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Biodentine Counteracts the Aging Process of Human Dental Pulp Stem Cells Through Wnt/β-Catenin Pathway

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ABSTRACT

Objectives: This study aimed to evaluate the effects of Biodentine on human Dental Pulp Stem Cells (hDPSCs) proliferation and senescence and explore the underlying anti-aging mechanism.

Methods: hDPSCs were isolated from two groups of patients aged 18 to 27 and 60 to 70 years and treated with Biodentine extract. The optimal concentration of Biodentine extract was determined using the CCK-8 assay. Biodentine treatment on hDPSCs proliferation and aging was assessed using qRT-PCR and Western blot. The small molecule inhibitor XAV939, targeting the Wnt/β-catenin signaling pathway, was added to investigate the anti-aging mechanism of Biodentine in hDPSCs.

Results: The isolated hDPSCs exhibited mesenchymal stem cells (MSCs) characteristics, and the proportion of S-phased cells decreased significantly in the aged group. Biodentine promoted hDPSCs proliferation, with the most pronounced effect observed at 0.2 mg/mL. qRT-PCR showed that Biodentine enhanced the expression of odonto/osteogenesis-related genes. Senescence-associated β-galactosidase staining and Western blot analysis further demonstrated the significant anti-aging effect of Biodentine on hDPSCs. Western blot revealed that Biodentine exerted its anti-aging effect through the Wnt/β-catenin signaling pathway.

Conclusion: The study demonstrated that Biodentine promotes proliferation and exerts anti-aging effects on hDPSCs through the Wnt/β-catenin pathway.

Introduction

With the concept of minimally invasive dentistry and the development of novel bioceramic materials, vital pulp therapy (VPT) has been emerging as a key research area in endodontics with asserted advantages: preserving vital pulp function and upholding fracture resistance.[1-3] Clinically, VPT involves excising the infected pulpal portion while retaining the beneath uninfected part with a minimal access cavity without the need for root canal interference, thus essentially maintaining the tooth integrity and its fracture resistance capacity.[2,4]

The efficacy of VPT depends on several factors, such as human dental pulp stem cells' (hDPSCs) proliferative and odonto/osteogenic differentiation ability,[5] pulp capping materials used,[6] etc. Mineral trioxide aggregate (MTA), the first generation of bioceramic material, is less utilized for pulp capping today due to its tooth discoloration, lengthy

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setting times, and poor handling properties.[7] Biodentine, a bioactive dentin replacement material, has increasingly been employed in pulp capping procedures.[8-10] Composed mainly of tricalcium silicate, dicalcium silicate, and calcium carbonate,[11,12] Biodentine offers several advantages over MTA, including reduced discoloration, shorter setting time, improved handling and mechanical properties, as well as adequate radiopacity.[13] It was also reported that Biodentine is superior for protecting pulp tissue from external stimuli and promoting hDPSCs' proliferation and odonto/osteogenic differentiation.[8,14]

Our previous study has revealed that VPT is comparable to root canal therapy for treating permanent mature teeth with pulpitis, and patient age is a key factor associated with the treatment outcomes.[15] In addition, our laboratory study demonstrated that Biodentine can significantly enhance hDPSCs' proliferation and differentiation, which are isolated from the elderly over 60 years old,[16] indicating that Biodentine may be able to rejuvenate the mature/aged hDPSCs for their regenerative capacity. Concurrently, Yi et al demonstrated that the proliferative capacity and differentiation potential of hDPSCs decreased by cell senescence.[17] Iezzi et al further confirmed that senescence was associated with the mineralization processes and decreased osteogenic potential of aging hDPSCs.[18] These suggest that the senescence of hDPSCs may have negative consequences in clinical applications, potentially leading to the failure of VPT. Therefore, targeting the senescence to rejuvenate the aged hDPSCs and uncovering the related anti-aging mechanisms could be able to improve the success rate of VPT.[19]

Thus, this study aimed to evaluate the biological characteristics of both young and aging hDPSCs and explore the effects of Biodentine on their proliferation and senescence and its anti-aging mechanisms on hDPSCs isolated from elderly individuals.

Materials and methods

Pulp samples

The Institutional Ethics Committee of Nanjing Medical University approved this study (IRB No. PJ2023-107-001). After obtaining informed consent, 12 molar or premolar samples were collected from patients of different ages at the Oral and Maxillofacial Surgery Department, Jiangsu Provincial Stomatological Hospital. All donors were free of systemic diseases, and the affected teeth had no pulpal lesions with fully developed apices. The samples were divided into two groups (6 donors per group): Group A (age 18-27) and Group B (age 60-70).

Cell culture

The primary culture of hDPSCs was carried out using an enzyme digestion method. Teeth were cleaned and cut transversely, and the pulp tissue was gently extracted. The tissue pieces were digested using 3 mg/mL type I collagenase (Gibco, Grand Island, NY, USA) with 4 mg/mL dispase (Gibco) for 1 hour at 37 °C. After centrifugation, the cells were cultured in α-MEM containing 10% fetal bovine serum (FBS, VivaCell, Shanghai, China) and 2% penicillin-streptomycin (Gibco) in a humidified 5% CO_2 incubator at 37 °C. The medium was refreshed every 3 days, and cell passages were performed when confluence reached 80-90%. The fourth to sixth passage cells were used for subsequent experiments, with growth monitored using an optical microscope (Leica, Germany).

Flow cytometry analysis

Flow cytometry was performed at the second passage to assess hDPSCs surface markers. Cells were rinsed with PBS, incubated with antibodies at 4 °C for 30 minutes, and analyzed using flow cytometry (BD, Franklin Lakes, NJ, USA). The following antibodies were used: CD34 FITC, CD90 PE (Invitrogen, USA), CD73 PE-Cy7, CD105 APC, and CD146 PE (BD).

Cell cycle assay

Cell cycle phases were analyzed by flow cytometry. After collecting the cells, their number was adjusted to 1×10^6. Cells were fixed with 70% ethanol overnight at 4 °C, then washed twice with PBS and stained with PI/RNase buffer (BD) in the dark for 15 minutes at room temperature.

Multipotent differentiation assay

For osteogenic and adipogenic induction, hDPSCs were seeded at 1×10^5 cells/well in 6-well plates. At 90% confluency, cells were treated with osteogenic or adipogenic induction medium. Osteogenic induction medium contained 10 mmol/L β-glycerophosphate sodium (Sigma, USA), 50 mg/L vitamin C (Solarbio, China), and 10 nmol/L dexamethasone (APExBIO, USA). Adipogenic induction medium contained 1 mg/mL insulin (APExBIO) , 2 mmol/L dexamethasone (APExBIO), 0.5 mmol/L IBMX (Biosharp, China), and 0.2 mmol/L indomethacin (Sigma). After 2 weeks osteogenic induction and 3 weeks adipogenic induction, cells were fixed with 4% paraformaldehyde (Biosharp) and stained with Alizarin Red S (Leagene, China) and Oil Red O (Solarbio) respectively, for 30 minutes at room temperature. For chondrogenic induction, 5×10^5 cells were transferred to a 15 mL centrifuge tube with 500 μL chondrogenic medium (Cyagen, China) and incubated for 24 hours to allow spheroid formation. After 3 weeks, frozen sections were prepared and stained with Alcian Blue (Cyagen) for 30 minutes, followed by dehydration and mounting.

