LETÍCIA ODAGUIRI WATANABE

SENESCÊNCIA DE CÉLULAS-TRONCO E REGENERAÇÃO TECIDUAL: DA EVIDÊNCIA SISTEMÁTICA AOS IMPACTOS NA CAPACIDADE REGENERATIVA DA PAPILA APICAL

BRASÍLIA, 2026

UNIVERSIDADE DE BRASÍLIA

FACULDADE DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

LETÍCIA ODAGUIRI WATANABE

SENESCÊNCIA DE CÉLULAS-TRONCO E REGENERAÇÃO TECIDUAL: DA EVIDÊNCIA SISTEMÁTICA AOS IMPACTOS NA CAPACIDADE REGENERATIVA DA PAPILA APICAL

Dissertação apresentada como requisito parcial para a obtenção do Título de Mestre em Ciências da Saúde pelo Programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

Orientadora: Prof.ª Dr.ª Taia Maria Berto Rezende

BRASÍLIA, 2026

LETÍCIA ODAGUIRI WATANABE

SENESCÊNCIA DE CÉLULAS-TRONCO E REGENERAÇÃO TECIDUAL: DA EVIDÊNCIA SISTEMÁTICA AOS IMPACTOS NA CAPACIDADE REGENERATIVA DA PAPILA APICAL

Dissertação apresentada como requisito parcial para a obtenção do Título de Mestre em Ciências da Saúde pelo Programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília.

Aprovado em 10 de fevereiro de 2026

BANCA EXAMINADORA

Prof.ª Dr.ª Taia Maria Berto Rezende (Presidente)
Universidade de Brasília

Prof. Dr. Jacy Ribeiro de Carvalho Júnior
Universidade de Brasília

Prof.ª Dr.ª Francine Benetti Faria
Universidade Federal de Minas Gerais

Prof.ª Dr.ª Stella Maris de Freitas Lima (Suplente)
Universidade Católica de Brasília

AGRADECIMENTOS

À **Prof.ª Dr.ª Taia Maria Berto Rezende**, pela orientação nesse trabalho, por ter me dado a oportunidade de fazer parte de seu grupo de pesquisa, por ser um exemplo de pesquisadora e profissional, e pelo apoio, confiança e valiosos ensinamentos ao longo desse período.

Aos meus pais, **Minoru e Sênia**, que sempre estiveram ao meu lado me apoiando, por terem abdicado de seus sonhos em favor dos meus e de minhas irmãs, e por todo o esforço e dedicação investidos em nossa educação.

Às minhas irmãs, **Larissa e Flávia**, pelo companheirismo e pelos momentos de descontração, e à minha sobrinha **Aya**, por trazer leveza e alegria à minha vida.

Aos colegas do grupo Biodonto, **Larissa, Johnny e Roberth**, pela companhia e apoio durante a realização dos experimentos. À **Luana,** por ter aceitado compor a equipe da revisão e por compartilhar generosamente todo o seu conhecimento. À **Maria Ester**, pela companhia nas idas às reuniões de grupo, pelo apoio constante e por compartilhar uma fase tão desafiadora, tornando esse momento mais leve. À **Salua** e à **Daianny**, pelas trocas acadêmicas e pelo compartilhamento de experiências ao longo do mestrado.

À **Ingrid** e à **Elisabete**, pela valiosa contribuição na realização e no suporte técnico dos ensaios de MEV e PCR.

Aos **Profs. Drs**. **José Correia** e **Felipe Saldanha**, pelo apoio e pelas contribuições neste trabalho.

À **Prof.ª Dr.ª Ana Carolina Acevedo**, por disponibilizar a sala de microscopia do Laboratório de Histopatologia Oral e por ser um exemplo de pesquisadora e profissional, referência em minha formação acadêmica desde a iniciação científica. Às **Prof.ª Dr.ª Eliete** e **Juliana Lott**, pelas valiosas contribuições neste trabalho e pela disponibilidade e apoio ao longo do desenvolvimento deste trabalho.

À **Larissa Di Carvalho**, pelos ensinamentos, paciência e generosidade ao longo da execução da revisão, e à **Vitória**, pela colaboração e auxílio na organização final dos resultados.

Aos membros da Banca Avaliadora, **Prof. Dr. Jacy Ribeiro de Carvalho Júnior, Prof.ª Dr.ª Francine Benetti Faria e Prof.ª Dr.ª Stella Maris de Freitas Lima**, por terem

aceitado o convite para compô-la e pela oportunidade de contar com suas relevantes contribuições neste trabalho.

Aos Laboratórios de Biotecnologia da Universidade Católica de Brasília e ao Laboratório de Farmacologia Molecular da Faculdade de Ciências da Saúde da Universidade de Brasília, pela infraestrutura e pelo apoio técnico.

Ao Programa de Pós-Graduação em Ciências da Saúde, pela oportunidade de realização deste trabalho.

Ao CNPq e à CAPES, pelo apoio financeiro para a realização deste trabalho.

“O futuro pertence àqueles que acreditam na beleza de seus sonhos.”

Eleanor Roosevelt

RESUMO

A senescência celular compromete funções essenciais das células-tronco/estromais mesenquimais (MSCs, *mesenchymal stem*/*stromal cells*), podendo limitar seu potencial terapêutico em processos de reparo tecidual e regeneração, especialmente em ambientes inflamatórios. Entretanto, seus efeitos sobre as células-tronco da papila apical (SCAPs, *stem cells from apical papilla*) e suas implicações para as terapias endodônticas regenerativas (RET, *regenerative endodontic therapy*) permanecem pouco esclarecidos. Esta dissertação teve como objetivo, realizar uma revisão sistemática ampla para investigar o impacto da senescência celular sobre as propriedades funcionais e imunomoduladoras de MSCs humanas. Posteriormente, foi conduzido um estudo experimental *in vitro* com células da papila apical (SCAPs), a fim de avaliar, de forma específica, as consequências da senescência sob estímulos inflamatórios relevantes à terapia endodôntica regenerativa (RET). Para isso, adotou-se uma abordagem metodológica integrada. Inicialmente, realizou-se uma revisão sistemática da literatura, conduzida conforme as diretrizes PRISMA 2020 e registrada no PROSPERO, que incluiu trinta e cinco estudos experimentais *in vitro* e dez estudos com modelos experimentais *in vitro* e *in vivo* avaliando os efeitos da senescência em MSCs humanas derivadas dos tecidos mais amplamente estudados, totalizando quarenta e cinco estudos incluídos. Em seguida, desenvolveu-se um estudo experimental *in vitro* utilizando SCAPs humanas, nas quais a senescência foi induzida com doxirrubicina e avaliadas sob estímulos inflamatórios, incluindo lipopolissacarídeo (LPS) e interferon-gama (IFN-γ). Foram analisados marcadores de senescência, atividade mitocondrial e morfologia celular, migração, proliferação e expressão de citocinas pró- e anti-inflamatórias. A revisão sistemática demonstrou que a senescência celular está consistentemente associada à redução da capacidade proliferativa e migratória das MSCs, ao comprometimento de desfechos regenerativos *in vivo* e ao desenvolvimento de um perfil secretório predominantemente pró-inflamatório, embora os efeitos sobre a diferenciação celular apresentem resultados heterogêneos. O estudo experimental *in vitro* corroborou esses achados ao demonstrar que SCAPs senescentes mantêm a atividade mitocondrial mesmo sob desafio inflamatório, porém exibem alterações morfológicas marcantes, redução significativa da proliferação e da migração e desequilíbrio no perfil imunomodulador, caracterizado pelo aumento da expressão de *IL-6* e redução de *TGF-β*. Conclui-se que a senescência celular representa uma barreira biológica relevante para a funcionalidade das SCAPs e para o sucesso das terapias endodônticas regenerativas, ressaltando a necessidade de incorporar a avaliação da

senescência celular e o desenvolvimento de estratégias senoterapêuticas como abordagens adjuvantes em protocolos regenerativos.

PALAVRAS-CHAVE: Endodontia Regenerativa; Senescência Celular; Células-Tronco Mesenquimais; Papila Apical; Fenótipo Secretor Associado à Senescência.

ABSTRACT

Cellular senescence compromises essential functions of mesenchymal stem/stromal cells (MSCs), potentially limiting their therapeutic potential in tissue repair and regeneration processes, particularly under inflammatory conditions. However, its effects on stem cells from the apical papilla (SCAPs) and the implications for regenerative endodontic therapy (RET) remain poorly understood. This dissertation aimed to investigate the impact of cellular senescence on the functional and immunomodulatory properties of human MSCs and, specifically, to evaluate its consequences on SCAPs under inflammatory stimuli relevant to RET. To this end, an integrated methodological approach was adopted. Initially, a systematic review of the literature was conducted in accordance with PRISMA 2020 guidelines and registered in PROSPERO, which included thirty-five experimental *in vitro* studies and ten studies with *in vitro* and *in vivo* experimental models evaluating the effects of senescence on human MSCs derived from the most widely studied tissues, totaling forty-five included studies. Subsequently, an *in vitro* experimental study was performed using human SCAPs, in which senescence was induced with doxorubicin and cells were evaluated under inflammatory stimuli, including lipopolysaccharide (LPS) and interferon-gamma (IFN-γ). Markers of senescence, cellular metabolism and morphology, migration, proliferation, and the expression of pro- and anti-inflammatory cytokines were analyzed. The systematic review demonstrated that cellular senescence is consistently associated with reduced proliferative and migratory capacities of MSCs, impaired *in vivo* regenerative outcomes, and the development of a predominantly pro-inflammatory secretory profile, although effects on cellular differentiation were heterogeneous. The experimental study corroborated these findings by demonstrating that senescent SCAPs maintain cellular metabolism even under inflammatory challenge; however, they exhibit marked morphological alterations, significant reductions in proliferation and migration, and an imbalance in the immunomodulatory profile, characterized by increased IL-6 expression and reduced TGF-β expression. It is concluded that cellular senescence represents a relevant biological barrier to SCAP functionality and to the success of regenerative endodontic therapies, highlighting the need to incorporate senescence assessment and the development of senotherapeutic strategies as adjuvant approaches in regenerative protocols.

KEYWORDS: Regenerative Endodontics; Cellular Senescence; Mesenchymal Stem/Stromal Cells; Apical Papilla; Senescence-Associated Secretory Phenotype.

LISTA DE FIGURAS

DISSERTAÇÃO

CAPÍTULO 1

CAPÍTULO 2

Figure 1 - Flowchart for Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines.

Figure 2 - Schematic illustration of metabolic alterations in senescent SCAPs (A). Representative images of non-senescent control cells (B; NS) and doxorubicin-induced senescent cells (S; 1μL.mL^{-1}) showing SA-β-Gal-positive cells (blue). Scale bar: 100 μm (C). Quantification of SA-β-Gal-positive SCAPs revealed significant increase in senescent cells ($p = 0.000041^{****}$, Mann–Whitney test; D). *P53* expression, represented as fold change, was significantly higher in senescent SCAPs compared with non-senescent controls ($p = 0.0368^{*}$, Student's t-test; E). MTT assay after 24-hour immunomodulatory stimulation with LPS (1 μg.mL^{-1}) or LPS plus IFN-γ (1 μg.mL^{-1}) showed higher metabolic activity in senescent cells under control conditions ($p = 0.0082$), whereas no significant differences were observed for LPS ($p = 0.2064^{**}$) or LPS plus IFN-γ ($p = 0.2282$) (F).

Figure 3 - SEM of SCAPs. Representative images of non-senescent (NS, A) and senescent (S, B) cells after immunomodulatory stimulation with LPS or LPS plus IFN-γ, shown at 1,500×, 3,000×, and 5,000× magnifications. Scale bar: 10 μm. Graphs show cell size (C) and number of cellular extensions (D) under each condition. Cell size was significantly increased in senescent cells across all conditions (control: $p = 0.0006^{***}$; LPS: $p = 0.0008^{***}$; LPS plus IFN-γ: $p = 0.0017^{**}$), while senescent cells showed a significant reduction in cellular extensions compared with NS cells (control: $p = 0.002^{**}$; LPS: $p = 0.0028^{**}$; LPS plus IFN-γ: $p = 0.0085^{**}$). Statistical analyses were performed using Student's *t*-test.

Figure 4 - Schematic representation of SCAP functions related to regenerative endodontic therapy. SCAP migration and proliferation are shown as essential processes for tissue regeneration (A). Representative micrographs of NS and S SCAPs in the wound-healing migration assay at 0, 24, and 48 hours under control, LPS, or LPS plus IFN-γ stimulation (B–C). Quantification of migrated cells (control: $p = 0.0000000036^{****}$; LPS: $p = 0.00000001^{****}$; LPS plus IFN-γ: $p = 0.0000000021^{****}$) (D). Proliferative activity evaluated by Trypan Blue exclusion at 24 and 48 hours (NS vs. S; control: $p = 0.0026^{***}$; LPS: $p = 0.0042^{**}$; LPS plus IFN-γ: $p = 0.0018^{**}$). Statistical analysis was performed using two-way ANOVA.

Figure 5 - Gene expression of pro- and anti-inflammatory mediators in senescent and non-senescent SCAP cultures following stimulation with LPS or LPS plus IFN-γ. *GAPDH* was used as the constitutive gene for normalization. Expression levels of *TNF-α* conditions (control p =

0.8; LPS p = 0.4; LPS plus IFN-γ p = 0.8) (A), *IL-6* (LPS p = 0.002**; LPS plus IFN-γ p = 0.000008****) (B), *TGF-β* (control p = 0.0003***; LPS p = 0.0004***; LPS plus IFN-γ p = 0.0005***) (C), and *IL-10* (control p = 0.8; LPS p = 0.002**; LPS plus IFN-γ p = 0.14) (D). Statistical analysis was performed using the Student's *t*-test for *TGF-β* and *IL-6* (LPS plus IFN-ɣ); the remaining groups did not pass the Shapiro–Wilk normality test and were analyzed by Mann–Whitney test.

Figure 6 - Schematic representation of the study framework. Inflammatory microenvironment of necrotic pulp, showing bacterial products, immune cell activation, cytokine release, and ROS generation, which contribute to SCAP senescence and potentially limiting pulp tissue regeneration and root maturation in immature teeth.

LISTA DE TABELAS

DISSERTAÇÃO

CAPÍTULO 1

CAPÍTULO 2

ANEXOS

Anexo 1. Parecer consubstanciado de aprovação do projeto no CEP/UnB.

Anexo 2. Parecer consubstanciado de aprovação do projeto no CEP/UCB.

Anexo 3. Trabalho em fase de submissão, elaborado durante o período de mestrado.

LISTA DE ABREVIATURAS E SIGLAS

AD-MSCs – *Adipose tissue-derived mesenchymal stem cells* (Células-tronco/estromais mesenquimais derivadas do tecido adiposo)

AI – *Artificial intelligence* (Inteligência artificial)

ALP – *Alkaline phosphatase* (Fosfatase alcalina)

ANOVA – *Analysis of variance* (Análise de variância)

BMP – *Bone morphogenetic protein* (Proteína morfogenética óssea)

BM-MSCs – *Bone marrow-derived mesenchymal stem cells* (Células-tronco/estromais mesenquimais derivadas da medula óssea)

BrdU – *Bromodeoxyuridine* (Bromodeoxiuridina)

CAAE – Certificado de Apresentação para Apreciação Ética

CCK-8 – *Cell Counting Kit-8*

CFU – *Colony-forming unit* (Unidade formadora de colônia)

COL1/COL2 – *Collagen type I/II* (Colágeno tipo I/II)

CPD – *Cumulative population doublings* (Duplicações populacionais cumulativas)

DPSCs – *Dental pulp stem cells* (Células-tronco da polpa dentária)

DT/PDT – *Doubling time/Population doubling time* (Tempo de duplicação/Tempo de duplicação populacional)

EDX – *Energy-dispersive X-ray spectroscopy* (Espectroscopia de raios X por dispersão de energia)

ELISA – *Enzyme-linked immunosorbent assay* (Ensaio de imunoabsorção enzimática)

GAPDH – *Glyceraldehyde-3-phosphate dehydrogenase* (Gliceraldeído-3-fosfato desidrogenase)

HE – *Hematoxylin and eosin* (Hematoxilina e eosina)

IFN-γ – *Interferon-gamma* (Interferon gama)

IL-1β/IL-6/IL-8/IL-10 – *Interleukin-1β/6/8/10* (Interleucina-1β6/8/10)

iNOS – *Inducible nitric oxide synthase* (Óxido nítrico sintase induzível)

ISCT – *International Society for Cell & Gene Therapy* (Sociedade Internacional de Terapia Celular e Gênica)

LPS – *Lipopolysaccharide* (Lipopolissacarídeo)

Micro-CT – *Micro-computed tomography* (Microtomografia computadorizada)

MSCs – *Mesenchymal stem/stromal cells* (Células-tronco/estromais mesenquimais)

MTT – *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* (Brometo de 3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazólio)

NR – *Not reported* (Não reportado)

OCN – *Osteocalcin* (Osteocalcina)

OD – *Optical density* (Densidade óptica)

OPN – *Osteopontin* (Osteopontina)

PBS – *Phosphate-buffered saline* (Solução salina tamponada com fosfato)

PCR – *Polymerase chain reaction* (Reação em cadeia da polimerase)

PD – *Population doublings* (Duplicações populacionais)

PDLSCs – *Periodontal ligament stem cells* (Células-tronco do ligamento periodontal)

PETRICCS – *Reporting Standards in Cell Culture Research* (Padrões de Relato em Pesquisa com Cultura de Células)

PGE2 – *Prostaglandin E2* (Prostaglandina E2)

PPAR-γ – *Peroxisome proliferator-activated receptor* γ (Receptor γ ativado por proliferador de peroxissoma)

PRILE – *Preferred Reporting Items for Laboratory Studies in Endodontology* (Itens Preferenciais para Relato de Estudos Laboratoriais em Endodontologia)

PRISMA – *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (Itens Preferenciais para Relato de Revisões Sistemáticas e Meta-análises)

PROSPERO – *International Prospective Register of Systematic Reviews* (Registro Prospectivo Internacional de Revisões Sistemáticas)

qPCR/RT-qPCR – *Quantitative polymerase chain reaction/Real-time quantitative PCR* (Reação em cadeia da polimerase quantitativa/Quantitativa em tempo real)

RET / TER – *Regenerative endodontic therapy* (Terapia Endodôntica Regenerativa)

ROS – *Reactive oxygen species* (Espécies reativas de oxigênio)

RUNX2 – *Runt-related transcription factor 2* (Fator de transcrição relacionado a Runt 2)

SA-β-Gal – *Senescence-associated β-galactosidase* (β-galactosidase associada à senescência)

SASP – *Senescence-associated secretory phenotype* (Fenótipo secretor associado à senescência)

SCAPs – *Stem cells from apical papilla* (Células-tronco da papila apical)

SD/SEM – *Standard deviation/Standard error of the mean* (Desvio padrão/Erro padrão da média)

SEM – *Scanning electron microscopy* (Microscopia eletrônica de varredura)

SYRCLE – *Systematic Review Centre for Laboratory animal Experimentation* (Centro de Revisão Sistemática para Experimentação Animal Laboratorial)

TGF-β – *Transforming growth factor β* (Fator de crescimento transformador β)

TNF-α – *Tumor necrosis factor α* (Fator de necrose tumoral α)

UC-MSCs – *Umbilical cord-derived mesenchymal stem cells* (Células-tronco/estromais mesenquimais derivadas do cordão umbilical)

UC-MSCs-CB – *Umbilical cord blood-derived mesenchymal stem cells* (Células-tronco/estromais mesenquimais derivadas do sangue do cordão umbilical)

UC-MSCs-WJ – *Wharton's jelly-derived mesenchymal stem cells* (Células-tronco/estromais mesenquimais derivadas da geleia de Wharton)

SUMÁRIO

PREFÁCIO

Esta dissertação de mestrado foi desenvolvida no âmbito do Programa de Pós-Graduação em Ciências da Saúde da Universidade de Brasília e está organizada em formato de capítulos, conforme as normas do programa. O estudo dá continuidade à linha de pesquisa do grupo sobre senescência celular, previamente explorada em investigações que resultaram na publicação dos artigos *"Senescence on Dental Pulp Cells: Effects on Morphology, Migration, Proliferation, and Immune Response"* e *"Impact of Cellular Senescence on the Immune-Inflammatory Response and Regenerative Capacity of Human Dental Pulp Cells"*.

Nessa perspectiva, o presente trabalho ampliou essa abordagem ao investigar outro tecido dentário, a papila apical. Diferentemente dos estudos anteriores, que analisaram os impactos morfofuncionais da senescência celular no tecido pulpar no contexto de tratamentos endodônticos conservadores, esta dissertação direciona seu foco à papila apical, considerando sua relevância biológica para os tratamentos endodônticos regenerativos de dentes com rizogênese incompleta. Além disso, o trabalho amplia essa abordagem ao examinar, de forma abrangente, a influência da senescência de células-tronco nos processos de reparo e regeneração de diferentes tecidos do corpo humano.

A escrita da dissertação seguiu o formato descrito no documento de Normas para a redação de teses e dissertações do Programa de Pós-graduação em Ciências da Saúde – Universidade de Brasília, sendo escolhido o formato em capítulos, para a apresentação dos elementos textuais. Assim, dois capítulos foram organizados de forma complementar. O Capítulo 1 consiste em uma revisão sistemática da literatura que reúne e analisa criticamente as evidências disponíveis acerca dos efeitos da senescência de células-tronco na capacidade de reparo e regeneração tecidual, estabelecendo uma base conceitual para a compreensão do tema. O Capítulo 2 apresenta um estudo experimental *in vitro* que investiga os efeitos da senescência celular em células-tronco da papila apical, com ênfase em parâmetros biológicos relevantes para a endodontia regenerativa. Em conjunto, os capítulos permitem uma abordagem integrada do tema, articulando o conhecimento já consolidado na literatura com a análise experimental de um tecido dentário ainda pouco explorado.

Ao integrar evidências da literatura com a investigação experimental de células-tronco da papila apical, esta dissertação contribui para o entendimento dos efeitos da senescência

celular como um fator limitante do reparo tecidual. A análise dos impactos da senescência sobre parâmetros celulares fundamentais, como metabolismo celular, migração, proliferação e resposta inflamatória, permite avançar na compreensão dos mecanismos biológicos que podem comprometer o sucesso de terapias regenerativas. Nesse contexto, os achados discutidos ao longo do trabalho oferecem subsídios para a reflexão crítica e o aprimoramento de estratégias clínicas futuras, particularmente aquelas baseadas na utilização de células-tronco nos tratamentos endodônticos regenerativos em pacientes jovens.

INTRODUÇÃO

As células-tronco mesenquimais (MSCs, *mesenchymal stem*/*stromal cells*) têm sido amplamente investigadas como uma fonte promissora para a engenharia tecidual e para terapias celulares, em virtude de sua capacidade de autorrenovação, diferenciação multipotente e propriedades imunomodulatórias (Pittenger et al., 1999). Essas células podem ser isoladas de diversos tecidos humanos, incluindo medula óssea, tecido adiposo, cordão umbilical e tecidos dentários, como a polpa dentária, ligamento periodontal e papila apical (Dagnino et al., 2020; Heo et al., 2016; Kang et al., 2019; Kern et al., 2006). Independentemente da origem tecidual, o potencial terapêutico das MSCs está fortemente relacionado à sua capacidade funcional de migrar para sítios de lesão, modular respostas inflamatórias e contribuir para o reparo tecidual, predominantemente por meio de mecanismos parácrinos, além de apresentarem potencial proliferativo e de diferenciação limitado (Zuk et al., 2001).

