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*Original Article*

# Effect of mesenchymal stem cell conditioned medium on human gingival fibroblast proliferation and collagen synthesis

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**Abstract:** As the major cell component of loose connective tissue, fibroblast is differentiated from mesenchymal cell in embryonic phase. It is of great significance to cell degeneration, necrosis, tissue defect, and bone traumatic repair. Human gingival fibroblast (HGF) is featured as self-repair and self-renew that could be treated as ideal seed cell for tissue repair. This study intends to investigate the effect of mesenchymal stem cell (MSC) conditioned medium on HGF proliferation and collagen synthesis. Primary HGFs were cultured *in vitro* and divided into two groups, including control maintained at complete DMEM medium containing 10% FBS and induction group treated by MSC conditioned medium. Cell proliferation was assessed by MTT assay. Cell apoptosis activity was evaluated by Caspase 3 activity. Type I and IV collagen expressions were detected by real-time PCR and ELISA. MSC conditioned medium induction significantly promoted HGFs proliferation, declined Caspase 3 activity, and enhanced type I and IV collagen expressions compared with control ($P < 0.05$). MSC conditioned medium facilitated HGFs proliferation and accelerated collagen synthesis, which was in favor of periodontium regeneration and traumatic repair.

**Keywords:** Human gingival fibroblast, mesenchymal stem cell, conditioned medium, proliferation, collagen synthesis

## Introduction

Fibroblast is the major cell component of loose connective tissue that differentiated from mesenchymal cell in embryonic phase [1]. Fibroblast is featured as high activity, addiction to weak alkaline, and significant protein synthesis and secretion effect [2]. It can mutually transform with fibrocyte under a certain condition. Fibroblast is of great significance to cell degeneration, necrosis, tissue defect, and bone traumatic repair [3, 4]. Burn, radiation injury, trauma, mucous membrane lesions, and physical or chemical factors may damage tissue structure and function [5]. The normal biological function of skin mucous membrane barrier is difficult to be recovered through self-repair, leading to irreversible injury [6]. Following the in-depth research of life science technology and the rapid development of engineering, tissue engineering technology has been widely used in clinic. Tissue engineering technology provides basis for the clinical application of damaged tissue and organ repair through separating seed cells [7, 8]. However, selection of seed cells is extremely important.

Mesenchymal stem cells (MSCs) are commonly used in tissue engineering, while bone mesenchymal stem cells (BMSCs) are the most common type. As a type of pluripotent stem cells, BMSCs can differentiate into bone, cartilage, and adipose in bone marrow. They can further differentiate into epithelioid cells, muscle, fat, bone, cartilage, and ligament tissue under suitable condition [9, 10]. However, BMSCs are featured as decreased proliferation rate and reduced differentiation following cultivation, which seriously restricts its application [11, 12]. It is showed that fibroblasts are of great significance in the process of trauma repair [13]. Regulation of fibroblasts function and biological behavior

**Table 1.** Primer sequences

| Gene | Forward, 5'-3' | Reverse, 5'-3' |
|---|---|---|
| GAPDH | AGTACCAGTCTGTTGCTGG | TAATAGACCCGGATGTCTGGT |
| Type I collagen | CCTACCTGCCTCTCTAGAAT | GTTAGGCAGACCTTTAATTT |
| Type IV collagen | TTGCTGCACCACTCCCA | GACCGTTATGACGAGTTTA |

can promote wound healing, inhibit scar dysplasia, and enhance healing ability [14, 15]. Gingival fibroblasts are characterized as self-healing and self-renew, thus could be treated as ideal seed cells for tissue repair [16]. This study aims to investigate the effect of MSC conditioned medium on HGF proliferation and collagen synthesis.

## Materials and methods

### *Main reagents and instruments*

MSC conditioned medium was bought from Yocon (China). High glucose DMEM medium, FBS, penicillin-streptomycin, and DispaseII digestive juice were gotten from Hyclone (USA). DMSO and MTT were obtained from Gibco (USA). Trypsin was derived from Sigma (USA). RNA extraction kit and reverse transcription kit were derived from Axygen (USA). Vimentin and Keratin immunohistochemistry kits were provided by Boster (Wuhan, China). Type I and IV collagen ELISA kits were purchased from R&D. Caspase 3 activity detection kit was purchased from Pall Life Sciences. Labsystem Version 1.3.1 microplate reader was provided by Bio-rad (USA). ABI 7700 Fast real-time PCR amplifier was derived from ABI (USA). Benchtop was provided by Sutai purification equipment engineering co., Ltd (Suzhou, China). Microsurgery instruments were bought from Shanghai Instrument factory. $CO_2$ incubator was got from SANYO (Japan). DNA amplifier was obtained from PE Gene Amp PCR System 2400. Other reagents were purchased from Sangon (China).

