Hydrogen Sulphide Alleviates Senescence of Human Periodontal Ligament Stem Cells by TRPV4 Channel Mediated Calcium Flux

Yi Kun ZHOU[1,2,3#], Rui Li YANG[1,2,3#], Xiao Mo LIU[1,2,3]

Objective: *To explore whether hydrogen sulphide (H_2S) could protect human periodontal ligament stem cells (PDLSCs) from senescence and the possible underlying mechanisms.*
Methods: *Cell cycle assay and Ki-67 assay were used to measure proliferation of PDLSCs. Real-time polymerase chain reaction (PCR) was used to measure cellular senescence–related p16 and p21. Calcium influx was detected by measurement of Ca^{2+} imaging. In addition, we analysed the possible mechanisms underlying H_2S acting on PDLSCs by microarray.*
Results: *The cell proliferation rate of aging PDLSCs decreased significantly. The expression of cellular senescence–related p16 and p21 significantly increased in aging PDLSCs. H_2S donor (GYY4137) treatment increased the proliferation rate of senescence PDLSCs. Furthermore, the donor of H_2S treatment effectively prevented cell cycle arrest of PDLSCs during the aging process and inhibited the expression of cellular senescence–related markers. Mechanically, H_2S donor treatment could activate the calcium influx in PDLSCs. Moreover, pretreatment with TRPV4 inhibitors significantly attenuated the calcium influx induced by H_2S donor treatment in PDLSCs. It also alleviated the protective effect of H_2S on the senescence of PDLSCs.*
Conclusion: *H2S alleviated the senescence of human PDLSCs by TRPV4 channel mediated calcium flux. These results provide a potential strategy to deal with cell aging and may facilitate cell therapy for oral diseases.*
Key words: *calcium flux, cell senescence, hydrogen sulphide, periodontal ligament stem cells, transient receptor potential cation channel subfamily V member 4*
Chin J Dent Res 2023;26(1):19–27; doi: 10.3290/j.cjdr.b3978645

Mesenchymal stem cells (MSCs) are pluripotent non-hematopoietic progenitor cells with self-renewal and multilineage differentiation, which play a key role in tissue regeneration and homeostasis[1]. Periodontal ligament stem cells (PDLSCs), one type of MSCs isolated from periodontal tissues, have self-renewal and multidirectional differentiation potential, which can provide a reliable source of cells for clinical applications such as periodontal regenerative therapy[2]; however, as MSCs increase in number during in vitro culture, their proliferation ability decreases, and they will eventually enter a state of replication stagnation[3]. This replication stagnation, called cell senescence, could be caused by various stressors, which are characterised by cell cycle arrest[4]. To provide references for cell therapy and tissue regeneration, it is urgent to explore new strategies to inhibit or control cell senescence.

Hydrogen sulphide (H_2S) is now considered the "third gasotransmitter" alongside nitric oxide and carbon monoxide. In recent years, it has been reported that H_2S mediates various biological processes through multiple

1 Department of Orthodontics, Peking University School and Hospital of Stomatology, Haidian District, Beijing, P.R. China.
2 National Clinical Research Centre for Oral Diseases & National Engineering Laboratory for Digital and Material Technology of Stomatology, Haidian District, Beijing, P.R. China.
3 Beijing Key Laboratory of Digital Stomatology, Haidian District, Beijing, P.R. China.
# These two authors contributed equally to this work.

Corresponding author: Dr Xiao Mo LIU, Department of Orthodontics, Peking University School and Hospital of Stomatology, 22# Zhongguancun South Avenue, Haidian District, Beijing 100081, P.R. China. Tel: 86-10-82195128; Fax: 86-10-82195577. Email: momo96@163.com

This work was supported by the National Natural Science Foundation of China (grant no. 81970940), the Ten-thousand Talents Programme QNBJ-2020 (RY) and the Programme for New Clinical Techniques and Therapies of Peking University School and Hospital of Stomatology (PKUSSNCT-21B11).

signalling pathways, and abnormal H_2S metabolism is related to the dysfunction of MSCs[5]. For example, H_2S epigenetically mitigates bone loss through OPG/RANKL regulation during hyperhomocysteinemia[6]. An H_2S deficiency in bone marrow mesenchymal stem cells (BMMSCs) will weaken osteogenesis and proliferation. Interestingly, the serum and intracellular H_2O_2 levels in cystathionine β-synthase (CBS) deficient mice was decreased, leading to severe osteoporosis phenotypes[7]. As observed in BMMSCs and dental pulp stem cells (DPSCs), our studies have reported the new function of H_2S in these dental stem cells[8]. H_2S is necessary for periodontal tissue homeostasis, and studies have confirmed that cystathionine-γ-lyase (CSE) is essential for mechanical load–induced bone remodelling[9]. The study also showed that H_2S treatment increased the tooth movement rate in vivo by promoting osteogenesis and osteoclast formation in alveolar bone[10]. Perridon et al[11] showed that H_2S has a direct or indirect protective effect on aging characteristics except telomere wear. NaHS protects human umbilical vein endothelial cells from cell aging, possibly by regulating SIRT1 activity and improving the function of aging cells[12,13]. H_2S also induces S-vulcanisation of MEK1, which leads to PARP-1 activation and DNA damage repair and protects cells from aging[14]. In addition, the lack of CSE in mouse embryonic fibroblasts leads to the early development of cell senescence[15]. Whether H_2S might be able to regulate the senescence of PDLSCs is still unclear.

Ca^{2+} signalling is central to driving morphological changes that are the hallmarks of senescence. A senescence-associated increase in cell volume is normally countered by regulatory volume decrease (RVD), which preserves the structural integrity of the cell[16]. Transient receptor potential cation channel V4 (TRPV4) is a member of the transient receptor potential (TRP) superfamily, which is characterised by a weak voltage-dependent nonselective cation channel. This channel has been proven to regulate the homeostasis of intracellular calcium concentration Ca^{2+} and participate in the integrity of osmotic adjustment, endothelial barrier, nociception and bone homeostasis[17-21]. Our previous study reported that the Ca^{2+} influx triggered by H_2S maintained the bone and MSC homeostasis, and TRPV1 played an important role in maintaining MSC capacity[22,23]. Whether H_2S could regulate the calcium influx in PDLSCs and detailed molecular mechanism needs to be elucidated.