Apesar de seu amplo potencial regenerativo, as MSCs são suscetíveis à senescência celular, um processo complexo caracterizado pela interrupção irreversível do ciclo celular em resposta a diferentes tipos de estresse, como dano ao DNA, estresse oxidativo e inflamação crônica (Wagner et al., 2008). A senescência é mediada pela ativação de vias regulatórias do ciclo celular, incluindo p16^INK4a^ e o eixo p53/p21, e está associada a alterações morfológicas, metabólicas e funcionais (Wenjing et al., 2024a). Entre as propriedades mais afetadas nas MSCs senescentes estão a redução da capacidade proliferativa, a diminuição do potencial de diferenciação osteogênica, adipogênica e condrogênica, amplamente utilizadas como indicadores de multipotência, e o comprometimento da capacidade migratória, resultando em reparo tecidual subótimo (Wenjing et al., 2024a).

Um dos principais marcos da senescência celular é o desenvolvimento do fenótipo secretor associado a senescência (SASP, *senescence-associated secretory phenotype*), caracterizado pela secreção sustentada de citocinas pró-inflamatórias, quimiocinas e enzimas proteolíticas (Coppé et al., 2008; Su et al., 2020). O SASP não apenas prejudica diretamente os processos de regeneração tecidual, como também contribui para a manutenção de um microambiente inflamatório crônico, promovendo a degradação da matriz extracelular e a propagação da senescência para células vizinhas (Wagner et al., 2008). Esse cenário é particularmente relevante no contexto das terapias regenerativas, uma vez que a presença de

células senescentes pode comprometer de forma significativa a funcionalidade das MSCs e, consequentemente, os desfechos clínicos.

No campo experimental, a senescência em MSCs tem sido investigada tanto no contexto do envelhecimento cronológico do doador, quanto por meio de modelos de envelhecimento biológico *in vitro*, como a senescência replicativa induzida por passagens seriadas e a senescência induzida por estresse, incluindo estímulos inflamatórios e agentes genotóxicos (Choudhery et al., 2014; X. Feng, Feng, et al., 2014; Gruber et al., 2012). No entanto, a diversidade de modelos experimentais, marcadores de senescência e tecidos de origem das MSCs contribui para uma literatura heterogênea, dificultando a comparação direta dos resultados e a compreensão integrada dos efeitos da senescência sobre as propriedades funcionais dessas células (Horibe et al., 2014a; Ma et al., 2019a; Zhang et al., 2024).

No contexto da odontologia, particularmente da endodontia regenerativa, a compreensão desses processos adquire relevância clínica direta. O tratamento de dentes com rizogênese incompleta, seja em decorrência de necrose pulpar, pulpite irreversível ou trauma dentário, representa um desafio clínico significativo devido à fragilidade estrutural e à presença do ápice aberto (Jamshidi et al., 2018). A terapia endodôntica regenerativa (TER) surge como uma abordagem promissora ao buscar não apenas a desinfecção do sistema de canais radiculares, mas também a possibilidade do contínuo desenvolvimento radicular e o fechamento apical (Adel & Asgari, 2025; Jena et al., 2023). O sucesso dessa abordagem depende fundamentalmente dos princípios da engenharia tecidual, os quais requerem a presença de uma população de MSCs viáveis e funcionalmente competentes (Ma et al., 2019a; Salem et al., 2025).

A papila apical é um tecido altamente vascularizado e rico em células-tronco (SCAPs, *stem cells from apical papilla*) que desempenha papel central nos processos regenerativos associados à RET (Q. Cheng et al., 2023; Smeda et al., 2022). Em condições favoráveis, as SCAPs podem ser recrutadas para o interior do canal radicular, onde proliferam e se diferenciam, contribuindo para a formação de tecidos semelhantes aos dentários (Tawfik et al., 2013; Temmerman et al., 2012). Assim, fatores que modulam o metabolismo celular e a funcionalidade dessas células tornam-se determinantes para a previsibilidade dos resultados clínicos.

Entretanto, as RET apresentam taxas de sucesso variáveis, e os fatores biológicos associados às falhas terapêuticas ainda não estão completamente elucidados. Evidências sugerem que a persistência de um microambiente inflamatório no interior do canal radicular pode induzir senescência em SCAPs, comprometendo sua capacidade regenerativa (Teawcharoensopa & Srisuwan, 2024a). Condições como necrose pulpar, pulpite irreversível e trauma dentário favorecem a liberação de produtos bacterianos, espécies reativas de oxigênio e citocinas pró-inflamatórias, incluindo IL-6, TNF-α e IFN-γ, criando um ambiente propício ao dano celular e à ativação de vias associadas à senescência (Hsu & Wen, 2002; Zhu et al., 2024). Embora mediadores regulatórios, como IL-10 e TGF-β, atuem na tentativa de conter a inflamação, esses mecanismos nem sempre são suficientes para impedir o estabelecimento de um estado de estresse celular persistente (Putra et al., 2018).

Do ponto de vista clínico, esse cenário ganha ainda mais relevância ao se considerar que muitos casos indicados para RET envolvem histórico de infecção prolongada, múltiplas intervenções endodônticas ou tempo decorrido significativo entre o diagnóstico e o tratamento (Adel & Asgari, 2025; Jena *et al*., 2023). Nessas situações, a permanência de mediadores inflamatórios e produtos bacterianos no interior do sistema de canais radiculares pode não apenas comprometer a desinfecção efetiva, mas também impactar diretamente a qualidade biológica das células residentes na papila apical (Zhu *et al*., 2024). Além disso, considerando que o sucesso da RET depende da sobrevivência, migração e diferenciação de SCAPs funcionalmente competentes, alterações nessas propriedades podem influenciar negativamente os desfechos clínicos (Cheng *et al*., 2023; Smeda *et al*., 2022; Tawfik *et al*., 2013). Assim, mesmo diante de protocolos clínicos adequadamente executados, a presença de SCAPs funcionalmente prejudicadas pode limitar o potencial regenerativo esperado, contribuindo para respostas clínicas insatisfatórias, como ausência de continuação do desenvolvimento radicular (Temmerman *et al*., 2012; Razghonova *et al*., 2022).

Apesar dessas hipóteses, ainda são escassos os estudos experimentais que avaliam diretamente como a senescência pode afetar as funções biológicas das SCAPs, relevantes para a endodontia regenerativa. Diferentes modelos de indução de senescência têm sido utilizados, incluindo peróxido de hidrogênio, radiação ultravioleta e agentes genotóxicos, sem consenso sobre qual deles melhor representa as condições clínicas (Cho et al., 2019a; Höfig et al., 2016a; Ma et al., 2019a). Entre esses modelos, a indução de senescência por agentes genotóxicos, como a doxorrubicina, tem se destacado por sua reprodutibilidade e robustez experimental

(Yaghoobi et al., 2020a). Embora não reproduza integralmente o caráter crônico da inflamação observada *in vivo*, esse modelo permite a investigação controlada dos mecanismos e consequências funcionais da senescência em SCAPs, especialmente quando associado a desafios imunoinflamatórios adicionais, como LPS e IFN-γ.

Dessa forma, compreender de maneira integrada como a senescência celular compromete as propriedades funcionais das MSCs, com ênfase nas SCAPs, torna-se essencial para o avanço do conhecimento biológico que sustenta as terapias endodônticas regenerativas. A articulação entre evidências da literatura e investigação experimental permite não apenas aprofundar a compreensão dos mecanismos envolvidos, mas também fornecer subsídios para o aprimoramento de estratégias clínicas futuras baseadas em abordagens regenerativas.

OBJETIVOS

Objetivo Geral

Investigar, por meio de uma revisão sistemática da literatura, o impacto da senescência celular sobre as propriedades funcionais, regenerativas e imunomoduladoras de células-tronco mesenquimais humanas, com ênfase nas implicações desse processo para a regeneração tecidual. Adicionalmente, avaliar por meio de experimentos *in* vitro como a senescência afeta a funcionalidade das células-tronco da papila apical no contexto das terapias endodônticas regenerativas.

Objetivos Específicos

1. Avaliar através de uma revisão sistemática os impactos da senescência celular sobre as células-tronco/estromais dos tecidos mais estudados, considerando diferentes métodos de indução da senescência e as principais alterações funcionais relacionadas à proliferação, migração, diferenciação, imunomodulação e capacidade regenerativa.
2. Induzir *in vitro* a senescência celular em células-tronco da papila apical humanas por meio do tratamento com doxorrubicina, visando utilizar um modelo experimental reprodutível de senescência.
3. Confirmar e caracterizar a indução do fenótipo senescente em células-tronco da papila apical por meio da avaliação de marcadores clássicos de senescência celular, incluindo a atividade da β-galactosidase associada à senescência e a expressão de *p53.*
4. Empregar, *in vitro*, estimulação com lipopolissacarídeo (LPS), isoladamente ou associado ao interferon-gama (IFN-γ), como modelo imunoinflamatório para reproduzir condições inflamatórias relevantes às terapias endodônticas regenerativas.
5. Avaliar *in vitro* as alterações morfológicas associadas à senescência em células-tronco da papila apical senescentes e não senescentes por meio de microscopia eletrônica de varredura (MEV).
6. Investigar *in vitro* os efeitos da senescência celular sobre a capacidade migratória, proliferativa e sobre a atividade mitocondrial de células-tronco da papila apical, em condições basais e sob estímulos inflamatórios.

7. Analisar o impacto da senescência celular sobre o perfil imunoinflamatório *in vitro* das células-tronco da papila apical, por meio da avaliação da expressão de mediadores inflamatórios e imunomoduladores, incluindo fator de necrose tumoral alfa *(TNF-α),* fator de crescimento transformante beta 1 *(TGF-β1)* e as interleucinas *IL-6 e IL-10.*

MATERIAIS E MÉTODOS

Delineamento Experimental

O presente estudo apresenta um delineamento metodológico misto, organizado em dois capítulos complementares. O primeiro capítulo consiste em uma revisão sistemática que buscou sintetizar e analisar criticamente a literatura acerca do impacto da senescência de células-tronco nos processos de reparo e regeneração tecidual, conduzida de acordo com as diretrizes do *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA 2020). O Segundo capítulo corresponde a um estudo experimental *in vitro*, no qual foi investigada o efeito da senescência sobre a capacidade de reparo das células-tronco da papila apical, considerando o papel da inflamação como modulador desse processo e seu impacto para a endodontia regenerativa (Figura 1).

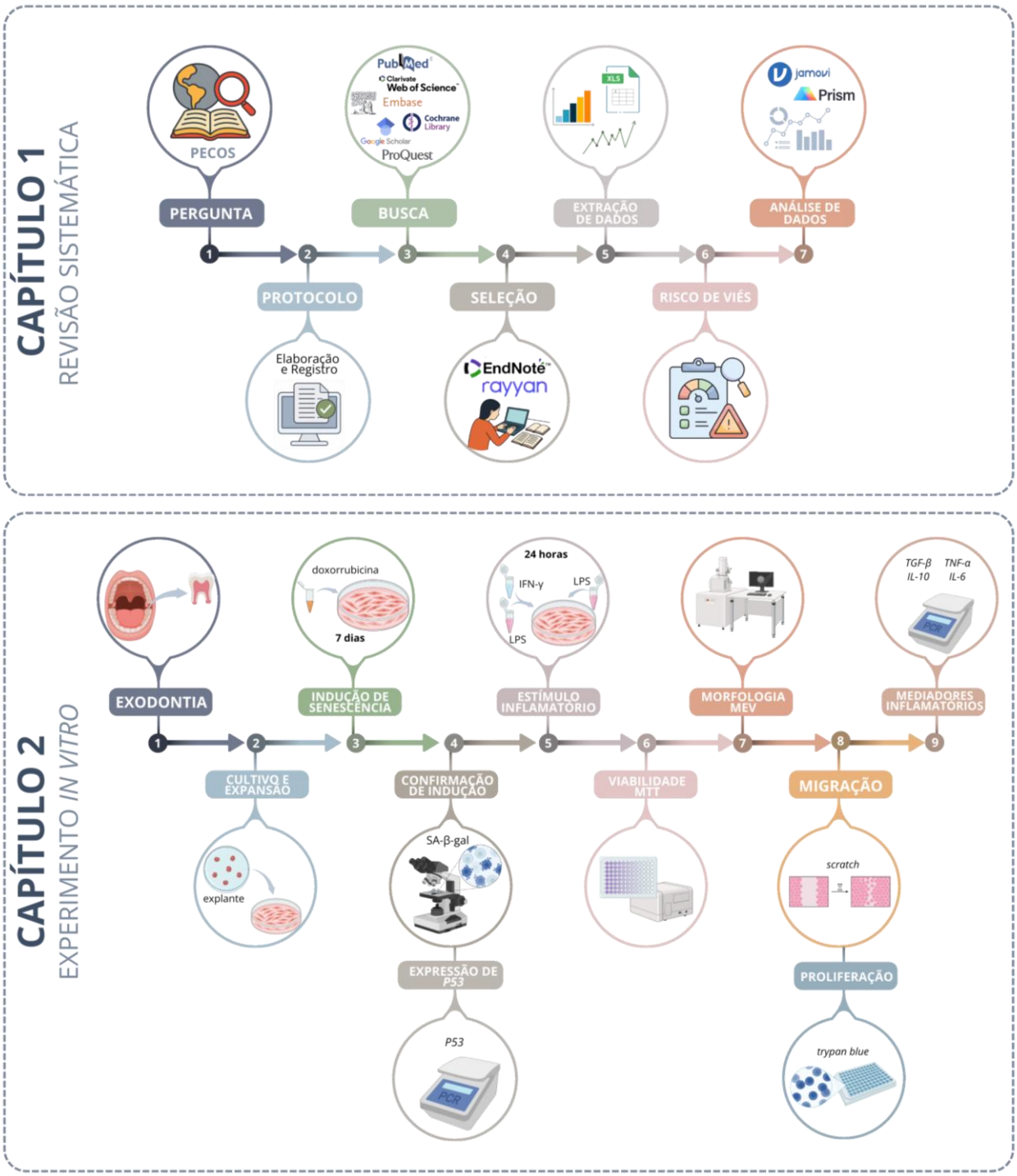


Figura 1. Fluxograma do delineamento do estudo.

Capítulo 1

Critérios de Elegibilidade

Os critérios de elegibilidade foram definidos com base na estratégia PECOS (*Population*, *Exposure*, *Comparator*, *Outcomes* e *Study design*), a fim de responder à pergunta da revisão: *"How does cellular senescence in stem cells from various tissues affect their*

regenerative potential and tissue repair mechanisms?". Foram incluídos estudos que avaliaram células-tronco mesenquimais humanas (*Population*) derivadas de tecido adiposo, medula óssea, polpa dentária, ligamento periodontal ou cordão umbilical, obtidas de fontes primárias e não geneticamente modificadas. A exposição de interesse (*Exposure*) foi a senescência celular induzida pelo envelhecimento cronológico do doador, por passagens seriadas (senescência replicativa) ou por estímulos inflamatórios, por representarem mecanismos fisiologicamente relevantes e amplamente descritos na literatura. A comparação (*Comparator*) deveria ser realizada com células-tronco não senescentes, incluindo células provenientes de doadores jovens, culturas em passagens iniciais ou controles não submetidos a estímulos indutores de senescência. Quanto aos desfechos (*Outcomes*), os estudos deveriam avaliar ao menos um parâmetro relacionado à capacidade regenerativa ou ao reparo tecidual, como taxa de proliferação, potencial de diferenciação, migração celular, modulação da resposta imune ou reparo tecidual, além de confirmar o estado de senescência por meio de pelo menos um marcador validado, incluindo atividade de SA-β-gal, expressão de CDKN2A (p16), CDKN1A (p21) ou TP53 (p53), atividade da telomerase ou encurtamento dos telômeros. Em relação ao delineamento (*Study design*), foram incluídos estudos experimentais *in vitro*, estudos *in vivo* e ensaios clínicos, sem restrição quanto ao ano de publicação ou idioma, sendo também considerada a literatura cinzenta. Foram excluídos estudos que investigaram outros tipos celulares, avaliaram a senescência em modelos de doença não relacionados a terapias regenerativas, utilizaram linhagens celulares imortalizadas ou células geneticamente modificadas, não apresentaram comparação direta entre grupos senescentes e não senescentes, não confirmaram o estado de senescência, abordaram exclusivamente mecanismos moleculares sem avaliar impacto funcional no reparo tecidual ou em terapias regenerativas, não estavam disponíveis na íntegra ou consistiam em revisões, meta-análises, resumos, protocolos, comunicações breves, cartas, pôsteres, resumos de conferências ou relatos de caso.

Fontes de Informação e Estratégia de Busca

A presente revisão sistemática foi conduzida de acordo com as diretrizes do *Preferred Reporting Items for Systematic Reviews and Meta-Analyses* (PRISMA) 2020 (Page et al., 2021) e registrada na plataforma PROSPERO (CRD42025638850). A estratégia de busca foi adaptada manualmente para cada base de dados, incluindo *MEDLINE*/*PubMed* (via *National Library of Medicine*), *EMBASE* (via *Elsevier*), *Cochrane Database* (via *Cochrane Library*) e

Web of Science Core Collection (via *Clarivate*). Buscas adicionais na literatura cinzenta foram realizadas por meio do *Google Scholar* e *ProQuest*, bem como busca manual nas listas de referências dos estudos incluídos. A busca inicial foi realizada no dia 28 de fevereiro de 2025 e atualizada em 12 de dezembro de 2025. As estratégias de busca completas estão disponíveis no material suplementar (S1), disponibilizado no capítulo 1 - *"The impact of stem cell senescence on wound repair and regeneration: A Systematic Review"*.

Processo de Seleção

A remoção de artigos duplicados foi realizada utilizando os *softwares EndNote* (Thomson Reuters, Philadelphia, PA) e *Rayyan*® (Rayyan Systems Inc., Doha, Qatar). A triagem inicial por título e resumo foi conduzida no *Rayyan* por dois revisores (L.O.W. e L.R.C.). Os artigos que avançaram nessa etapa foram avaliados na íntegra por dois revisores para confirmação da elegibilidade, com eventuais conflitos ou incertezas resolvidos por um terceiro revisor (J.C.S.). O fluxograma PRISMA foi utilizado para resumir o número de estudos em cada etapa do processo de seleção (Page et al., 2021).

Processo de Coleta de Dados e Itens de Dados

A extração de dados foi realizada de forma independente por dois revisores (L.O.W. e L.R.C.). A ferramenta *DeepSeek AI* (2025) foi utilizada para a extração automática de informações gerais dos estudos (autor, ano, país, tipo de estudo e periódico) e de características da população (tipo de célula-tronco, fonte tecidual, espécie, idade do doador e número de amostras ou doadores), a partir dos textos completos, sendo todas as informações posteriormente validadas pelos revisores. A extração dos dados referentes aos desfechos principais foi realizada manualmente. Quando valores quantitativos não estavam descritos no texto, estes foram obtidos a partir de gráficos utilizando o *software PlotDigitizer* (Versão 3.1.6, 2025). Artigos publicados em idiomas diferentes do inglês foram traduzidos com o auxílio da ferramenta *DeepL* (DeepL GmbH, 2025).

Os dados extraídos incluíram exposição (método de indução da senescência, método de confirmação, tempo de indução), comparação (grupo comparador, descrição), desfechos principais (proliferação, diferenciação osteogênica, adipogênica e condrogênica, migração,

reparo tecidual e resposta imune) e desfechos adicionais (vias moleculares, alterações epigenéticas, expressão gênica e estratégias de rejuvenescimento ou mitigação). Para cada desfecho, quando múltiplos ensaios foram reportados, foi aplicada uma hierarquia previamente definida para selecionar um único resultado para síntese: para proliferação, foram priorizados tempo de duplicação populacional e ensaios relacionados (por exemplo, tempo de duplicação cumulativo); para diferenciação, foram extraídos dados de ensaios colorimétricos (por exemplo, *Alizarin Red, Oil Red O, Alcian Blue*), sendo a expressão gênica de marcadores de diferenciação utilizada apenas quando a quantificação colorimétrica não estava disponível; para migração celular, foram extraídos dados de ensaios de *scratch* ou *transwell*; para resposta imune, foram extraídos dados de expressão gênica e/ou proteica provenientes de PCR, ELISA ou *western blot*; e, para reparo tecidual, apenas dados de ensaios *in vivo* foram considerados.

Medidas de Efeito e Métodos de Síntese

Devido à substancial heterogeneidade metodológica entre os estudos (diferentes modelos de senescência, células-tronco provenientes de diferentes tecidos e distintos tipos de ensaios), não possível realizar uma meta-análise. Assim, os resultados foram sintetizados de forma narrativa e descritiva. Os dados foram organizados e apresentados em tabelas-resumo estruturadas por desfecho principal (proliferação, diferenciação osteogênica, adipogênica, condrogênica, migração, entre outros) e agrupadas de acordo com o tipo de senescência e o tipo de célula-tronco. Para estudos que reportaram análises estatísticas comparativas diretas entre grupos senescentes e controles não senescentes, a significância do efeito foi baseada nos valores de *p* informados no artigo original. Quando os grupos de interesse não foram submetidos a um teste estatístico direto ou quando os dados necessários para tal comparação não estavam disponíveis, a diferença foi registrada como NR, independentemente de aparentes diferenças visuais nos gráficos. As medidas de efeito brutas reportadas (por exemplo, média e desvio padrão, valores de *fold-change*) foram extraídas quando disponíveis. Devido à heterogeneidade dos estudos, os resultados foram analisados por meio de síntese narrativa, considerando fatores previamente definidos, como o tipo de célula-tronco e o método de indução da senescência. A análise descritiva dos dados foi realizada utilizando o *software* estatístico *Jamovi* (Versão 2.6), enquanto as representações gráficas foram elaboradas no *software GraphPad Prism* (Versão 8.0.2).

Avaliação do Risco de Viés dos Estudos

O risco de viés foi avaliado de forma independente por dois revisores (L.R.C. e L.O.W.) utilizando uma ferramenta de avaliação crítica baseada na diretriz PETRICCS para estudos *in vitro* (Monteiro et al., 2025) e na ferramenta *SYRCLE's* para avaliação de risco de viés em estudos *in vivo* (Hooijmans et al., 2014), com a consulta de um terceiro revisor (J.C.S.) para obtenção de consenso em caso de divergências. As avaliações foram realizadas por meio de um instrumento com respostas "*yes*", "*no*" e "*unclear*". Os estudos foram categorizados de acordo com a porcentagem de respostas "*yes*": alto risco (<50%), risco moderado (50–69%) e baixo risco (≥70%).