Preparation of Biodentine extracts

Biodentine (Septodont, Saint-Maur-des-Fossés, France) was mixed according to the manufacturer's instructions and solidified for 2 hours at room temperature. The solidified material was ground into powder, sterilized under a UV lamp (365 nm), and 600 mg of powder was added to 15 mL of α-MEM. The solution was incubated at 37 °C for 72 hours to prepare the extracts (40 mg/mL). After filtration through a 0.22 mm filter (Millipore, MA, USA), the extracts were diluted to concentrations of 20, 2, 0.2, and 0.02 mg/mL and stored at 4 °C for further use.

Cell proliferation

The optimal concentration of Biodentine for subsequent experiments was determined using the CCK-8 assay (APExBIO). Young and aging hDPSCs were treated with different concentrations of Biodentine (0.02, 0.2, 2, and 20 mg/mL) and MTA (0.2 mg/mL). The control group was cultured in a medium without Biodentine. Cells were seeded at a density of 1×10^3/well in 96-well plates and incubated at 37 °C, 5% CO_2 for 1 week. CCK-8 reagent was added and incubated with cells for 2 hours at days 1, 3, 5, and 7. The optical density (OD) was measured at 450 nm using a plate reader (Tecan, Switzerland).

Alizarin red staining (ARS)

The mineralization capacity of hDPSCs was assessed using ARS. Cells were seeded at 1×10^5/well in 6-well plates and treated with Biodentine extracts at 90% confluency. Basal medium served as the negative control, and MTA extracts were used as the positive control. Mineralization induction medium was prepared as described in section 2.5. After 2 weeks of induction, cells were fixed with 4% paraformaldehyde (Biosharp) and stained with Alizarin Red solution (Leagene) at room temperature for 30 minutes. Mineralization was quantified by destaining with 10% cetylpyridinium chloride (Solarbio), and absorbance was measured at 562 nm (Tecan).

qRT-PCR

The experimental groups were the same as in 2.8, and the cells were cultured for 1 week at 90% confluency. qRT-PCR was utilized to detect the mRNA expression levels of odonto/osteogenesis related genes, including dentin sialophosphoprotein (DSPP), runt-related transcription factor 2(RUNX2), alkaline phosphatase (ALP), and type I collagen (COL-1). Seven days after culturing, total RNA was extracted by a FastPure Cell/Tissue Total RNA Isolation Kit (Vazyme, NJ, China). cDNA was synthesized by reverse transcription using Hiscript IIQ RT SuperMix For qPCR (Vazyme). Equal amounts of cDNA were used for real-time amplification of the target genes using ChamQ Universal SYBR qPCR Master Mix (Vazyme). The reactions were performed on a lightcycler 480II Real-Time PCR System (Roche, Basel, Switzerland) at 95 °C for 30 seconds for one cycle, and then 95 °C for 10 seconds, 60 °C for 30 seconds for 40 cycles, with a final extension at 95 °C for 15 seconds, 60 °C for 1 minute, and 95 °C for 15 seconds. The $2^{-\Delta\Delta Ct}$ method was applied to calculate the relative expression of odonto/osteogenic genes. Primer sequences are shown in Table.

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

SA-β-gal staining was conducted using the SA-β-gal staining kit (Beyotime, Shanghai, China) to detect senescence in both young and aging hDPSCs. The hDPSCs were grouped according to the experiment and cultured in the corresponding media for 72 hours. The culture medium was aspirated, and the cells were washed with PBS three times, then fixed at room temperature for 15 minutes using a fixative solution. After removing the fixative solution, the fixed cells were washed with PBS three times and incubated with SA-β-gal staining solution overnight at 37 °C. The cells were then rinsed with 70% ethanol and observed under a microscope. SA-β-gal-positive cells appeared as blue-stained cells, and the percentage of aging hDPSCs was calculated as the ratio of positive hDPSCs to the total number of hDPSCs, counted from five randomly selected fields of view.

Table – Real-time PCR primer sequences of the odonto/osteogenesis-related genes.

Gene	Primer sequence (5'-3')
GAPDH	F:5'-GAAGGTGAAGGTCGGAGTC-3'
	R:5'-GAGATGGTGATGGGATTTC-3'
DSPP	F:5'-TGTCGCTGTTGTCCAAGAAG-3'
	R:5'-CATCACCAGAACCCTCGTCT-3'
RUNX2	F:5'-TGGTTACTGTCATGGCGGGTA -3'
	R:5'- TCTCAGATCGTTGAACCTTGCTA-3'
ALP	F:5'-GACCTCCTCGGAAGACACTC -3'
	R:5'-TGAAGGGCTTCTTGTCTGTG -3'
COL-1	F:5'-AAAGATGGACTCAACGGTCTC-3'
	R:5'-CATCGTGAGCCTTCTCTTGAG-3'

F: Forward, R: Reverse.

Western blot analysis

Western blot was performed to detect the effects of Biodentine on senescence-related genes and Wnt/β-catenin pathway-related proteins in hDPSCs. After one week of culture, the cells were lysed with RIPA buffer (Beyotime) for protein extraction. Protein separation was performed using polyacrylamide gel electrophoresis, followed by electrophoretic transfer of proteins to PVDF membranes (Millipore). The membranes were blocked with skim milk at room temperature for 2 hours and incubated with primary antibodies overnight at 4 °C. The following primary antibodies were used: anti-p21 (1:1000; Proteintech, Chicago, IN, USA), anti-p16 (1:1000; Abbkine, Wuhan, China), anti-p53 (1:1000; Proteintech), anti-β-catenin (1:1000; Affinity, USA), anti-p-β-catenin (1:1000; Affinity), anti-Axin1 (1:1000; Proteintech) and anti-GAPDH (1:1000; Proteintech). After incubation with primary antibodies, the membranes were treated with secondary antibodies (1:10000; Proteintech) for 1 hour. The protein bands were visualized using ECL chemiluminescent reagents (NCM, Suzhou, China) under a chemiluminescence imaging system (VILBER, Paris, France). The relative expression levels of senescence genes were normalized to GAPDH as a reference protein.

