

Human Gingiva-derived Mesenchymal Stem Cells Promote Osteogenic Differentiation via Suppressing T Cell Biological Activity

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Research

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Abstract

Background: Accumulating evidence has revealed that human gingiva-derived mesenchymal stem cells (GMSCs) are emerging as a new line of mesenchymal stem cells and have a robust immune regulatory function and regenerative ability. However, the relationship between immune regulatory function and regenerative ability is unclear, whether GMSCs promoted osteoblastic differentiation by regulating immune cells.

Methods: In this study, we investigated the effect of GMSCs regulating T-cell biological activity on osteoblasts in a direct contact co-culture system. GMSCs and T-cell were co-cultured, the co-culture supernatant was collected and acted on MC3T3-E1 cells, then the alkaline phosphatase (ALP) staining, Alizarin Red staining, Immunofluorescence staining, and real-time quantitative PCR (RT-qPCR) analysis of osteogenic genes were detected to evaluate the osteogenesis differentiation of MC3T3-E1 cells.

Results: Our results demonstrated that GMSCs could suppress the activated T-cell function by downregulation pro-inflammatory cytokines (Interleukin-1 β [IL-1 β] and Tumor necrosis factor- α [TNF- α]) and the upregulation of anti-inflammatory cytokines (IL-10). Meanwhile, the co-culture supernatant significantly increased osteogenic differentiation of MC3T3-E1.

Conclusion: GMSCs could promote the osteogenic differentiation of MC3T3-E1 cells by inhibiting the biological activity of activated T-cell.

Background

Periodontitis is an immunoinflammatory disease caused by a wide range of bacteria and their products presenting as progressive destruction of periodontal support tissue finally leading to tooth loss [1]. At present, the most commonly used clinical treatments for periodontitis include scaling, root planning, adjunctive pharmacological therapy, surgical treatment, among others [2]. However, these methods cannot achieve ideal periodontal tissue regeneration. Periodontal tissue engineering is a promising method to treat periodontitis and regenerate periodontal tissue. Finding an ideal stem cell source is needed to achieve this process.

In recent years, mesenchymal stem cells (MSCs), a group of stem cells with self-renewal capacity and multi-differentiation potential, have been widely used in tissue engineering technology. Mesenchymal stem cells were first isolated from bone marrow and can be induced to differentiate into bone tissue [3]. However, the widespread clinical application of bone marrow MSCs (BMSCs) in periodontal therapy is severely influenced due to its limited access and difficulty in isolation. Compared to BMSCs, MSCs isolated from gingival tissue (Gingival MSCs [GMSCs]) maintain normal karyotype and telomerase activity in long-term *in vitro* cultures and are not tumorigenic [4]. In oral treatment, the excised gingival tissue is often treated as discarded tissue. Gingival resection not only has negligible impact on the patients, but also enables accelerated scarless wound healing without any sequelae. Obtaining adequate

biological activity of MSCs from gingival tissue appears to be more feasible. Furthermore, similar to other MSCs, GMSCs have extraordinary immunomodulatory characteristics.

Bacteria and their toxic products in chronic inflammatory tissues activate host immune response, release inflammatory factors, mediate or directly stimulate osteoblasts and osteoclasts, and affect their biological activities, eventually leading to bone remodeling in inflammatory areas [5]. Studies have shown that there are many pro-inflammatory cytokines in the gingiva from patients with periodontitis, such as Interleukin (IL)-1 β , IL-6, IL-10, tumor necrosis factor (TNF)- α , IFN- γ , among others [6,7]. These inflammatory biomolecules regulate periodontal cell survival, behavior, and activity, thereby influencing the duration, extent, and outcome of disease or treatment [8]. Most of the periodontal tissue regeneration by tissue engineering occurs under the inflammatory microenvironment. Therefore, the study of the interaction between the inflamed microenvironment, GMSCs and osteoblasts is pivotal to further optimize and guide the clinical periodontal regeneration methods.

A variety of cells and factors are involved in the periodontal healing process, and previous experiments on GMSCs involvement in this process mostly focused on some single aspects, including immunosuppressive function of GMSCs, inflammatory environment on osteogenic differentiation of GMSCs, and others. In addition to differentiating into osteoblasts and secreting bone matrix proteins to repair bone defects after GMSCs transplantation into the microenvironment of periodontal defects, the bioactive factors secreted by GMSCs can also regulate other cellular functions. In this study, we isolated and identified human gingival GMSCs to investigate the interaction between GMSCs and T-cell, and their effect on osteogenesis. Co-culture with activated Jurkat T cells revealed that GMSCs inhibited the proliferation and secretion of inflammatory factors. Upon culturing osteogenic precursor cells with 50% diluent of co-culture supernatant, it was found that GMSCs could improve the inhibitory effect of inflammatory factors secreted by T-cell on osteogenesis.

Methods

Cell preparation

Human gingival samples were collected from discarded tissues of third molar extractions at the Qingdao Municipal Hospital. All healthy donors were aged between 18-25 years and provided signed informed consent. All procedures were performed according to the ethical standards and were approved by the Institutional Review Board of Qingdao Municipal Hospital. GMSCs were isolated as described in a previously study [9]. GMSCs obtained by the limited dilution method were cultured in a Minimum Essential Medium (α -MEM) (Hyclone laboratories, Logan, UT) containing 10% fetal bovine serum (FBS) (Hyclone laboratories, Logan, UT). The cells were sub-cultured at 80% confluence using 0.25% trypsin/EDTA solution (Hyclone laboratories, Logan, UT). Cells (passages 3-6) were used for the following experiments. In addition, Jurkat T cells were provided by the microbiology laboratory of Qingdao university. Murine calvarial cell line (MC3T3-E1) cells were purchased from Gefan (Shanghai, China).