Here, we demonstrated that H_2S alleviates senescence of PDLSCs shown as increased cell proliferation and inhibited cell cycle arrest, which was mediated by TRPV4 channel–mediated calcium flux.

Materials and methods

Reagents and cell culture

Cell viability was confirmed by toluidine blue staining (Sigma-Aldrich, St Louis, MO, USA). Chemicals were purchased from Sigma-Aldrich, and TRIzol and RevertAid Reverse Transcriptase were purchased from Thermo Fisher Scientific (Carlsbad, CA, USA). TRPV4 siRNA was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). Transfection reagent was purchased from Invitrogen (Waltham, MA, USA). PDLSCs were provided by the Oral Stem Cell Bank (Beijing, China) and isolated as previously reported. PDLSCs were cultured in α-MEM supplemented with 15% foetal bovine serum, 100 U/ml penicillin and 100 U/ml streptomycin. This study was approved by the Animal Care and Use Committee of the Health Science Centre, Peking University (no. 2015-186).

Real-time PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA) and LightCycler 96 (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer's instruction. The qPCR primers were designed as follows: p21 Forward:5`-AGGTGGACCTGGAGACTCTCAG-3`,Reverse:5`-TCCTCTTGGAGAAGATCAGCCG-3`;p16Forward:5`-CTCGTGCTGA TGCTACTGAGGA-3`,Reserve:5`-GGTCGGCGCAGTTGGGCTCC-3`; IL-6 Forward:5`-TTCTGCGCAGCTTTAAGGAG-3`,Reverse:5`-AGGTGCCCATGCTACATTTG-3`;IL-8 Forward:5`- ATGACTTCCAAGCTGGCCGTG-3`,Reserve:5`- TGTGTTGGCGCAGTGTGGTC-3`;MCP1 Forward:5`- AGGGAACTTGAGCGTGAATC -3`, Reserve: 5`- TCACTTGTCTGTTGCACACG -3`;GAPDH Forward:5`-AGCCGCATCTTCTTTTGCGTC-3`,Reserve: 5`-TCATATTTGGCAGGTTTTTCT-3`.

Senescence-associated β-galactosidase (SA-β-gal) staining

Senescence-associated β-galactosidase (SA-β-gal) expression was visualised using an SA-β-gal staining kit (Beyotime Institute of Biotechnology, Shanghai, China) according to the manufacturer's protocol. Cells were washed with phosphate buffer solution (PBS) twice and fixed using a fixative solution at room temperature for 15 minutes, and then stained with X-gal solution for 24 hours at 37°C (without CO_2). Cells were observed using a light microscope (Leica, Wetzlar, Germany) with a magnification of ×100, and the percentage of SA-β-gal-positive cells in 10 random fields was calculated.

Western blotting

Western blotting was performed as previously described[22]. The antibodies used included p21, p16 and Actin (Thermo Fisher Scientific).

Senescence-associated heterochromatin foci (SAHF) analysis

To determine that SAHF formation had occurred, cells were cultured directly on glass cover slips and then fixed with 4% paraformaldehyde. After washing with PBS, cells were permeabilised with 0.2% Triton X-100/PBS for 10 minutes. DNA was visualised by 4′-6′-diamidino-2-phenylindole (DAPI) staining (1 mg/ml) for 1 minute and then washed with PBS twice. Cover slips were mounted in a 90% glycerol PBS solution and examined under a laser confocal microscope.

Flow cytometric analysis

To detect the levels of Ki-67, PDLSCs were washed twice and incubated with Ki-67 antibodies at 4°C for 40 minutes. Isotype antibodies served as controls. Cells were analysed by flow cytometry using a BD FACSVia flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Each analysis included 20,000 events.

Cell cycle assay

To determine the effect of H_2S on cell cycle progression, PDLSCs from the log phase were grown in 6-well plates and treated with 50 μM GYY4137 for 12 hours. Cells were collected using trypsinisation and centrifugation for 5 minutes at 300 × g centrifuge force and fixed with 70% ethanol at 4°C overnight. They were stained using a Cell Meter Fluorimetric Fixed Cell Cycle Assay Kit (AAT Bioquest, Pleasanton, CA, USA) following the manufacturer's instructions, then subjected to flow cytometric analyses with FACSCalibur and CellQuest software (both BD Biosciences). Cell cycles were analysed and the proportion of cells in the G0/G1, S and G2/M phases was recorded.

Microarray

Total RNAs were extracted from the control and 50 μM GYY4137 treated PDLSCs with an RNeasy kit (QIAGEN, Hilden, Germany). Microarray assays were performed at the Genome Centre at Children's Hospital Los Angeles using 1.0 ST arrays (Affymetrix, Santa Clara, CA, USA). The gene expression difference was analysed using Partek Genomics Suite (Partek, St Louis, MA, USA) and Ingenuity Pathway Analysis (IPA) software (QIAGEN). Global gene expression profiles rank ordered by relative fold-change values were analysed using Gene Set Enrichment Analysis software (Broad Institute, MIT, Cambridge, MA, USA).

Measurement of Ca^{2+} imaging

PDLSCs (1×10^5) were seeded onto 60-mm culture dishes and cultured for 24 hours at 37 °C in 5% CO_2. Next, PDLSCs were stimulated by GYY4137 with a concentration of 50 μm for 24 hours. Then, the cells were loaded with fura-2 AM (Invitrogen) and incubated for 1 hour at 37°C in 5% CO_2 in the dark. Ca^{2+} levels were measured by the ratio of emission in response to excitation at 340 and 380 nm on an Olympus Optical IX71 microscope (Olympus Life Science, Tokyo, Japan).

Statistical analysis

P values were analysed using a two-tailed Student *t* test for the difference between two groups or one-way analysis of variance to compare the difference from more than two groups using SPSS 18.0 software (IBM, Armonk, NY, USA). $P < 0.05$ was considered significant.