Capítulo 2

Isolamento e cultivo de células-tronco da papila apical (SCAPs)

Terceiros molares hígidos com rizogênese incompleta indicados para extração foram obtidos de três pacientes sistemicamente saudáveis (dois do sexo masculino, ambos com 18 anos, e uma do sexo feminino, com 15 anos), submetidos a tratamento odontológico na unidade de cirurgia bucomaxilofacial da Unidade de Saúde Bucal do Hospital Universitário de Brasília. O consentimento livre e esclarecido foi obtido de todos os participantes ou de seus responsáveis legais. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da Universidade de Brasília e da Universidade Católica de Brasília (CAAE: 86052124.4.0000.0030 e 86052124.4.3002.0029). Os tecidos da papila apical foram cuidadosamente removidos do terço apical das raízes com o auxílio de lâmina de bisturi estéril, e então fragmentados e transferidos para placas de seis poços (Costar Corp., Cambridge, MA, EUA) para cultivo por explante. Os fragmentos teciduais foram cultivados em meio *Dulbecco's Modified Eagle Medium* (DMEM; Gibco, Invitrogen, Carlsbad, CA, EUA), suplementado com 20% (v/v) de soro fetal bovino (Bionutrientes, São Paulo, Brasil), 100 $U.mL^{-1}$ de penicilina (Invitrogen, Grand Island, Nova York, EUA), 100 $\mu g.mL^{-1}$ de estreptomicina (Invitrogen) e 1% de anfotericina B (Invitrogen) (Guirado et al., 2019). As culturas foram mantidas a 37 °C, em atmosfera umidificada contendo 5% de CO_2, com troca de meio a cada dois dias, até atingirem aproximadamente 90% de confluência. Em seguida, as células foram subcultivadas para expansão. Para prevenir

senescência replicativa, todos os experimentos foram realizados utilizando células entre as passagens 2 e 5.

Indução de senescência celular e estímulo imunoinflamatório com LPS e LPS associado ao IFN-γ

Para a indução da senescência, as células foram tratadas com doxorrubicina (cloridrato de doxorrubicina; Sigma-Aldrich, St. Louis, MO, EUA) na concentração de 1 μL.mL^{-1}, em DMEM suplementado, por 24 horas. Após o tratamento, o meio de cultura foi substituído a cada dois dias por um período de sete dias sem a doxorrubicina, conforme descrito previamente (da Silva et al., 2025; de Farias et al., 2024a). As células não senescentes (NS) foram mantidas em DMEM completo sob condições padrão de cultivo. Ao final do período de indução, tanto as células não senescentes quanto as células senescentes (S) foram submetidas à estimulação imunoinflamatória com lipopolissacarídeo (LPS; *Escherichia coli*, 1 μg.mL^{-1}; Sigma-Aldrich) e interferon-gama (IFN-γ, 1 μg.mL^{-1}; Sigma-Aldrich) por 24 horas(Hong et al., 2021).

Ensaio de β-galactosidase associada à senescência

O ensaio de β-galactosidase associada à senescência (SA-β-gal) foi realizado nos grupos S e não senescente NS para avaliar a eficácia da indução da senescência. As SCAPs (2 × 10^5 células/poço) foram semeadas em placas de seis poços (Costar Corp., Cambridge, MA, EUA). Após o período de incubação os poços foram aspirados e lavados duas vezes com solução tampão fosfato (PBS), seguidos de fixação com formaldeído a 2% (Sigma-Aldrich) por 5 minutos, à temperatura ambiente. Após a fixação, as células foram novamente lavadas e incubadas com 1 mL da solução de coloração X-Gal (40 mM de tampão ácido cítrico/fosfato de sódio, 5 mM de ferrocianeto de potássio, 150 mM de cloreto de sódio, 2 mM de cloreto de magnésio e 1 mg.mL^{-1} de X-Gal). As placas foram protegidas da luz com papel laminado e incubadas a 37 °C, em incubadora umidificada e sem CO_2, por 12 horas. Após a incubação, a solução corante foi removida, e os poços foram lavados duas vezes com PBS por 30 segundos e uma vez com metanol (Sigma-Aldrich), conforme descrito previamente (Yaghoobi et al., 2020a). As células coradas foram quantificadas a partir de cinco campos microscópicos selecionados aleatoriamente por poço, obtidos em microscópio de contraste de fase (Axio Observer D1, Zeiss, Oberkochen, Alemanha), em objetiva de 5×. A análise das imagens e a

contagem celular foram realizadas utilizando o *software ImageJ* (National Institutes of Health, Bethesda, MD, EUA).

Ensaio de atividade mitocondrial (MTT)

A atividade mitocondrial foi avaliada pelo ensaio de MTT. Os grupos NS e S foram semeados em placas de 96 poços (1×10^4 células/poço; Costar Corp., Cambridge, MA, EUA) e submetidos aos estímulos imunoinflamatórios previamente descritos. Após o período de incubação, o meio foi removido, e 100 μL de DMEM contendo 10 μL da solução de MTT (5 $mg.mL^{-1}$; Sigma-Aldrich) foram adicionados a cada poço. As placas foram protegidas da luz e incubadas por 4 horas a 37 °C, em atmosfera contendo 5% de CO_2 e 95% de umidade. Em seguida, o meio foi aspirado, e 100 μL de dimetilsulfóxido (DMSO; Sigma-Aldrich) foram adicionados a cada poço para solubilização dos cristais de formazan, com agitação suave por 30 minutos. A absorbância foi medida a 570 nm em leitor de microplacas (SpectraMax Plus 384, Molecular Devices, San Jose, CA, EUA) (Van De Loosdrecht et al., 1994). O controle negativo consistiu em células tratadas com tampão de lise (10 mM Tris, pH 7,4; 1 mM EDTA; 0,1% Triton X-100).

Microscopia eletrônica de varredura (MEV)

Para avaliação das alterações morfológicas, lamínulas circulares de vidro (13 mm × 13 mm; Fisher Scientific, Suwanee, GA, EUA) foram posicionadas em placas de 12 poços, e 5×10^4 células foram semeadas por poço. Após a exposição aos estímulos inflamatórios, as células foram fixadas em solução de Karnovsky 0,1 M (2% glutaraldeído e paraformaldeído) por uma noite, seguida de lavagem com tampão cacodilato de sódio 0,1 M. Posteriormente, as amostras foram pós-fixadas com tetróxido de ósmio a 1% por 30 minutos e lavadas duas vezes com água destilada. A desidratação foi realizada em série crescente de acetona (50%, 70%, 90% e 100%). As amostras foram secas em equipamento de ponto crítico (CPD 030, Balzers, Liechtenstein) e metalizadas com ouro em equipamento EM SCD 500 (Leica, Áustria). As lamínulas foram então analisadas em microscópio eletrônico de varredura (JSM-7001F, JEOL Ltd., Tóquio, Japão), com aquisição de imagens nos aumentos de 1500×, 3000× e 5000× (dos Santos et al., 2019). A quantificação do tamanho celular e do número de extensões celulares foi realizada no

software ImageJ (NIH, Bethesda, Maryland, EUA), analisando-se aproximadamente 15 células por lamínula.

Ensaio de migração celular

A migração celular foi avaliada por meio do ensaio de *scratch*, que simula uma lesão artificial em uma monocamada celular confluente. As SCAPs foram semeadas em placas de seis poços (2×10^5 células/poço; Prolab, São Paulo, Brasil) e, após atingirem confluência, uma linha foi realizada utilizando a ponta estéril de uma ponteira de 1000 μL para simular uma ferida. As células desprendidas foram removidas através de lavagens com PBS por 3 vezes, e o meio de cultura sem soro foi adicionado para evitar interferência da proliferação celular durante o processo migratório. As imagens foram capturadas imediatamente após a realização do *scratch* (0 h) e após 24 h e 48 h, utilizando microscópio de contraste de fase (Axio Observer D1, Zeiss, Oberkochen, Alemanha), em objetiva de 5× (Martinotti & Ranzato, 2020). O fechamento da área da lesão foi analisado no *software ImageJ* (NIH, Bethesda, Maryland, EUA), sendo a migração quantificada pela contagem de células presentes na área do *scratch*.

Ensaio de proliferação celular

A proliferação celular foi avaliada por meio da coloração com Azul de *Tripan*. As células (5×10^4 células/poço) foram semeadas em placas de 24 poços e expostas às condições experimentais em meio livre de soro, a fim de evitar interferências de fatores de crescimento presentes no soro fetal bovino. Após 24 h e 48 h, as células foram desaderidas com tripsina (Tripsina-EDTA; Gibco, Invitrogen, Grand Island, NY, EUA) a 0,25%, ressuspendidas e misturadas à solução de Azul de *Tripan* a 0,4% (Sigma Aldrich) (1:1, v/v) (Crowley et al., 2016). As células viáveis foram contadas em câmara de *Neubauer* sob microscopia óptica, excluindo-se as células coradas, consideradas não viáveis.

Análise da expressão gênica de marcadores inflamatórios, anti-inflamatórios e de senescência

A expressão gênica foi quantificada por PCR em tempo real utilizando o sistema *StepOnePlus™ Real-Time PCR* (Applied Biosystems, Thermo Fisher Scientific). Foram

avaliados os níveis de expressão dos genes associados a processos inflamatórios, anti-inflamatórios e à senescência celular, incluindo *TNF-α, IL-6, IL-10, TGF-β1* e *p53*, utilizando o gene *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* como controle endógeno. As reações de amplificação foram realizadas com o SYBR™ Green PCR Master Mix (Thermo Fisher Scientific), e as sequências específicas dos primers para cada gene estão descritas na Tabela 1. A aquisição da fluorescência ocorreu ao final de cada ciclo de amplificação. A expressão gênica relativa foi calculada pelo método $2^{-}\Delta\Delta CT$, utilizando o grupo controle como calibrador (Livak & Schmittgen, 2001).

GENE	*FOWARD*	*REVERSE*
GAPDH	TCAACGACCACTTTGTCAAGCTCAGCT	GGTGGTCCAGGGGTCTTAC
TGF-β1	GCTGTATTTAAGGACACCGTGC	TGACACAGAGATCCGCAGTC
TNF-α	CACAGTGAAGTGCTGGCAAC	GATCAAAGCTGTAGGCCCCA
IL-10	GGTGGTCCAGGGGTCTTAC	ACTCTGCTGAAGGCATCTCG
IL-6	TCAATATTAGAGTCTCAACCCCCA	TTCTCTTTCGTTCCCGGTGG
p53	AGAAAACCTACCAGGGCAGC	ACATCTTGTTGAGGGCAGGG

Tabela 1. Sequências dos primers de cada gene utilizados no ensaio de qPCR.

Análise Estatística

O teste *t* de Student (para dados paramétricos) ou o teste de Mann–Whitney (para dados não paramétricos) foram utilizados. Comparações envolvendo múltiplos grupos foram analisadas por ANOVA de uma ou duas vias, seguida dos testes *post hoc* apropriados, conforme indicado nas legendas das figuras. Os dados são apresentados como média ± desvio-padrão (DP). Valores de $p < 0,05$ foram considerados estatisticamente significativos. Todas as análises foram realizadas utilizando o *software GraphPad Prism* versão 8 (GraphPad Software, CA, EUA).

CAPÍTULO 1 (Intended submission: Wound Repair and Regeneration; IF: 3.4)

The impact of mesenchymal stem/stromal cell senescence on wound repair and regeneration: A Systematic Review

ABSTRACT

Mesenchymal stem/stromal cells (MSCs) are key mediators of wound repair and tissue regeneration due to their proliferative, differentiation, migratory, and immunomodulatory capacities. However, cellular senescence induced by donor aging, *in vitro* expansion, or inflammatory stress may impair these functions and limit therapeutic efficacy. Thus, this systematic review aimed to evaluate the impact of cellular senescence on functional properties of human MSCs relevant to wound repair and regeneration. This systematic review will include experimental *in vitro* and *in vivo* studies that assessed senescence in primary human MSCs induced by chronological aging, serial passaging, or inflammatory stimuli. Following PRISMA 2020 guidelines and registered in PROSPERO, searches were conducted in MEDLINE (via PubMed), Embase, Web of Science, Cochrane Library, and grey literature without language restrictions (updated December 2025). Risk of bias was assessed using PETRICCS-based criteria for *in vitro* studies and SYRCLE's tool for *in vivo* studies. Forty-five studies met the inclusion criteria, with thirty-five using *in vitro* designs and ten combining *in vitro* and *in vivo* methodologies. Across MSC sources, senescence was consistently associated with reduced proliferative capacity, while effects on osteogenic, adipogenic, and chondrogenic differentiation were heterogeneous. Migratory capacity and *in vivo* regenerative outcomes were generally impaired in senescent MSCs, and senescence was frequently associated with a pro-inflammatory immunomodulatory profile. Overall, cellular senescence adversely affects key MSC functions critical for wound repair and regeneration, highlighting the need for standardized senescence screening and quality control in regenerative therapies.

INTRODUCTION

Mesenchymal stem/stromal cells (MSCs) have emerged as a promising cell source for tissue engineering and cell-based therapies due to their capacity for self-renewal, multilineage differentiation, and immunomodulatory effects(Pittenger et al., 1999). These cells can be

isolated from a wide range of human tissues, including bone marrow (BM-MSCs), adipose tissue (AD-MSCs), dental tissues, such as dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs), as well as umbilical cord cells (UC-MSCs), including Wharton's jelly–derived MSCs (UC-MSCs-WJ) and umbilical cord blood–derived MSCs (UC-MSCs-CB)(Heo et al., 2016; Kern et al., 2006). The therapeutic potential of MSCs is closely linked to their functional properties, including their capacity to migrate to sites of injury, modulate immune responses, and promote tissue repair predominantly through paracrine signaling, in addition to limited proliferative and differentiation potential(Zuk et al., 2001).

Despite their considerable therapeutic potential, MSCs are susceptible to cellular senescence, a complex process characterized by irreversible cell cycle arrest, accompanied by morphological and metabolic changes, such as the acquisition of a senescence-associated secretory phenotype (SASP)(Kirkland & Tchkonia, 2017). Key functional impairments include reduced proliferative capacity, decreased migratory ability, and compromised osteogenic, adipogenic, and chondrogenic differentiation — standard indicators of MSC multipotency(Wenjing et al., 2024a). In addition, senescence is associated with the development of SASP, characterized by the sustained release of pro-inflammatory mediators, which impair tissue regeneration and perpetuate a chronic inflammatory microenvironment, further exacerbating MSC functional decline(Coppé et al., 2008; Su et al., 2020). In experimental MSC research, senescence has been investigated in the context of chronological aging, as well as through *in vitro* models of biological aging, including replicative senescence induced by repeated cell passaging and stress-induced senescence triggered by inflammatory stimuli(Choudhery et al., 2014; X. Feng, Feng, et al., 2014; Gruber et al., 2012). However, the extent to which these distinct contexts of senescence differentially affect MSC functional outcomes remains insufficiently synthesized. Understanding these changes is important for the clinical translation of MSC-based therapies, as the administration of senescent cells may not only reduce therapeutic efficacy but also pose safety concerns due to their pro-inflammatory secretome.

Although numerous studies have examined senescence-associated changes in MSCs, findings remain fragmented and difficult to compare across experimental models and outcome measures. This inconsistency stems from the wide variety of markers and methods that are used to confirm the senescent state, including senescence-associated β-galactosidase activity, the upregulation of cell cycle-related proteins (p16, p21, and p53), telomere length or activity(Horibe et al., 2014a; Zhang et al., 2024). Beyond differences in senescence models

and markers, the tissue source of MSCs represents an additional layer of biological variability that may influence senescence-associated functional outcomes(Ma et al., 2019a).

Given the heterogeneity of experimental models, senescence contexts, and MSC tissue sources, a comprehensive synthesis of the available evidence is warranted. Rather than focusing on a single tissue source or senescence model, this systematic review integrates findings from multiple MSC origins and aging contexts to provide an overarching understanding of how cellular senescence affects their functional properties. Therefore, this review aims to evaluate the effects of cellular senescence on key functional characteristics of mesenchymal stem cells derived from adipose tissue, bone marrow, dental pulp, periodontal ligament, Wharton's jelly, and umbilical cord blood, considering chronological donor aging, serial in vitro passaging, and inflammatory stimulation as relevant senescence contexts.

METHODS

Eligibility Criteria

This review was conducted in accordance with PRISMA 2020(Page et al., 2021) guidelines and the protocol was registered in PROSPERO (CRD42025638850). This systematic review addressed the question: "How does cellular senescence in stem cells from various tissues affect their regenerative potential and tissue repair mechanisms?" The eligibility criteria were defined based on the PECO framework (Population, Exposure, Comparison, and Outcome). The Population (P) included human MSCs derived from adipose tissue, bone marrow, dental pulp, periodontal ligament, umbilical cord blood, or Wharton's jelly. The Exposure (E) was defined as cellular senescence induced by donor aging, serial passaging, or exposure to inflammatory stimuli. These contexts were compared against Comparators (C) consisting of non-senescent counterparts (derived from young donors, early-passage cultures, or unstimulated controls) used as comparators. These three senescence induction contexts were selected based on their distinct biological and translational relevance to MSC-based therapies: chronological aging reflects donor-related variability; serial passaging express *in vitro* expansion during manufacturing; and inflammatory stimuli mimic the chronic wound microenvironment where MSCs are therapeutically applied. Studies were required to assess at least one Outcome (O) related to regenerative capacity of the investigated MSCs, including proliferation, differentiation, migration, immune modulation, or *in vivo* repair outcomes. and

to confirm the cellular senescence state using at least one established marker (e.g., SA-β-gal activity, *CDKN2A* (p16)*/CDKN1A* (p21)*/TP53* (p53) expression, or telomere-related measures). Studies were only included if investigating primary, non-genetically modified stem or progenitor cells. No restrictions on language or publication year were applied. Reviews, conference materials, case reports, studies using immortalized or genetically modified cells, studies without a non-senescent comparator, or without confirmation of senescence were excluded.

Information Sources and Search Strategy

Searches were performed in MEDLINE/PubMed (via the National Library of Medicine), EMBASE (via Elsevier), Web of Science Core Collection (via Clarivate), and the Cochrane Database (via the Cochrane Library). Grey literature was searched using Google Scholar and ProQuest, and reference lists of included studies were manually screened. The initial search was conducted on 28 February 2025 and updated on 12 December 2025. Full search strategies for all databases are provided in Supplementary Table 1.

Selection Process

Duplicate records were removed using EndNote and Rayyan®. Titles and abstracts were independently screened by two reviewers, followed by full-text assessment for eligibility. Disagreements were resolved by a third reviewer. The study selection process is summarized using a PRISMA flow diagram(Page et al., 2021).

Data Collection Process and Data Items

Data extraction was independently performed by two reviewers using a standardized form. The Deep-Seek AI-assisted tool was used to support extraction of general study characteristics and population data, with all outputs manually verified. Non-English articles were translated using automated translation software. Extracted data included MSC tissue source, senescence induction and confirmation methods, comparator group characteristics, and functional outcomes related to proliferation, differentiation, migration, immune response, and tissue repair. When multiple assays were reported for a given outcome, a predefined hierarchy was applied to select one representative measure for synthesis. Quantitative data not reported in the text were extracted from figures using PlotDigitizer (Version 3.1.6, 2025).

Effect Measures and Synthesis Methods

Due to substantial heterogeneity in senescence models, MSC sources, and outcome assessments, a quantitative meta-analysis was not performed. Results were synthesized narratively and summarized in tables organized by outcome category, senescence induction method, and MSC tissue source. Statistical significance was reported as described in the original studies; when comparative statistics were unavailable, outcomes were recorded as not reported (NR). Data handling and tabulation were performed using Jamovi Statistical Software (Version 2.6).

Study Risk of Bias Assessment

Risk of bias was independently assessed by two reviewers using a critical appraisal tool based on the PETRICCs guideline for *in vitro* studies and SYRCLE's tool for *in vivo* studies(Hooijmans et al., 2014; Monteiro et al., 2025). Discrepancies were resolved by consensus with a third reviewer. Studies were classified as high, moderate, or low risk of bias based on predefined thresholds.

RESULTS

Study Selection

The study selection process is summarized in the PRISMA 2020 flow diagram (Figure 1). Database searches identified 4,857 records, of which 1,127 duplicates were removed. After title and abstract screening, 113 full-text reports were assessed for eligibility. Of these, 76 were excluded based on predefined criteria (Figure 1; Supplementary Table S2). An additional eight records were identified through citation searching and included. In total, 45 studies met the inclusion criteria and were included in the systematic review, with 35 *in vitro* studies and 10 combining *in vitro* and *in vivo* methodologies.

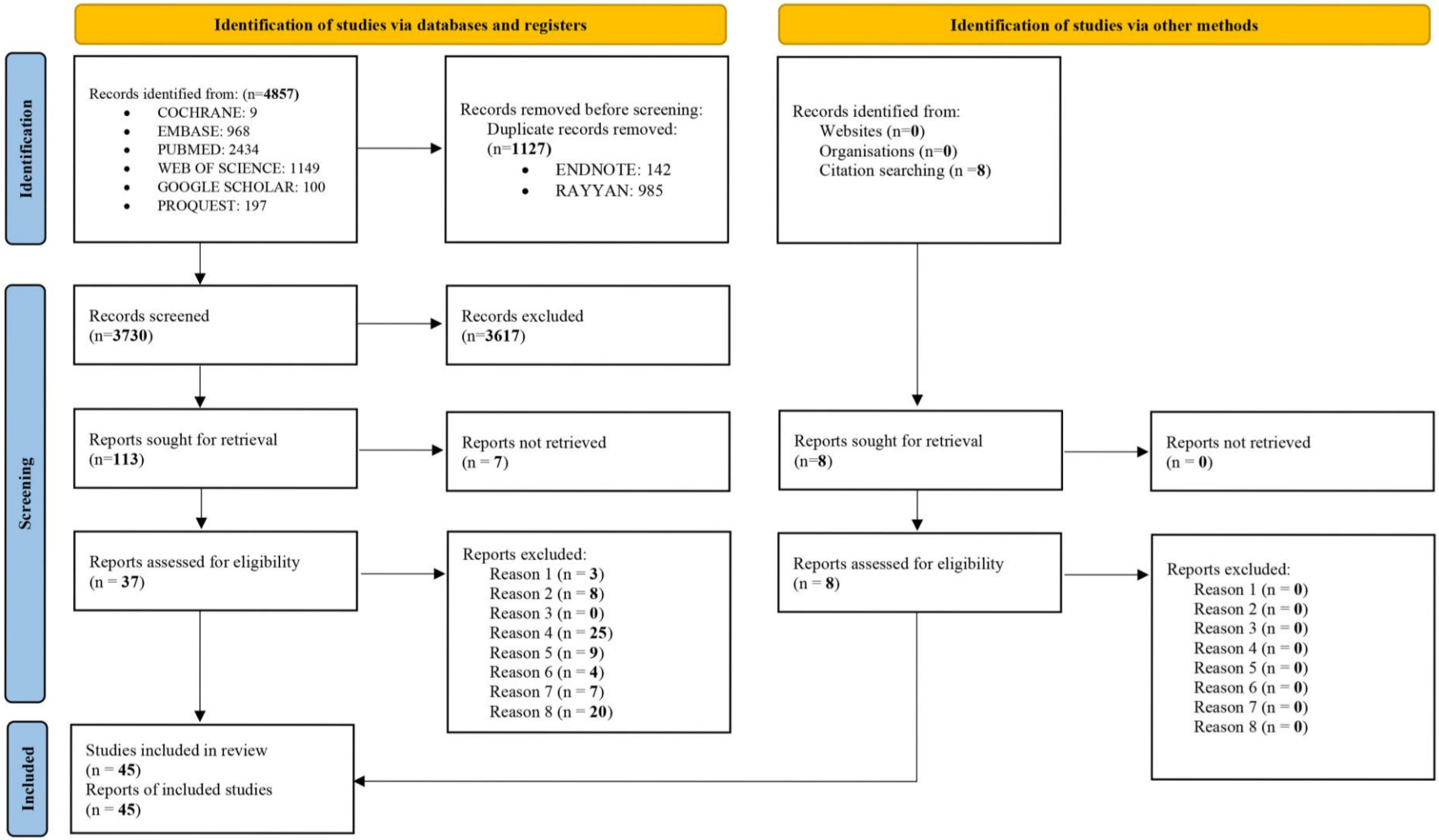


Figure 1 - Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Flowchart.