Immunophenotype and differentiation capacity of GMSCs

The immunophenotypic characterization of GMSCs was performed as described in a previous study [10]. The surface antigen expression of the molecules CD34, CD73, CD105, and CD14 (BioLegend, San Diego, CA, USA), was analyzed by flow cytometry (Beckman Coulter, Brea, CA, USA).

To evaluate the multidirectional differentiation of GMSCs. For adipocyte differentiation, GMSCs (5×10^5 cells/well) were incubated in 6-well plates in adipogenic medium (α -MEM containing 10% FBS, 0.1 μ M dexamethasone, 60 μ M indomethacin and 50 mg/ml ascorbate-2-phosphate; Sigma-Aldrich, St. Louis, Mo, USA). The medium was changed every three days. After 14 days, the plates were staining with Oil Red O solution. For osteoblast differentiation, GMSCs (5×10^5 cells/well) were incubated in 6-well plates in osteogenic medium (α -MEM containing 5% FBS, 0.1 μ M dexamethasone, 10 mM β -glycerophosphate, and 50 mg/ml ascorbate-2-phosphate; Sigma-Aldrich, St. Louis, Mo, USA). After 28 days, the mineralized nodules were stained with 2% Alizarin red Solarbio, Beijing, China.

The colony forming units-fibroblast (CFU-F) assay was performed to evaluate the colony forming efficiency of GMSCs. Five hundred cells were seeded in a 60mm culture dish and cultured for 14 days at 37 °C in 5% CO₂. After 14 days, cells were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Solarbio, Beijing, China).

Activation of T-cell

A 50 μ l antibody solution of anti-CD3 (5 μ g/ml; Bioss, Beijing, China) was dispensed into each microwell of the 96-well assay plate and kept at 4°C overnight to coat the culture plates with antibodies. Before adding the T-cell, the antibody solution was decanted and each microwell was rinsed. Soluble anti-CD28mAb (5 μ g/ml; Bioss, Beijing, China) was added to cells at 2 μ g/mL. Cells were incubated for two days, and harvested for subsequent experiments. To determine the optimal culture time, unactivated cells were used as controls. Optical density at 450 nm, that is OD₄₅₀ of both the groups was detected using Cell Counting kit-8 (CCK-8; Beyotime, Shanghai, China), following manufacturer's instructions.

Cell proliferation of Jurkat T cells

The effect of GMSCs and Jurkat T cells co-culture times on Jurkat T cell proliferation was estimated by a stimulation index (SI) assay. GMSCs (5×10^3 cells/well) were plated in a 96-well plate for direct co-culture. After 24h, confirming that the GMSCs was adherent to the wall, the cells were thoroughly washed twice with phosphate-buffered saline (PBS, HyClone laboratories, Logan, UT) and seeded with activated Jurkat T cells. Jurkat T cells were cultured with GMSCs for 24h, 48h, 72h and 96h. After culture, non-adherent Jurkat T cells were collected and treated with CCK-8. Phosphate-buffered saline was used as a control and each sample had 6 wells. The absorbance at 450nm was measured with a microplate spectrophotometer. The stimulation index (SI) was calculated as follows: $(OD_{450} \text{ of Jurkat T cells with MSCs} - OD_{450} \text{ of PBS control}) / (OD_{450} \text{ of Jurkat T} - OD_{450} \text{ of PBS control})$.

The effect of GMSCs and Jurkat T cells co-culture proportions on Jurkat T cell proliferation was estimated by a stimulation index (SI) assay too. Jurkat T cells were co-cultured with GMSCs for 0:1,0.1:1,

0.5:1 and 1:1 ratio. The method was the same as described above.

Immunomodulation capacity of GMSCs

To confirm the possible effect of GMSCs on the inflammatory process, the mRNA expression levels of pro-inflammatory molecules (IL-1 β and TNF- α) and anti-inflammatory factors (IL-10) were measured by real time RT-qPCR. TRIzol reagent (Sigma-Aldrich, St. Louis, MO, USA) was used to extract the RNA, which was then immediately reverse transcribed to cDNA using the PrimeScript™ RT reagent Kit (Takara, Dalian, China). The primer sequences used are given in Table1. β -Actin was used as an internal control, and the 2^{-Delta Delta C[T]} ($2^{-\Delta\Delta C_t}$) method was used to evaluate gene expression levels. The experiments were repeated three times and RT-qPCR was performed three times for all samples.

Preparation of Co-culture supernatant

GMSCs (5×10^3 cells/well) were plated in a 6-well plate for 24h, and subsequently, activated Jurkat T cells were added. As a control, activated Jurkat T cells were incubated alone in the same medium. After 1 and 3 days, the Jurkat T cells were collected for osteogenic analysis and the supernatants were collected and stored at -20°C for the following experiments.

MC3T3-E1 cells (2.0×10^5 cells/well) were seeded in a 6-well plate. Based on the different contents of medium, three groups were considered, which include the supernatants of GMSCs and Jurkat T cells co-culture, Jurkat T cells culture alone and the normal medium. After 3 and 7 days, the cells were collected for analysis.