Results

Hydrogen sulphide alleviated senescence of PDLSCs

Firstly, we compared the cell viability of PDLSCs from passage 5 (P5) and passage 15 (P15) and found that when the cells were passaged to P15, the PDLSCs became flat and the number of cells was significantly reduced, which may be because the cells differentiated. GYY4137, a slow-release agent of H_2S, treatment could increase cell density, especially in the PDLSCs in the P15 group (Figs 1a and b). The expression of p21 and p16, which are cyclin-dependent kinase inhibitors and play a vital role in cell senescence, increased significantly when PDLSCs expanded to passage 15, while the addition of H_2S donor could partially decrease the expression of p16 and p21 (Figs 1c, d and g). Besides, the staining results showed that the expression of β-galactosidase in senescent cells was obviously upregulated compared with PDLSCs at P5 (Figs 1e and f). As shown in Fig 1h, senescence also led to pronounced DNA SAHF formation, which was visualised by DAPI staining (Figs 1h and i). Compared with the control, GYY4137 significantly suppressed the senescence-induced SA-β-gal activity and SAHF forma-

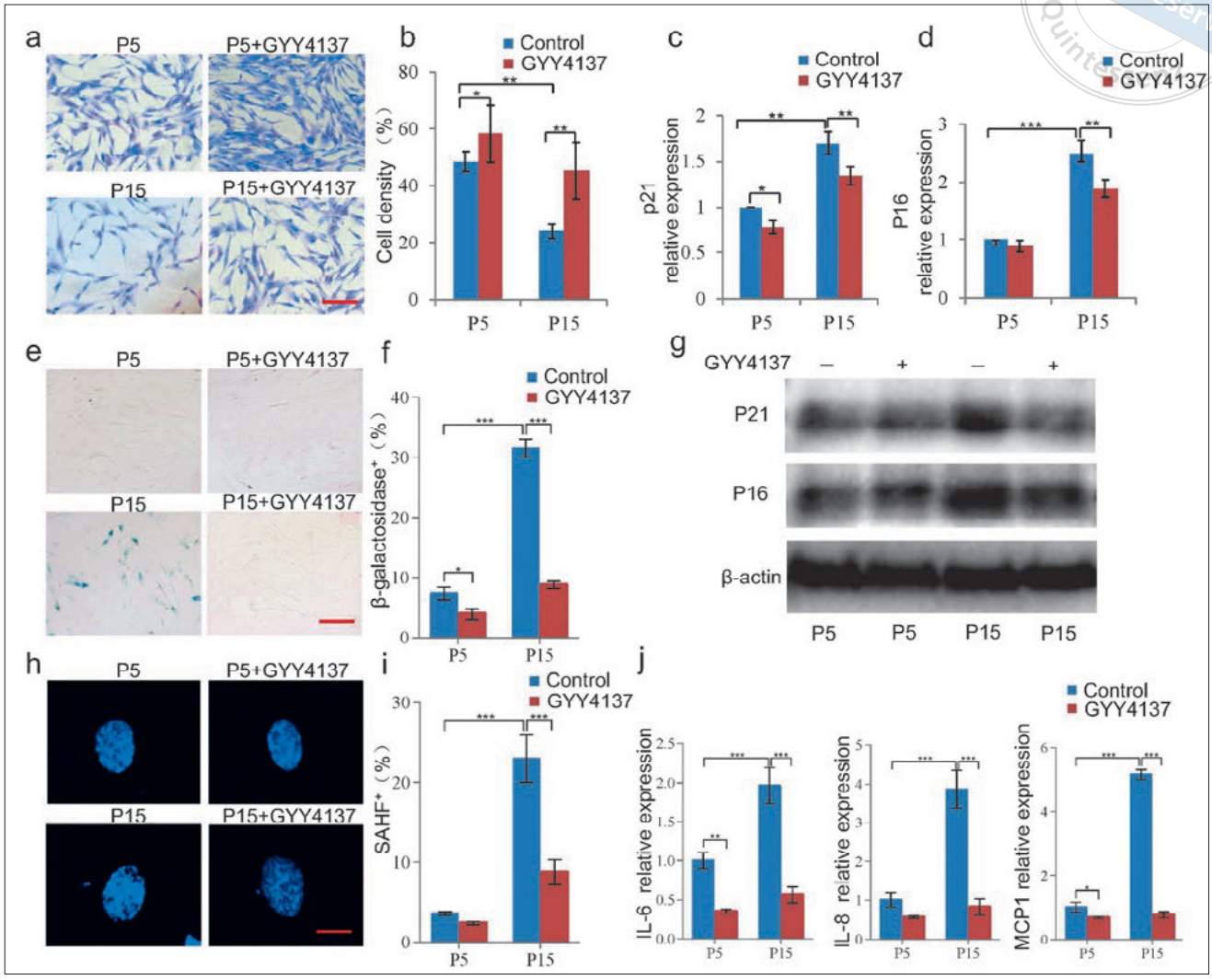

Fig 1 Hydrogen sulphide alleviates senescence of PDLSCs. **(a)** Toluidine blue staining of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137. Scale bar: 50 μm. **(b)** Semi-quantitative analysis of cell density of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137. **(c-d)** Expression of p21 **(c)** and p16 **(d)** in PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by qPCR. **(e)** β-galactosidase staining of PDLSCs at P5 and P15 treated with or without 50μM GYY4137. Scale bar: 50 μm. **(f)** Semi-quantitative analysis of β-galactosidase positive of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137. **(g)** Expression of p21 and p16 in PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by western blot. **(h)** Cells were stained for DAPI to visualise SAHF formation. Scale bar: 10 μm. **(i)** Semi-quantitative analysis of SAHF positive of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137. **(j)** Expression of SASP (IL-6, IL-8 and MCP1) in PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by qPCR. Data are presented as mean ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$), n = 3.

tion in PDLSCs at P15. Senescence associated secretory phenotype (SASP) is a phenomenon whereby senescent cells increase the expression and secretion of certain cytokines, chemokines and other proteins. Notably, the SASP-related gene expression (IL-6, IL-8, MCP1) was significantly higher in the PDLSCs in the P15 group compared to those in the P5 group. Moreover, the expression of SASP-related cytokine genes was also inhibited by the H_2S donor treatment (Fig 1j).