Study Characteristics

A summary of study characteristics is presented in Figure 2 and Supplementary Table 3. Included studies were published between 2006 and 2025, with a higher concentration of publications observed from 2014 onwards. Most studies originated from China (n = 17; 37.8%), followed by South Korea (n = 5; 11.1%) and the United States (n = 4; 8.9%). AD-MSCs (28%), BM-MSCs (22%), and DPSCs (22%) were the most frequently studied cell types, while UC-MSCs and PDLSCs were less commonly evaluated (Figure 2A). Serial passaging was the most common method used to induce senescence (n = 24, 53%), followed by chronological donor aging (n = 11, 24%); 16% (n = 7) of studies assessed both approaches. Inflammatory stimuli–induced senescence was investigated only in 3 studies (4%) (Figure 2B). Senescence was most frequently confirmed using SA-β-gal staining (91.1%), followed by *CDKN2A* (p16) (53.3%)*/CDKN1A* (p21) (48.9%)*/TP53* (p53) (26.7%) expression (Figure 2C). Proliferation (86.7%) and osteogenic (73.3%) differentiation were the most frequently assessed functional outcomes, whereas migration (6.7%), immune response (8.9%), and *in vivo* tissue repair (8.9%) were evaluated in relatively few studies (Figure 2D).

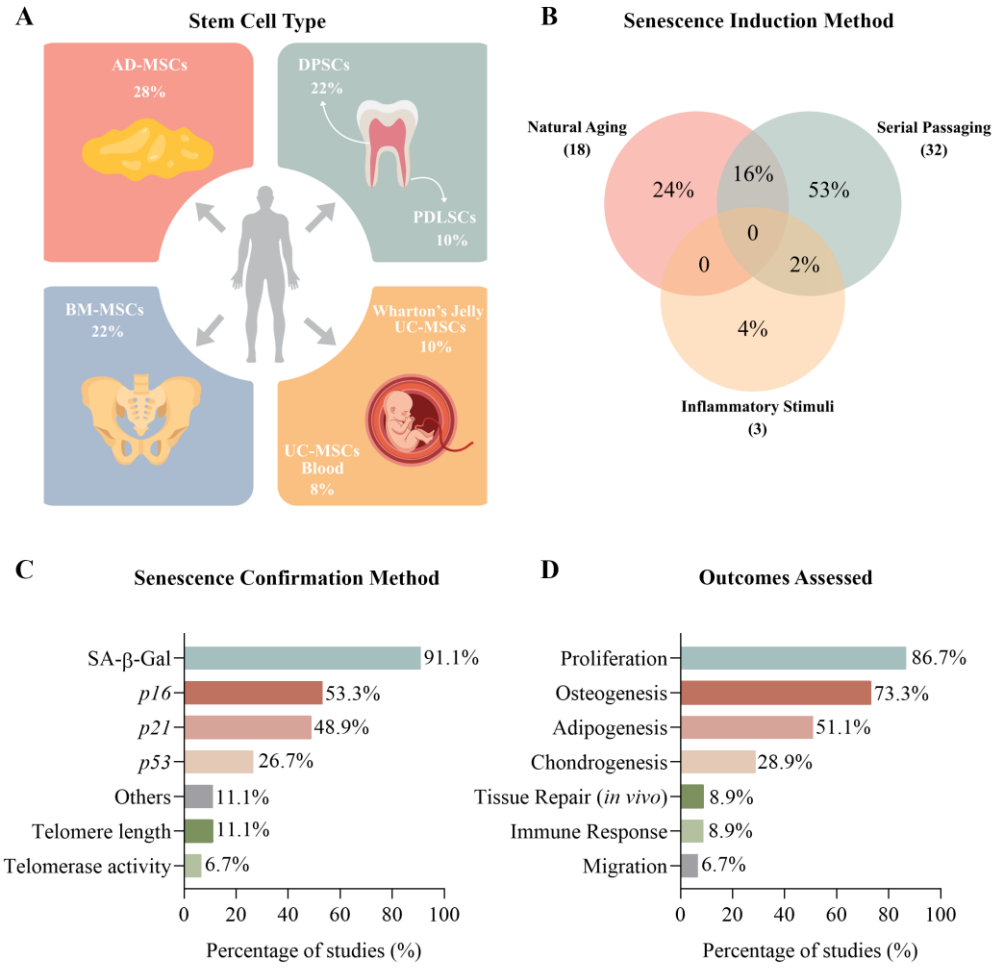


FIGURE 2 Characteristics of the included studies. (A) Distribution of mesenchymal stem cell (MSC) types analyzed across studies. (B) Venn diagram of senescence induction methods. (C) Methods used to confirm cellular senescence, highlighting the predominance of SA-β-gal staining and molecular markers. (D) Outcomes assessed in the included studies. In graphs C and D, the x-axis represents the number of included studies, with percentages indicate the proportion of studies evaluating each variable (n = 45).

Results of Individual Studies

Results were synthesized according to senescence induction method (chronological aging, serial passaging, or inflammatory stimuli). Due to limited data, immune response, *in vivo* tissue repair, and inflammatory stimulus–induced senescence were analyzed descriptively. Detailed outcome-specific data are provided in Supplementary Tables 4–10. A graphical summary of proliferation and differentiation outcomes according to chronological aging and serial passaging is presented in Figure 3.

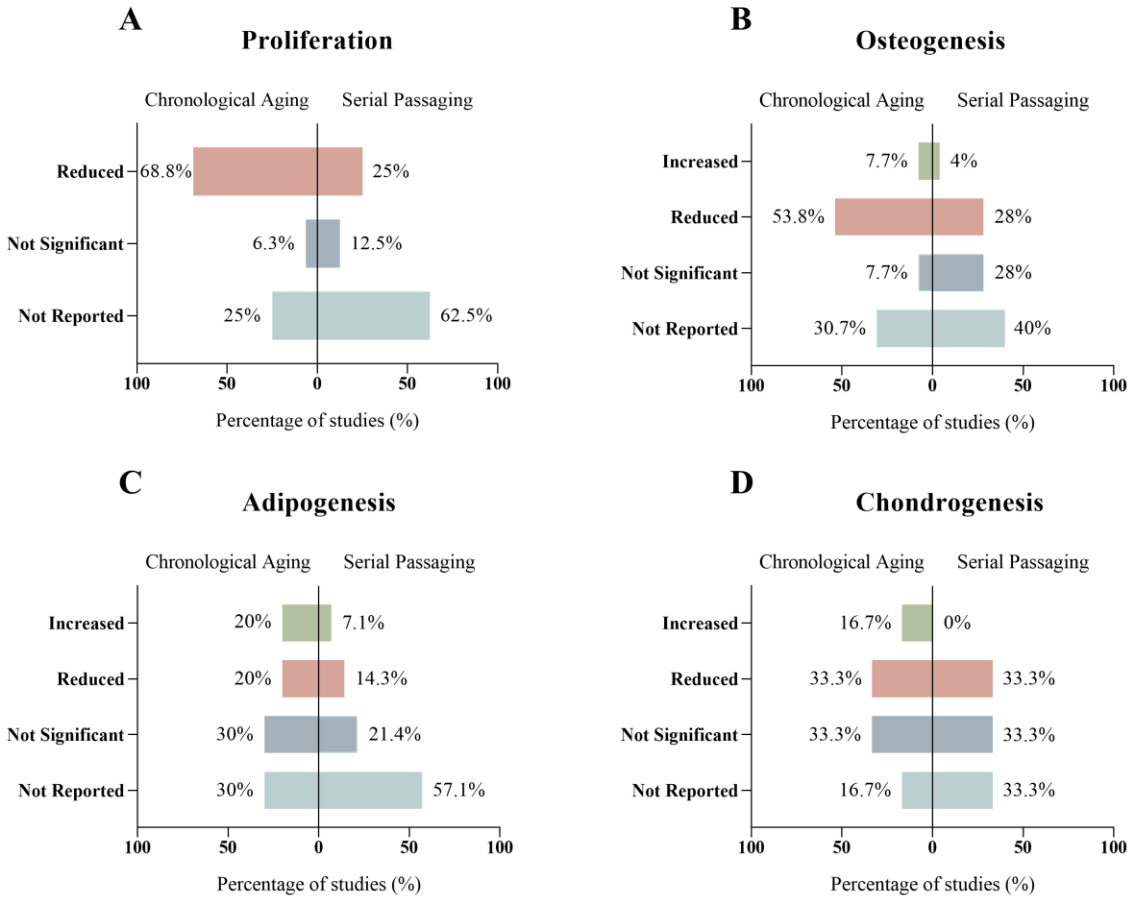


FIGURE 3 Comparative effects of chronological aging versus serial passaging on MSC functional capacities. Mirrored horizontal bar charts display the percentage of studies reporting increased, reduced, not significant, or not reported outcomes: (A) proliferation, (B) osteogenesis, (C) adipogenesis, and (D) chondrogenesis. Left-side bars represent chronological aging models; right-side bars represent serial passaging models. All values are expressed as percentages of total studies for each functional assay.

Proliferation

Inflammatory stimuli, chronological aging, and serial passaging were frequently associated with reduced proliferative capacity, although this outcome was not uniformly reported across studies (Supplementary Table 4). Repeated inflammatory stimulation led to diminished proliferation in DPSCs(G. Feng et al., 2018a; X. Feng, Feng, et al., 2014). Similarly, chronological aging was predominantly associated with reduced proliferation in AD-MSCs(Alt et al., 2012; Choudhery et al., 2014; Gruber et al., 2012; Kornicka et al., 2015; S. H. Wu et al., 2022), BM-MSCs(Huang et al., 2023), and DPSCs(Chen et al., 2024; X. Feng, Xing, et al., 2014; Iezzi et al., 2019; X. Wang et al., 2025), although several studies did not perform direct statistical comparisons(Katsube et al., 2008; Stolzing et al., 2008; Wagner et al., 2008) (n = 10, 66.7%). Serial passaging showed a variable but generally negative impact on proliferation (n =

6, 25%), with significant reductions more frequently reported in AD-MSCs(Danisovic L et al., 2017; Zhang et al., 2024) and PDLSCs(Hu et al., 2024; Jia et al., 2025), while studies on UC-MSCs rarely performed formal comparative analyses(Christodoulou et al., 2013; Jin et al., 2016; S. N. Kim et al., 2021; Zhuang et al., 2015). Studies using serial passaging to induce senescence were included only when independent measures of proliferative capacity were reported, as cumulative population doublings primarily reflect replicative history rather than proliferative function.

Osteogenic Differentiation

Osteogenic differentiation was variably affected by senescence depending on the induction method and cell source (Supplementary Table 5). In a single study evaluating BM-MSCs and DPSCs under inflammatory stimulation, osteogenic differentiation appeared reduced in senescent cells; however, the absence of statistical analysis precludes confirmation of this finding(Ma et al., 2019a). Under chronological aging conditions, 7 studies (53.8%) reported reduced osteogenic potential in AD-MSCs(Choudhery et al., 2014; Kornicka et al., 2015; W. Wu et al., 2013; Ye et al., 2016), BM-MSCs(Stolzing et al., 2008), and DPSCs(X. Feng, Xing, et al., 2014; Iezzi et al., 2019), with occasional reports of preserved (n = 1, 7.7%) or increased (n = 1, 7.7%) differentiation depending on donor characteristics(Horibe et al., 2014a; S. H. Wu et al., 2022). Serial passaging demonstrated heterogeneous effects: while 7 studies (28%) reported reduced osteogenesis in AD-MSCs(Ma et al., 2019a), BM-MSCs(H. Cheng et al., 2011a; Ma et al., 2019a), PDLSCs(Jia et al., 2025; Yang et al., 2021), and UC-MSCs(Govarthanan et al., 2025), one study (4%) reported increased osteogenic differentiation in late-passage cells(H. Cheng et al., 2011a), and others found no significant differences (n = 7, 28%), indicating heterogeneous osteogenic outcomes(Bertolo et al., 2016; Diomede et al., 2017; Gruber et al., 2012; Jin et al., 2016; Ma et al., 2019a; Wenjing et al., 2024a).

Adipogenic Differentiation

The effects of senescence on adipogenic differentiation were variable (Supplementary Table 6). Chronological aging was associated with both increased(Kornicka et al., 2015; S. H. Wu et al., 2022) (n = 2, 20%) and reduced(X. Feng, Xing, et al., 2014; Ye et al., 2016) (n = 2, 20%) adipogenic potential in AD-MSCs, whereas BM-MSCs and DPSCs generally showed non-significant(Horibe et al., 2014a; Stolzing et al., 2008) (n = 3, 30%) or no reported changes(Iezzi et al., 2019) (n = 3, 30%). Following serial passaging, qualitative or non-

comparative analyses predominated, with 8 studies (57.1%) not reporting direct statistical comparisons(Bonab et al., 2006; Jin et al., 2016; S. N. Kim et al., 2021; Legzdina et al., 2016a; Scheers et al., 2013; Truong et al., 2019; Wagner et al., 2008; J. Wang et al., 2025a). Among studies providing comparative data, one study (14.3%) reported reduced adipogenic differentiation in senescent cells, with this finding observed in two different MSC populations analyzed within the same study[39]. In contrast, another single study (7.1%) described increased adipogenic differentiation in senescent cells[42].

Chondrogenic Differentiation

Few studies evaluated chondrogenic differentiation of senescent MSCs (Supplementary Table 7). Chronological aging was generally associated with reduced chondrogenic capacity(Choudhery et al., 2014; Ye et al., 2016) (n = 2, 33.3%), with occasional reports of preserved(Iezzi et al., 2019; Stolzing et al., 2008) (n = 2, 33.3%) or enhanced(S. H. Wu et al., 2022) (n = 1, 16.7%) differentiation. Serial passaging more consistently impaired chondrogenic differentiation in AD-MSCs(Truong et al., 2019; J. Wang et al., 2025a), BM-MSCs(Bertolo et al., 2016), and UC-MSCs(Govarthanan et al., 2025), although the limited number of studies precludes firm conclusions.

Migration

Cell migration was rarely assessed (Supplementary Table 8). However, the available evidence suggests that both chronological aging and serial passaging reduce migratory capacity in DPSCs(Chen et al., 2024), BM-MSCs(Wenjing et al., 2024a), and PDLSCs(Yang et al., 2021) (n = 3, 100%).

Immune Response

Data on immunomodulatory function were scarce (Supplementary Table 9). Chronological aging was associated with a phenotypic shift towards a pro-inflammatory profile in BM-MSCs(Huang et al., 2023), although direct comparisons were limited. Serial passaging altered immune-related marker expression in UC-MSCs, generally favoring increased expression of immunosuppressive mediators, such as PGE2, although comparative statistical analyses were not consistently performed(Yu et al., 2014).

***In Vivo* Tissue Repair**

Evidence regarding *in vivo* tissue repair capacity was limited (Supplementary Table 10). Inflammation-induced, aging-related, and serial passaging–induced senescence was generally associated with impaired tissue regeneration properties across different MSC sources; however, most studies relied on qualitative or non-comparative analyses(Chen et al., 2024; Horibe et al., 2014a; Ma et al., 2019a; Yang et al., 2021) (n =4, 80%).

Additional Narrative Evidence

One study assessing replicative senescence was not included in the summary tables because cells were grouped according to high versus low proliferative potential rather than donor age, passage number, or inflammatory exposure. Results from this study were therefore synthesized only in narrative form. High-proliferative clones sustained more than 80 population doublings. Osteogenic differentiation, assessed by Alizarin Red staining, was positive at 30 PDs but markedly reduced at 75 PDs, whereas adipogenic differentiation, evaluated by Oil Red O staining, was positive at 30 PDs and increased at 75 PDs. All differentiation outcomes were evaluated qualitatively, without statistical analysis(Alraies et al., 2017).

Risk of Bias in Studies

Most *in vitro* studies demonstrated low overall risk of bias according to the PETRICCS-based assessment, with high reporting adequacy and no studies classified as high risk (Supplementary Figure 1). Common limitations included insufficient reporting of blinding, randomization, and replicate numbers. *In vivo* studies assessed using SYRCLE's risk of bias tool demonstrated low to moderate methodological quality. Frequent deficiencies were observed in domains related to blinding of caregivers/investigators and outcome assessors, random housing procedures, and selective outcome reporting, which may compromise internal validity and limit translational relevance (Supplementary Figure 2).

DISCUSSION

Cellular senescence is a fundamental biological process induced by chronological and biological aging, as well as diverse cellular stressors. Beyond its physiological role in development and tissue homeostasis, senescence has emerged as a key driver of organismal aging and age-related diseases, a promising target for therapeutic strategies aimed at modulating aging and aging-associated conditions, and a potential determinant of cell therapy product quality(Kirkland & Tchkonia, 2017). Accumulating evidence indicates that cellular senescence is associated with a set of well-defined molecular and functional events; however, investigations across different cell types suggest the existence of cell-type–specific features and responses.

The synthesis of the current literature reveals that the most consistent hallmark of MSC senescence is a marked reduction in proliferative capacity. This decline was observed across all tissue sources and was independent of the induction model employed (chronological aging, serial passaging, or inflammatory stress). This universal loss of proliferation capacity confirms that the core machinery of senescence—primarily the activation of p16 and p21 pathways—is robustly conserved in MSCs(Gruber et al., 2012; Ma et al., 2019a). In contrast, the impact of senescence on other functional properties, such as differentiation and immunomodulation, appears far more heterogeneous.

Osteogenic differentiation shows heterogeneous responses, largely dependent on cellular origin and experimental context. While many studies report impaired osteogenesis,

others describe preserved or even enhanced osteodifferentiation, particularly in DPSCs and UC-MSCs(Bertolo et al., 2016; Diomede et al., 2017; Ma et al., 2019a). Similarly, adipogenic and chondrogenic differentiation outcomes range from reduced to increased potential, with no consistent pattern across senescence models or cell types, with findings ranging from functional impairment to unexpected gain-of-function(Kornicka et al., 2015; Ye et al., 2016). This lack of consistency suggests that the multilineage differentiation program may be more resilient to senescence-associated changes than the cell cycle machinery. The predominance of non-significant findings suggests that differentiation capacity may be less sensitive to senescence than proliferation(Diomede et al., 2017; Horibe et al., 2014a; Stolzing et al., 2008; Wenjing et al., 2024a). Furthermore, age-group definitions significantly influence outcomes; for instance, studies labeling 'mature adult' donors as senescent rather than focusing on truly elderly populations may mask the full extent of age-related functional decline(S. H. Wu et al., 2022).

Although fewer studies evaluated migration, available evidence consistently indicates impaired migratory capacity in senescent MSCs derived from DPSCs, BM-MSCs, and PDLSCs, using both chronological aging and serial passaging models(Chen et al., 2024; Wenjing et al., 2024a; Yang et al., 2021). This convergence suggests that migration is a senescence-sensitive function, with potential implications for tissue homing and regenerative efficacy in cell-based therapies and wound repair(Akram et al., 2013). In parallel, senescence is associated with marked alterations in the immunomodulatory profile of MSCs, characterized by increased expression or secretion of pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-8, alongside variable regulation of anti-inflammatory mediators including IL-10, IDO-1, and TGF-β1(Horibe et al., 2014a; Huang et al., 2023; Yu et al., 2014; Zhuang et al., 2015). These findings support the concept that senescence shifts the MSC secretome towards SASP, modifying immune regulatory functions rather than uniformly suppressing them(Coppé et al., 2008; Rodier et al., 2009).

Evidence from *in vivo* studies, although limited, consistently indicates reduced regenerative capacity of senescent MSCs. Across different senescence models, MSCs derived from bone marrow, dental pulp, and periodontal ligament demonstrated impaired mineralization, reduced bone formation, and compromised neo-angiogenesis in regenerative settings(Chen et al., 2024; Horibe et al., 2014a; Ma et al., 2019a; Yang et al., 2021). In particular, aged/senescent DPSCs exhibited significantly decreased pulp regeneration and vascularized tissue formation compared with young/non-senescent cells, highlighting the functional consequences of senescence in complex tissue environments(Horibe et al., 2014a).

This compromised reparative potential likely stems not only from the loss of proliferative and differentiation capacity but also from the deleterious effects of the SASP(Gnani et al., 2019; Lopes-Paciencia et al., 2019). In a physiological context, senescent MSCs can actively impair the local microenvironment through a 'bystander effect,' where the secretion of pro-inflammatory cytokines and reactive oxygen species propagates senescence to neighboring healthy cells and inhibits the endogenous regenerative response(Acosta et al., 2013). Despite frequent lack of statistical significance, the consistent direction of these findings suggests compromised reparative potential of senescent MSCs *in vivo*.

Despite these insights, several elements explain the inconsistencies found in the literature and represent limitations of the current evidence. Donor-related factors such as chronological and biological age, genetic background, and underlying inflammatory status contribute to inter-study variability. Methodological differences, including the senescence induction model employed, the level of senescence-induction achieved (i.e. the lack of unified senescence level thresholds), culture conditions, and timing of analysis, might result in divergent molecular and functional outcomes. Furthermore, the lack of standardized criteria for senescence definition—reflected in variability in marker selection and functional readouts—complicates direct comparisons between studies. Such substantial heterogeneity in senescence induction methods, donor age definitions, passages evaluated, and functional assays preclude quantitative synthesis. Small sample sizes and also limited direct statistical comparisons further constrain interpretation. Two studies from the same research group reported highly similar proliferation data(G. Feng et al., 2018a; X. Feng, Feng, et al., 2014), which may limit independence for this outcome. Furthermore, our search strategy may have introduced a selection bias. Although the term "mesenchymal stromal cell" is currently recommended by the International Society for Cell & Gene Therapy (ISCT) (Viswanathan et al., 2019), the search strategy relied on the historically predominant term "mesenchymal stem cell". Consequently, studies that exclusively used the term "stromal" without referencing "stem" may have been missed, which represents a limitation of this review.

Although some of these factors are inherent to primary cell culture research, collectively, they likely underlie the diverse and sometimes conflicting results reported in the literature regarding the impact of senescence on MSC biology and therapeutic potential. Furthermore, given that most included studies were based on *in vitro* experiments, with few investigations involving *in vivo* models and no clinical trials being included, a moderate to high risk of bias in several *in vivo* studies suggests that the translational interpretation of the findings

is indeed limited at this point. Due to these limitations, results were synthesized narratively, and some data extracted from figures may introduce minor imprecision. Overall, the consistent functional impairment observed in senescent MSCs contributes to the understanding of how these cells act throughout aging and underscores donor age and passage number as critical quality parameters in cell manufacturing(Danisovic L et al., 2017; Truong et al., 2019).

Senescence-associated changes in inflammatory and immunomodulatory profiles further emphasize the need for careful control of inflammatory stimuli during cell expansion, especially for therapeutic application purposes(G. Feng et al., 2018a; X. Feng, Feng, et al., 2014; Ma et al., 2019a). These findings support the implementation of standardized senescence screening, inflammatory profiling, functional quality control criteria, and upper passage limits for clinical-grade MSCs to ensure therapeutic efficacy and reproducibility(Chen et al., 2024; Yang et al., 2021). Future studies should prioritize standardized senescence models, direct comparisons across MSC tissue sources, and greater use of *in vivo* and translational models with clinically relevant endpoints, as well as strategies to prevent or mitigate senescence-related functional decline.