Alkaline Phosphatase and Alizarin red staining

MC3T3-E1 cells (2.0×10^5 cells/well) were plated in a 12-well plate for 24h. After adherent growth to 70-80%, the culture medium was removed, and the supernatants of GMSCs and Jurkat T cells co-culture, Jurkat T cells culture alone and the normal medium were respectively added to the cells. On days 3 and 7, cells were washed with PBS and fixed in 4% paraformaldehyde for 10 min at 4°C, and incubated with a mixture of naphthol AS-MX phosphate and fast blue BB salt (ALP Kit, Hongqiao, Shanghai, China). Areas stained as purple were designated as positive. Alizarin red (AL) staining was performed on days 7 and 14. Cell layers were washed with PBS and fixed in 95% alcohol for 15 minutes at room temperature, and later incubated with the AL solution (1%, pH 4.2, Merck Chemicals Ltd, Shanghai, China) for 30 min at 37°C. After aspiration of the overflow, the cells were washed three times with PBS and observed using a microscope (Leica, Hamburg, Germany). All experiments were performed in triplicate.

Immunofluorescence

After 3 and 7 days of culture, the expression of osteopontin (OPN) and COL-1 in MC3T3-E1 cells was detected by immunofluorescence. Adherent cells were fixed in 4% paraformaldehyde at 4 °C, and frozen sections were then permeabilized with 0.5% Triton X-100 for 20 min at room temperature. They were washed with PBS, and incubated with primary antibodies against OPN and COL-1 overnight, followed by

fluorescence-tagged secondary antibodies against IgG. The cell nuclei were stained with DAPI (Invitrogen, Carlsbad, CA, USA) before observation with a confocal laser scanning microscope (CLSM, Leica).

Real-time quantitative PCR

After 3 and 7 days in culture, the expression of osteogenic genes were measured, and the mRNA expression of ALP, COL1, and RUNX2 was analyzed using real time RT-qPCR. The primers' sequences are displayed as follows (Table2). The method was the same as described in 2.4.

Statistical Analysis

All measurements were conducted at least in triplicate, and all quantitative data are presented as means \pm standard deviations. One-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons was selected for statistical analysis using the SPSS 20.0 statistical software package. The levels of significance were determined at * $P < 0.05$, ** $P < 0.01$.

Results

Isolation and characteristics of human GMSCs

Human GMSCs isolated from gingival tissues were long spindle-like shaped cells (Fig.1b). As illustrated in Figure 1E for flow cytometry, the cells were negative for CD34 (0.46%) and CD45 (1.36%), but were positive for CD29 (96.56%) and CD44 (97.78%). Oil red O and Alizarin red staining showed that under induction culture conditions, GMSCs could be differentiated into adipocytes and osteoblasts (Fig.1c, 1d). In addition, GMSCs formed cell colonies as shown in the CFU-F assay (Fig. 1a).

Jurkat T cells activity was inhibited by co-culture with GMSCs

We tested whether co-culture with GMSCs has a direct effect on Jurkat T cells proliferation.

The initial experiments showed that GMSCs and Jurkat T cells were co-cultured for 48h had a higher SI index (Fig.2a). Therefore, we considered 48h as the appropriate co-culture time. In addition, Jurkat T cells proliferation was different with the different co-culture ratio. The results showed that GMSCs inhibited the proliferation of Jurkat T cells in a cell dose-dependent manner, when the ratio of GMSCs and Jurkat T cells was 1:1, the SI index was the highest. (Fig.2b).

To confirm whether GMSCs can reduce the inflammatory process, we measured the mRNA expression levels of inflammatory factors. The results of RT-qPCR revealed that the expression of pro-inflammatory molecules (IL-1 β , TNF- α) was markedly reduced in the GMSCs co-culture groups. Meanwhile, the expression of anti-inflammatory factors (IL-10) was significantly upregulated (Fig.2c). The results indicated that co-culture with GMSCs reduced the release of pro-inflammatory factors in activated T-cell and increased the release of anti-inflammatory factors.

ALP activity assay and AL staining analysis

Alkaline phosphatase (ALP) staining was observed on days 3 and 7 (Fig. 3a). Staining was more intensive in MC3T3-E1 cells treated with co-culture supernatant group than in the Jurkat T cells supernatant group, be in on days 3 or days 7. Upon staining with Alizarin red on days 7 and 14 (Fig. 3b), MC3T3-E1 cells treated with the co-culture supernatant showed an increase in mineralized deposition compared to the cells treated with the normal medium. However, the cells treated with Jurkat T cells supernatant alone showed the lowest mineralized deposition. This result suggested that GMSCs promoted the MC3T3-E1 differentiation into osteoblastic cells. Meanwhile GMSCs improve the inhibitory effect by T-cell on osteogenesis.

Immunofluorescence staining analysis

Osteopontin is a gene associated with the maturation stage of osteoblasts during attachment and matrix synthesis before mineralization. Collagen type I is a matrix protein synthesized by osteoblasts, and is mineralized with hydroxyapatite during the later stages of osteogenesis. The expression of OPN and COL-1 was detected using immunofluorescence on days 3 and 7. As shown in Figure 4a, the staining was highest in MC3T3-E1 cells treated with the co-culture supernatants from the three groups. A similar trend was observed in the COL-1 (Fig.4b), and the expression of COL-1 was highest in co-culture supernatants. Meanwhile, COL-1 expression was significantly decreased in MC3T3-E1 cells treated with the supernatants of Jurkat T cells culture alone. The results of this study demonstrated that GMSCs had a synergistic effect on directing the differentiation of stem cells into osteogenic cells.