To further characterise the effects of H_2S on the senescence of PDLSCs, we analysed the proliferation of PDLSCs and found that it was markedly decreased when passaged to P15 compared with P5, while GYY4137 treatment could increase the proliferation of PDLSCs at P15, which was assessed by the expression level of Ki67 using flow cytometry analysis (Figs 2a and b). Cell cycle arrest during the G1 phase is a characteristic exhibited by senescent cells. Our results demonstrated that

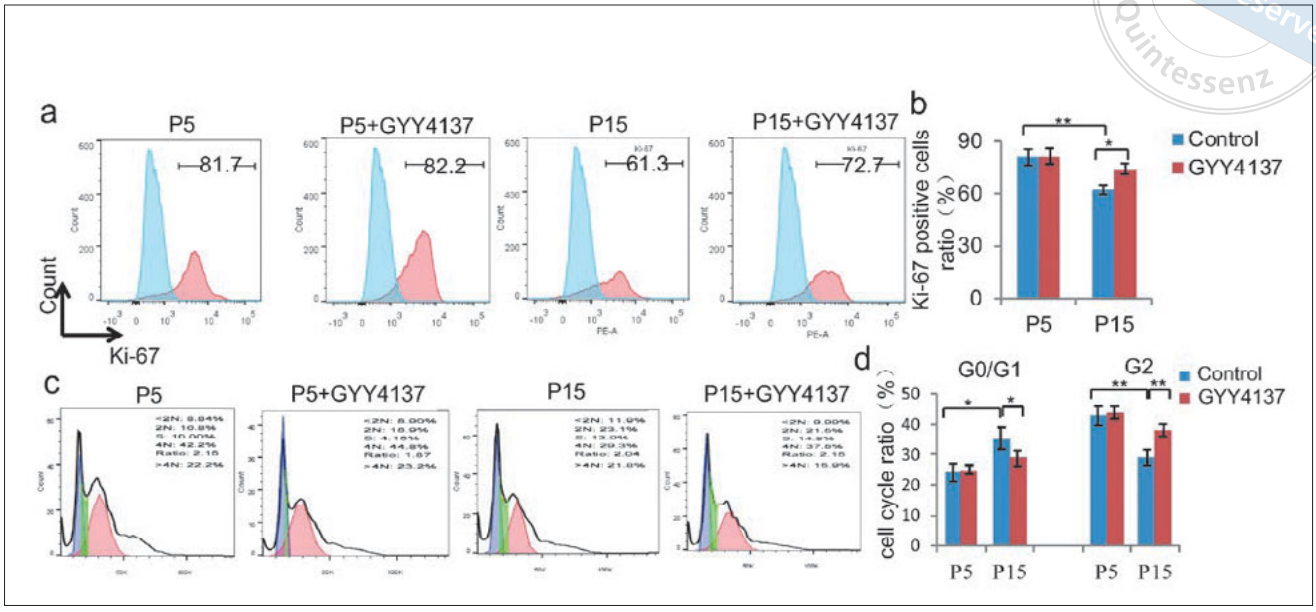

Fig 2 Hydrogen sulphide treatment increased the proliferation of PDLSCs **(a-b).** The proliferation rate of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by flow cytometry. **(c-d)** Cell cycle of PDLSCs at P5 and P15 treated with or without 50 μm GYY4137, as assessed by flow cytometry. Data are presented as mean ± SD (*P < 0.05, **P < 0.01, ***P < 0.001), n = 3.

PDLSCs at P15 stagnated in the G0/G1 phase as the proportion of cells in the G0/G1 phase increased compared to those at P5. Moreover, the proportion of PDLSCs in the G2 phase decreased at P15 compared with the ones at P5. H_2S treatment reduced the proportion of PDLSCs during the G0/G1 phase and increased the ratio of cells during the G2 phase in the P15 group (Figs 2c and d).

Hydrogen sulphide treatment increased the calcium pathway in PDLSCs

To investigate the stem cell biology regulated by H_2S, we performed a microarray using PDLSCs with or without 50 μm GYY4137 treatment. The heatmap depicting the significant upregulated and downregulated genes (fold change > 2, P < 0.05) is shown in Fig 2a. The calcium pathway was one of the top 10 significantly enriched signalling clusters after H_2S treatment, analysed by Kyoto Encyclopaedia of Genes and Genomes (KEGG) (Figs 3a and b). Most of the genes related to the calcium pathway were significantly upregulated after H_2S treatment in PDLSCs (Fig 3c). Furthermore, H_2S donor treatment induced Ca^{2+} influx in PDLSCs (Fig 3d). Sequencing results showed that the calcium-related signalling pathway increased significantly after H_2S stimulation. Next, we analysed the differential genes using Gene Set Enrichment Analysis and the results showed that the TRPV channel was one of the top enriched clusters (Fig 3e).

Hydrogen sulphide activated TRPV4-channel mediated Ca^{2+} influx

Calcium entry could be activated through TRPV4 channels[19,24]. We found that the Ca^{2+} influx induced by H_2S was partially blocked by TRPV4 siRNA treatment (Figs 4a and b). In addition, compared with the control group, treatment with TRPV4 inhibitor GSK2193874A also blocked the calcium influx induced by H_2S (Fig 4c). Moreover, the H_2S donor treatment induced significantly higher Ca^{2+} in PDLSCs from P5 compared with the ones from P15 (Fig 4d). To confirm the role of H_2S treatment, we analysed the expression level of TRPV4. The results showed that TRPV4 mRNA were increased after H_2S treatment (Fig 4e). These results indicated that H_2S activated Ca^{2+} influx via the TRPV4 channel in PDLSCs.