Statistical analysis

All experiments were performed independently at least three times, and the data are expressed as the mean ± standard deviation (SD). To ensure the robustness and generalizability of our findings, the hDPSCs used in this study were derived from pooled samples. Statistical calculations were carried out using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). One-way ANOVA was used for multiple group comparisons, and Student's t-test was applied to compare the means of two groups. A P-value of $<.05$ was considered statistically significant.

Results

Isolation, culture, and characterization of hDPSCs

Human pulp tissues were isolated from freshly extracted donor teeth, and hDPSCs were successfully cultured using the enzymatic digestion method. Adherent cells could be observed under the microscope after 3 to 8 days of incubation. Under the inverted optical microscope, the morphology of wall-adherent cells was long, spindle-shaped, or polygonal. These hDPSCs were attached well after passing at the same density (Figure 1A).

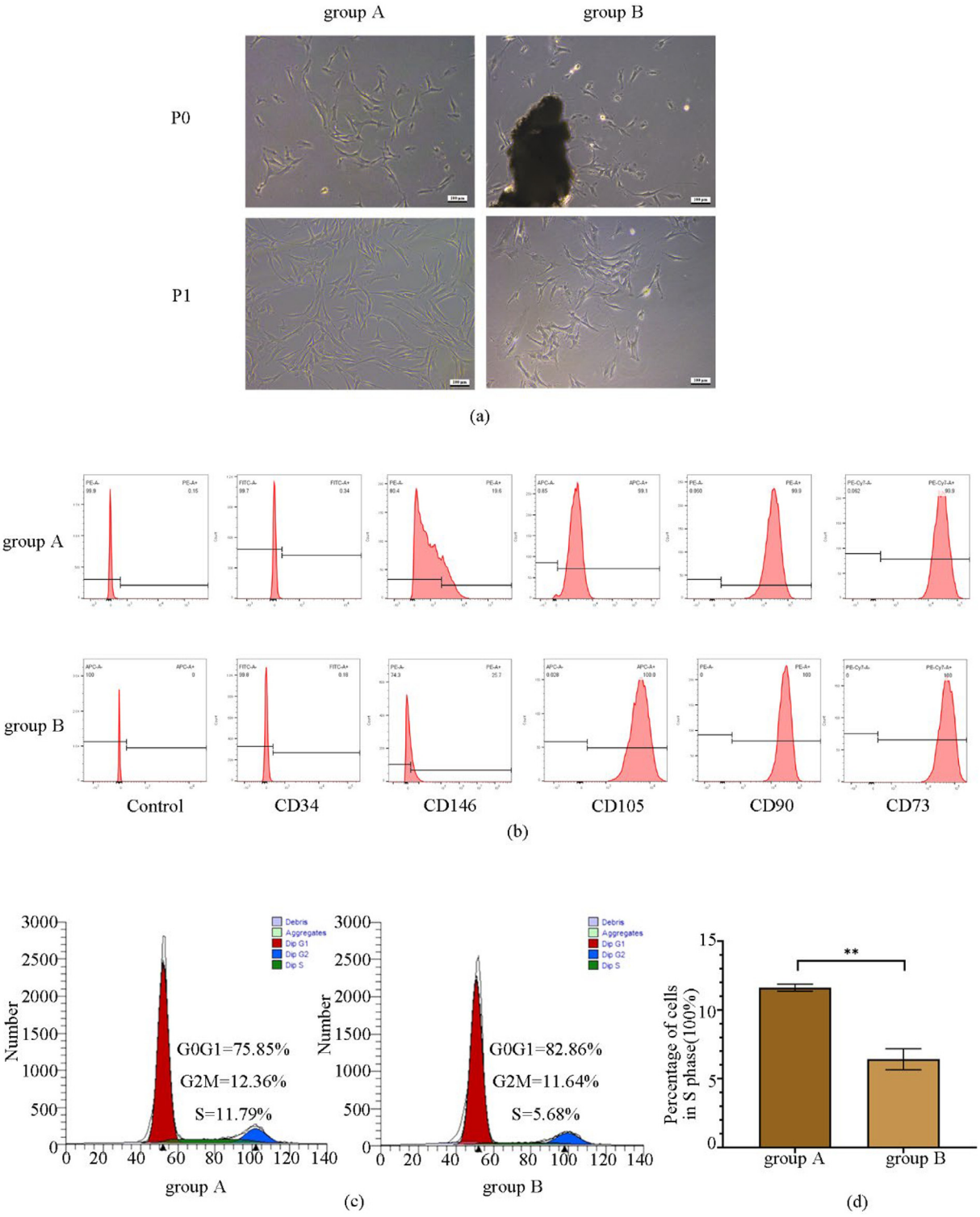

Fig. 1 – Isolation and identification of hDPSCs. A, Morphological features of hDPSCs at primary and passage 1 under an inverted microscope (scale bar: 100 μm). B, Surface marker profiling of young and aged hDPSCs was evaluated by flow cytometry. C, Cell cycle of each group was detected by flow cytometry. D, Percentage of hDPSCs in S phase. ** $P < .01$.