Effects of supernatant of GMSCs and Jurkat T cells on osteogenic differentiation of MC3T3-E1 cells

To reveal the influence of different supernatants on the osteogenic differentiation of MC3T3-E1 cells, we used real time RT-qPCR to measure the mRNA expression of osteogenesis genes in MC3T3-E1 cells after culturing in different media. Alkaline phosphatase and COL-I are early markers of osteoblastic differentiation and are used to check the initiation of mineralization [11]. Runt-related transcription factor 2 is a marker of osteoblastic differentiation and plays an important role in the maturation of osteoblasts [12]. Our results showed that the expression of osteogenic related genes (COL-I, ALP, and RUNX2) were upregulated in the co-culture supernatant group of days 3 and 7 compared with Jurkat T cells supernatant group. However, the osteogenic-related gene expression in both two groups were lower than that in the normal culture medium group (Fig.5)

Discussion

The process of the periodontal tissues healing is complex because it must occur in an open inflammatory environment exposed to bacterial contamination. GMSCs show a high immunomodulation capacity and can exert anti-inflammatory effects through cell contact, growth factor, and cytokine secretion [13]. Several studies have shown that GMSCs inhibit proliferation effects on peripheral blood mononuclear cells activated with phytohemagglutinin by secreting soluble factors and direct cell-cell contact [9]. GMSCs suppress T-cell proliferation in multiple pathways, including the CD39/CD73 pathway, Fas/FasL coupling pathway, and IDO signaling pathway [14,15,16]. Animal experiments have confirmed the

therapeutic effect of GMSCs on inflammatory diseases by regulating T-cell subset differentiation, including graft-versus-host disease, arthritis and hypersensitivity [17,18]. To evaluate the immunosuppressive effect of GMSCs on Jurkat T cells, the two groups of cells were directly co-cultured. The results suggest that GMSCs suppressed activated Jurkat T cells proliferation and activation. The lymphocyte SI of all the GMSC-co-cultured groups was significantly lower than that of the control group. In addition, the inhibition effect of GMSCs is cell-dose dependent. Real-time RT-qPCR results showed that co-culture with GMSCs had an inhibitory effect on the release of pro-inflammatory factors (IL-1 β , TNF- α), however, it promoted the secretion of anti-inflammatory factors (IL-10).

The pathological process of periodontitis can be summarized as follows: microorganisms attach to the root surface to activate the immune system, leading to the release and propagation of a range of inflammation cytokines, driving the destruction of the connective tissue and bone [19,20]. Immune cells and cytokines exert a crucial effect in alveolar bone resorption during the course of experimental periodontitis. The proportion of receptor activator of nuclear factor-kappa ligand (RANKL)-positive T-cell and B-cell was much higher in gingival tissue of periodontitis [21], than that in healthy gingival tissue. Severe combined immunodeficient mice showed lower bone loss following oral infection than immunocompetent mice, suggesting that B- and T-lymphocytes contribute to this process [22]. Inflammatory cytokines in the periodontal inflammatory microenvironment can influence the progression of periodontitis [23,24]. The interaction of pro-inflammatory and anti-inflammatory cytokines is highly important in the progression of periodontitis. The levels of pro-inflammatory cytokines including IL-1 β , IL-6, and TNF- α in gingival crevicular fluid of chronic periodontitis were higher than those in healthy subjects [25]. IL-1 β has a wide range of biological activities and can mediate a variety of inflammatory responses, including secretion of PGE2 and collagenase, and activation of T- and B- lymphocytes [26,27]. In addition, it can induce the secretion of proteinases, resulting in bone resorption and periodontal tissue loss. IL-1 β has been shown to induce bone resorption in mice both *in vitro* and *in vivo* [28,29]. TNF- α plays an important role in inhibiting osteoblastic differentiation and inducing osteoclast formation [30]. Higher concentrations of TNF- α could inhibit the osteogenic differentiation potential of odontogenic mesenchymal stem cells [31,32]. Several studies have shown that pro-inflammatory receptor antagonists can inhibit bone loss from periodontitis [33,34] IL-10 is an anti-inflammatory factor that influences the development and function of regulatory T-cell and downregulates the production of certain pro-inflammatory factors [35]. Moreover, IL-10 is involved in bone remodeling. It has been shown that IL-10 promotes osteogenic differentiation of MC3T3-E1 [36]. The absence of IL-10 could lead to accelerated resorption of alveolar bone [37].

The immunomodulatory effect of GMSCs in the microenvironment of periodontal regeneration could influence bone remodeling. Bone remodeling is composed of different phases during which osteoclasts remove mineralized bone and osteoblasts form new bone, with the osteoblast cell playing a key role. MC3T3-E1 cells culture is the most commonly used *in vitro* model for studying bone matrix mineralization [38]. To evaluate the effect of GMSCs and T-cell interaction on osteogenesis, MC3T3-E1 cells were cultured with the supernatant of GMSCs and Jurkat T cells co-culture. Our results suggest that the GMSCs co-culture increased the MC3T3-E1 cells' ALP activity, Alizarin red staining, and

immunofluorescence staining of OPN and COL-1. Meanwhile, on days 3 or 7, the GMSCs co-culture increased the MC3T3-E1 cells mRNA expressions levels of osteogenic parameters, including COL-I, ALP, and Runx2. However, the mRNA expressions of osteogenic-related genes in the two groups were lower than that in the common medium. This may be related to the cell culture ratio selected in the study that may not completely inhibit T-cell biological activity.

