, Green, & Rudland, 2001; Lee & Bai, 2002; Migliaccio et al., 2000). Therefore, maintaining a reasonable E2 level is essential for condylar cartilage remodeling and orthodontics.

Condylar cartilage carries and transmits loads on joints (Bouvier & Zimny, 1987). Under the stimulation of direct or indirect mechanical stress, the condylar cartilage will undergo a series of functional remodeling, which will affect the mandible growth direction and the rate (Ng, Chiu, Rabie, & Hagg, 2006; Ruggiero et al., 2015; Sriram et al., 2009). Appropriate MCCs could promote the proliferation and differentiation of condylar cartilage, producing adaptive remodeling. It functions through affecting the mandible growth direction and growth rate. In contrast, inappropriate MCCs would cause degenerative disease of condylar cartilage (Ghislanzoni et al., 2011). A large number of clinical studies have found that if the applied stress belongs to the physiological category, it can promote the adaptation of condylar cartilage and change the growth direction of the mandible to achieve the desired orthodontic position (Ghislanzoni et al., 2011, 2013; Slade, Diatchenko, Ohrbach, & Maixner, 2008; Thilander, Rubio, Pena, & de

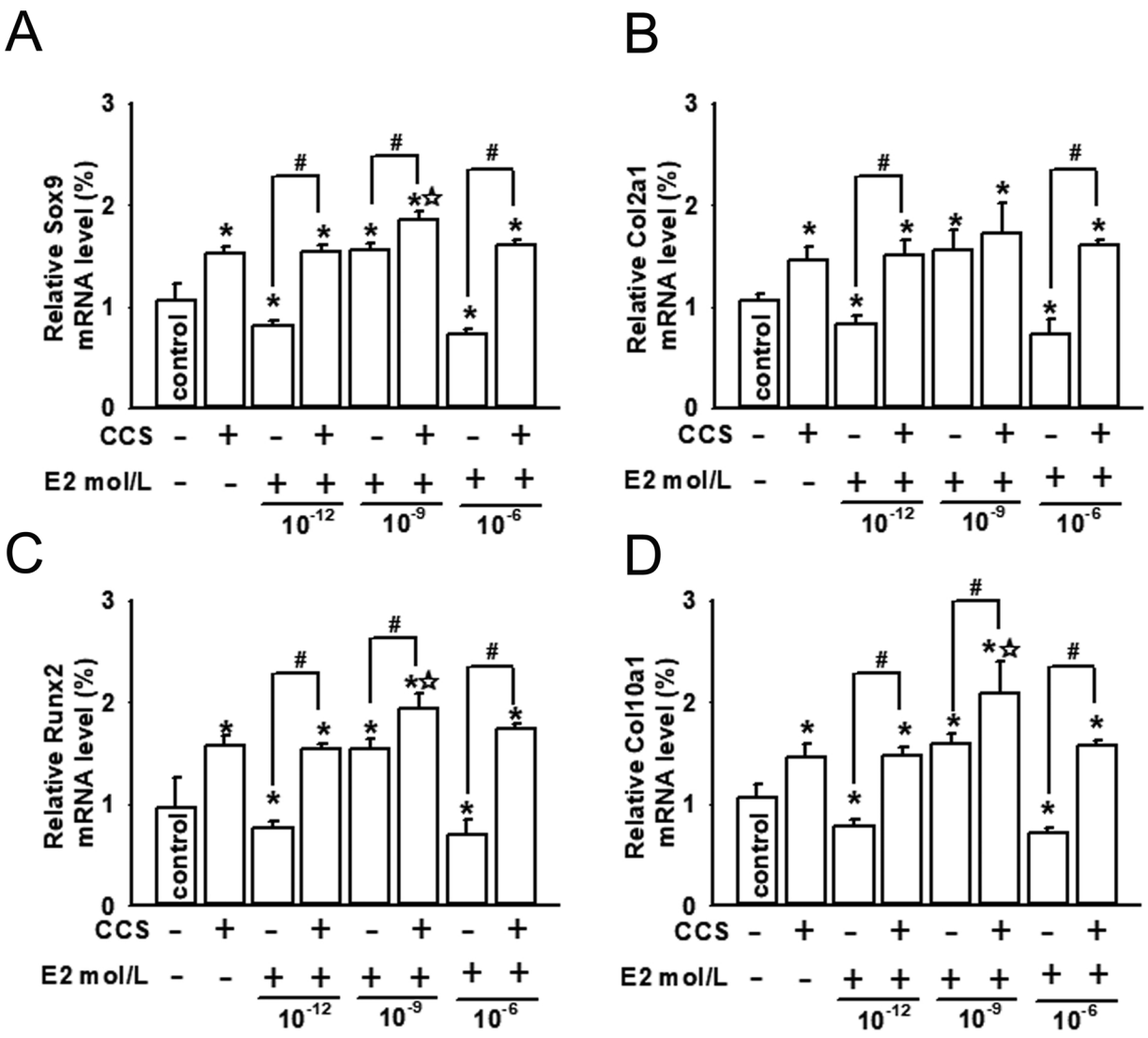


Fig. 5. Effects of different concentrations of E2 combined with CCS on differentiation-related gene mRNA expression at 72 h.
(A) Effects of different concentrations of E2 combined with CCS on Sox9 mRNA at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$; ⋆$P < 0.05$ *vs* CCS group. (B) Effects of different concentrations of E2 combined with CCS on Col2a1 mRNA at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$. (C) Effects of different concentrations of E2 combined with CCS on Runx2 mRNA at 72h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$; ⋆$P < 0.05$ *vs* CCS group. (D) Effects of different concentrations of E2 combined with CCS on Col10a1 mRNA at 72 h. Values represent the means ± standard errors, n = 6, *$P < 0.05$ *vs* control group; #$P < 0.05$; ⋆$P < 0.05$ *vs* CCS group. MCCs: mandibular condylar chondrocytes; CCS: cyclical compressive stress; E2: 17β-Estradiol.