Hydrogen sulphide alleviated senescence of PDLSCs by TRPV4 channel-mediated calcium flux

To further verify the role of TRPV4 mediated by calcium on senescence of PDLSCs, we pretreated PDLSCs with TRPV4 siRNA and found that the effects of GYY4137 against senescence of PDLSCs were attenuated by TRPV4 siRNA treatment. For instance, the cell density and viability of PDLSCs at P15 reversed by H_2S treatment were decreased after TRPV4 siRNA pretreatment (Figs 5a and b). The expression of p16 and p21 in PDLSCs at P15 decreased by H_2S donor treatment was partially

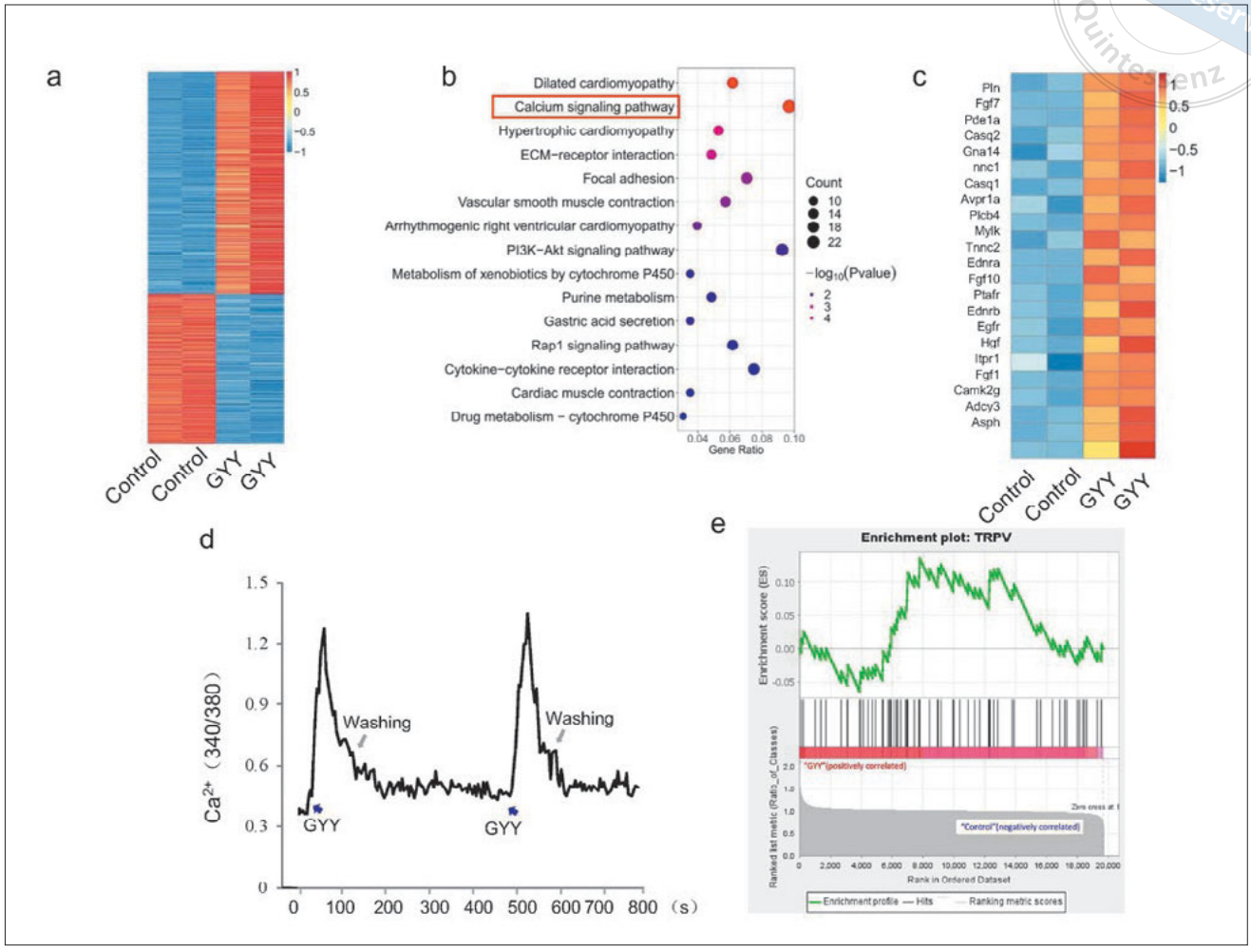

Fig 3 Hydrogen sulphide treatment increased the calcium pathway in PDLSCs. **(a)** Heatmap of genes differentially expressed in control and GYY4137 treated PDLSCs, as analysed by microarray. **(b)** The calcium signalling pathway is one of the top ten enriched clusters between control and GYY4137 treated PDLSCs as assessed by KEGG pathway analysis. **(c)** The different expression genes related with the calcium signalling pathway between control and GYY4137 treated PDLSCs. **(d)** The Ca^{2+} influx induced by GYY4137 treatment in PDLSCs, as assessed by Ca^{2+} levels using the ratio of emission in response to excitation at 340 and 380 nm. **(e)** The TRPV channels were significantly enriched based on the differential genes, as assessed by GSEA.

attenuated after TRPV4 siRNA pretreatment (Figs 5c and d). The proliferation rate of PDLSCs at P15 increased by GYY4137 stimulation was alleviated after TRPV4 siRNA pretreatment (Fig 5e). Furthermore, the cell cycle arrest reversed by GYY4137 was partially attenuated when PDLSCs received TRPV4 siRNA pretreatment (Fig 5f). These results demonstrated that inhibition of TRPV4 attenuated the protective effect of H_2S against senescence in PDLSCs.

Discussion

Cell senescence is a process whereby cells irreversibly withdraw from the cell cycle and stop dividing in response to various stresses[25]. In this study, we obtained senescent cells through continuous passaging of stem cells and found that when PDLSCs were passaged to P15, the cell viability and proliferation were decreased significantly compared with PDLSCs at P5. The ratio of the β-galactosidase positive cells increased significantly when passaged to P15. In addition, the senescence-related markers SAHF and SASP also increased remarkably at P15 compared to P5, and the PDLSCs at P15 showed cell cycle arrest with the higher expression of cyclin-dependent kinase inhibitor p16 and p21. All these results demonstrated that long-term in vitro culturing and expansion caused cell senescence in PDLSCs.