Conclusions

Our study suggests that GMSCs can indirectly promote bone regeneration through its immunosuppressive function. GMSCs as a seed cell for periodontal tissue regeneration, have broad application prospects. However, growth factors and scaffolds also play important roles in bone tissue engineering. Since Ideal growth factors and scaffolds are also being explored, there is still a long way to go before GMSCs can be used as a seed cell in tissue engineering for periodontal tissue regeneration.

List Of Abbreviations

GMSCs, gingival-derived mesenchymal stem cells;

IL-1 β , Interleukin-1 β ;

TNF- α , tumor necrosis factor- α ;

ALP, alkaline phosphatase;

AL, Alizarin red \square

COL-I, collagen type I;

RUNX2, Runt-related transcription factor 2;

OPN \square osteopontin;

MSCs, mesenchymal stem cells;

BMSCs, bone marrow mesenchymal stem cells;

α -MEM, Minimum Essential Medium;

FBS, fetal bovine serum;

MC3T3-E1, Murine calvarial cell line;

PBS, phosphate-buffered saline;

RT-qPCR, Real-time quantitative PCR.

Declarations

Ethics approval and consent to participate

The study was approved by the ethic's board of the Qingdao Municipal Hospital, china, study code 2020–097.

Consent for publication

Not applicable.

Availability of data and materials

The data sets examined for this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

As contributors, we certify that all the authors have participated adequately in the intellectual content, conception, and design of this work or the analysis and interpretation of the data as well as the writing, approving and agreeing with the content in the manuscript at the submitted version.

All persons who have made a substantial contribution to the work reported in the manuscript. But each author contributed to a part of the project, Jing Zhao participated at the experimental operation. Rui Liu and Jing Zhu participated in the test application and data analysis. Professor Shulan Chen and Ling Xu, article's preparation and orientation for the paper.

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Tables

Table1 Primer sequences for Jurkat T cells

Gene	Forward	Reverse
β -Actin	CGCCGCCAGCTCACC	TCGATGGGGTACTTCAGGGT
IL-1 β	AGCTGGAGAGTGTAGATCCC	GGGAACTGGGCAGACTCAA
TNF- α	TGCACTTTGGAGTGATCGGC	AGCTTGAGGGTTTGCTACAAC
IL-10	ATGCCTTTAATAAGCTCCAAGAGA	GTTTCTCAAGGGGCTGGGTC

Table2 Primer sequences for MC3T3-E1 cells

Gene	Forward	Reverse
β -Actin	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
ALP	CCAACCTTTTTGTGCCAGAGA	GGCTACATTGGTGTGAGCTTTT
COL-1	ACGTCCTGGTGAAGTTG	CAGGGAAGCCTCTTTCTCCT
Runx2	TCGGAGAGGTACCAGATGGG	AACTCTTGCCTCGTCCACTC