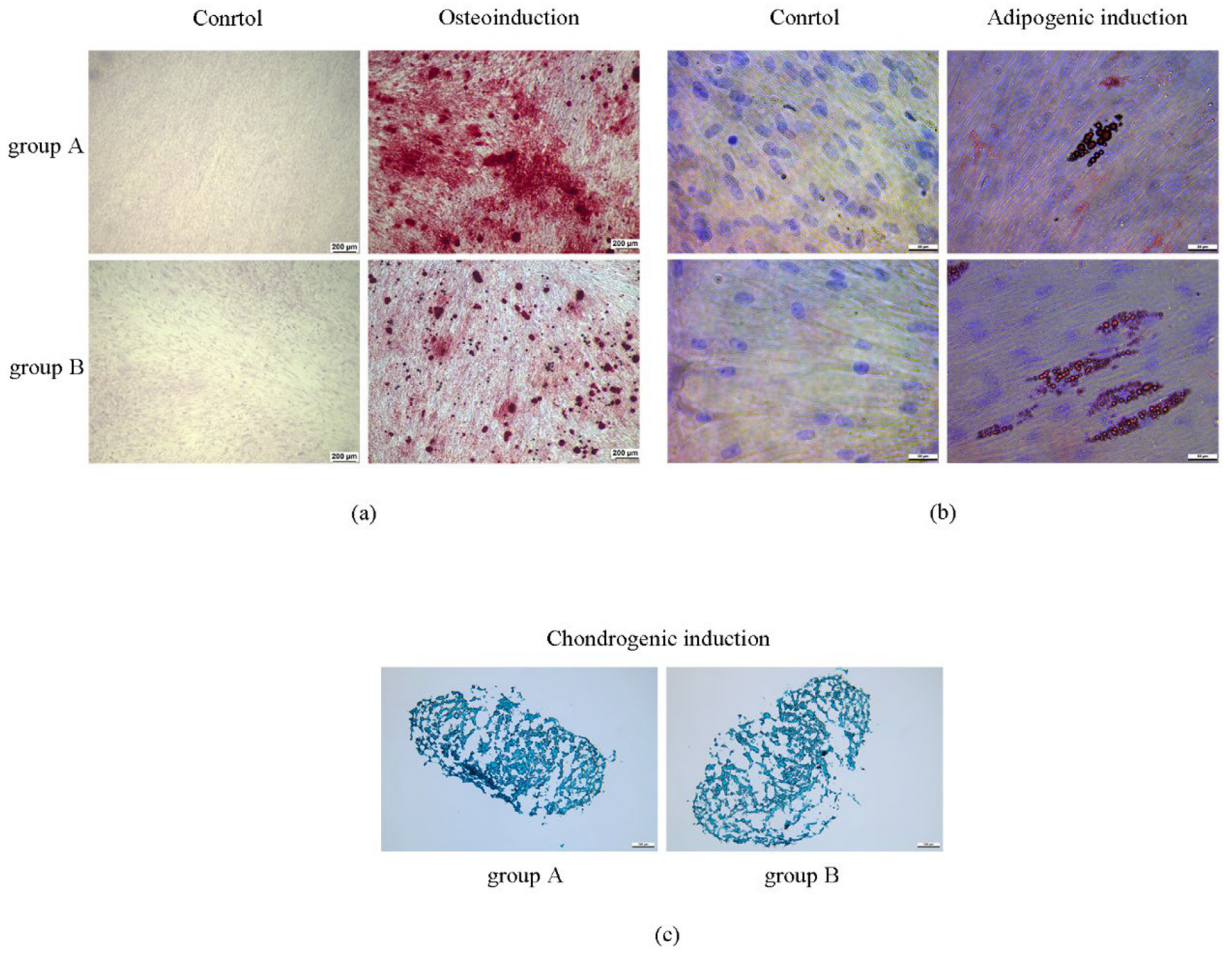

Fig. 2 – Multipotent Differentiation of hDPSCs. A, Osteogenic induction (scale bar: 200 μm). B, Adipogenic induction (scale bar: 30 μm). C, Chondrogenic induction (scale bar: 100 μm).

Flow cytometry demonstrated that both young and aging hDPSCs positively expressed human mesenchymal stem cells (MSCs) surface markers CD73 (> 90%), CD90 (> 90%), CD105 (> 90%), with low expression of CD146 (< 30%), while negatively expressing CD34 (< 1%) (Figure 1B). These results suggest that the cultured cells exhibited the characteristics of MSCs. The flow cytometry analysis also revealed that the percentage of S-phase cells was markedly lower in aging hDPSCs compared to young hDPSCs ($P < .05$) (Figure 1C and 1D). After osteogenic induction, widespread mineralized nodules were observed. Adipogenic induction resulted in red lipid droplets, and chondrogenic induction showed Alcian Blue-stained cells (Figure 2). These findings confirmed the multipotent differentiation ability of hDPSCs.

Biodentine on hDPSCs proliferation

The effect of Biodentine on cell proliferation was evaluated to determine the optimal concentration for further experiments. Compared with the control group, 0.2 mg/mL of Biodentine significantly promoted the proliferation of aging hDPSCs during the first 3 days ($P < .01$), followed by a steady increase (Figure 3B). In group A, 0.2 mg/mL Biodentine accelerated hDPSCs after 5 days ($P < .01$) (Figure 3A). Compared to the MTA group, 0.2 mg/mL Biodentine significantly promoted proliferation in Group A from days 3 to 7 ($P < .01$), while no significant difference was observed in Group B. However, high concentrations (20 mg/mL) significantly inhibited the proliferation of hDPSCs within 3-7 days ($P < .01$), while the other two groups showed no significant difference compared to the control group (Figure 3A and B). Based on these findings, 0.2 mg/mL Biodentine was used in subsequent experiments.

Biodentine promoted mineralization of hDPSCs

ARS showed positive results in both groups. Compared with the control group and MTA group, A and B groups of hDPSCs cultured with Biodentine for 2 weeks showed more aggregated nodules ($P < .01$, Figure 4A and B).

Biodentine promoted odonto/ osteogenesis of hDPSCs

The mRNA levels of DSPP, RUNX2, ALP, and COL-1 were significantly upregulated ($P < .05$) compared with the control group and MTA group (Figure 5A-D). These findings indicated that Biodentine substantially promoted the odonto/osteogenic gene expression in young and aging hDPSCs.

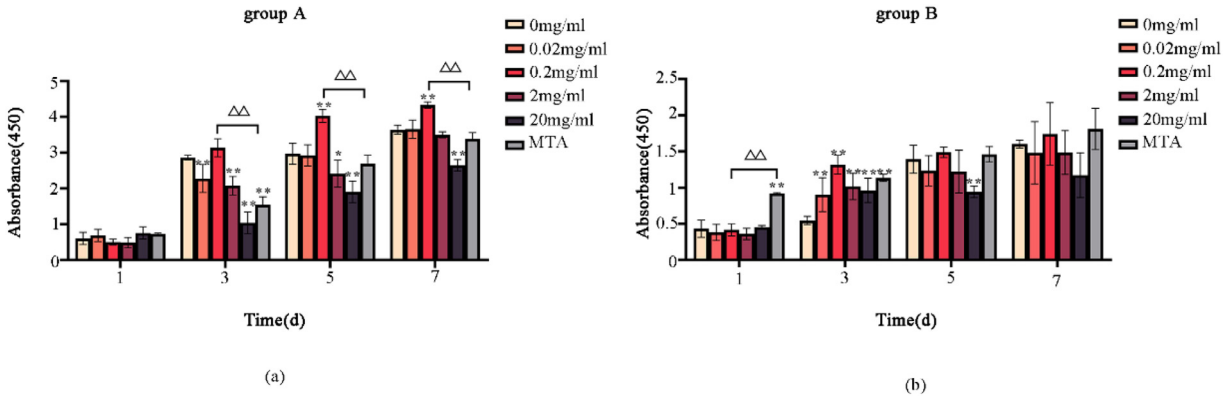

Fig. 3 – The Influence of Biodentine on hDPSCs Proliferation. A, The influence of Biodentine on young and B, aged hDPSCs proliferation. The CCK-8 assay was performed after stimulation with different concentrations of Biodentine and MTA on days 1, 3, 5, and 7. ∗ P < .05, ∗∗ P < .01 versus control, △△ P < .01 compared with MTA.