Our previous study showed that H_2S could promote

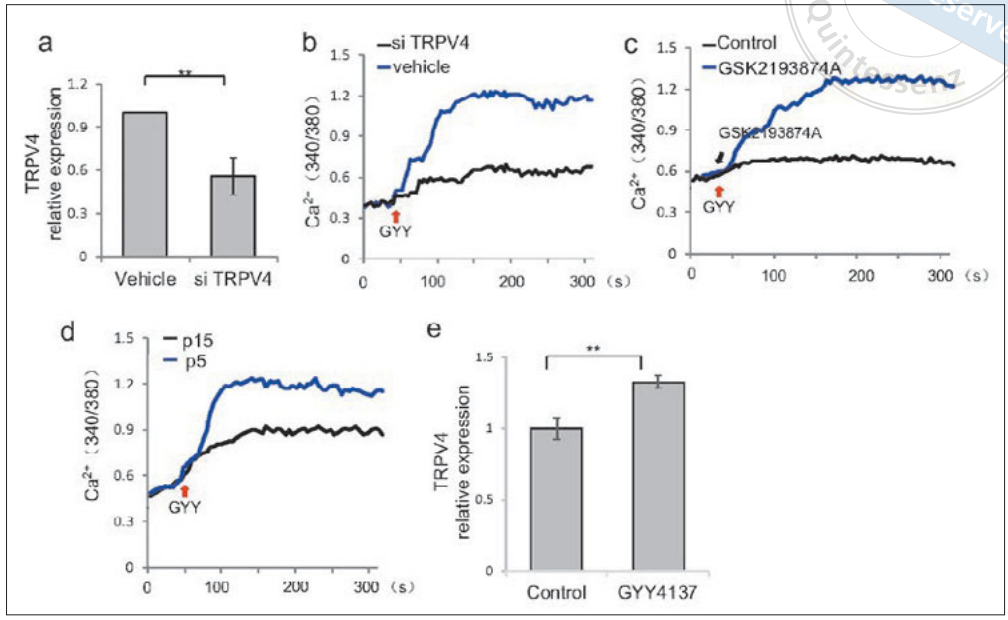

Fig 4 Hydrogen sulphide activated TRPV4 channel–mediated Ca^{2+} influx. **(a)** Efficacy of TRPV4 siRNA, as assessed by qPCR. **(b)** Ca^{2+} influx induced by NaHS treatment in PDLSCs with or without TRPV4 siRNA treatment. **(c)** The Ca^{2+} influx induced by NaHS treatment in PDLSCs was inhibited with TRPV4 inhibitor GSK2193874A treatment. **(d)** Ca^{2+} influx induced by NaHS treatment in PDLSCs of P5 and P15. **(e)** Expression of TRPV4 in control and GYY4137 treated PDLSCs, as analysed by qPCR. Data are presented as mean ± SD ($^{**}P < 0.01$).

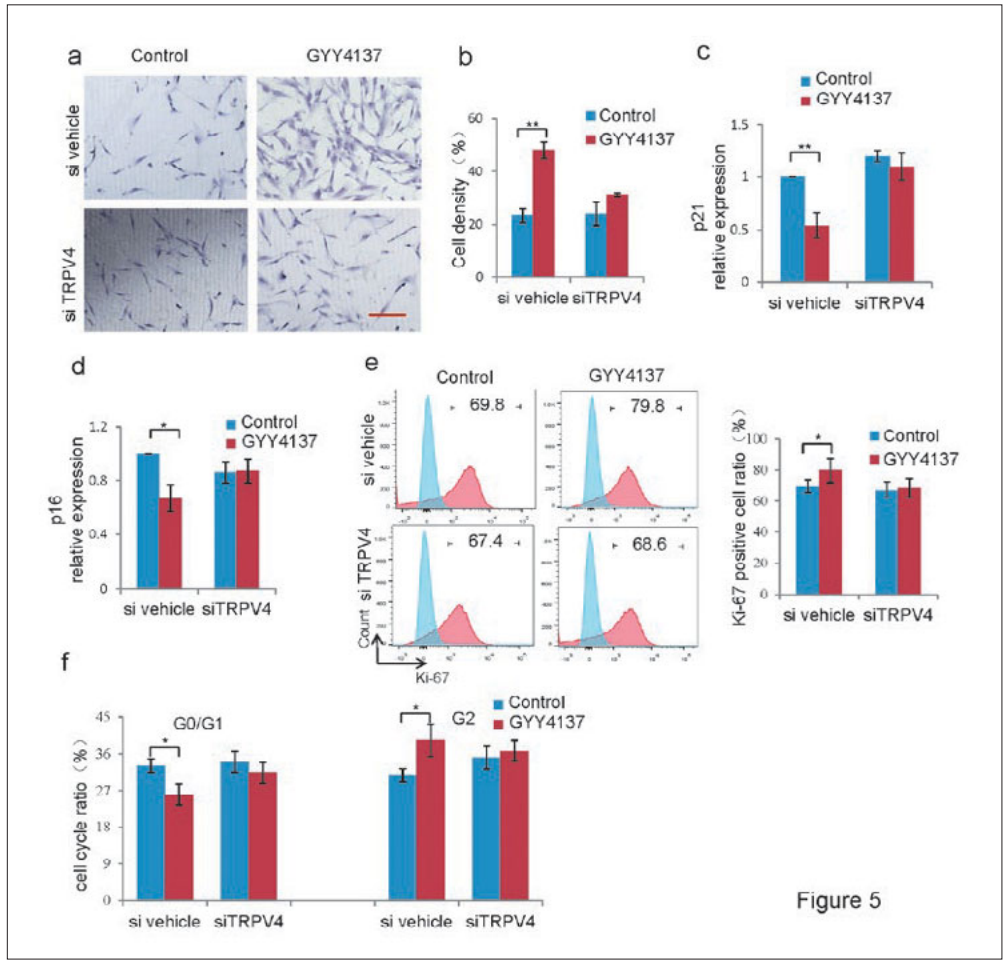

Figure 5

Fig 5 The capacity of H_2S to alleviate senescence of PDLSCs was inhibited by TRPV4 siRNA treatment. **(a)** Toluidine blue staining of control and TRPV4 siRNA treated PDLSCs with or without 50 μm GYY4137 stimulation. **(b)** Semi-quantitative analysis of cell density of control and TRPV4 siRNA treated PDLSCs with or without 50 μm GYY4137 stimulation. **(c-d)** Expression of p21 **(c)** and p16 **(d)** in control and TRPV4 siRNA treated PDLSCs with or without 50μM GYY4137 stimulation, as assessed by qPCR. **(e)** Proliferation rate of control and TRPV4 siRNA treated PDLSCs with or without 50 μm GYY4137 stimulation, as assessed by flow cytometry. **(f)** Cell cycle of control and TRPV4 siRNA treated PDLSCs with or without 50 μm GYY4137 stimulation, as assessed by flow cytometry. Data are presented as mean ± SD ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