Figures

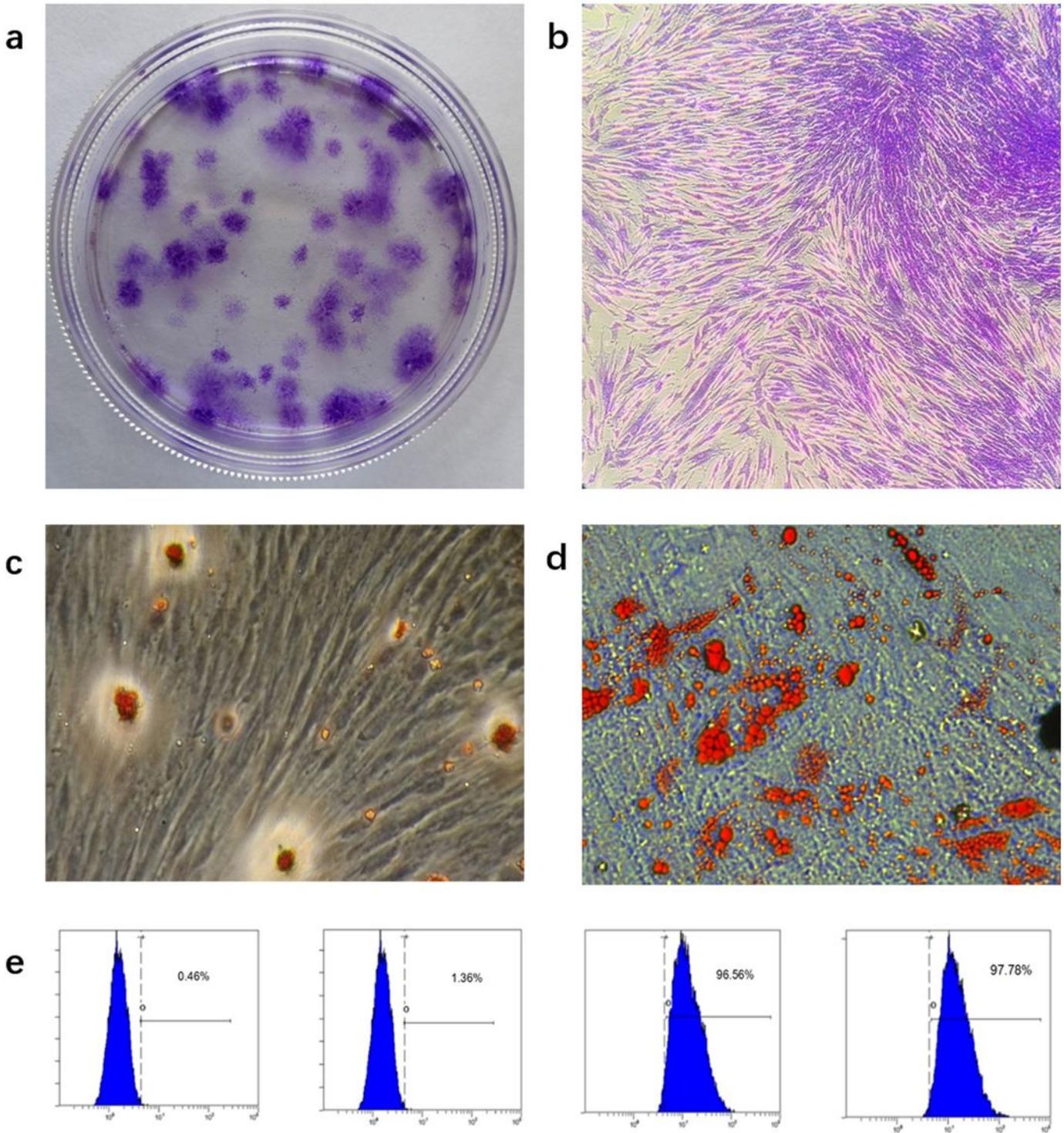


Figure 1

Isolation and characteristics of human gingival mesenchymal stem cells (GMSCs) a: Images of colony-forming units of GMSCs at 14 days. b: Long spindle-like shaped GMSCs (x100). c: Alizarin red staining confirm the formation of mineralized nodules (x200). d: Oil red O staining confirm the formation of lipid droplets (x200). e: The surface markers of GMSCs expressed through flow-cytometric analysis.

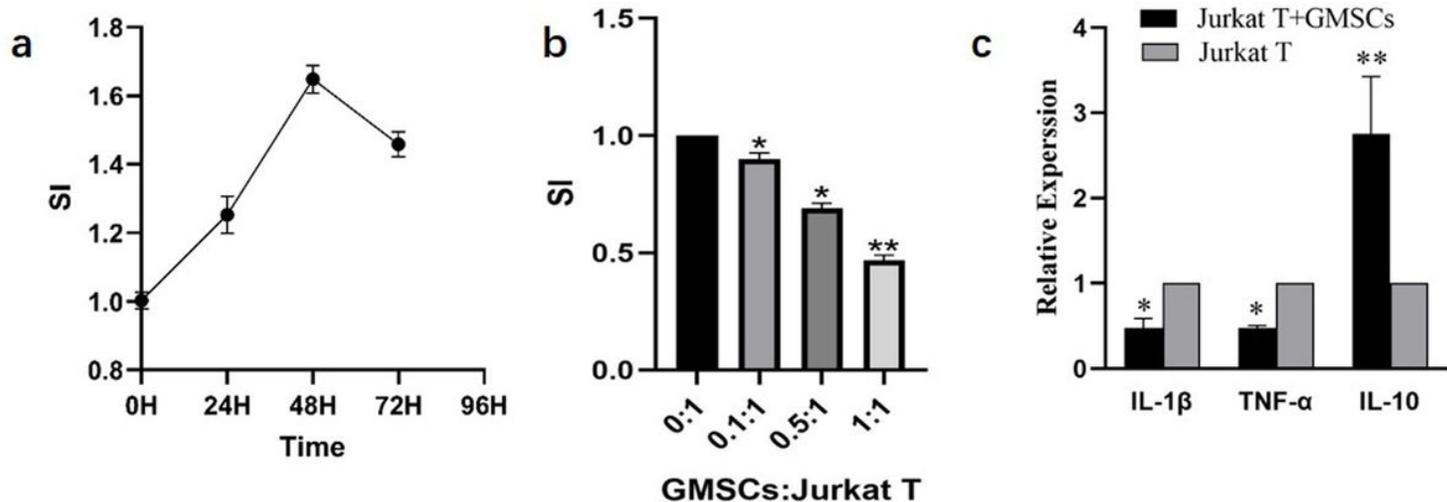


Figure 2

Gingival mesenchymal stem cells (GMSCs) inhibit the biological activity of activated Jurkat T cells a: The SI indexes of Jurkat T cell proliferation with different co-cultured times. b: The SI indexes of Jurkat T cell proliferation with different co-cultured proportions. c: The expressions of IL-1 β , TNF- α and IL-10 genes in Jurkat T cells co-cultured with GMSCs analyzed by real time RT-qPCR. (*p < .05, **p < .01 compared with the control, Jurkat T cell cultured without GMSCs was regarded as control group).