CONCLUSION

This systematic review based on pre-clinical studies demonstrates that cellular senescence consistently affects key MSC properties essential for wound repair and tissue regeneration. Proliferative and migratory capacities are most consistently impaired, while differentiation and immunomodulatory functions vary according to cell source and senescence model. Available *in vivo* evidence indicates reduced regenerative efficacy of senescent MSCs, contributing to a better understanding of how aged MSCs might contribute to organismal aging. Despite limitations related to methodological heterogeneity and the predominance of *in vitro* studies, these findings identify donor age, culture expansion, and inflammatory exposure as critical determinants of MSC functional quality. Standardized senescence screening and thresholds, functional quality control measures, and defined passage limits are therefore essential to improving the safety, efficacy, and reproducibility of MSC-based regenerative therapies.

REFERENCES

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage Potential of Adult Human Mesenchymal Stem Cells. Science (1979) [Internet]. 1999 Apr 2;284(5411):143–7.

2. Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. Int J Mol Med. 2016 Jan 1;37(1):115–25.

3. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue. Stem Cells. 2006 May;24(5):1294–301.

4. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. Vol. 7, TISSUE ENGINEERING. Mary Ann Liebert, Inc; 2001.

5. Kirkland JL, Tchkonia T. Cellular Senescence: A Translational Perspective. Vol. 21, EBioMedicine. Elsevier B.V.; 2017. p. 21–8.

6. Wenjing Z, Baikun L, Qiulian L, Xi C. Effects of long-term subculture on biological characteristics of bone marrow mesenchymal stem cells. Chinese Journal of Tissue Engineering Research. 2024 Nov 8;28(31):4926–30.

7. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, et al. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008;6(12).

8. Su X, Yang H, Shi R, Zhang C, Liu H, Fan Z, et al. Depletion of SNRNP200 inhibits the osteo−/dentinogenic differentiation and cell proliferation potential of stem cells from the apical papilla. BMC Dev Biol. 2020 Dec 1;20(1).

9. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med [Internet]. 2014 Jan 7;12:8.

10. Gruber HE, Somayaji S, Riley F, Hoelscher GL, Norton HJ, Ingram J, et al. Human adipose-derived mesenchymal stem cells: Serial passaging, doubling time and cell senescence. Biotechnic and Histochemistry. 2012;87(4):303–11.

11. Feng X, Feng G, Xing J, Shen B, Tan W, Huang D, et al. Repeated lipopolysaccharide stimulation promotes cellular senescence in human dental pulp stem cells (DPSCs). Cell Tissue Res. 2014;356(2):369–80.

12. Horibe H, Murakami M, Iohara K, Hayashi Y, Takeuchi N, Takei Y, et al. Isolation of a stable subpopulation of Mobilized Dental Pulp Stem Cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. PLoS One. 2014 May 28;9(5).

13. Zhang Y, Zhang J, Lesani P, Lu Z, Zreiqat H. Osteopontin Rejuvenates Senescent Adipose-Derived Stem Cells and Restores their Bone Tissue Regenerative Function. Stem Cell Rev Rep. 2024 May 1;20(4):1106–20.

14. Ma L, Hu J, Cao Y, Xie Y, Wang H, Fan Z, et al. Maintained properties of aged dental pulp stem cells for superior periodontal tissue regeneration. Aging Dis. 2019;10(4):793–806.

15. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Vol. 372, BMJ. BMJ Publishing Group; 2021.

16. Monteiro MM, dos Santos JA, Paiva Barbosa V, De Luca Canto G, Squarize CH, Coletta RD, et al. PETRICCS Guideline Protocol: A Call to Improve Reporting Standards in Cell Culture Research. Journal of Oral Pathology and Medicine. 2025 Feb 1;54(2):112–9.

17. Hooijmans CR, Rovers MM, De Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014 Mar 26;14(1).

18. Feng G, Zheng K, Cao T, Zhang J, Lian M, Huang D, et al. Repeated stimulation by LPS promotes the senescence of DPSCs via TLR4/MyD88-NF-κB-p53/p21 signaling. Cytotechnology. 2018 Jun 1;70(3):1023–35.

19. Alt EU, Senst C, Murthy SN, Slakey DP, Dupin CL, Chaffin AE, et al. Aging alters tissue resident mesenchymal stem cell properties. Stem Cell Res. 2012 Mar;8(2):215–25.

20. Kornicka K, Marycz K, Tomaszewski KA, Marędziak M, Smieszek A. The Effect of Age on Osteogenic and Adipogenic Differentiation Potential of Human Adipose Derived

Stromal Stem Cells (hASCs) and the Impact of Stress Factors in the Course of the Differentiation Process. Oxid Med Cell Longev. 2015;2015.

21. Wu SH, Yu JH, Liao YT, Liu KH, Chiang ER, Chang MC, et al. Comparison of the Infant and Adult Adipose-Derived Mesenchymal Stem Cells in Proliferation, Senescence, Anti-oxidative Ability and Differentiation Potential. Tissue Eng Regen Med. 2022 Jun 1;19(3):589–601.

22. Huang X, Liu B, Liang Y, Mai C, Shen Y, Huang X, et al. TRAF3IP2 drives mesenchymal stem cell senescence via regulation of NAMPT-mediated NAD biosynthesis. Heliyon. 2023 Sep 1;9(9).

23. Chen D, Tang Q, Song W, He Y. Platelet-derived exosomes alleviate tendon stem/progenitor cell senescence and ferroptosis by regulating AMPK/Nrf2/GPX4 signaling and improve tendon-bone junction regeneration in rats. J Orthop Surg Res. 2024 Dec 1;19(1).

24. Feng X, Xing J, Feng G, Huang D, Lu X, Liu S, et al. P16INK4A mediates age-related changes in mesenchymal stem cells derived from human dental pulp through the DNA damage and stress response. Mech Ageing Dev. 2014 Nov 1;141–142:46–55.

25. Iezzi I, Cerqueni G, Licini C, Lucarini G, Mattioli Belmonte M. Dental pulp stem cells senescence and regenerative potential relationship. J Cell Physiol. 2019 May 1;234(5):7186–97.

26. Wang X, Wang L, Zhou L, Chen L, Shi J, Ge J, et al. NUP62 alleviates senescence and promotes the stemness of human dental pulp stem cells via NSD2-dependent epigenetic reprogramming. International Journal of Oral Science . 2025 Dec 1;17(1).

27. Katsube Y, Hirose M, Nakamura C, Ohgushi H. Correlation between proliferative activity and cellular thickness of human mesenchymal stem cells. Biochem Biophys Res Commun. 2008 Apr 4;368(2):256–60.

28. Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies. Mech Ageing Dev. 2008 Mar;129(3):163–73.

29. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, et al. Replicative senescence of mesenchymal stem cells: A continuous and organized process. PLoS One. 2008 May 21;3(5).

30. Danisovic L, Oravcova L, Krajciova L, Varchulova Novakova Z, Bohac M, Varga I, et al. Effect of long-term culture on the biological and morphological characteristics of human adipose tissue-derived stem cells. Journal of Physiology and Pharmacology [Internet]. 2017 Feb [cited 2025 Dec 17];68(1):149–58.

31. Hu M, Liu R, Chen X, Yan S, Gao J, Zhang Y, et al. Metabolomics Dysfunction in Replicative Senescence of Periodontal Ligament Stem Cells Regulated by AMPK Signaling Pathway. Stem Cells Dev. 2024 Nov 1;33(21–22):607–15.

32. Jia L, Xiao H, Hao Z, Sun S, Zhao W, Gong Z, et al. Senolytic elimination of senescent cells improved periodontal ligament stem cell-based bone regeneration partially through inhibiting YAP. Biochim Biophys Acta Mol Cell Res. 2025 Mar 1;1872(3).

33. Christodoulou I, Kolisis FN, Papaevangeliou D, Zoumpourlis V. Comparative evaluation of human mesenchymal stem cells of fetal (Wharton's Jelly) and adult (adipose tissue) origin during prolonged in vitro expansion: Considerations for cytotherapy. Stem Cells Int. 2013 Mar 3;2013:246134.

34. Jin HJ, Kwon JH, Kim M, Bae YK, Choi SJ, Oh W, et al. Downregulation of Melanoma Cell Adhesion Molecule (MCAM/CD146) Accelerates Cellular Senescence in Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells. Stem Cells Transl Med. 2016 Apr 1;5(4):427–39.

35. Kim SN, Choi B, Lee CJ, Moon JH, Kim MK, Chung E, et al. Culturing at Low Cell Density Delays Cellular Senescence of Human Bone Marrow-Derived Mesenchymal Stem Cells in Long-Term Cultures. Int J Stem Cells. 2021 Feb;14(1):103–11.

36. Zhuang Y, Li D, Fu J, Shi Q, Lu Y, Ju X. Comparison of biological properties of umbilical cord.derived mesenchymal stem cells from early and late passages: Immunomodulatory ability is enhanced in aged cells. Mol Med Rep. 2015 Jan 1;11(1):166–74.

37. Wu W, Niklason L, Steinbacher DM. The effect of age on human adipose-derived stem cells. Plast Reconstr Surg. 2013 Jan;131(1):27–37.

38. Ye X, Liao C, Liu G, Xu Y, Tan J, Song Z. Age-related changes in the regenerative potential of adipose-derived stem cells isolated from the prominent fat pads in human lower eyelids. PLoS One. 2016 Nov 1;11(11).

39. Cheng H, Qiu L, Ma J, Zhang H, Cheng M, Li W, et al. Replicative senescence of human bone marrow and umbilical cord derived mesenchymal stem cells and their differentiation to adipocytes and osteoblasts. Mol Biol Rep. 2011 Nov;38(8):5161–8.

40. Yang Y, Wang T, Zhang S, Jia S, Chen H, Duan Y, et al. Vitamin C alleviates the senescence of periodontal ligament stem cells through inhibition of Notch3 during long-term culture. J Cell Physiol. 2021 Feb 1;236(2):1237–51.

41. Govarthanan K, Meenakshi Sundaram RS, Richard AS, Chabathula SC, Rupert S, Sathyanesan J, et al. Inhibition of GSK-3β Restores Differentiation Potential of Late-Passage Mesenchymal Stem Cells. Pharmaceuticals. 2025 Apr 1;18(4).

42. Bertolo A, Mehr M, Janner-Jametti T, Graumann U, Aebli N, Baur M, et al. An in vitro expansion score for tissue-engineering applications with human bone marrow-derived mesenchymal stem cells. J Tissue Eng Regen Med. 2016 Feb 1;10(2):149–61.

43. Diomede F, Rajan TS, Gatta V, D'Aurora M, Merciaro I, Marchisio M, et al. Stemness maintenance properties in human oral stem cells after long-term passage. Stem Cells Int. 2017;2017.

44. Legzdina D, Romanauska A, Nikulshin S, Kozlovska T, Berzins U. Characterization of senescence of culture-expanded human adipose-derived mesenchymal stem cells. Int J Stem Cells. 2016;9(1):124–36.

45. Truong NC, Bui KHT, Van Pham P. Characterization of Senescence of Human Adipose-Derived Stem Cells After Long-Term Expansion. In: Advances in Experimental Medicine and Biology. Springer New York LLC; 2019. p. 109–28.

46. Wang J, Jian K, Yang Q, Gu C, Sheng J, Zhou Y, et al. Retarding human adipose-derived MSCs senescence and promoting tendon repair using cell sheet engineering with a histone methyltransferase inhibitor. Sci Rep. 2025 Dec 1;15(1).

47. Bonab MM, Alimoghaddam K, Talebian F, Ghaffari SH, Ghavamzadeh A, Nikbin B. Aging of mesenchymal stem cell in vitro. BMC Cell Biol. 2006 Mar 10;7.

48. Scheers I, Lombard C, Paganelli M, Campard D, Najimi M, Gala JL, et al. Human Umbilical Cord Matrix Stem Cells Maintain Multilineage Differentiation Abilities and Do Not Transform during Long-Term Culture. PLoS One. 2013 Aug 9;8(8).

49. Yu KR, Lee JY, Kim HS, Hong IS, Choi SW, Seo Y, et al. A p38 MAPK-mediated alteration of COX-2/PGE2 regulates immunomodulatory properties in human mesenchymal stem cell aging. PLoS One. 2014 Aug 4;9(8).

50. Alraies A, Alaidaroos NYA, Waddington RJ, Moseley R, Sloan AJ. Variation in human dental pulp stem cell ageing profiles reflect contrasting proliferative and regenerative capabilities. BMC Cell Biol. 2017 Feb 2;18(1).

51. Akram KM, Samad S, Spiteri MA, Forsyth NR. Mesenchymal stem cells promote alveolar epithelial cell wound repair in vitro through distinct migratory and paracrine mechanisms. Respir Res. 2013 Jan 25;14(1).

52. Rodier F, Coppé JP, Patil CK, Hoeijmakers WAM, Muñoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol. 2009;11(8):973–9.

53. Gnani D, Crippa S, della Volpe L, Rossella V, Conti A, Lettera E, et al. An early-senescence state in aged mesenchymal stromal cells contributes to hematopoietic stem and progenitor cell clonogenic impairment through the activation of a pro-inflammatory program. Aging Cell. 2019 Jun 1;18(3).

54. Lopes-Paciencia S, Saint-Germain E, Rowell MC, Ruiz AF, Kalegari P, Ferbeyre G. The senescence-associated secretory phenotype and its regulation. Cytokine. 2019 May 1;117:15–22.

55. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janich P, Morton JP, et al. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol. 2013 Aug;15(8):978–90.

56. Viswanathan S, Shi Y, Galipeau J, Krampera M, Leblanc K, Martin I, et al. Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT®) Mesenchymal Stromal Cell committee position statement on nomenclature. Cytotherapy. 2019 Oct 1;21(10):1019–24.

57. Figiel-Dabrowska A, Krzesniak NE, Noszczyk BH, Domanska-Janik K, Sarnowska A. Efficiency assessment of irrigation as an alternative method for improving the regenerative potential of non-healing wounds. Wound Repair and Regeneration. 2022 May 1;30(3):303–16.

58. Zhang L, Xia D, Wang C, Gao F, Hu L, Li J, et al. Pleiotrophin attenuates the senescence of dental pulp stem cells. Oral Dis. 2023 Jan 1;29(1):195–205.

59. Jeoung JY, Nam HY, Kwak J, Jin HJ, Lee HJ, Lee BW, et al. A decline in Wnt3a signaling is necessary for mesenchymal stem cells to proceed to replicative senescence. Stem Cells Dev. 2015 Apr 15;24(8):973–82.

60. Kim M, Bae YK, Um S, Kwon JH, Kim GH, Choi SJ, et al. A Small-Sized Population of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells Shows High Stemness Properties and Therapeutic Benefit. Stem Cells Int. 2020;2020.

61. Li B, Xu M, Huang J, Jia R. FTO Suppresses Dental Pulp Stem Cell Senescence by Destabilizing NOLC1 mRNA. Biomolecules. 2025 Nov 1;15(11).

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY TABLE 1 Search Strategy.

SEARCH	QUERY	ITEMS FOUND
PUBMED	("Stem Cells"[MeSH Terms] OR "Stem Cells"[All Fields] OR "Stem Cell"[All Fields] OR "Mother Cell"[All Fields] OR "Progenitor Cell"[All Fields]) AND ("Cellular Senescence"[MeSH Terms] OR "Cellular Senescence"[All Fields] OR "Cell Senescence"[All Fields] OR "Cell Aging"[All Fields] OR "Cellular Aging"[All Fields] OR "Cell Ageing"[All Fields] OR "Cellular Ageing"[All Fields]) AND ("Regenerative Medicine"[MeSH Terms] OR "Regenerative Medicine"[All Fields] OR "Tissue Engineering"[MeSH Terms] OR "Tissue Engineering"[All Fields] OR "Cell Engineering"[MeSH Terms] OR "Cell Engineering"[All Fields] OR "cell and tissue based therapy"[MeSH Terms] OR "cell and tissue based therapy"[All Fields] OR "Regeneration"[MeSH Terms] OR "Regeneration"[All Fields] OR "Wound Healing"[MeSH Terms] OR "Wound Healing"[All Fields] OR "Wound Repair"[All Fields] OR "Tissue Therapy"[All Fields])	2,434
EMBASE	('stem cells'/exp OR 'stem cells' OR 'stem cell'/exp OR 'stem cell' OR 'mother cell'/exp OR 'mother cell' OR 'progenitor cell'/exp OR 'progenitor cell') AND ('cellular senescence' OR 'cell senescence' OR 'cell aging' OR 'cellular aging' OR 'cell ageing' OR 'cellular ageing') AND ('regenerative medicine' OR 'tissue engineering' OR 'cell engineering' OR 'cell- and tissue-based therapy' OR 'regeneration' OR 'wound healing' OR 'wound repair' OR 'tissue therapy') AND ([embase]/lim NOT ([embase]/lim AND [medline]/lim) OR [preprint]/lim)	968

COCHRANE	“Stem Cells” OR “Stem Cell” OR “Mother Cell” OR “Progenitor Cell” in Title Abstract Keyword AND “Cellular Senescence” OR “Cell Senescence” OR “Cell Aging” OR “Cellular Aging” OR “Cell Ageing” OR “Cellular Ageing” in Title Abstract Keyword AND “Regenerative Medicine” OR "Tissue Engineering" OR "Cell Engineering" OR "Cell- and Tissue-Based Therapy" OR "Regeneration" OR "Wound Healing" OR “Wound Repair” OR “Tissue Therapy” in Title Abstract Keyword - (Word variations have been searched)	9
WEB OF SCIENCE	((ALL=(“Stem Cells” OR “Stem Cell” OR “Mother Cell” OR “Progenitor Cell”)) AND ALL=(“Cellular Senescence” OR “Cell Senescence” OR “Cell Aging” OR “Cellular Aging” OR “Cell Ageing” OR “Cellular Ageing”)) AND ALL=(“Regenerative Medicine” OR “Tissue Engineering” OR "Cell Engineering" OR "Cell- and Tissue-Based Therapy" OR "Regeneration" OR "Wound Healing" OR “Wound Repair” OR “Tissue Therapy”)	1,149
GOOGLE SCHOLAR	"stem cell" AND "cell senescence" AND "regeneration"	100
PROQUEST	(“Stem Cells” OR “Stem Cell” OR “Mother Cell” OR “Progenitor Cell”) AND (“Cellular Senescence” OR “Cell Senescence” OR “Cell Aging” OR “Cellular Aging” OR “Cell Ageing” OR “Cellular Ageing”) AND (“Regenerative Medicine” OR "Tissue Engineering" OR "Cell Engineering" OR "Cell- and Tissue-Based Therapy" OR "Regeneration" OR "Wound Healing" OR “Wound Repair” OR “Tissue Therapy”)	197

Supplementary Table 1. Detailed search strategies used in each database. The initial search was conducted on 28 February 2025 and updated on 12 December 2025; all databases were searched on the same day for each search.

SUPPLEMENTARY TABLE 2 Excluded articles and reasons for exclusion (n=80).

Reference	Reason for exclusion
Flores I, Blasco MA. A p53-dependent response limits epidermal stem cell functionality and organismal size in mice with short telomeres. PLoS One. 2009;4(3):e4934. doi: 10.1371/journal.pone.0004934. Epub 2009 Mar 19. PMID: 19295915; PMCID: PMC2654505.	1
Madonna R., De Caterina R., Willerson J.T., Geng Y.-J. (2011). Adipose tissue-derived mesenchymal stem cells with myocardin-a and telomerase co-expression possess augmented capacity of proliferation and promyogenic activities, American Heart Association's Scientific Sessions 2011. Circulation.	8
Scheers I., Paganelli M., Lombard C., Najimi M., Sokal E.M. (2011). Umbilical cord matrix stem cells maintain a stable genotype after long-term in vitro culture, 62nd Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2011. Hepatology. http://dx.doi.org/10.1002/hep.24666	8
Goichberg P., Kannappan R., Bai Y., Fiorini C., D'Amario D., Sanada F., Pesapane A., Arranto C., Kim J., Anastasia L., Gurusamy N., D'Alessandro D.A., Michler R.E., Zheng H., Hosoda T., Rota M., Kajstura J., Bolli R., Anversa P., Leri A. (2011). Alterations in ephrin A1/EphA2 signaling causes defects in the migration of senescent human cardiac stem cells, American Heart Association's Scientific Sessions 2011. Circulation.	8
Scheers I., Maerckx C., Decottignies A., Paganelli M., Najimi M., Sokal E. (2011). Long-term in vitro cultured umbilical cord matrix stemcells maintain a stable genotype after long-term in vitro culture, European Society for Paediatric Gastroenterology, Hepatology, and Nutrition Annual Meeting 2011. Journal of Pediatric Gastroenterology and Nutrition. http://dx.doi.org/10.1097/MPG.0b013e318224e326	8
Klotz B, Mentrup B, Regensburger M, Zeck S, Schneidereit J, Schupp N, Linden C, Merz C, Ebert R, Jakob F. 1,25-dihydroxyvitamin D3 treatment delays cellular aging in human mesenchymal stem cells while maintaining their multipotent capacity. PLoS One. 2012;7(1):e29959. doi: 10.1371/journal.pone.0029959. Epub 2012 Jan 5. PMID: 22242193; PMCID: PMC3252365.	4
Lapasset L., Milhavet O., Pellestor F., Prieur A., Aït-Hamou N., Lagnado A., Schwerer H., Besnard E., Babled A., Ramirez J.M., De Vos J., Lehmann S., Lemaitre J.-M. (2013). Exploring strategies for cell rejuvenation and tissues regeneration, 23rd Annual Meeting of the European Tissue Repair Society. Wound Repair and Regeneration.	8
Chen C., Sun K., Geng S., Liu J., Zhou Y., Wang L., Wang J.-W., Huang G.-N., Wang Y.-P. (2013). Effect of ginsenoside Rg1 on radiation-induced senescence of hematopoietic stem/progenitor cells and relevant mechanism. Chinese Journal of Biologicals, 26(11), 1604-1609+1616.	7
Cipriani P, Di Benedetto P, Liakouli V, Del Papa B, Di Padova M, Di Ianni M, Marrelli A, Alesse E, Giacomelli R. Mesenchymal stem cells (MSCs) from scleroderma patients (SSc) preserve their immunomodulatory properties although senescent and normally induce T regulatory cells (Tregs) with a functional phenotype: implications for cellular-based therapy. Clin Exp Immunol. 2013 Aug;173(2):195-206. doi: 10.1111/cei.12111. PMID: 23607751; PMCID: PMC3722920.	2