bone regeneration by increasing osteogenic differentiation of MSCs[22]. Here we showed that H_2S donor GYY4137 treatment could protect PDLSCs from cell senescence with continuous passage. It has been reported that endogenous H_2S signalling maintains the proliferation ability of PDLSCs and neural stem cells[26-28]. The aging of MSCs is a complex process with comprehensive mechanisms. There is an urgent need for a strategy to produce a large number of MSCs that retains stemness and pedigree plasticity. Thus, many alternative methods have been tried to prevent or reverse aging and improve the clinical application of MSCs. Antioxidants such as ascorbic acid, cirsium setidens, lactoferrin and N-acetyl-l-cysteine can slow the aging of MSCs by inhibiting the production of reactive oxygen stress (ROS) in MSCs[29-31]. Some genetic engineering methods can also effectively slow the aging of MSCs. It was reported that knocking out migration inhibitory factor (MIF) could induce the aging of young MSCs, while overexpression of MIF led to regeneration of old MSCs[32]. In addition, some molecular compounds that activate endogenous telomerase, such as aspirin, vitamin C and FGF-2, were also used to save the proliferation potential of aged MSCs and restore their osteogenic ability[33-35]. Our results suggested that H_2S might be responsible for retarding the aging process, but the mechanisms for such properties need further investigation.

Furthermore, we investigated the possible underlying mechanism for the protective role of H_2S in PDLSCs senescence. First, we found that the expression of calcium influx–related pathway proteins in cells treated with H_2S was upregulated significantly by sequencing. Up to now, only a few studies have clarified the effect of calcium on cell senescence. One study revealed that calcium chelation could protect cells from premature senescence by reducing the DNA damage response (DDR) activation with a subsequent decrease in the p53/p21/Rb pathway[36]. It was reported that the basal calcium level of replicative aging fibroblasts was higher compared with non-aging cells[36]. Calcium protease is activated in the aging cells and leads to the enhancement of IL-1α processing. Chelating Ca^{2+} can inhibit the activation of calpain and the processing of IL-1α and alleviate cell senescence[37]. Our studies showed that Ca^{2+} influx was necessary to maintain the proliferation activity and steady state of MSCs, which was consistent with previous results[22,23]. Activation of TRPV4 channels appears to cause calcium events that result in the opening of eBK channels, endothelial hyperpolarisation and subsequent vasodilation[38]. Our results showed that H_2S protected cells against senescence through TRPV4-mediated calcium influx. To further confirm the role of TRPV4-mediated calcium influx on stem cell senescence, we used TRPV4 inhibitor or SiRNA to reduce the expression of TRPV4. We found that TRPV4 inhibition attenuated the protective effect of H_2S on cell senescence. It was reported that the transient receptor potential TRP) family mediated Ca^{2+} channels were sulfhydrylated by H_2S[22].The decrease in H_2S levels leads to cascade reactions in BMMSCs, including altered Ca^{2+} channel (at least including TRPV6, TRPV3 and TRPM4) sulfyhydration, and osteogenic differentiation[22]. H_2S-mediated vasodilation involves the activation of TRPV4-dependent Ca^{2+} influx and eBK channel activation in vascular endothelial cells[38]. H_2S also promotes the activation of T cells and differentiation of Th-cells (Treg cells) to control the homeostasis of the immune system. The specific molecular mechanisms that show H_2S regulated calcium influx in PDLSCs need to be investigated further.

Conclusion

The current research illustrates that H_2S could inhibit the senescence of PDLSCs and determines the intermediary role of TRPV4-mediated calcium ion influx in the protective effect of H_2S on senescence. It provides a potential strategy to prevent cell aging and may facilitate cell therapy for oral diseases.

Acknowledgements

The authors thank the Oral Stem Cell Bank for the PDLSCs provided by Beijing Tason Biotech (http://www.kqgxb.com).

Conflicts of interest

The authors declare no conflicts of interest related to this study.

Author contribution

Drs Yi Kun ZHOU and Rui Li YANG contributed to the collection and assembly of data, data analysis and interpretation, and manuscript drafting; Drs Rui Li YANG and Xiao Mo LIU contributed to the overall design of the study, critical editing of the manuscript, and financial support. All authors read and approved the final manuscript.

(Received Aug 03, 2022; accepted Nov 07, 2022)