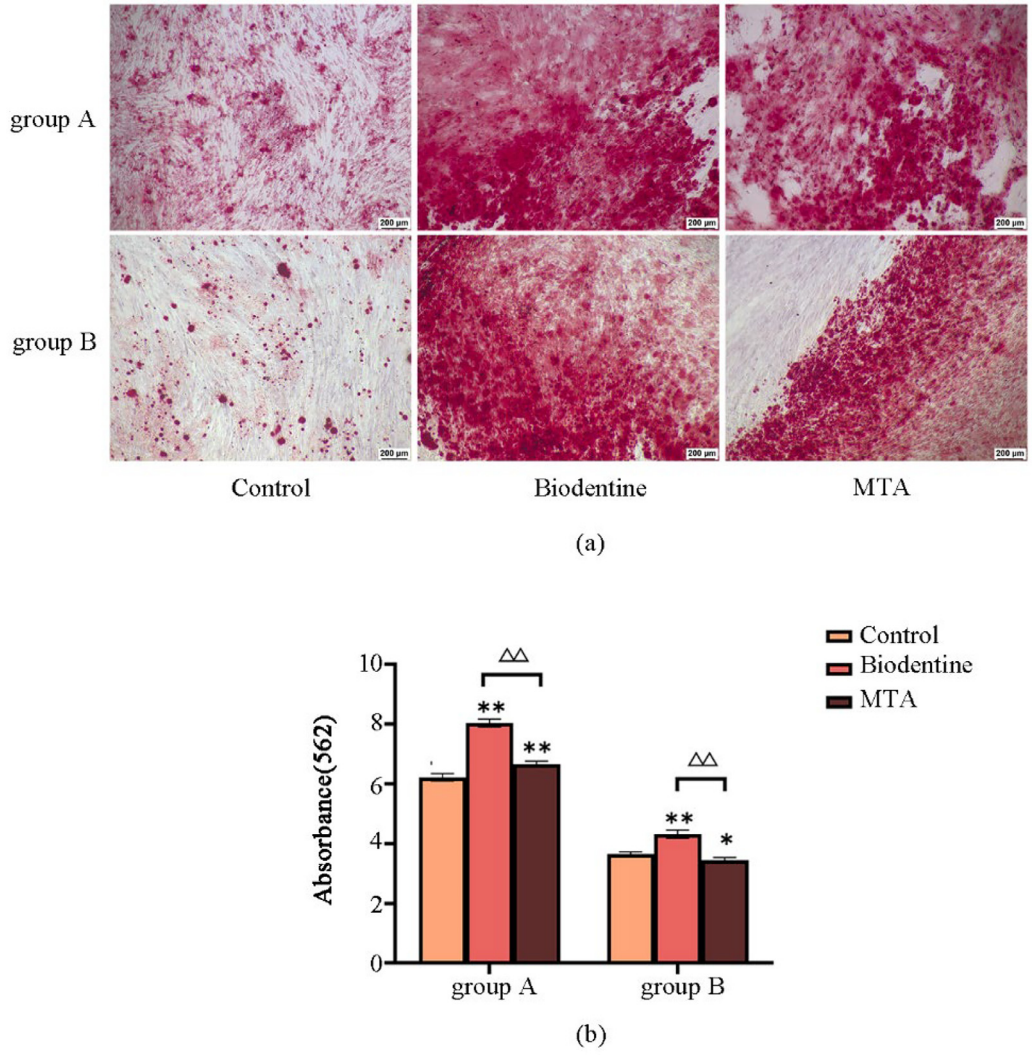

Fig. 4 – Biodentine promoted mineralization of hDPSCs. A, Images of alizarin red staining (scale bar: 200 μm). B, Quantitative measurement of alizarin red staining of hDPSCs. ∗ P <.05, ∗∗ P < .01 versus control, △△ P <.01 compared with MTA.

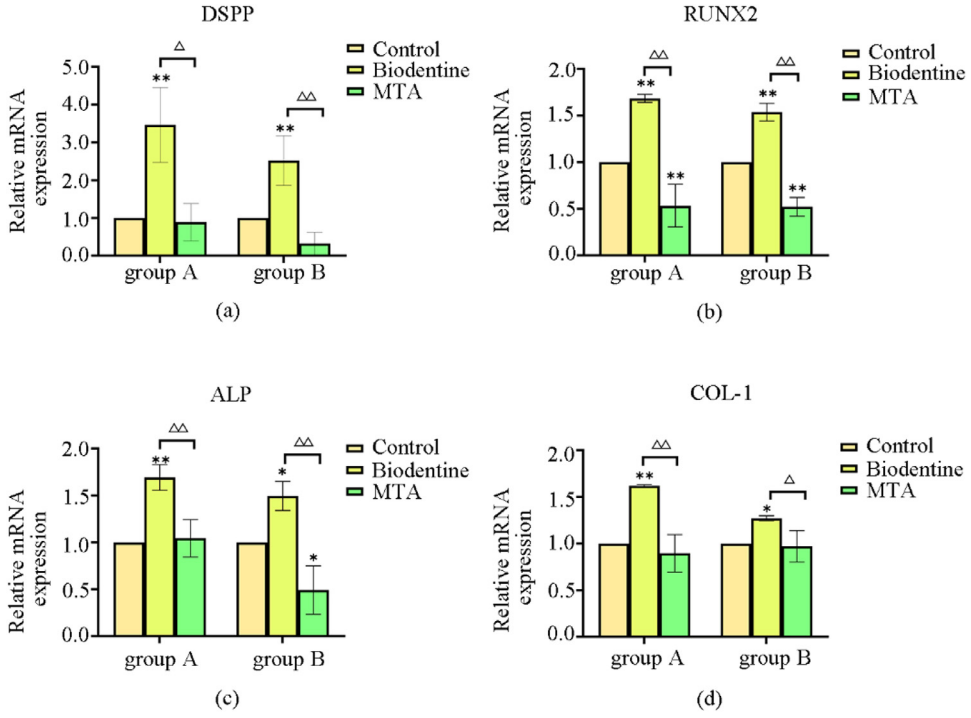

Fig. 5 – Biodentine promoted odonto/ osteogenesis of hDPSCs. The mRNA expression levels of DSPP A, RUNX2 B, ALP C, and COL-1 D, were detected by qRT-PCR analysis. $*$ $P < .05$, $**$ $P < .01$ versus control, △ $P < .05$, △△ $P < .01$ compared with MTA.

Biodentine inhibited senescence in hDPSCs

SA-β-gal staining was used to identify the effect of Biodentine on the senescence of young and aging hDPSCs. A higher percentage of SA-β-gal-positive cells was found in aging hDPSCs compared to young hDPSCs ($P < .01$, Figure 6A and B). However, when treated with Biodentine, the percentage of SA-β-gal-positive cells was markedly reduced in both groups ($P < .01$, Figure 6A and B), indicating that Biodentine can inhibit the senescence of hDPSCs.