Gullo F, De Bari C. Prospective purification of a subpopulation of human synovial mesenchymal stem cells with enhanced chondro-osteogenic potency. Rheumatology (Oxford). 2013 Oct;52(10):1758-68. doi: 10.1093/rheumatology/ket205. Epub 2013 Jun 26. PMID: 23804221.	4
Jun HS, Dao LT, Pyun JC, Cho S. Effect of cell senescence on the impedance measurement of adipose tissue-derived stem cells. Enzyme Microb Technol. 2013 Oct 10;53(5):302-6. doi: 10.1016/j.enzmictec.2013.07.001. Epub 2013 Jul 16. PMID: 24034428.	5
He X., Li X., Bao H.-J., Wang R.-F., Liu Y.-D., Song S.-W. (2014). Effects of three-dimensional spheroid culture system on biological characteristics of mouse bone marrow mesenchymal stem cells. Chinese Journal of Tissue Engineering Research, 18(45), 7227-7232. http://dx.doi.org/10.3969/j.issn.2095-4344.2014.45.003	7
Klinkhammer BM, Kramann R, Mallau M, Makowska A, van Roeyen CR, Rong S, Buecher EB, Boor P, Kovacova K, Zok S, Denecke B, Stuettgen E, Otten S, Floege J, Kunter U. Mesenchymal stem cells from rats with chronic kidney disease exhibit premature senescence and loss of regenerative potential. PLoS One. 2014 Mar 25;9(3):e92115. doi: 10.1371/journal.pone.0092115. PMID: 24667162; PMCID: PMC3965415.	2
Kumar D., Rooge S.B., Shubham S., Anand L., Mohanty S., Bihari C., Kumar A., Sarin S.K. (2015). Bone marrow mesenchymal stem cells (BMSCS) from decompensated cirrhotics show premature senescence and limited tissue repair and regenerative potential, 66th Annual Meeting of the American Association for the Study of Liver Diseases: The Liver Meeting 2015. Hepatology. http://dx.doi.org/10.1002/hep.28216	2
Machado AK, Cadoná FC, Azzolin VF, Dornelles EB, Barbisan F, Ribeiro EE, *et al*. **Guaraná (Paulinia cupana) improves the proliferation and oxidative metabolism of senescent adipocyte stem cells derived from human lipoaspirates. Food Res Int. 2015;67:426-33.**	5
Cho JG, Lee JH, Hong SH, Lee HN, Kim CM, Kim SY, Yoon KJ, Oh BJ, Kim JH, Jung SY, Asahara T, Kwon SM, Park SG. **Tauroursodeoxycholic acid, a bile acid, promotes blood vessel repair by recruiting vasculogenic progenitor cells. Stem Cells. 2015 Mar;33(3):792-805. doi: 10.1002/stem.1901. PMID: 25407160.**	1
Windmolders S, Willems L, Daniëls A, Linsen L, Fanton Y, Hendrikx M, Koninckx R, Rummens JL, Hensen K. Clinical-scale in vitro expansion preserves biological characteristics of cardiac atrial appendage stem cells. Cell Prolif. 2015 Apr;48(2):175-86. doi: 10.1111/cpr.12166. Epub 2015 Jan 29. PMID: 25630660; PMCID: PMC6495491.	4
Li Y., Zhou Y., Wang Y.-P., Wang J.-W., He Y.-H., Ding J.-C., Zhao W.-Z., Yang Y.-Q., Wang H.-J. (2016). Effect of SIRT1/NF-κB signal axis on delaying hematopoietic stem cell and progenitor cell senescence with ginsenoside R1 in aging model rat induced by D-galactose. Chinese Traditional and Herbal Drugs, 47(22), 4016-4020. http://dx.doi.org/10.7501/j.issn.0253-2670.2016.22.014	7
Tsai T.-L., Li W.-J. (2016). Soluble factors in bone marrow niche enhance human mesenchymal stem cell-based tissue regeneration, 2016 Annual Meeting of the Orthopaedic Research Society. Journal of Orthopaedic Research. http://dx.doi.org/10.1002/jor.23247	8
Marycz K, Tomaszewski KA, Kornicka K, Henry BM, Wroński S, Tarasiuk J, Maredziak M. Metformin Decreases Reactive Oxygen Species, Enhances Osteogenic Properties of Adipose-Derived Multipotent Mesenchymal Stem Cells In Vitro, and Increases Bone Density In Vivo. Oxid Med Cell Longev.	4

2016;2016:9785890. doi: 10.1155/2016/9785890. Epub 2016 Apr 18. Erratum in: Oxid Med Cell Longev. 2017;2017:5923818. doi: 10.1155/2017/5923818. PMID: 27195075; PMCID: PMC4852347.	
Tsai T., Li W. (2016). Bone marrow-extracted soluble factors maintain properties of human mesenchymal stem cells, 2016 TERMIS - Americas Conference. Tissue Engineering - Part A. http://dx.doi.org/10.1089/ten.tea.2016.5000.abstracts	8
Lee J, Byeon JS, Lee KS, Gu NY, Lee GB, Kim HR, Cho IS, Cha SH. Chondrogenic potential and anti-senescence effect of hypoxia on canine adipose mesenchymal stem cells. Vet Res Commun. 2016 Mar;40(1):1-10. doi: 10.1007/s11259-015-9647-0. Epub 2015 Dec 10. PMID: 26661466.	5
Maciel R.C., Jiang J., Leong N.L., Yamaguchi K.T., Kabir N., Petrigliano F.A., McAllister D.R. (2017). Adipose derived perivascular stem cells are a superior cell source for ligament tissue engineering, 2017 Annual Meeting of the Orthopaedic Research Society. Journal of Orthopaedic Research.	8
Gilpin S.E., Li Q., Evangelista-Leite D., Frey B.L., Ott H.C. (2017). Enhanced epithelial regeneration on human neonatal lung derived scaffolds is mediated by tenascin-C, American Thoracic Society International Conference, ATS 2017. American Journal of Respiratory and Critical Care Medicine. http://dx.doi.org/10.1164/ajrccm-conference.2017.A19	8
OConnor K., Madsen S., Russell K., Tucker A., Glowacki J., Bunnell B. (2017). CD264: A predictor of MSC regenerative potential, Tissue Engineering and Regenerative Medicine International Society's - Americas Annual Conference, TERMIS 2017. Tissue Engineering - Part A. http://dx.doi.org/10.1089/ten.tea.2017.29003.abstracts	8
Rasmus M., Maj M., Balcerczyk D., Buhl M., Warda K., Jundzill A., Buchholz L., Kloskowski T., Pokrywczynska M., Drewa T. (2017). Adipose derived stem cell properties in long-term culture-an implications for regenerative medicine, 4th Baltic Meeting in Conjunction with the EAU. European Urology, Supplements. http://dx.doi.org/10.1016/S1569-9056(17)31372-6	8
Tsai TL, Li WJ. Identification of Bone Marrow-Derived Soluble Factors Regulating Human Mesenchymal Stem Cells for Bone Regeneration. Stem Cell Reports. 2017 Feb 14;8(2):387-400. doi: 10.1016/j.stemcr.2017.01.004. Epub 2017 Feb 2. PMID: 28162996; PMCID: PMC5312426.	4
Mobarak H, Fathi E, Farahzadi R, Zarghami N, Javanmardi S. L-carnitine significantly decreased aging of rat adipose tissue-derived mesenchymal stem cells. Vet Res Commun. 2017 Mar;41(1):41-47. doi: 10.1007/s11259-016-9670-9. Epub 2016 Dec 10. PMID: 27943151.	4
Jiang T, Xu G, Wang Q, Yang L, Zheng L, Zhao J, Zhang X. In vitro expansion impaired the stemness of early passage mesenchymal stem cells for treatment of cartilage defects. Cell Death Dis. 2017 Jun 1;8(6):e2851. doi: 10.1038/cddis.2017.215. Erratum in: Cell Death Dis. 2019 Sep 26;10(10):716. doi: 10.1038/s41419-019-1939-9. PMID: 28569773; PMCID: PMC5520885.	4
Sanap A, Chandravanshi B, Shah T, Tillu G, Dhanushkodi A, Bhonde R, Joshi K. Herbal pre-conditioning induces proliferation and delays senescence in Wharton's Jelly Mesenchymal Stem Cells. Biomed Pharmacother. 2017 Sep;93:772-778. doi: 10.1016/j.biopha.2017.06.107. Epub 2017 Jul 15. PMID: 28724259.	4
Gilpin SE, Li Q, Evangelista-Leite D, Ren X, Reinhardt DP, Frey BL, Ott HC. Fibrillin-2 and Tenascin-C bridge the age gap in lung epithelial regeneration. Biomaterials. 2017 Sep;140:212-219. doi: 10.1016/j.biomaterials.2017.06.027. Epub 2017 Jun 22. PMID: 28662401; PMCID: PMC5553709.	4

Colasuonno F, Borghi R, Niceforo A, Muzzi M, Bertini E, Di Giulio A, Moreno S, Compagnucci C. Senescence-associated ultrastructural features of long-term cultures of induced pluripotent stem cells (iPSCs). Aging (Albany NY). 2017 Oct 23;9(10):2209-2222. doi: 10.18632/aging.101309. PMID: 29064821; PMCID: PMC5680563.	6
Gao S, Xiang C, Qin K, Sun C. Mathematical Modeling Reveals the Role of Hypoxia in the Promotion of Human Mesenchymal Stem Cell Long-Term Expansion. Stem Cells Int. 2018 May 14;2018:9283432. doi: 10.1155/2018/9283432. PMID: 29861746; PMCID: PMC5976908.	6
Conley S.M., Hickson L.J., Tang H., Jordan K.L., Abumoawad A.M., Kim S.R., Zhu X.-Y., Tchkonia T., Kirkland J.L., Lerman L.O. (2019). Obesity induces mesenchymal stem cell senescence and dysfunction, Midwest Clinical and Translational Research Meeting 2019. Journal of Investigative Medicine. http://dx.doi.org/10.1136/jim-2019-midwestern2019.98	2
Chen B, Sun Y, Zhang J, Zhu Q, Yang Y, Niu X, Deng Z, Li Q, Wang Y. Human embryonic stem cell-derived exosomes promote pressure ulcer healing in aged mice by rejuvenating senescent endothelial cells. Stem Cell Res Ther. 2019 May 21;10(1):142. doi: 10.1186/s13287-019-1253-6. PMID: 31113469; PMCID: PMC6528288.	1
Cui H, Tang D, Garside GB, Zeng T, Wang Y, Tao Z, Zhang L, Tao S. Wnt Signaling Mediates the Aging-Induced Differentiation Impairment of Intestinal Stem Cells. Stem Cell Rev Rep. 2019 Jun;15(3):448-455. doi: 10.1007/s12015-019-09880-9. PMID: 30790135; PMCID: PMC6534527.	5
Theys N., Episkopou H., Pierard C., Decottignies A. (2020). Assessment of senescence in adipose mesenchymal stem cells for optimized ex vivo expansion and therapeutic potential, ECTS Congress 2020. Bone Reports. http://dx.doi.org/10.1016/j.bonr.2020.100367	8
Ghannoum D., Roeder E., El Ouafy M., Cauchois G., Charif N., de Isla N. (2020). Evaluation of fetal mesenchymal stromal/stem cells senescence during in vitro amplification for therapeutic purpose: choice of cell quality parameters, ISCT 2020 Annual Meeting. Cytotherapy. http://dx.doi.org/10.1016/j.jcyt.2020.03.141	8
Hu W, Zhu S, Fanai ML, Wang J, Cai J, Feng J. 3D co-culture model of endothelial colony-forming cells (ECFCs) reverses late passage adipose-derived stem cell senescence for wound healing. Stem Cell Res Ther. 2020 Aug 14;11(1):355. doi: 10.1186/s13287-020-01838-w. PMID: 32795343; PMCID: PMC7427858.	5
Zhang WT, Zhou Q, He C. Effects of Hydrogen Peroxide on the Senescence of Human Mesenchymal Stem Cells Through Fatty Acid 2-Hydroxylase. J Biomater Tissue Eng. 2020 Nov 1;10(11):1634-8. DOI: 10.1166/jbt.2020.2474	7
Hong T, Kim MY, Da Ly D, Park SJ, Eom YW, Park KS, Baik SK. Ca2+-activated mitochondrial biogenesis and functions improve stem cell fate in Rg3-treated human mesenchymal stem cells. Stem Cell Res Ther. 2020 Nov 4;11(1):467. doi: 10.1186/s13287-020-01974-3. PMID: 33148318; PMCID: PMC7640456.	4
Li X, Ma X, Chen Y, Peng D, Wang H, Chen S, Xiao Y, Li L, Zhou H, Cheng F, Gao Y, Chang J, Cheng T, Liu L. Coinhibition of activated p38 MAPKα and mTORC1 potentiates stemness maintenance of HSCs from SR1-expanded human cord blood CD34+ cells via inhibition of senescence. Stem Cells Transl Med. 2020 Dec;9(12):1604-1616. doi: 10.1002/sctm.20-0129. Epub 2020 Jun 29. PMID: 32602209; PMCID: PMC7695631.	6
Fu S, Zhang C, Yan X, Li D, Wang Y, Dong C, Cao Z, Ning Y, Shao C, Yang T. Primary Cilia as a Biomarker in Mesenchymal Stem Cells Senescence: Influencing	5

Osteoblastic Differentiation Potency Associated with Hedgehog Signaling Regulation. Stem Cells Int. 2021 Jan 26;2021:8850114. doi: 10.1155/2021/8850114. PMID: 33574852; PMCID: PMC7857927.	
Sun Y, Zhang J, Chen B, Yang Y, Li H, Niu X, *et al*. **Small extracellular vesicles secreted by urine-derived stem cells enhanced wound healing in aged mice by ameliorating cellular senescence. J Mater Sci Technol. 2021;63:216-27. doi: 10.1016/j.jmst.2020.03.014.**	1
Kim SR, Zou X, Tang H, Puranik AS, Abumoawad AM, Zhu XY, Hickson LJ, Tchkonia T, Textor SC, Kirkland JL, Lerman LO. Increased cellular senescence in the murine and human stenotic kidney: Effect of mesenchymal stem cells. J Cell Physiol. 2021 Feb;236(2):1332-1344. doi: 10.1002/jcp.29940. Epub 2020 Jul 13. PMID: 32657444; PMCID: PMC7900892.	2
Labusca L, Herea DD, Emanuela Minuti A, Stavila C, Danceanu C, Plamadeala P, Chiriac H, Lupu N. Magnetic Nanoparticles and Magnetic Field Exposure Enhances Chondrogenesis of Human Adipose Derived Mesenchymal Stem Cells But Not of Wharton Jelly Mesenchymal Stem Cells. Front Bioeng Biotechnol. 2021 Oct 18;9:737132. doi: 10.3389/fbioe.2021.737132. PMID: 34733830; PMCID: PMC8558412.	4
Le HM, Nguyen LT, Hoang DH, Bach TQ, Nguyen HTN, Mai HT, Trinh DP, Nguyen TD, Nguyen LT, Than UTT. Differential Development of Umbilical Cord-Derived Mesenchymal Stem Cells During Long-Term Maintenance in Fetal Bovine Serum-Supplemented Medium and Xeno- and Serum-Free Culture Medium. Cell Reprogram. 2021 Dec;23(6):359-369. doi: 10.1089/cell.2021.0050. Epub 2021 Nov 8. PMID: 34748399.	7
Chuan T., Xiangqing Z., Zailing Y., Donghai Y., Ye L., Yanying W., Yukun Y., Jie H., Guanke L., Xuemin C., Liping S., Zhixu H., Xinghua P. (2022). Bone marrow mesenchymal stem cells regulate ovarian aging in macaques. Chinese Journal of Tissue Engineering Research, 26(7), 1033-1039. http://dx.doi.org/10.12307/2022.133	4
Choi D.H., Oh S.-Y., Lee K.E., Jo I., Park Y.S. (2022). Changes in the integrin a3 expression controls tonsil derived mesenchymal stem cell proliferation and senescence, Tissue Engine International Society Asia Engineering and Regenerative Medicine Asia-Pacific Chapter Conference, TERMIS-AP 2022. Tissue Engineering - Part A. http://dx.doi.org/10.1089/ten.tea.2022.29037.abstract.index	8
Gao Y., Chi Y., Chen Y., Li H.Y., Wang W., Zheng W., Zhu P., An J., Duan Y., Sun T., Xiaofan L., Xue F., Liu W., Fu R., Han Z., Zhang Y., Yang R., Tao C., Wei J., ... Zhang L. (2022). Multi-Omics Analysis of Human Mesenchymal Stem Cell Guides Perinatal MSC Based Cellular Therapy for Splenectomy-Nonresponsive Refractory Immune Thrombocytopenia: A Phase I Clinical Trial, 64th ASH Annual Meeting. Blood. http://dx.doi.org/10.1182/blood-2022-156764	8
Khorraminejad-Shirazi M., Sani M., Talaei-Khozani T., Dorvash M., Mirzaei M., Faghihi M.A., Monabati A., Attar A. (2022). AICAR AND NAM SYNERGISTICALLY ATTENUATE SENESCENCE-ASSOCIATED CHANGES IN MESENCHYMAL STEM CELLS: THE INTERPLAY OF AUTOPHAGY AND MTORC1, 6th Tissue Engineering and Regenerative Medicine International Society's - Americas Annual Conference, TERMIS 2021. Tissue Engineering - Part A. http://dx.doi.org/10.1089/ten.tea.2022.29025.abstracts	8

Narcisi R., Voskamp C., Koevoet W.J., van Osch G.J. (2022). CELLULAR SENESCENCE IMPAIRS CHONDROGENIC DIFFERENTIATION OF MESENCHYMAL STEM/STROMAL CELLS BY INHIBITING THE TRASFORMING GROWTH FACTOR-BETA SIGNALING, 2022 OARSI World Congress on Osteoarthritis. Osteoarthritis and Cartilage. http://dx.doi.org/10.1016/j.joca.2022.02.570	8
Cruciani S, Garroni G, Pala R, Barcessat ARP, Facchin F, Ventura C, Fozza C, Maioli M. Melatonin finely tunes proliferation and senescence in hematopoietic stem cells. Eur J Cell Biol. 2022 Jun-Aug;101(3):151251. doi: 10.1016/j.ejcb.2022.151251. Epub 2022 Jun 22. PMID: 35772322.	2
Choi D., Lee K., Oh S., Lee S., Hwang J., Jo B., Lee J., Park Y., Park K., Jo I. (2023). Tissue Engineering: tonsil-derived mesenchymal stem cells incorporated in reactive oxygen species releasing hydrogel promote rejuvenation and bone formation through increased regulation of cell surface GRP78, 29th Annual ISCT Meeting. Cytotherapy. http://dx.doi.org/10.1016/S1465-3249(23)00712-0	8
Go Y.-Y., Lee C.-M., Chae S.-W., Song J.-J. (2023). Regenerative capacity of trophoblast stem cell-derived extracellular vesicles on mesenchymal stem cells. Biomaterials Research, 27(1). http://dx.doi.org/10.1186/s40824-023-00396-5	4
Dadashpour M., Mahmoudi H., Rahimi Z., Janghorbanian Poodeh R., Mousazadeh H., Firouzi-Amandi A., Yazdani Y., Nezami Asl A., Akbarzadeh A. (2023). Sustained in vitro delivery of metformin-loaded mesoporous silica nanoparticles for delayed senescence and stemness preservation of adipose-derived stem cells. Journal of Drug Delivery Science and Technology, 87. http://dx.doi.org/10.1016/j.jddst.2023.104769	7
Ishiuchi N, Nakashima A, Maeda S, Miura Y, Miyasako K, Sasaki K, Uchiki T, Sasaki A, Nagamatsu S, Nakao N, Nagao M, Masaki T. Comparison of therapeutic effects of mesenchymal stem cells derived from superficial and deep subcutaneous adipose tissues. Stem Cell Res Ther. 2023 May 4;14(1):121. doi: 10.1186/s13287-023-03350-3. PMID: 37143086; PMCID: PMC10161523.	4
Elsied MA, Sharawi ZW, Al-Amrah H, Hegazy RA, Mohamed AE, Saleh RM, El-Kholy SS, Farrag FA, Fayed MH, El-Magd MA. **Walnut Kernel Oil and Defatted Extracts Enhance Mesenchymal Stem Cell Stemness and Delay Senescence. Molecules. 2023 Aug 28;28(17):6281. doi: 10.3390/molecules28176281. PMID: 37687109; PMCID: PMC10488345.**	4
Hejazi, Sajjad1,2; Maleki, Masoud3; Rasekh, Morteza4. Study of the Cellular Senescence Process in Human Umbilical Cord Wharton's Jelly-derived Mesenchymal Stem Cells. Advances in Human Biology 13(4):p 361-366, Oct–Dec 2023. | DOI: 10.4103/aihb.aihb_19_23	6
Mapping aged stem cell states associated with decline in skeletal muscle regeneration. Nat Aging 4, 1680–1681 (2024). https://doi.org/10.1038/s43587-024-00768-z	8
Pei YA, Patel J, Pei M. Extracellular Matrix Tunes the Regenerative Potential of Fetal Stem Cells. Applied Sciences. 2024; 14(5):1932. https://doi.org/10.3390/app14051932	4
Rajput SN, Naeem BK, Ali A, Salim A, Khan I. Expansion of human umbilical cord derived mesenchymal stem cells in regenerative medicine. World J Stem Cells. 2024 Apr 26;16(4):410-433. doi: 10.4252/wjsc.v16.i4.410. PMID: 38690517; PMCID: PMC11056638.	4
Sun Y, Yu Y, Ma S, Liao C, Yang J, Lyu Y, Zhang X, Zhang J, Tian W, Liao L. Nanotube topography rejuvenates the senescence of mesenchymal stem cells by	7

activating YAP signalling. J Mater Chem B. 2024 Jul 17;12(28):6917-6926. doi: 10.1039/d3tb02828c. PMID: 38904147.	
Li F, Zhang F, Shi H, Xia H, Wei X, Liu S, Wu T, Li Y, Shu F, Chen M, Li J, Duan R. Aerobic exercise suppresses CCN2 secretion from senescent muscle stem cells and boosts muscle regeneration in aged mice. J Cachexia Sarcopenia Muscle. 2024 Oct;15(5):1733-1749. doi: 10.1002/jcsm.13526. Epub 2024 Jun 26. PMID: 38925632; PMCID: PMC11446704.	5
Kang GH, Shin YK, Lim KM, Kim SJ, Song M, Song K, Kim JH, Kim DY, Shin HC, Shin HJ, Cho SG. Thermostable bFGF Improves Cell Lifespan by Enhancing Cell Activity in the Long-Term Culture of Human Orbicularis Oculi Stem Cells. Int J Stem Cells. 2025 Aug 30;18(3):301-310. doi: 10.15283/ijsc24039. Epub 2024 Oct 23. PMID: 39439110; PMCID: PMC12394077.	4
Sun H, Zhai H, Han K, Ma H, Tan Y, Li S, Liu Z, Cheng L, Lu Q, Zhou L, Liu P. Clinical outcomes of autologous adipose-derived mesenchymal stem cell combined with high tibial osteotomy for knee osteoarthritis are correlated with stem cell stemness and senescence. J Transl Med. 2024 Nov 18;22(1):1039. doi: 10.1186/s12967-024-05814-3. PMID: 39558365; PMCID: PMC11575038.	2
Xu L, Ren W, Long Y, Yang B, Chen L, Chen W, Chen S, Cao Y, Wu D, Qu J, Li H, Yu Y, Zhang A, Wang S, Wang H, Chen T, Fan G, Li Q, Chen Z. Antisenescence Expansion of Mesenchymal Stem Cells Using Piezoelectric β-Poly(vinylidene fluoride) Film-Based Culture. ACS Appl Mater Interfaces. 2024 Nov 20;16(46):63207-63224. doi: 10.1021/acsami.4c12725. Epub 2024 Nov 6. PMID: 39503875.	4
Gu K, Feng XM, Sun SQ, Hao XY, Wen Y. Yes-associated protein-mediated melatonin regulates the function of periodontal ligament stem cells under oxidative stress conditions. World J Stem Cells. 2024 Nov 26;16(11):926-943. doi: 10.4252/wjsc.v16.i11.926. PMID: 39619874; PMCID: PMC11606347.	4
Liu DY, Wu J, Zhou HY, Lv JX, Cai KZ, Tang CB. **Phytic acid improves osteogenesis and inhibits the senescence of human bone marrow mesenchymal stem cells under high-glucose conditions via the ERK pathway. Chem Biol Interact. 2024 Jan 5;387:110818. doi: 10.1016/j.cbi.2023.110818. Epub 2023 Nov 22. PMID: 38000455.**	2
Li XY, Zhang L, Sun Y. [Mitochondrial transfer contributes to the odontogenic differentiation of dental mesenchymal stem cells]. Zhonghua Kou Qiang Yi Xue Za Zhi. 2025 Jan 9;60(1):43-53. Chinese. doi: 10.3760/cma.j.cn112144-20240926-00360. PMID: 39743364.	4
Kaokaen P, Pangjantuk A, Kunhorm P, Promjantuek W, Chaicharoenaudomrung N, Noisa P. Conditioned medium of human umbilical cord-mesenchymal stem cells cultivated with human cord blood serum enhances stem cell stemness and secretome profiles. Toxicol In Vitro. 2025 Mar;103:105973. doi: 10.1016/j.tiv.2024.105973. Epub 2024 Nov 17. PMID: 39561911.	4
Habibi Roudkenar H., Bahmani B., Halabian R. (2014). Over expression of neutrophil associated lipocalin in bone marrow derived-mesenchymal stem cells improve their regenerative potential trough reducing of senescent cells, 33rd International Congress of the International Society of Blood Transfusion, in Conjunction with the 33rd Congress of the KSBT and the 2014 Congress of the Korean Hematology Societies. Vox Sanguinis. http://dx.doi.org/10.1111/vox.12155	8