References

1. Weng Z, Wang Y, Ouchi T, et al. Mesenchymal stem/stromal cell senescence: Hallmarks, mechanisms, and combating strategies. Stem Cells Transl Med 2022;11:356–371.
2. Seo BM, Miura M, Gronthos S, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149–155.
3. Yang YK, Ogando CR, Wang See C, Chang TY, Barabino GA. Changes in phenotype and differentiation potential of human mesenchymal stem cells aging in vitro. Stem Cell Res Ther 2018;9:131.
4. Sethe S, Scutt A, Stolzing A. Aging of mesenchymal stem cells. Ageing Res Rev 2006;5:91–116.
5. Hao Y, Wang H, Fang L, Bian J, Gao Y, Li C. H2S donor and bone metabolism. Front Pharmacol 2021;12:661601.
6. Behera J, George AK, Voor MJ, Tyagi SC, Tyagi N. Hydrogen sulfide epigenetically mitigates bone loss through OPG/RANKL regulation during hyperhomocysteinemia in mice. Bone 2018;114:90–108.
7. Behera J, Kelly KE, Voor MJ, Metreveli N, Tyagi SC, Tyagi N. Hydrogen sulfide promotes bone homeostasis by balancing inflammatory cytokine signaling in CBS-deficient mice through an epigenetic mechanism. Sci Rep 2018;8:15226.
8. Yang R, Liu Y, Shi S. Hydrogen sulfide regulates homeostasis of mesenchymal stem cells and regulatory T cells. J Dent Res 2016;95:1445–1451.
9. Mo S, Hua Y. Cystathionine gamma lyase-H2S contributes to osteoclastogenesis during bone remodeling induced by mechanical loading. Biochem Biophys Res Commun 2018;501:471–477.
10. Pu H, Hua Y. Hydrogen sulfide regulates bone remodeling and promotes orthodontic tooth movement. Mol Med Rep 2017;16:9415–9422.
11. Perridon BW, Leuvenink HGD, Hillebrands JL, van Goor H, Bos EM. The role of hydrogen sulfide in aging and age-related pathologies. Aging (Albany NY) 2016;8:2264–2289.
12. Zheng M, Qiao W, Cui J, et al. Hydrogen sulfide delays nicotinamide-induced premature senescence via upregulation of SIRT1 in human umbilical vein endothelial cells. Mol Cell Biochem 2014;393:59–67.
13. Suo R, Zhao ZZ, Tang ZH, et al. Hydrogen sulfide prevents H_2O_2-induced senescence in human umbilical vein endothelial cells through SIRT1 activation. Mol Med Rep 2013;7:1865–1870.
14. Zhao K, Ju Y, Li S, Altaany Z, Wang R, Yang G. S-sulfhydration of MEK1 leads to PARP-1 activation and DNA damage repair. EMBO Rep 2014;15:792–800.
15. Yang G, Zhao K, Ju Y, et al. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. Antioxid Redox Signal 2013;18:1906–1919.
16. Lang F, Busch GL, Ritter M, et al. Functional significance of cell volume regulatory mechanisms. Physiol Rev 1998;78:247–306.
17. Pairet N, Mang S, Fois G, et al. TRPV4 inhibition attenuates stretch-induced inflammatory cellular responses and lung barrier dysfunction during mechanical ventilation. PLoS One 2018;13:e0196055.
18. Nilius B, Droogmans G. Ion channels and their functional role in vascular endothelium. Physiol Rev 2001;81:1415–1459.
19. Sonkusare SK, Bonev AD, Ledoux J, et al. Elementary Ca2+ signals through endothelial TRPV4 channels regulate vascular function. Science 2012;336:597–601.
20. Phuong TTT, Redmon SN, Yarishkin O, Winter JM, Li DY, Križaj D. Calcium influx through TRPV4 channels modulates the adherens contacts between retinal microvascular endothelial cells: TRPV4 modulates adherens contacts between retinal endothelial cells. J Physiol 2017;595:6869–6885.
21. Liedtke W, Friedman JM. Abnormal osmotic regulation in trpv4-/- mice. Proc Natl Acad Sci U S A 2003;100:13698–13703.
22. Liu Y, Yang R, Liu X, et al. Hydrogen sulfide maintains mesenchymal stem cell function and bone homeostasis via regulation of Ca(2+) channel sulfhydration. Cell Stem Cell 2014;15:66–78.
23. Yang R, Liu Y, Yu T, et al. Hydrogen sulfide maintains dental pulp stem cell function via TRPV1-mediated calcium influx. Cell Death Discov 2018;4:69.
24. Bagher P, Beleznai T, Kansui Y, Mitchell R, Garland CJ, Dora KA. Low intravascular pressure activates endothelial cell TRPV4 channels, local Ca2+ events, and IKCa channels, reducing arteriolar tone. Proc Natl Acad Sci U S A 2012;109:18174–18179.
25. Childs BG, Durik M, Baker DJ, van Deursen JM. Cellular senescence in aging and age-related disease: from mechanisms to therapy. Nat Med 2015;21:1424–1435.
26. Su Y, Liu D, Liu Y, Zhang C, Wang J, Wang S. Physiologic levels of endogenous hydrogen sulfide maintain the proliferation and differentiation capacity of periodontal ligament stem cells. J Periodontol 2015;86:1276–1286.
27. Wang Z, Liu DX, Wang FW, et al. l-Cysteine promotes the proliferation and differentiation of neural stem cells via the CBS/H2S pathway. Neuroscience 2013;237:106–117.
28. Liu D, Wang Z, Zhan J, et al. Hydrogen sulfide promotes proliferation and neuronal differentiation of neural stem cells and protects hypoxia-induced decrease in hippocampal neurogenesis. Pharmacol Biochem Behav 2014;116:55–63.
29. Park SY, Jeong AJ, Kim GY, et al. Lactoferrin protects human mesenchymal stem cells from oxidative stress-induced senescence and apoptosis. J Microbiol Biotechnol 2017;27:1877–1884.
30. Yang M, Teng S, Ma C, Yu Y, Wang P, Yi C. Ascorbic acid inhibits senescence in mesenchymal stem cells through ROS and AKT/mTOR signaling. Cytotechnology 2018;70:1301–1313.
31. Lee JH, Jung HK, Han YS, et al. Antioxidant effects of Cirsium setidens extract on oxidative stress in human mesenchymal stem cells. Mol Med Rep 2016;14:3777–3784.
32. Zhang Y, Zhu W, He H, et al. Macrophage migration inhibitory factor rejuvenates aged human mesenchymal stem cells and improves myocardial repair. Aging (Albany NY) 2019;11:12641–12660.
33. Pietrocola F, Castoldi F, Markaki M, et al. Aspirin recapitulates features of caloric restriction. Cell Rep 2018;22:2395–2407.
34. Wei F, Qu C, Song T, et al. Vitamin C treatment promotes mesenchymal stem cell sheet formation and tissue regeneration by elevating telomerase activity. J Cell Physiol 2012;227:3216–3224.
35. Gharibi B, Hughes FJ. Effects of medium supplements on proliferation, differentiation potential, and in vitro expansion of mesenchymal stem cells. Stem Cells Transl Med 2012;1:771–782.
36. Yu X, Li X, Jiang G, et al. Isradipine prevents rotenone-induced intracellular calcium rise that accelerates senescence in human neuroblastoma SH-SY5Y cells. Neuroscience 2013;246:243–253.
37. McCarthy DA, Clark RR, Bartling TR, Trebak M, Melendez JA. Redox control of the senescence regulator interleukin-1α and the secretory phenotype. J Biol Chem 2013;288:32149–32159.
38. Naik JS, Osmond JM, Walker BR, Kanagy NL. Hydrogen sulfide-induced vasodilation mediated by endothelial TRPV4 channels. Am J Physiol Heart Circ Physiol 2016;311:H1437–H1444.