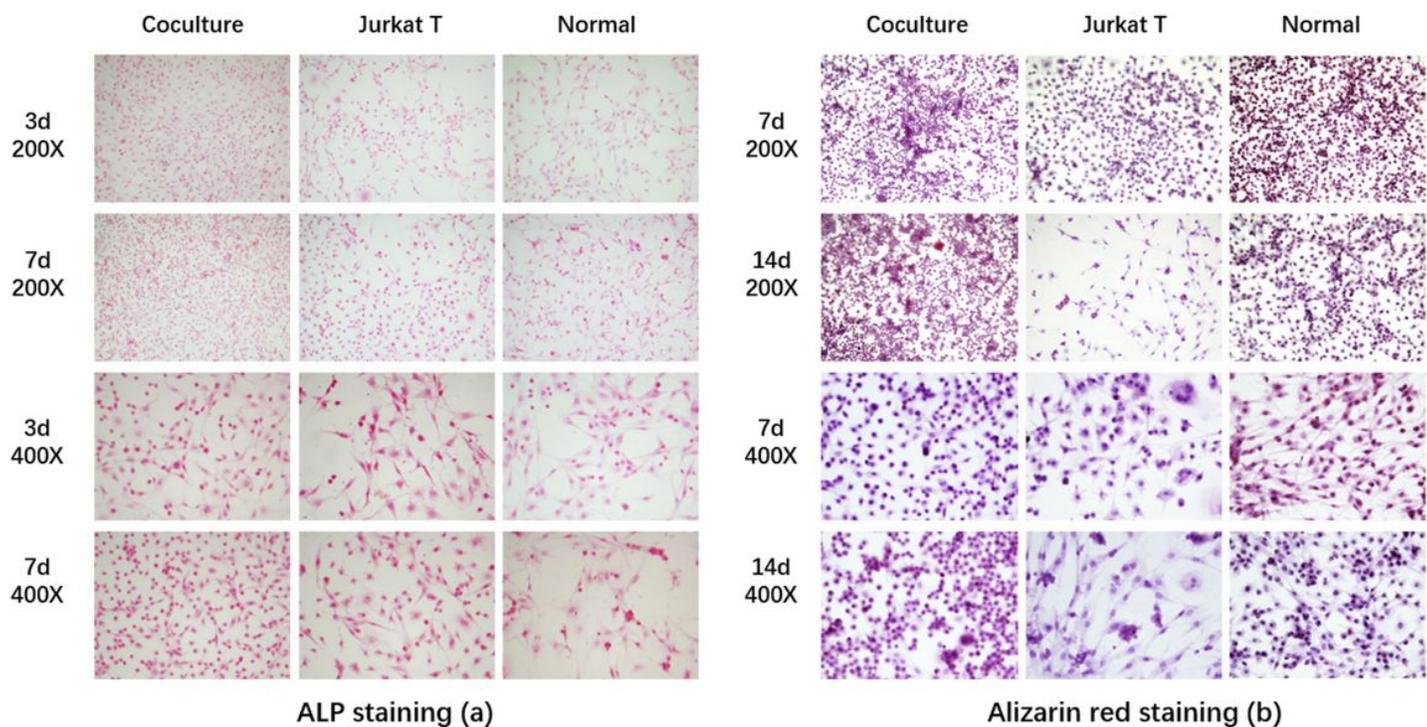


Figure 3

Gingival mesenchymal stem cells (GMSCs) promote the MC3T3-E1 osteogenic differentiation a: ALP staining observed on days 3 and 7. b: Alizarin red staining observed on days 7 and 14. Based on the different contents of medium, three groups were considered which include the supernatants of GMSCs

and Jurkat T cells co-culture (coculture), Jurkat T cells culture alone (Jurkat T), and the normal medium (Normal).