Lee H.C., Wong C.Y., Nguyen Phan N.N., Cheong S.K., Choo K.B., Kamarul T. (2013). Establishment and characterization of replicate senescence study models of human mesenchymal stem cells, 19th Annual Meeting of the International Society for Cellular Therapy, ISCT 2013. Cytotherapy. http://dx.doi.org/10.1016/j.jcyt.2013.01.054	8
Ohgo S, Hasegawa S, Hasebe Y, Mizutani H, Nakata S, Akamatsu H. Senescent dermal fibroblasts enhance stem cell migration through CCL2/CCR2 axis. Exp Dermatol. 2015 Jul;24(7):552-4. doi: 10.1111/exd.12701. Epub 2015 May 4. PMID: 25808810.	4
Roudkenar M.H., Roushandeh A.M. (2017). Ngal protects bone marrow-derived mesenchymal stem cells under sub-lethal doses of oxidative stress conditions by regulation of senescence, 2nd National Festival and International Congress on Stem Cell and Regenerative Medicine. Iranian Journal of Biotechnology. http://dx.doi.org/10.15171/ijb.2017.s1-334	8
Severino V., Alessio N., Farina A., Galderisi U., Chambery A. (2013). Secretome profiling of senescent mesenchymal stem cells (MSC) by high resolution LC-MS analysis, 38th FEBS Congress. FEBS Journal. http://dx.doi.org/10.1111/febs.12340	8
Yi Q, Liu O, Yan F, Lin X, Diao S, Wang L, Jin L, Wang S, Lu Y, Fan Z. Analysis of Senescence-Related Differentiation Potentials and Gene Expression Profiles in Human Dental Pulp Stem Cells. Cells Tissues Organs. 2017;203(1):1-11. doi: 10.1159/000448026. Epub 2016 Sep 15. PMID: 27627434.	5
Ambrosi TH, Goodnough LH, Steininger HM, Hoover MY, Kim E, Koepke LS, Marecic O, Zhao L, Seita J, Bishop JA, Gardner MJ, Chan CKF. Geriatric fragility fractures are associated with a human skeletal stem cell defect. Aging Cell. 2020 Jul;19(7):e13164. doi: 10.1111/acel.13164. Epub 2020 Jun 14. PMID: 32537886; PMCID: PMC7370785.	5
Das M, Das A, Barui A, Paul RR. Comparative evaluation of proliferative potential and replicative senescence associated changes in mesenchymal stem cells derived from dental pulp and umbilical cord. Cell Tissue Bank. 2022 Mar;23(1):157-170. doi: 10.1007/s10561-021-09926-8. Epub 2021 Apr 26. PMID: 33900487.	4
Cheng Y, Lin KH, Young TH, Cheng NC. The influence of fibroblast growth factor 2 on the senescence of human adipose-derived mesenchymal stem cells during long-term culture. Stem Cells Transl Med. 2020 Apr;9(4):518-530. doi: 10.1002/sctm.19-0234. Epub 2019 Dec 16. PMID: 31840944; PMCID: PMC7103622.	4

Exclusion Criteria

1. Studies that evaluate the impact of senescence on other cell types (e.g., somatic cells, fibroblasts, or epithelial cells).
2. Studies that evaluate the impact of senescence in disease models unrelated to regenerative therapies (e.g., cancer, aging-related diseases, etc.).
3. Studies using immortalized/cancer cell lines or genetically modified cells (e.g., knock-in/knock-out for senescence-related genes).
4. Studies without a direct comparison between non-senescent and senescent groups.
5. Studies that do not confirm the senescent state of the cells.
6. Studies that focus solely on the mechanistic pathways of senescence without assessing its direct impact on tissue repair or regenerative therapies.
7. Full paper copy not available or inaccessible.
8. Reviews articles, systematic reviews, meta-analyses, abstracts, protocols, short communications, personal opinions, letters, posters, conference abstracts, and case report.

SUPPLEMENTARY TABLE 3 Characteristics of the included studies (n=45).

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
Alraies *et al.*, 2017 UK[50]	Experimental (*in vitro*)	DPSCs	18-30 years	3	NR (>80 PD)	Serial Passaging	SA-β-Gal staining, *P16, P21* and *P53* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Short telomeres: ↓proliferation ↓osteogenesis ↑adipogenesis ↓chondrogenesis
Alt *et al.*, 2012 USA[19]	Experimental (*in vitro*)	AD-MSCs	Group 1: <20 years Group 2: 30-40 years Group 3: >50 years	Group 1: 15 Group 2: 17 Group 3: 8	P1-P5	Chronological Aging	*P16* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Group 3: ↓proliferation ↓osteogenesis ↓adipogenesis ↓chondrogenesis
Bertolo *et al.*, 2016 Switzerland[42]	Experimental (*in vitro*)	BM-MSCs	17-57 years (Mean: 34 ± 13 years)	7	P7-P11	Serial Passaging	SA-β-Gal staining	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	Late-Passage: ↓proliferation ↓osteogenesis ↑adipogenesis ↓chondrogenesis
Bonab *et al*., 2006 Iran[47]	Experimental (*in vitro*)	BM-MSCs	2.5-63 years (Mean: 25 years)	11	P1-P10	Serial Passaging	Telomere shortening	Proliferation and Differentiation (osteogenic and adipogenic)	Late-Passage: ↓proliferation ↓osteogenesis ↓adipogenesis
Chen *et al*., 2024 China[23]	Experimental (*in vitro, in vivo*)	DPSCs	16-70 years	25	P3-P5	Chronological Aging	SA-β-gal staining, *P21* and *P53* expression	Proliferation, Differentiation (osteogenic), Migration and Bone Repair (*in vivo).*	Aged Group: ↓proliferation ↓osteogenesis ↓migration; ↓bone repair

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
Cheng *et al.*, 2011 China[39]	Experimental (*in vitro*)	BM-MSCs UC-MSCs-WJ	Median: 19 years	5 4	P2-P13 P2-P25	Serial Passaging	SA-β-Gal staining	Proliferation and Differentiation (osteogenic and adipogenic)	<u>Late-Passage:</u> ↓proliferation ↓adipogenesis ↑osteogenesis
Choudhery *et al.*, 2014 USA[9]	Experimental (*in vitro*)	AD-MSCs	Group 1: <30 years (mean 25.5 ± 1.6) Group 2: 35-55 years (mean 46.4 ± 2.1) Group 3: >60 years (mean 66.0 ± 1.4)	Group 1: 8 Group 2: 10 Group 3: 11	P1-P3	Chronological Aging	SA-β-Gal staining, *P16* and *P21* expression	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	<u>Group 3:</u> ↓proliferation ↓osteogenesis →adipogenesis ↓chondrogenesis
Christodoulou *et al.*, 2013 Greece[33]	Experimental (*in vitro*)	UC-MSCs-WJ AD-MSCs	Fetal (full-term) 44 ± 11 years	5 3	P2-P20 P2-P10	Serial Passaging	SA-β-Gal staining	Proliferation and Differentiation (osteogenic)	<u>Late-Passage:</u> ↓proliferation ↓osteogenesis
Danisovic *et al.*, 2017 Slovakia[30]	Experimental (*in vitro*)	AD-MSCs	Mean: 34 years	5	P1-P30	Serial Passaging	Telomere activity	Proliferation	<u>Late-Passage:</u> ↓proliferation
Diomede *et al.*, 2017 Italy[43]	Experimental (*in vitro*)	DPSCs PDLSCs	NR	NR	P2-P15	Serial Passaging	SA-β-Gal staining, *P16* and *P21* expression	Proliferation and Differentiation (osteogenic and adipogenic)	<u>Late-Passage:</u> →proliferation →osteogenesis →adipogenesis
Feng *et al.*, 2014 China[11]	Experimental (*in vitro*)	DPSCs	13-23 years	9	P3	Inflammatory Stimuli – LPS (1x, 3x, 6x)	SA-β-Gal staining	Proliferation	<u>LPS – 3x/6x:</u> ↓proliferation

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
Feng *et al*., 2014 China[24]	Experimental (*in vitro*)	DPSCs	5–12 years 12–20 years 20–35 years 35–50 years >50 years	33	P3	Chronological Aging	SA-β-gal staining and *p16* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Aged Group: ↓proliferation ↓osteogenesis ↓adipogenesis
Feng *et al*., 2018 China[18]	Experimental (*in vitro*)	DPSC	13-23 years	9	P3	Inflammatory Stimuli – LPS (1x, 3x, 6x)	SA-β-Gal staining	Proliferation	LPS – 3x/6x: ↓proliferation
Figiel-Dabrowska et al., 2022[57]	Experimental (*in vitro*)	AD-MSCs	NR	NR	P3-P22	Serial Passaging	SA-β-Gal staining	Proliferation	Late-Passage: ↓proliferation
Gao *et al.*, 2023 China[58]	Experimental (*in vitro, in vivo*)	BM-MSCs	NR	NR	P3-P4	Chronological Aging	SA-β-Gal staining, *P16* and *P21* expression	Proliferation and Cardiac Repair (*in vivo*)	Aged Group: ↓proliferation ↓cardiac repair (vs young group)
Govarthanan *et al.*, 2025 India[41]	Experimental (*in vitro*)	UC-MSCs-WJ	NR	NR	P3-P15	Serial Passaging	SA-β-Gal staining	Differentiation (osteogenic, adipogenic and chondrogenic)	Late-Passage: ↓osteogenesis ↓chondrogenesis
Gruber *et al.*, 2012 USA[10]	Experimental (*in vitro*)	AD-MSCs	30-73 years (Mean: 52.6 ± 12.8 years)	11	P1-P13	Chronological Aging and Serial Passaging	SA-β-Gal staining	Differentiation (osteogenic and chondrogenic)	Aged Group and Late-Passage: ↓proliferation →osteogenesis →chondrogenesis
Horibe *et al.*, 2014 Japan[12]	Experimental (*in vitro, in vivo*)	DPSCs	Young: 19-30 years Aged: 44-70 years	6	P5-P20	Chronological Aging and Serial Passaging	SA-β-gal staining, *p16*, *p21*, telomerase	Proliferation, Differentiation (osteogenesis and	Aged Group and Late-Passage: ↓proliferation →osteogenesis

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
							activity, telomere length	adipogenesis), Pulp Regeneration and Immune Response	→adipogenesis ↓pulp regeneration ↑IL-1β, IL-6, IL-8 and Groα
Hu *et al.*, 2024 China[31]	Experimental (*in vitro*)	PDLSCs	18-26 years	10	P4-P20	Serial Passaging	SA-β-Gal staining, *P16* and *P21* expression	Proliferation	Late-Passage: ↓proliferation
Huang *et al.*, 2023 China[22]	Experimental (*in vitro*)	BM-MSCs	NR	NR	P3-P12	Chronological Aging and Serial Passaging	SA-β-Gal staining, *P16* and *P21* expression	Proliferation and Immune Response	Aged Group and Late-Passage: ↓proliferation ↑TNF-α and IL-6 ↓IL-10
Iezzi *et al.*, 2019 Italy[25]	Experimental (*in vitro*)	DPSCs	Group A (Young): 20-23 years (mean 21) Group B (Middle): 42-45 years (mean 43) Group C (Old): 62-66 years (mean 64)	12	NR	Chronological Aging	SA-β-Gal staining, *P16* expression and telomere length	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	Aged Group: ↓proliferation ↓osteogenesis ↓adipogenesis →chondrogenesis
Jia *et al.*, 2025 China[32]	Experimental (*in vitro, in vivo*)	PDLSCs	NR	NR	P5-P20	Serial Passaging	SA-β-gal staining and *P21* expression	Proliferation, Differentiation (osteogenic) and	Late-Passage ↓proliferation ↓osteogenesis

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
								Bone Repair (*in vivo*)	
Jeoung *et al.*, 2015 Korea[59]	Experimental (*in vitro*)	UC-MSCs-CB	31-34 years (Mothers)	2	P5-P17	Serial Passaging	SA-β-gal staining, Telomerase activity, Expression of *p53, p21, p27*	Proliferation	Late-Passage ↓proliferation
Jin *et al.*, 2016 Korea[34]	Experimental (*in vitro*)	UC-MSCs-CB	NR	27	P5-P15	Serial Passaging	SA-β-Gal staining, *P16, P21* and *P53* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Late-Passage ↓proliferation ↓osteogenesis ↓adipogenesis
Katsube *et al.*, 2008 Japan[27]	Experimental (*in vitro*)	BM-MSCs	1, 25, and 74 years	3	P3	Chronological Aging	SA-β-gal staining and expression of p*16, p21*, and *MMP1*	Proliferation	Aged Group: ↓proliferation
Kim *et al.*, 2020 Korea[60]	Experimental (*in vitro, in vivo*)	UC-MSCs-CB	NR	10	P2-P15	Serial Passaging	SA-β-Gal staining, *P16, P21* and *P53* expression	Proliferation	Late-Passage ↓proliferation
Kim *et al*., 2021 Korea[35]	Experimental (*in vitro*)	BM-MSCs	NR	1	P5-P15	Serial Passaging	SA-β-gal staining, *P15* and *P16* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Late-Passage ↓proliferation ↓osteogenesis →adipogenesis
Kornicka *et al.*, 2015 Poland[20]	Experimental (*in vitro*)	AD-MSCs	>20 (age range 20–29, mean age 24 ± 1.4 years)	28	P1-P3	Chronological Aging	SA-β-Gal staining, *P16* and *P53* expression	Proliferation and Differentiation (osteogenic and adipogenic)	Aged Group: ↓proliferation ↓osteogenesis ↓/↑adipogenesis

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
			>50 (age range 50–60, mean age 57.5±0.7) >60 (age range 60–69, age 67) >70 (age range 70–79, mean age 75 ± 2.8)						
Legzdina *et al.*, 2016 Latvia[44]	Experimental (*in vitro*)	AD-MSCs	27-63 years (27, 38, 38, 43, 47, 57, 61, 63)	8	P2-P14	Chronological Aging and Serial Passaging	SA-β-Gal staining and relative telomere length analysis	Proliferation and Differentiation (osteogenic and adipogenic)	Aged Group and Late-Passage: ↓proliferation ↓osteogenesis ↓adipogenesis
Li *et al.*, 2025 China[61]	Experimental (*in vitro*)	DPSCs	NR	NR	P3-P12	Serial Passaging	SA-β-gal staining, *P16* expression ROS levels	Differentiation (osteogenic)	Late-Passage ↓osteogenesis
Ma *et al.*, 2019 China[14]	Experimental (*in vitro, in vivo*)	DPSCs[a] PDLSCs[a] BM-MSCs[b] AD-MSCs[b]	16-20 years	5	P3-P6	Serial Passaging and Inflammatory Stimuli – LPS and TNF- α	SA-β-Gal staining, *P16, P21* and *P53* expression	Proliferation, Differentiation (osteogenic) and Bone/Periodontal Repair	Late-Passage ↓proliferation →[a]/↓[b]osteogenesis ↓bone/periodontal repair LPS and TNF- α ↓ osteogenesis ↓ bone/periodontal repair

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
Scheers *et al.*, 2013 Belgium[48]	Experimental (*in vitro, in vivo*)	UC-MSCs-WJ	Full-term newborns	14	P1-P21	Serial Passaging	SA-β-Gal staining	Proliferation and Differentiation (osteogenic and adipogenic)	Late-Passage ↓proliferation ↓osteogenesis ↓adipogenesis
Stolzing *et al.*, 2008 UK[28]	Experimental (*in vitro*)	BM-MSCs	Young: 7-18 years Adult: 19-40 years Aged: >40 years	33	P1-P5	Chronological Aging	SA-β-Gal staining, *P21* and *P53* expression	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	Aged Group: ↓ proliferation ↓osteogenesis ↓adipogenesis ↓chondrogenesis
Truong, *et al.*, 2019 Vietnam[45]	Experimental (*in vitro*)	AD-MSCs	NR	3	P5-P15	Serial Passaging	SA-β-gal staining	Differentiation (osteogenic, adipogenic and chondrogenic)	Late-Passage →osteogenesis →adipogenesis →chondrogenesis
Wagner *et al.*, 2008 Germany[29]	Experimental (*in vitro*)	BM-MSCs	8, 23, 25, 28, 29, 31, 32, 59 years	8	P2-P12	Chronological Aging and Serial Passaging	SA-β-gal staining	Proliferation and Differentiation (osteogenic and adipogenic)	Aged Group and Late-Passage: ↓proliferation ↑osteogenesis ↓adipogenesis
Wang *et al.*, 2025 China[46]	Experimental (*in vitro, in vivo*)	AD-MSCs	NR	NR	P4-P10	Serial Passaging	SA-β-Gal staining, *P16* and *P21* expression	Differentiation (osteogenic, adipogenic and chondrogenic)	Late-Passage: ↑osteogenesis ↓adipogenesis ↓chondrogenesis
Wang *et al.*, 2025 China[26]	Experimental (*in vitro*)	DPSCs	16-70 years	NR	P3-P12	Chronological Aging and Serial Passaging	SA-β-gal staining, γH2AX immunofluorescence,	Proliferation and Differentiation (osteogenic)	Aged Group and Late-Passage: ↓proliferation ↓osteogenesis

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
							P16/P21 expression		
Wenjing *et al.*, 2024 China[6]	Experimental (*in vitro*)	BM-MSCs	NR	NR	P5-P15	Serial Passaging	SA-β-gal staining, *p16/p21/p53* expression	Proliferation, Differentiation (osteogenic, adipogenic and chondrogenic) and Migration	<u>Late-Passage:</u> ↓proliferation ↓osteogenesis ↓adipogenesis ↓chondrogenesis ↓migration
Wu *et al.*, 2013 Taiwan[37]	Experimental (*in vitro*)	AD-MSCs	Infant: <1 year (3 mo, 4 mo, 6 mo, 1 yr) Adult: 20-54 years (15, 27, 35, 37, 44, 45) Elderly: >55 years (51, 55, 71)	13	P0-P1	Chronological Aging	Telomere length	Differentiation (osteogenic)	<u>Aged Group:</u> ↓osteogenesis
Wu *et al.*, 2022 Taiwan [21]	Experimental (*in vitro*)	AD-MSCs	NR	6	P3-P10	Chronological Aging and Serial Passaging	SA-β-gal staining, *p16/p21/p53* expression	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	<u>Aged Group and Late-Passage:</u> ↓proliferation ↑osteogenesis ↓adipogenesis ↓chondrogenesis
Yang *et al.*, 2021 China[40]	Experimental (*in vitro, in vivo*)	PDLSCs	11-16 years	NR	P3-P12	Serial Passaging	SA-β-gal staining, *p16 and p21* expression	Proliferation, Differentiation (osteogenic) and Migration	<u>Late-Passage:</u> ↓proliferation ↓osteogenesis ↓migration

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
									↓bone formation
Ye *et al.*, 2016 China[38]	Experimental (*in vitro*)	AD-MSCs	Group A (Young): 20–38 years (mean 26.33 ± 5.48); Group B (Old): 50–67 years (mean 56.44 ± 5.83)	20	P0-P9	Chronological Aging	SA-β-Gal staining, *P16* and *P53* expression	Proliferation and Differentiation (osteogenic, adipogenic and chondrogenic)	<u>Aged Group:</u> ↓osteogenesis ↓adipogenesis ↓chondrogenesis
Yu *et al.*, 2014 Korea[49]	Experimental (*in vitro, in vivo*)	UC-MSCs-CB	NR	3	P5-P20	Serial Passaging and Inflammatory Stimuli – IFN-ɣ and TNF- α	SA-β-gal staining and *p16* expression	Proliferation and Immune Response	<u>Late-Passage:</u> ↓proliferation ↓PGE2 ↓COX-2 →TGF-β1 →NO
Zhang *et al.*, 2024 Australia[13]	Experimental (*in vitro*)	AD-MSCs	NR	NR	P4-P10	Serial Passaging	SA-β-gal staining, *p16/p21/p53* expression	Proliferation and Differentiation (osteogenic)	<u>Late-Passage:</u> ↓proliferation ↓osteogenesis
Zhuang *et al.*, 2015 China[36]	Experimental (*in vitro*)	UC-MSCs-WJ	Full-term deliveries	10	P3-P15	Serial Passaging	SA-β-Gal staining	Proliferation and Immune Response	<u>Late-Passage:</u> ↓proliferation ↑HMOX-1 ↑IL-10 ↑iNOS ↑IL-6 ↓IL-1α

Author /year/Country	Study Design	Stem Cell Source	Donor age (as reported)	n (donors)	Passage Number	Senescence Induction Method	Senescence Confirmation Method	Outcomes Assessed	Key Findings (Senescent group)
									↓IL-1β ↓IFN-ɣ

SUPPLEMENTARY TABLE 3 Methodological characteristics and main findings of included studies on senescence in MSC. The table summarizes the experimental designs, cell types, senescence induction and confirmation methods, assessed outcomes, and key results from the selected studies. Abbreviations used include: DPSCs (dental pulp stem cells), AD-MSCs (adipose-derived mesenchymal stem cells), BM-MSCs (bone marrow mesenchymal stem cells), UC-MSCs[1] (umbilical cord mesenchymal stem cells derived from Wharton's jelly), UC-MSCs[2] (umbilical cord mesenchymal stem cells derived from cord blood), PDLSCs (periodontal ligament stem cells), SA-β-Gal (senescence-associated β-galactosidase), LPS (lipopolysaccharide), NR (not reported). The symbols ↓, ↑, and → denote, respectively, a decrease, increase, or no significant change in the assessed parameter in the senescent group compared to controls.