Western blot analysis further demonstrated that Biodentine downregulated p53, p21, and p16 in both young and aging hDPSCs ($P < .01$, Figure 6C and D), suggesting that Biodentine inhibits hDPSCs senescence by reducing the expression of these senescence-associated proteins.

Biodentine inhibited senescence via the Wnt/β-catenin pathway

After the addition of the Wnt/β-catenin pathway inhibitor XAV939, the expression levels of senescence-related proteins significantly increased ($P < .01$, Figure 7A and B). At the same time, the expression of β-catenin was suppressed, while p-β-catenin and Axin1 were significantly elevated ($P < .01$, Figure 7A and C). Biodentine treatment reversed these effects by reducing senescence-related protein expression and increasing β-catenin levels ($P < .01$, Figure 7A-C). These results illustrated that Biodentine inhibited hDPSCs senescence by activating the Wnt/β-catenin pathway.

In SA-β-gal staining, the proportion of SA-β-gal-positive cells significantly increased after the addition of XAV939 ($P < .01$). Following Biodentine treatment, the proportion of SA-β-gal-positive cells was markedly reduced ($P < .01$, Figure 8), consistent with the Western blot results. This further confirmed that Biodentine exerted its anti-aging effects through the Wnt/β-catenin pathway.

Discussion

The reduction of pulp tissue volume and regenerative capacity hinder the application of VPT in old individuals.[20] It was reported that cellular senescence caused by aging may lead to cell function loss and subsequent disruption of tissue homeostasis and repair.[21] In another aspect, the efficacy of stem cell-based therapies was undermined due to its senescence.[22] In this study, we investigated Biodentine treatment on the biological characteristics of both young and aged hDPSCs and found that it significantly enhances hDPSCs proliferation. Additionally, Biodentine demonstrated anti-aging effects by activating the Wnt/β-catenin pathway, providing a potential target on cell senescence to rejuvenate the aged hDPSCs.

The isolated aged hDPSCs expressed typical mesenchymal surface markers (CD73, CD90, and CD105) and exhibited multipotent differentiation potential, which were aligned with the established criteria for MSCs, confirming the MSCs identity of the isolated hDPSCs.[23] Additionally, we observed a progressive reduction in the proportion of S-phase cells with age, indicating inhibited cell cycle progression from G0/G1 to S phase. To explore whether bioceramic material can reverse the aging process of hDPSCs, Biodentine was used to treat the isolated cells. It was found that Biodentine can significantly enhance the

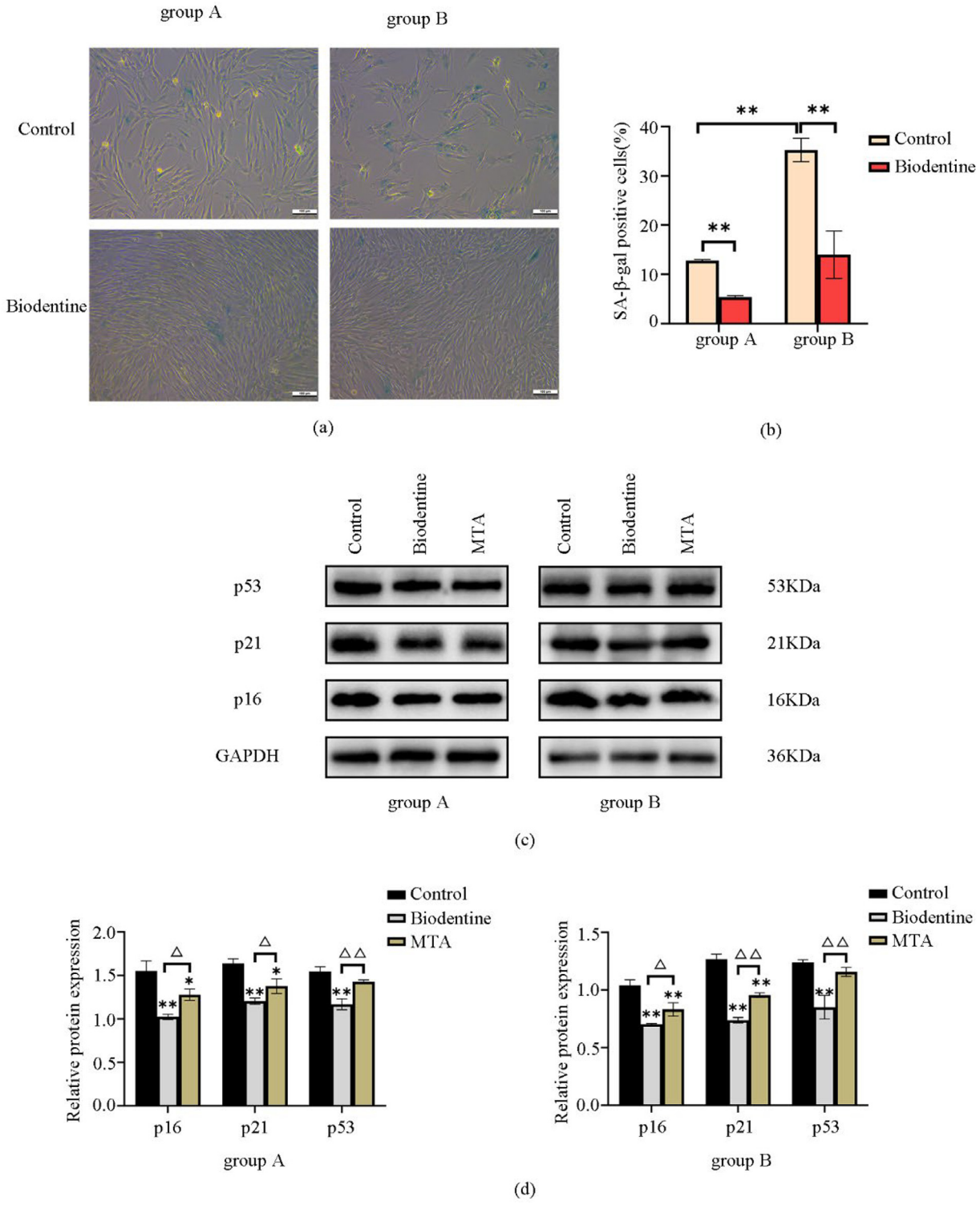

Fig. 6 – Biodentine inhibited senescence in hDPSCs. A, Cellular senescence was determined using SA-β-gal staining. representative images are shown (scale bar: 100 μm). B, Quantitative analysis of SA-β-gal-positive cells. $**P < .01$. C, Representative blots of p53, p21, and p16 protein expression levels by western blot. D, Quantitative analysis of p53, p21, and p16 proteins. $* P < .05$, $** P < .01$ versus control, $\triangle P < .05$, $\triangle\triangle P < .01$ compared with MTA.

proliferation of both young and aged hDPSCs.[16] 0.2 mg/mL was the optimal concentration, while higher concentrations (such as 20 mg/mL) exerted an inhibitory effect. These findings support the idea that the appropriate dosage/concentration must be optimized for clinical translational application.