### *Methods*

*HGFs isolation and grouping:* The impacted mandibular tooth and accessory gingival tissue were collected from the healthy adult. Inclusion criteria [17]: healthy volunteers without acute or chronic inflammation during tooth extraction. Exclusion criteria [17]: subjects combined with other diseases, such as infectious diseases, malignant tumor, severe liver and kidney disease, pulmonary fibrosis, bone metabolic disease, secondary renal hypertension, and systemic autoimmune disease. This study was approved the ethic committee and all the subjects had signed informed consent.

The impacted mandibular tooth and accessory gingival tissue removed from the healthy adult were washed by PBS containing 2% penicillin-streptomycin and digested by $2.4 \times 10^3$ U/ml Dispase II at 4°C overnight. Next, the tissue was cut into 1 $cm^3$ and seeded in the 25 $cm^2$ cell culture flask at 37°C and 5% $CO_2$. After 2 h, the flask was turned over and tissue was removed when the cells around the tissue reached 1/3. Then the cells were digested by 0.25% EDTA-trypsin and passaged when the cell fusion reached 80-90%. The cells in $2^{nd}$-$3^{rd}$ generation and logarithmic phase were used for experiments. HGFs were cultured in vitro and divided into two groups, including control maintained at complete DMEM medium containing 10% FBS and induction group treated by MSC conditioned medium.

*HGFs identification:* Immunochemistry was applied to test Vimentin and Keratin expression in HGFs. After growing on the glass slide, the cells were washed by PBS and fixed by 4% paraformaldehyde for 30 min. Next, the cells were penetrated by Triton X-100 at room temperature for 30 min and treated by 3% $H_2O_2$ for 30 min. Then the cells were blocked by goat serum for 20 min and incubated in Vimentin (1:200) and Keratin (1:400) primary antibodies at 37°C overnight. After incubated in biotin labeled secondary antibody at 37°C for 20 min, the slides were incubated in HRP humidifying box for 20 min. At last, the slides were developed by DAB and observed under the microscope.

*MTT assay:* HGFs in logarithmic phase were added with 20 μL MTT for 4 h. Then, the plate was added with 150 μL DMSO for 10 min and tested at 570 nm to obtain the absorbance value. Each experiment was repeated for three times.

*Caspase 3 activity detection:* Caspase 3 activity was tested according to the manual. The cells were digested by trypsin and centrifuged at 600 g and 4°C for 5 min. Next, the cells were lysed on ice for 15 min and centrifuged at 20000 g and 4°C for 5 min. At last, the cells

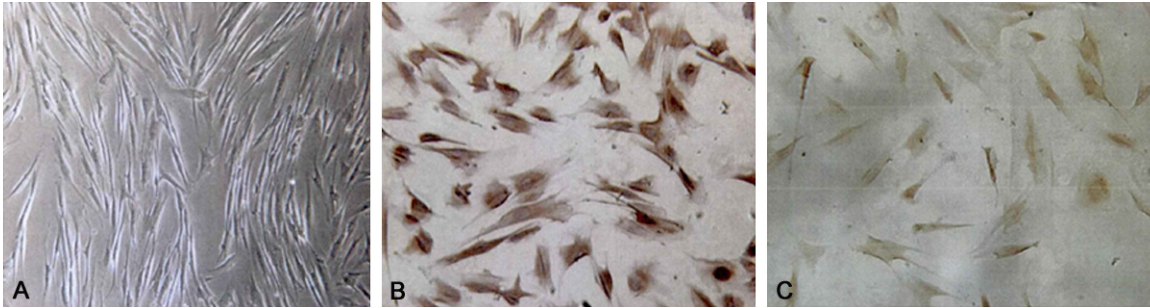


**Figure 1.** HGFs cultivation and identification (×200). A: The 4th generation of HGFs morphology; B: Immunochemistry staining of Vimentin; C: Immunochemistry staining of Keratin.