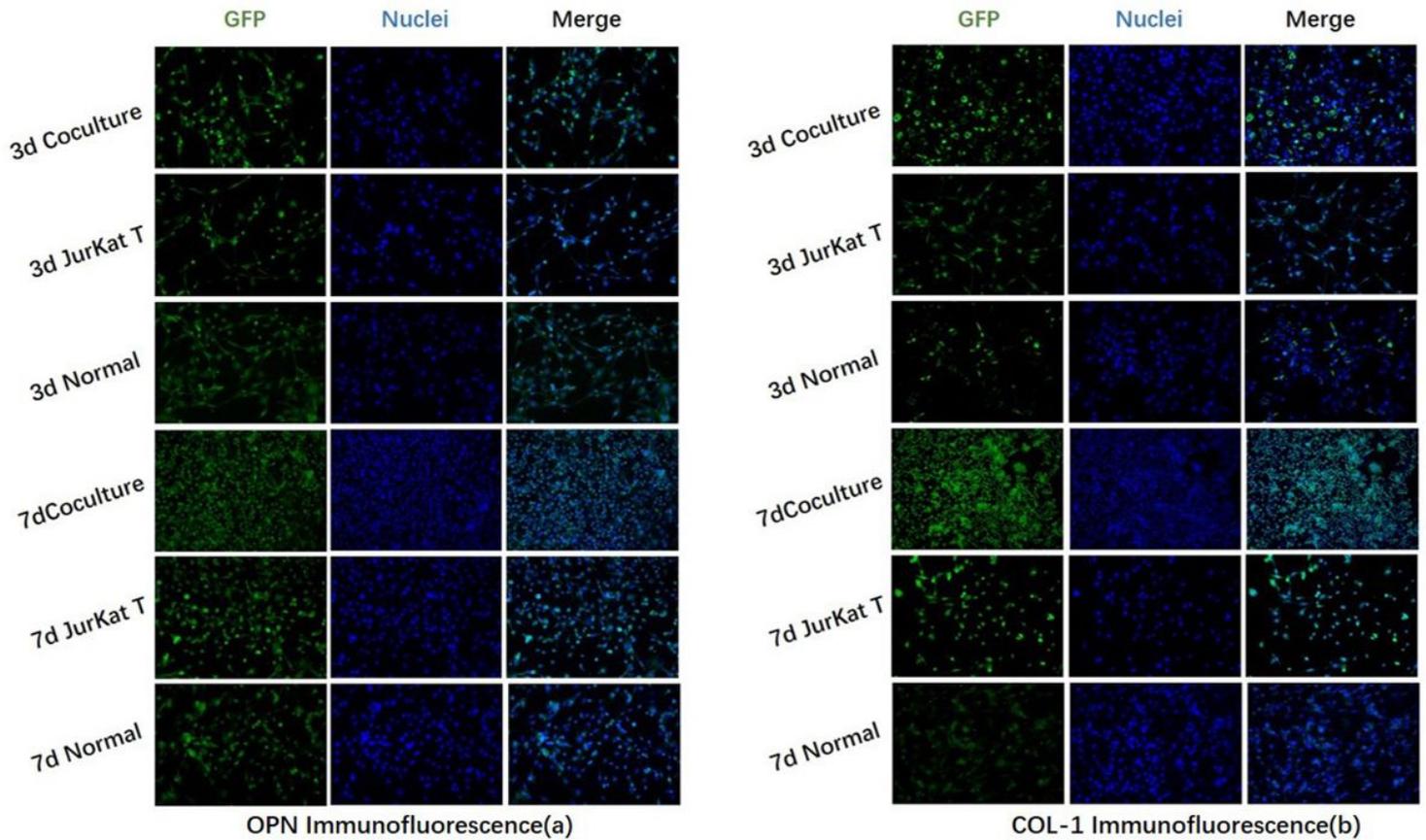


Figure 4

Gingival mesenchymal stem cells (GMSCs) promote osteogenic expression of MC3T3-E1 a: Immunofluorescence staining of Osteopontin (OPN), b: Immunofluorescence staining of collagen type 1 (COL-1). "Merge" represents the merged images of OPN-COL-1 and nuclei. Based on the different contents of medium, three groups were considered, which include the supernatants of GMSCs and Jurkat T cells co-culture (coculture), Jurkat T cells culture alone (Jurkat T), and the normal medium (Normal).

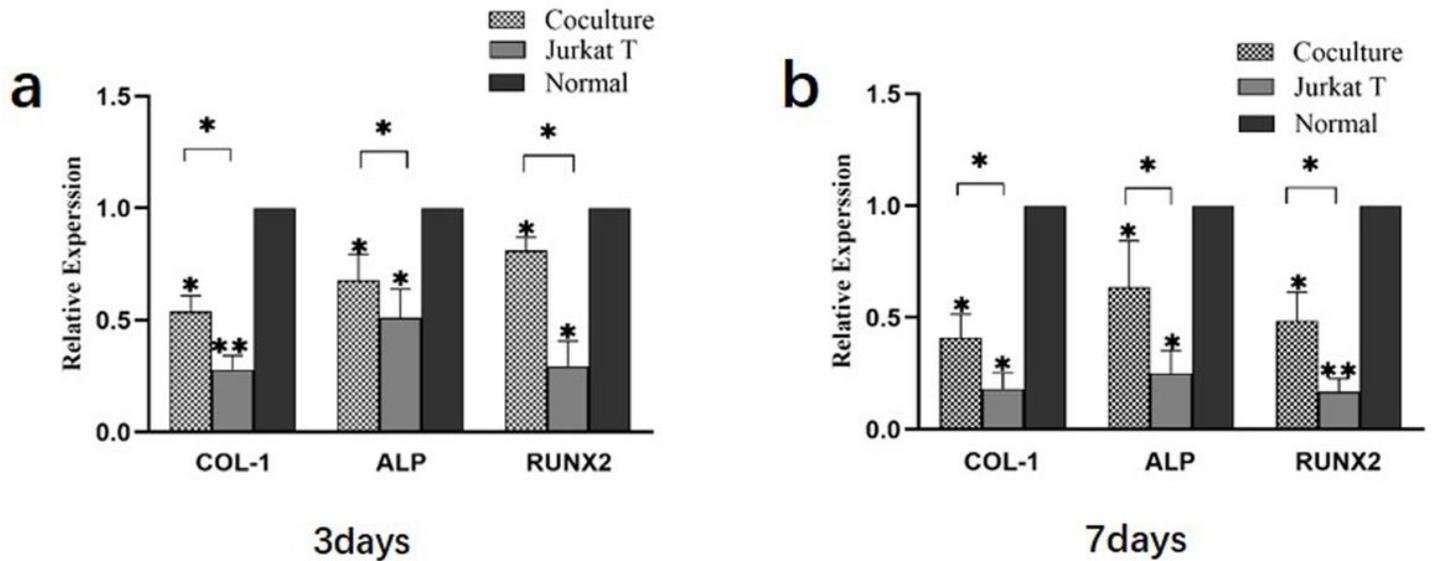


Figure 5

Osteogenic related gene expression of MC3T3-E1 after 3 and 7 days of induction a: Real time RT-qPCR analysis on days 3. b: Real time RT-qPCR analysis on days 7. Real time RT-qPCR analysis of gene expression of COL-1, ALP, RUNX2 in MC3T3-E1 treated with co-culture supernatant or Jurkat T cells supernatant compared with the gene expression of normal medium treated cells (*p < .05, **p < .01 compared with the normal group).