Consistent with other studies,[6,8,16] we found that Biodentine promoted odonto/osteogenic differentiation of hDPSCs

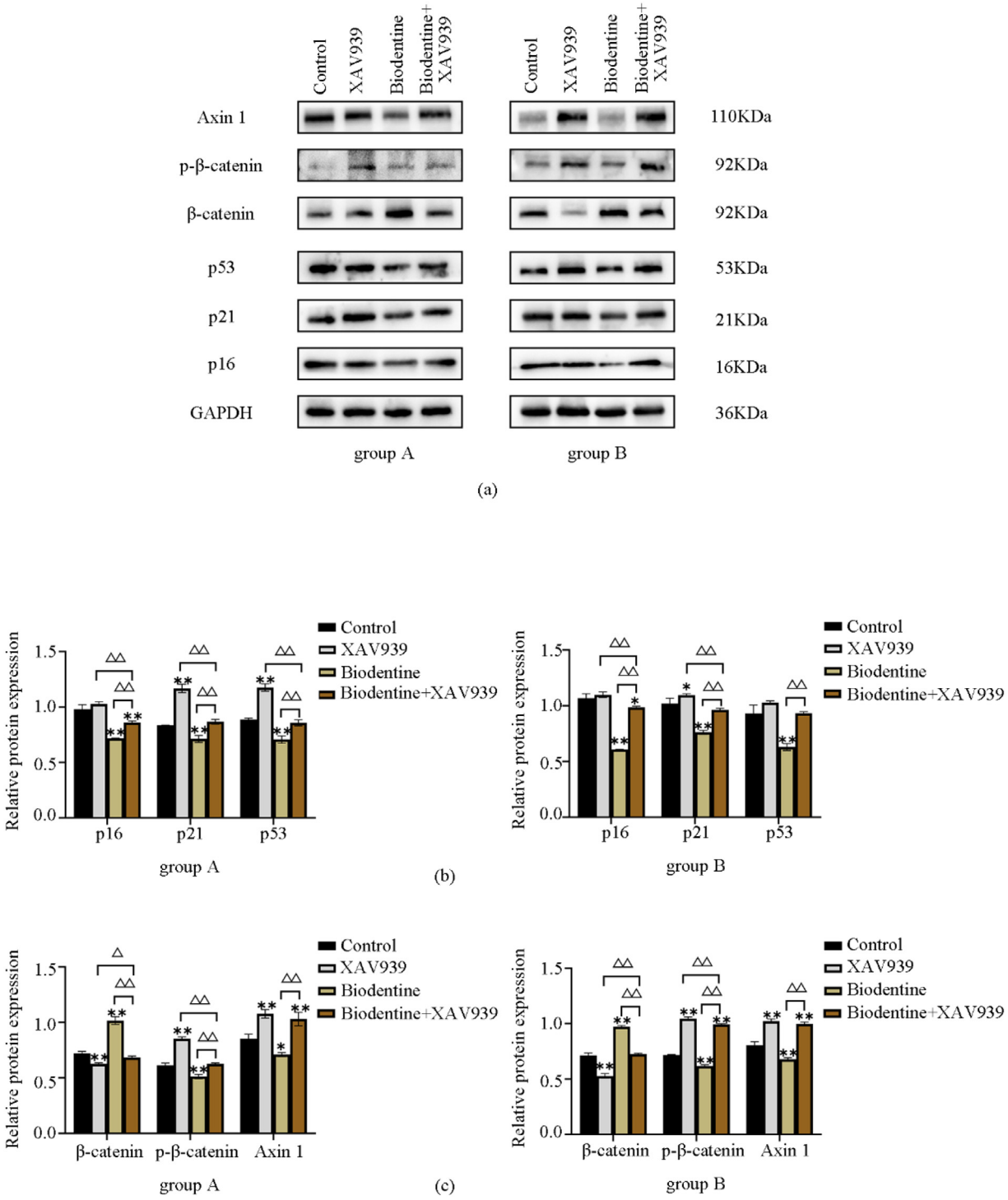

Fig. 7 – Biodentine inhibited senescence via the Wnt/β-catenin Pathway. A, Representative blots of p53, p21, p16, β-catenin, p- β-catenin and Axin1 protein expression levels by western blot. B, Quantitative analysis of p53, p21, and p16 proteins. C, Quantitative analysis of β-catenin, p- β-catenin and Axin1 proteins. ∗ $P < .05$, ∗∗ $P < .01$ versus control, △ $P < .05$, △△ $P < .01$ compared with Biodentine+XAV939.

and suppressed cell aging, as shown by downregulating SA-β-gal activity and senescence-associated protein expression. Various drugs have been investigated to reduce the aging effects of hDPSCs, such as Metformin and Visfatin, demonstrating the feasibility of hDPSCs' anti-aging treatment for their enhanced regenerative capacity.[24-27] In such context, Biodentine may be preferable, especially for elderly patients with dental pulp exposure or inflammation caused by caries or abrasion.

Gomes et al co-cultured embryonic chick femurs with various materials and found that Biodentine had good osteogenic potential, slightly lower than ProRoot MTA.[28] Yan's