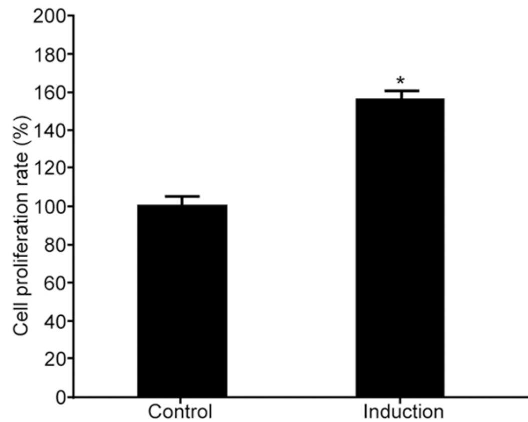


**Figure 2.** The impact of MSC conditioned medium on HGFs proliferation. *P < 0.05, compared with control.

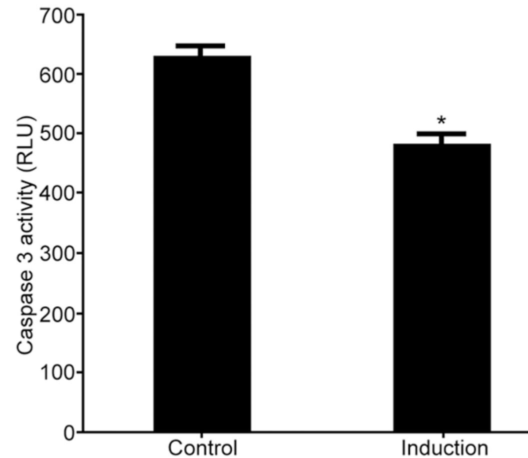


**Figure 3.** The effect of MSC conditioned medium on HGFs apoptosis. *P < 0.05, compared with control.

were added with 2 mM Ac-DEVD-pNA and tested at 405 nm to calculate Caspase 3 activity.

*Real-time PCR:* Total RNA was extracted from the HGFs and reverse transcribed to cDNA. The primers were designed using PrimerPremier 6.0 software and synthetized by Invitrogen (Shanghai, China) (**Table 1**). Real-time PCR was performed at 56°C for 1 min, followed by 35 cycles of 92°C for 30 s, 58°C for 45 s, and 72°C for 35 s. GAPDH was selected as internal reference. The relative expression of mRNA was calculated by $2^{-\Delta Ct}$ method.

*ELISA:* ELISA was used to test type I and IV collagen contents in the supernatant. A total of 50 μL diluted standard substance were added to each well to establish standard curve. Next, the plate was added with 50 μL sample and washed for five times. Then the plate was incubated in 50 μL conjugate reagent at 37°C for 30 min. After washed for five times, the plate was treated by 50 μL color agent A and B at 37°C avoid of light for 30 min. At last, the plate was added with 50 μL stop buffer to stop the reaction and tested at 450 nm to obtain the OD value. The OD value of standard substance was used to prepare the linear regression equation, which was adopted to calculate the concentration of samples.

*Statistical analysis*

All data analyses were performed on SPSS16.0 software. All data were presented as mean ± standard deviation and compared by one-way ANOVA. $P < 0.05$ was depicted as statistical significance.

## Results

*HGFs cultivation and identification*

Primary cells presented numerous impurity and other cells. After three times passage, the cells showed uniform cellular morphology, stability,

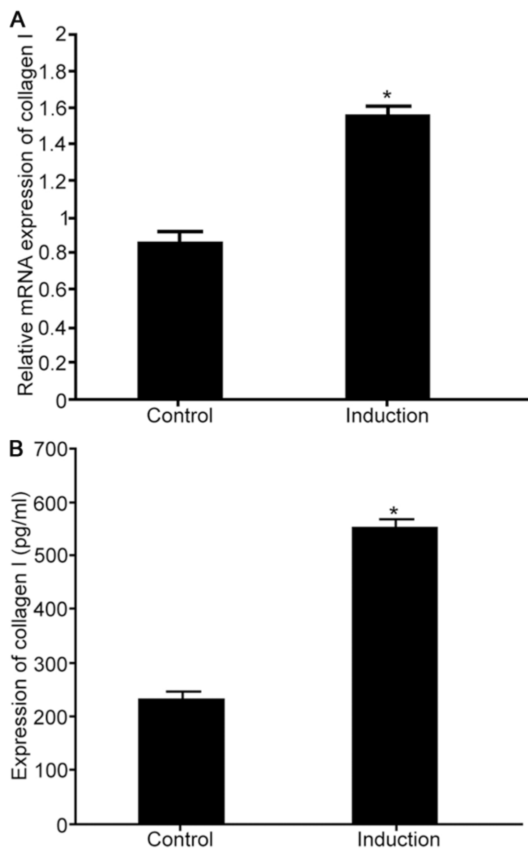


**Figure 4.** The influence of MSC conditioned medium on type I collagen synthesis in HGFs. A: Real-time PCR detection of type I collagen mRNA; B: ELISA detection of type I collagen secretion. *P < 0.05, compared with control.