Mayorga, 2002). In our present study, we show that 0.5 Hz 2000 μstrain cyclical compressive stress could not only promote MCCs proliferation, but also promote MCCs differentiation, which also suggest that cyclical compressive stress could play an important part in condylar cartilage remodeling and orthodontics. Under mechanical stress, a series of biological changes occur in MCCs. How do MCCs recognize and react to exogenous mechanical signals? Studies have shown that the mechanism is very complex, involving many links, such as integrin-mediated signal transduction pathways, receptor tyrosine protein kinase signal transduction pathways, and second messenger-mediated signal transduction pathways (Turner, Ball, & Balmforth, 2001). However, the underlying mechanism is still unclear. We will carry out different studies targeting this problem in the future.

Although the effects of single estrogen stimulation or single mechanical stimulation on condylar cartilage have been extensively and thoroughly studied, in the microenvironment of cartilage proliferation and differentiation, biomechanical stimulation and biochemical stimulation exist at the same time or even interact with each other (Kuroda et al., 2009). The relationship between them has an important impact on the formation of condylar cartilage and the maintenance of its normal function. Unfortunately, the study of the combined effects of estrogen and mechanical stimulation is still in its infancy, and the experimental results are different. It is still unclear whether there is a real biological integration effect between estrogen and mechanical stimulation, which is just what we mainly focused on in this study. The results showed that the effects of different concentrations of estrogen combined with cyclical compressive stress on the proliferation and differentiation of MCCs cultured *in vitro* was totally different: cyclical compressive stress combined with E2 at physiological concentration shows synergistic effect in proliferation and differentiation; cyclical compressive stress combined with E2 at non-physiological concentration has no synergistic effect in MCCs proliferation, but reverses the inhibition in MCCs differentiation provoked by E2 at non-physiological concentration. We also know that it is not that simple to extrapolate the findings of an *in vitro* study to the clinical setting, but at least, the present conclusion could give orthodontist some hints that they should

take fully consideration of the individual conditions of patients, especially for the levels of E2. According to the changes of E2, cyclical compressive stress could be used to adjust the effects of E2. We think this kind of comprehensive strategy might be more beneficial for the proliferation and differentiation of MCCs.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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