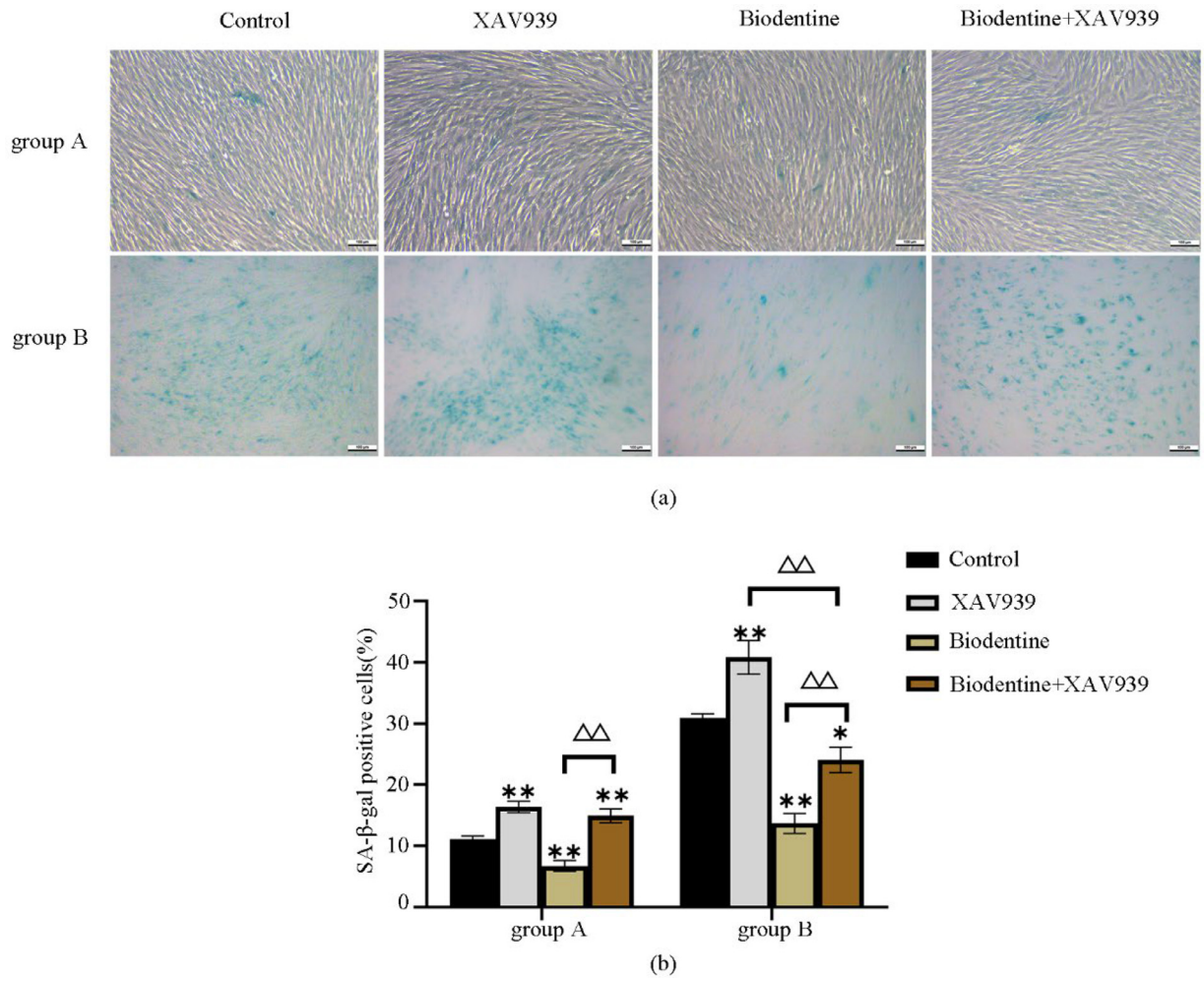

Fig. 8 – Biodentine delayed hDPSCs senescence through the Wnt signaling pathway. A, Microscopic images of cellular senescence are shown (scale bar: 100 μm). B, Quantitative analysis of SA-β-gal-positive cells. $*$ P < .05, $**$ P < .01 versus control, △ P < .05, △△ P < .01 compared with Biodentine+XAV939.

study showed that MTA at 2 mg/mL could enhance the odonto/osteogenic differentiation of stem cells from the apical papilla (SCAPs).[29] Variations in MTA brands and the use of different cell or tissue sources may affect the osteogenic activity of the material. More importantly, Gomes et al applied solid material in direct contact with tissue rather than using extracts, resulting in different effective concentrations. In our study, both MTA and Biodentine were used at 0.2 mg/mL, and the low concentration of MTA did not yield the expected osteogenic effect. Biodentine is commonly used clinically for direct pulp capping, root end filling, and perforation repair.[30] It functions similarly to MTA but has shown improved success rates in preserving vital pulp in cases of irreversible pulpitis.[31,32] Additionally, Biodentine released significantly more free calcium ions.[33] However, as Biodentine has recently been used clinically in China, further experiments are necessary to validate its clinical efficacy.

Wnt signaling plays an essential role in age-related changes in stem cells. Many studies have shown that inhibiting Wnt pathway can improve disease progression, reduce inflammation, and offer anti-cancer benefits.[34-36] Inhibiting Wnt signaling can also initiate senescence of human WI38 fibroblasts,[37] propelling us to hypothesize that Wnt/β-catenin pathway is associated with age-related functional decline in hDPSCs. In this study, we found that XAV939, which can block Wnt/β-catenin pathway by stabilizing Axin1 and leading to degradation of β-catenin,[38] negatively affected the anti-aging effects of Biodentine. Biodentine was able to partially counteract XAV939′s inhibitory effects. These experiments demonstrated that Biodentine exhibits anti-aging properties through Wnt signaling pathway, supporting its potential application in VPT for aging pulp tissue. Enhancing Wnt signaling may further improve the regenerative potential of aging pulp as shown in Cheng et al study, which demonstrated that Wnt10a overexpression promoted hDPSCs proliferation by increasing G2/M and S-phase cells through the canonical Wnt/β-catenin signaling pathway.[39] LiCl, as a Wnt pathway agonist, enhanced the accumulation of β-catenin in hDPSCs, simulating the activation of the Wnt pathway and enhancing the formation of calcium nodules and the

expression of odontogenic/osteogenic genes.[40,41] Treatment with Wnt3a promoted genomic DNA demethylation, enhancing the formation of calcified matrix and lipid droplets, thereby improving the stemness and differentiation capacity of hDPSCs.[42,43] Furthermore, Wnt3a stimulated mitochondrial metabolism and lipid synthesis, reprogramming the metabolism of hDPSCs to maintain their stemness and pluripotency.[44] These studies demonstrated that manipulating the Wnt signaling pathway can enhance the stem cell characteristics of hDPSCs. We hypothesize that Biodentine may contain components similar to LiCl or act on Wnt3a, enhancing the stem cell traits of hDPSCs through activation of the Wnt pathway, thereby enhancing their odontogenic/osteogenic capabilities and achieving anti-aging effects. Given the complexity of the Wnt/β-catenin pathway, further studies are needed to investigate whether Biodentine's anti-aging effects on aging hDPSCs might also involve non-canonical pathways.

Conclusions

This study demonstrated that Biodentine not only promoted the proliferation of aged hDPSCs but also effectively counteracted the aging process through the Wnt/β-catenin pathway. Considering its significant anti-aging effects on aged hDPSCs, Biodentine could be a viable pulp-capping material for vital pulp therapy in clinical practice. However, further research is needed to validate the clinical efficacy of Biodentine comprehensively.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of Nanjing Medical University (PJ2023-107-001).

Author contributions

Conceptualization, Q.Z. and S.C; methodology, M.Z. and S.C.; data curation, Q.Z.; writing—original draft preparation, Q.Z. and S.C; writing—review and editing, D.W. and C.Z.; supervision, J.L. All authors read and approved the final manuscript.

Data availability

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Conflict of interest

None disclosed.

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