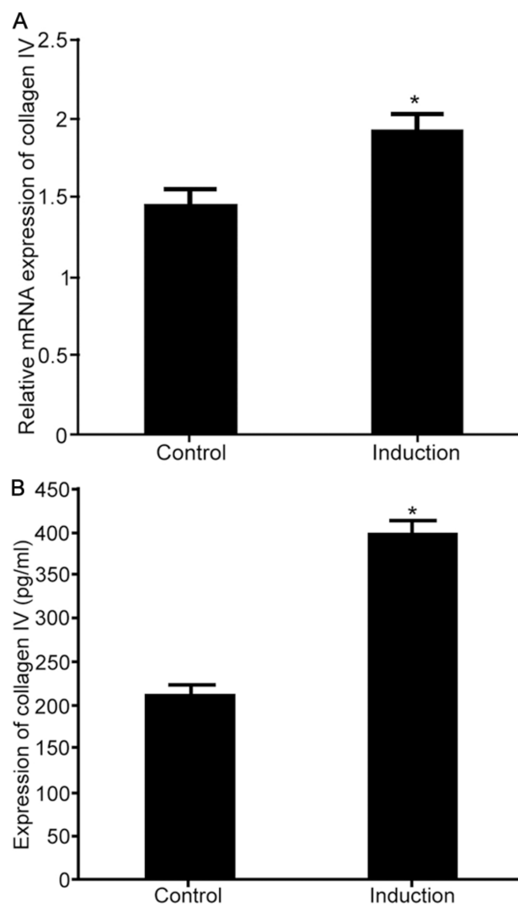


**Figure 5.** The influence of MSC conditioned medium on type IV collagen synthesis in HGFs. A: Real-time PCR detection of type IV collagen mRNA; B: ELISA detection of type IV collagen secretion. *P < 0.05, compared with control.

and high purity (**Figure 1A**). Cell immunochemistry staining exhibited that Keratin was negative, while Vimentin was positive in cytoplasm but not nucleus (**Figure 1B** and **1C**).

*The impact of MSC conditioned medium on HGFs proliferation*

MTT assay was adopted to test the impact of MSC conditioned medium on HGFs proliferation. MSC conditioned medium obviously promoted HGFs proliferation compared with control ($P < 0.05$) (**Figure 2**).

*The effect of MSC conditioned medium on HGFs apoptosis*

Caspase 3 activity detection was selected to assess the effect of MSC conditioned medium on HGFs apoptosis. MSC conditioned medium markedly restrained Caspase 3 activity in HGFs ($P < 0.05$) (**Figure 3**).

*The influence of MSC conditioned medium on type I collagen synthesis in HGFs*

Real-time PCR and ELISA were applied to determine the influence of MSC conditioned medium on type I collagen synthesis in HGFs. MSC conditioned medium significantly facilitated type I collagen mRNA synthesis and secretion in HGF supernatant compared with control ($P < 0.05$) (**Figure 4**).

*The influence of MSC conditioned medium on type IV collagen synthesis in HGFs*

Real-time PCR and ELISA were applied to determine the influence of MSC conditioned medium on type IV collagen synthesis in HGFs. MSC conditioned medium significantly facilitated type IV collagen mRNA synthesis and secretion in HGF supernatant compared with control ($P < 0.05$) (**Figure 5**).

## Discussion

As the major component of gingival connective tissue, HGFs can promote gingival tissue to perform protective and repair functions. HGFs have the differentiation and proliferation ability, thus to play a role in traumatic repair through migration, proliferation, and collagen secretion [17, 18]. Because of its easily sampling, rapid proliferation, and multi-directional differentiation ability to fat, bone, and cartilage, HGFs have the similar phenotype with BMSCs [19, 20]. In addition, the gingival tissue is characterized as rapid healing and reduced scar formation, thus is considered to be advantage in epithelial repair [21].

Traumatic repair is a complex process involving interaction among multiple cells, growth factors, and extracellular matrix (ECM). HGFs maintain ECM function via regulating collagen synthesis and degradation [22, 23]. ECM facilitates cell proliferation and differentiation through microenvironment. As the main components of ECM, type I and IV collagen can accelerate the synthesis of different cell types. Meanwhile, they are the major components of tooth, bone, and cartilage tissues to promote tissue repair [24]. This study confirmed that MSC conditioned medium induction promoted HGFs proliferation, reduced Caspase 3 activity, and elevated type I and IV collagen expressions. We aimed to explore the related mechanism in the future. MSCs conditioned medium is a type of human MSC medium without heterologous animal component that can promote human MSCs proliferation and differentiation, including BMSCs and umbilical cord MSCs [25].

MSC conditioned medium facilitated HGFs proliferation and accelerated collagen synthesis, which was in favor of periodontium regeneration and traumatic repair.

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## Disclosure of conflict of interest

None.

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