SUPPLEMENTARY TABLE 4 Individual results for the proliferation outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
(Feng et al., 2014)a	Inflammatory Stimuli	DPSCs	3	BrdU assay (370 nm) Cell Number (10^3)	~0.89 ± 0.1 ~65.13 ± 5.9	~0.37 ± 0.04 ~34.97 ± 3.06	p<0.05 p<0.05	Senescent	S: LPS 10 ng/mL, 6x
(Feng et al., 2018)b	Inflammatory Stimuli	DPSCs	See (Feng et al., 2014)a					-	Proliferation data identical of Feng *et al.,* 2014.
(Alt et al., 2012)	Chronological Aging	AD-MSCs	8	PDT (hours)	~46.19 ± 8.14	~62.67 ± 3.87	p<0.01	Senescent	The CFU assay showed a significantly higher colony-forming percentage in the young group.
(Choudhery et al., 2014)	Chronological Aging	AD-MSCs	3	PDT (hours)	62.0 ± 5.9	89.1 ± 26.6	p<0.05	Senescent	CFU assay showed ~30% reduced colony formation in aged donors; quantitative values could not be extracted. TPD was also assessed, with higher values

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									observed in the younger group.
(Gruber et al., 2012)	Chronological Aging	AD-MSCs	3	Mean DT (hours)	~55.56 ± 4.2	~89.41 ± 5.84	p<0.0001	Senescent	S: 40-75 years
(Kornicka et al., 2015)	Chronological Aging	AD-MSCs	3	PDT (hours)	55 ± 9.8	~130 ± 5.5	p<0.01	Senescent	S: >70 years. CFU-E assay was performed, but quantitative data were not extracted for synthesis; however, the aged group showed a lower percentage of CFU-E.
(Legzdina et al., 2016b)	Chronological Aging	AD-MSCs	2-3	CPD	28.97*	23.28*	not significant	not significant	NS: CS-8 (27 years), S: CS-3 (63 years). Cells from eight donors showed pronounced donor-dependent heterogeneity in proliferation kinetics (PD/PDT). CFU and MTT assays and PDT curves

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									were performed, with no significant differences observed between younger and older donors.
(S. H. Wu et al., 2022)	Chronological Aging	AD-MSCs	3	PDT (days)	~1 ± 0.03	~1.26 ± 0.09	p<0.01	Senescent	S: adult. A growth curve assay was performed; however, quantitative data were not extracted for synthesis. The fold increases in the cell numbers of the infant were significantly higher than those of the adult.
(Gao et al., 2023)	Chronological Aging	BM-MSCs	3	Ki-67 (%)	~49.78 ±4.08 ~11.42*	~9.92 ± 1.9 ~6.33*	p<0.001	Senescent	The CPD analysis revealed that the proliferative capacity was exhausted by passage 7 in the aged group, while remaining active until at least

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									passage 12 in the young group.
(Huang et al., 2023)	Chronological Aging	BM-MSCs	3	Ki-67 (%)	~74.15 ± 2.25	~23.66 ± 2.09	NR	Senescent	NS: Lenti-Control (fig.:2C), S: Control-siRNA (fig.: 3C). Groups were not directly compared.
(Katsube et al., 2008)	Chronological Aging	BM-MSCs	4	Growth Rate (fold increase)	~20*	~5*	NR	NR	NS: "High" (1 year), S: "Low" (74 years).
(Stolzing et al., 2008)	Chronological Aging	BM-MSCs	NR	CPD	~32.25*	~21.69*	NR	NR	NS: mean of two 18-year-old donors; S: mean of two 50-year-old donors. Cell cultures from donors ≤9 years old exhibited early proliferative arrest (CPD). CFU-f assay was performed and a significant decline was seen in the older age group (p=0.007).
(Wagner et al., 2008)	Chronological Aging	BM-MSCs	NR	CPD	~9.06*	~7.84*	NR	NR	NS: donor 7 (25 years), S: mean of

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									44- and 42-years old donors.
(Chen et al., 2024)	Chronological Aging	DPSCs	3	CCK-8 (450 nm)	~2.28*	~1.79*	p<0.001	Senescent	Data were extracted from day 9.
(Feng et al., 2014)b	Chronological Aging	DPSCs	3	BrdU (370 nm)	~0.87 ± 0.15	~0.32± 0.05	p<0.05	Senescent	NS: 5-12 years, S: >50 years. Growth curve was performed and after day 4, cells from younger group exhibited more rapid proliferation rates compared to other groups. These results indicated that the capacity of proliferation was gradually inhibited by age.
(Horibe et al., 2014a)	Chronological Aging	DPSCs	6	Cumulative Cell Number	~10^{12}*	~10^{11}*	NR	NR	-
(Iezzi et al., 2019)	Chronological Aging	DPSCs	3	PDT (days)	~4.84 ± 0.48	~13.95 ± 1.06	p<0.05	Senescent	S: Group C (62-66 years).
(Wang et al., 2025)	Chronological Aging	DPSCs	5	CCK-8 (450 nm)	~3.23 ± 0.18	~1.29 ± 0.13	p<0.001	Senescent	Data were extracted from day 7.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
(Christodoulou et al., 2013)	Serial Passaging	AD-MSCs	NR	PDT (hours)	~210 ± 55,38	~623.07 ± 83.08	NR	NR	NS: P3, S: P10. CPD and Proliferation Index was performed and exhibited severely limited proliferative capacity, with an early plateau in cumulative PD and a rapid decline in proliferation index after passage 2.
(Danisovic et al., 2017)	Serial Passaging	AD-MSCs	5	Proliferation Index (%)	~40.72 ± 0.43	~34.78 ± 0.26	$p<0.01$	Senescent	NS: P1, S: P30. The proliferation index increased until P10, slightly decreased, and remained stable until P25; by P30, proliferative activity was reduced to 32%.
(Figiel-Dabrowska et al., 2022)	Serial Passaging	AD-MSCs	4-6	PDT (days)	~3.81 ± 0.37	~13.53 ± 0.03	NR	NR	NS: P3, S: P20. The study evaluated different O_2 concentrations,

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									however only data from the 21% O_2 group (standard culture conditions) were extracted for analysis. Data from the 5% O_2 group were documented but excluded as an experimental intervention outside the scope of this review. Additional assays were performed but focused on different O_2 concentrations.
(Gruber et al., 2012)	Serial Passaging	AD-MSCs	3	Mean DT (hours)	~55.56 ± 4.2	~89.07 ± 10.63	NR	NR	NS: P1-P4, S: P5-P11.
(Ma et al., 2019a)	Serial Passaging	AD-MSCs	5	Cell Count (10^5)	~10.16*	~18.14*	NR	NR	NS: P3, S: P6. Data from day 3.
(Wu et al., 2022)	Serial Passaging	AD-MSCs	3	PDT (days)	~1 ± 0.03 ~1.26 ± 0.09	~1.48 ± 0.04 ~2.67 ± 0.51	NR	NR	NS: P3; S: P10. Infant and adult donors. Growth curve analysis showed higher

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									fold increases in cell number at early versus late passages.
(Zhang et al., 2024)	Serial Passaging	AD-MSCs	4	BrdU (360 nm)	~0.25 ± 0.008	~0.21 ± 0.006	$p<0.05$	Senescent	NS: P4, S: P10.
(Bertolo *et al.*, 2016)	Serial Passaging	BM-MSCs	7	PDT (hours)	~4,309.63 ± 376.57	~7,405.85 ± 1,317.99	$p<0.05$	Senescent	NS: P3; S: P10. P3 vs P9 ($p<0.05$). CPD and CFU assays were performed, revealing marked inter-donor variability in cumulative population doublings and CFU counts, with progressive decline in colony numbers from P3 to P11.
(Bonab *et al.*, 2006)	Serial Passaging	BM-MSCs	NR	PD (passage)	2.6 ± 0.68	P10: 1.2 ± 0.73	not significant	not significant	NS: P3; S: P10. CPD analysis was performed and revealed a gradual decline in proliferative potential. After

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									120 days in culture, proliferative potential decreased more rapidly.
(Kim et al., 2021)	Serial Passaging	BM-MSCs	NR	PDT (days)	LD: 1.3±0.02 MD: 1.9±0.03 HD: 1.8±0.05	LD: 3.4±0.10 MD: 5.6±0.82 HD: 22.2±3.48	NR	NR	NS: P5, S: P15. The acronyms LD, MD, and HD refer to Low-Density (50 cells/cm²), Medium-Density (1,000 cells/cm²), and High-Density (4,000 cells/cm²) cultures, respectively.
(Ma et al., 2019a)	Serial Passaging	BM-MSCs	5	Cell Count (10^5)	~6.94*	~7.96*	NR	NR	NS: P3, S: P6. Data from day 3.
(Wenjing et al., 2024)	Serial Passaging	BM-MSCs	3	MTT (490 nm)	~0.743	~0.447	NR	NR	NS: P5, S: P15. Data from day 7.
(Diomede et al., 2017)	Serial Passaging	DPSCs	3	MTT (650 nm)	~18,666.45 ± 444.45	~17.925.92 ± 641.98	not significant	not significant	NS: P2; S: P15. Data from day 7. The Trypan Blue exclusion test was performed and

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									revealed no statistically significant differences between passages.
(Ma et al., 2019)	Serial Passaging	DPSCs	5	Cell Count (10^5)	~27.79 ± 2.04	~37.38*	NR	NR	NS: P3, S: P6. Data from day 3.
(Diomede et al., 2017)	Serial Passaging	PDLSCs	3	MTT (650 nm)	~10,447.59 ± 425.77	~9661.57 ± 425.76	not significant	not significant	NS: P2; S: P15. Data from day 7. The Trypan Blue exclusion test was performed and revealed no statistically significant differences between passages.
(Hu et al., 2024)	Serial Passaging	PDLSCs	3	CCK-8 (450 nm)	~0.43 ± 0.03	~0.27 ± 0.02	p<0.001	Senescent	NS: P4, S: P20. Data from day 7.
(Jia et al., 2025)	Serial Passaging	PDLSCs	3	CCK-8 (450 nm)	~3.2*	~0.31 ± 0.05	p<0.001	Senescent	NS: P5, S: P20. Data from day 6.
(Ma et al., 2019)	Serial Passaging	PDLSCs	5	Cell Count (10^5)	~20.67 ± 2.71	~27.87 ± 1.77	NR	NR	NS: P3, S: P6. Data from day 3.
(Yang et al., 2021)	Serial Passaging	PDLSCs	3	CCK-8 (450 nm)	~2.46 ± 0.1	~1.5*	NR	NR	NS: 3, S: P12. Data from day 7.
(Christodoulou et al., 2013)	Serial Passaging	UC-MSCs-WJ	NR	PDT (hours)	~34.46 ± 7.5	~38.98 ± 6.25	NR	NR	NS: P3, S: P10. CPD and Proliferation Index analysis

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									demonstrated a high and sustained proliferative capacity, with continuous accumulation of PD over at least 15 passages and a peak Proliferation Index at passage 6.
(Zhuang et al., 2015)	Serial Passaging	UC-MSCs-WJ	3	MTT (570 nm)	~0.64 ± 0.04	~0.42 ± 0.04	NR	NR	NS: P3, S: P15. Data from day 6.
(Jin et al., 2016)	Serial Passaging	UC-MSCs-CB	2	Fold Increase	MSC1: ~1 ± 0.03 MSC2: ~1 ± 0.1 MSC3: ~1 ± 0.0	MSC1: ~0.34 ± 0.04 MSC2: ~0.22 ± 0.03 MSC3: ~0.37 ± 0.09	NR	NR	NS: P5, S: P13.
(Kim et al., 2020)	Serial Passaging	UC-MSCs-CB	4	PD	~4.05 ± 1.3	~1.74 ± 0.46	NR	NR	NS: P3, S: P14. Cells were grouped by size (heterogeneous, large, and small); the reported data

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Proliferative Potential	Notes
									refer to the heterogeneous group.

Supplementary Table 4. Individual results for the proliferation outcome. This table summarizes individual study results evaluating proliferation-related outcomes in non-senescent (NS) and senescent (S) stem cell populations. Studies are first grouped according to the type of senescence induction (inflammatory stimuli, Chronological aging, and serial passaging) and then sub-grouped by cell type (AD-MSCs, BM-MSCs, DPSCs, PDLSCs, UC-MSCs-WJ and UC-MSCs-CB). Data are presented as mean ± SD or SEM, as reported in the original studies. Different assays were used to assess proliferative capacity, including BrdU incorporation, cell number, population doublings (PD), doubling time (DT), population doubling time (PDT), cumulative population doublings (CPD), and colony-forming unit (CFU) assays. When more than one assay was used to assess the same outcome, only one was included in the table. NR indicates not reported. Values preceded by a tilde (~) indicate data extracted manually from figures when numerical results were not reported in the text. An asterisk (*) indicates that SD or SEM was not reported in the original study. In some studies, NS and S groups represent different experimental controls (e.g., Lenti-Control or Control-siRNA) and were not directly compared.

SUPPLEMENTARY TABLE 5 Individual results for the osteogenic differentiation outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Ma et al., 2019)	Inflammatory Stimuli – LPS and TNF- α	BM-MSCs	5	Alizarin Red (area - %)	~69.38 ± 5.28	LPS: ~25.33 ± 2.32 TNF-α: ~22.24 ± 1.76	NR	NR	LPS: 1 µg/mL TNF-α: 100 ng/mL 2 weeks.
(Ma et al., 2019)	Inflammatory Stimuli – LPS and TNF- α	DPSCs	5	Alizarin Red (area - %)	~84.36 ± 8.15	LPS: ~39.64 ± 4.41 TNF-α: ~19.82 ± 3.52	NR	NR	LPS: 1 µg/mL TNF-α: 100 ng/mL 2 weeks.
(Alt et al., 2012)	Chronological Aging	AD-MSCs	5	ALP Concentration (ng)	~48.73 ± 1.66	~21.34 ± 1.18	NR	NR	NS: group 1 (<20 years), S: group 3 (>50 years). 12 days. Significantly decreased with age; although the p-value was not reported, this finding was described in the results.
(Choudhery et al., 2014)	Chronological Aging	AD-MSCs	3	Von Kossa staining (% positive area)	20.0% ± 1.7	8.9% ± 2.2	p<0.001	Senescent	NS: group 1 (<30 years), S: group 3 (>60 years). Similar results were obtained when gene

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									expression of OST and ALP was analyzed through quantitative RT-PCR. Induction time was not reported.
(Kornicka et al., 2015)	Chronological Aging	AD-MSCs	≥2	SEM/EDX (nº of bone nodules)	~42.82 ±2.46	~4.51 ± 1.18	$p<0.001$	Senescent	NS: >20 years, S: >70 years. 16 days. Cells from young donors exhibited greater osteogenic outcomes, including increased calcium and phosphorus deposition in the extracellular matrix, enhanced formation of mineralized nodules, higher ALP activity, and elevated expression of osteogenic markers (such as *OPN* and *OCN*). In contrast, these osteogenic features were progressively reduced in cells

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									isolated from older donors.
(Legzdina et al., 2016b)	Chronological Aging	AD-MSCs	NR	Alizarin Red S staining (Calcium deposits)	positive	positive	NR	NR	NS: CS-8 (27 years), S: CS-8 (63 years). 30 days. Alkaline phosphatase activity was positive in both age groups.
(Wu et al., 2013)	Chronological Aging	AD-MSCs	NR	Calcium content (µmol/µg)	~6.41 ± 0.59	~4.0.3 ± 0.13	$p<0.05$	Senescent	NS: infant, S: adult. Data are shown for day 21; days 7 and 14 were also evaluated. Osteogenic differentiation decreased with age, with higher mineralized matrix deposition and *RUNX-2* expression in infant donors and reduced calcium phosphate content in elderly donors. Paracrine osteoinductive factor expression, including *BMP-2*

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									and *TGF-β*, was preserved across ages.
(Wu et al., 2022)	Chronological Aging	AD-MSCs	3	RT-qPCR - *OCN*	~1.96 ± 0.51	~27.14 ± 3.08	p<0.001	Non-Senescent	NS: Infant, S: Adult. 7 days. *RUNX* expression was higher in the adult group.
(Ye et al., 2016)	Chronological Aging	AD-MSCs	6	Alizarin Red Staining (% positive area)	86.7 ± 10.0	65.1 ± 15.1	p<0.05	Senescent	2 weeks. Alizarin Red S solubilization (OD 562 nm) revealed a significant reduction in the S group (p < 0.01).
(Stolzing et al., 2008)	Chronological Aging	BM-MSCs	NR	ALP activity (nmol p-NP/min/10^4 cells)	1.45 ± 0.1	0.54 ± 0.04	p<0.001	Senescent	10 days.
(Chen et al., 2024)	Chronological Aging	DPSCs	NR	Alizarin Red S staining	positive	reduced	NR	NR	21 days. No quantitative analysis was performed.
(Feng et al., 2014b)	Chronological Aging	DPSCs	3	Alizarin Red Staining (OD)	~1 ± 0.08	~0.29 ± 0.04	p<0.05	Senescent	21 days.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Horibe et al., 2014a)	Chronological Aging	DPSCs	6	Alizarin Red S staining	positive	positive	Not significant	not-significant	28 days. *OCN* expression assessed by RT-PCR was similar in both groups.
(Iezzi et al., 2019)	Chronological Aging	DPSCs	3	Alizarin Red Staining (540 nm)	~5.40 ± 0.29	~2.32 ± 0.13	$p<0.05$	Senescent	NS: group A (21 years), S: group C (64 years). 14 days. 3 weeks. Reduced ALP staining and significantly decreased osteogenesis-related gene expression were observed in aged group (*RUNX2, BGLAP, BMP2, β-catenin, DMP1, DSPP*).
(Wang et al., 2025)b	Chronological Aging	DPSCs	NR	Alizarin red S staining	positive	reduced	NR	NR	7 days.
(Christodoulou et al., 2013)	Serial Passaging	AD-MSCs	3	Alizarin Red Staining	>50 nodules	<20 nodules	NR	NR	NS: P2, S: P10. 21 days. Early passage showed robust osteogenic capacity assessed by calcium deposition, and CFU-ALP assays,

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									whereas late passage (P10) cells exhibited reduced osteogenic outcomes.
(Gruber et al., 2012)	Serial Passaging	AD-MSCs	NR	Alizarin Red Staining	positive	positive	Not significant	not-significant	NS: P1, S: P12. No quantitative analysis was performed; however, both passages analyzed tested positive for osteogenic potential by forming mineralized nodules in vitro. Induction time was not reported.
(Legzdina et al., 2016b)	Serial Passaging	AD-MSCs	NR	Alizarin Red S staining (Calcium deposits)	positive	reduced	NR	NR	NS: P3, S: P9. CS-8 donor (27 years) Alkaline phosphatase activity was positive in both passages. Induction time was not reported.
(Ma et al., 2019a)	Serial Passaging	AD-MSCs	5	Alizarin Red (area - %)	~65.26 ± 9.92	~43.92 ± 1.73	$p \leq 0.01$	Senescent	NS: P3, S: P6. 2 weeks.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Truong, et al., 2019)	Serial Passaging	AD-MSCs	NR	Alizarin Red staining	positive	reduced	NR	NR	NS: P5, S: P15. 30 days.
(Wang et al., 2025)a	Serial Passaging	AD-MSCs	3	Alizarin Red Staining (405 nm)	~2.77 ± 0.89	~3.12 ± 0.18	NR	NR	NS: P4, S: P8. 3 weeks.
(Bertolo et al., 2016)	Serial Passaging	BM-MSCs	7	RT-PCR - *OPN*	~1 ± 0.01	~0.58 ± 0.63	Not significant	not-significant	NS: P3. S: P11. 21 days. Mineralized matrix deposition assessed by von Kossa staining was higher in early passages and decreased with in vitro aging, with significant inter-donor variability (P3 vs P9).
(Bonab et al., 2006)	Serial Passaging	BM-MSCs	NR	Von Kossa and ALP (%)	100	20	NR	NR	NS: P1-4, S: P10. 2-3 weeks.
(Cheng et al., 2011a)	Serial Passaging	BM-MSCs	3	Alizarin Red (595 nm)	~0.32 ± 0.03	~0.54 ± 0.04	$p<0.01$	Senescent	Ns: P4, S: P12. 21 days. *ALPL* expression was higher in late passage group.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Kim et al., 2021)	Serial Passaging	BM-MSCs	NR	Alizarin Red (405 nm)	LD: ~0.49 ± 0.05 MD: ~0.54 ± 0.1 HD: ~0.48 ± 0.03	LD: ~0.23 ± 0.03 MD: ~0.24 ± 0.02 HD: ~0.11 ± 0.02	NR	NR	NS: P5, S: P15. Alizarin Red S mineralization decreased in a passage-dependent manner, with reduced *ALPP* and *SPP1* in late passage; no direct statistical test between P5 and P15 was performed (described in text). LD, MD, and HD denote low-, medium-, and high-density cultures. Induction time was not reported.
(Ma et al., 2019a)	Serial Passaging	BM-MSCs	5	Alizarin Red (area - %)	~83.63 ± 6.94	~52.60 ± 6.95	$p \leq 0.01$	Senescent	NS: P3, S: P6. 2 weeks.
(Wagner et al., 2008)	Serial Passaging	BM-MSCs	NR	Alizarin Red Staining (595 nm)	~0.22 ± 0.02	~0.42 ± 0.13	NR	NR	NS: P3, S: P11. 21 days. Von Kossa Staining showed higher mineralization in late passage.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Wenjing et al., 2024)	Serial Passaging	BM-MSCs	3	ALP Staining (Positive Cell Rate-%)	94.1 ± 3.3	82.4 ± 8.7	Not significant	not-significant	NS: P5, S: P15. Induction time was not reported.
(Diomede et al., 2017)	Serial Passaging	DPSCs	2	qRT-PCR - *ALP*	~0.9 ± 0.05	~1.1 ± 0.08	Not significant	not-significant	NS: P2, S: P15. 28 days. *RUNX* expression was assessed and showed higher levels in early passage; however, no significant difference was observed. Alizarin Red S staining revealed mineralized nodules in both passages.
(Li et al., 2025)	Serial Passaging	DPSCs	3	Alizarin Red Staining (562 nm)	~2.24 ± 0.34	~0.66 ± 0.12	p<0.001	Senescent	NS: P3, S: P12. 21 days.
(Ma et al., 2019a)	Serial Passaging	DPSCs	5	Alizarin Red (area - %)	~80.14 ± 5.21	~71.46 ± 5.71	Not significant	not-significant	NS: P3, S: P6. 2 weeks.
(Diomede et al., 2017)	Serial Passaging	PDLSCs	2	qRT-PCR - *ALP*	~0.98 ± 0.04	~1.06 ± 0.09	Not significant	not-significant	*RUNX* expression was assessed and showed higher

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									levels in late passage; however, no significant difference was observed. Alizarin Red S staining revealed mineralized nodules in both passages.
(Jia et al., 2025)	Serial Passaging	PDLSCs	3	ALP Activity Assay (U/mg protein)	~81.94*	~14.85 ± 0.96	p<0.001	Senescent	NS: P5, S: P20. 21 days. *COL1, ALP, and RUNX2* expression was assessed and showed decreased levels at late passages, along with fewer mineralized nodules observed in the Alizarin Red S staining assay.
(Ma et al., 2019a)	Serial Passaging	PDLSCs	5	Alizarin Red (area - %)	~70.22 ± 6.2	~55.83 ± 3.97	Not significant	not-significant	NS: P3, S: P6.
(Yang et al., 2021)	Serial Passaging	PDLSCs	3	Alizarin Red S Staining (562 nm)	~1 ± 0.09	~0.46 ± 0.07	p<0.01	Senescent	NS: P3, S: P12. 28 days. ALP activity was reduced, as indicated by quantitative

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									assessment and weaker staining. Expression of osteogenic genes (*ALP, RUNX2, OCN, COL-I*) was lower compared with late passage.
(Cheng et al., 2011a)	Serial Passaging	UC-MSCs-WJ	NR	Alizarin Red (595 nm)	~0.43 ± 0.03	~1.18 ± 0.03	$p<0.01$	Non-Senescent	NS: P4, S: P23. 21 days. *ALPL* expression was higher in late passage group.
(Christodoulou et al., 2013)	Serial Passaging	UC-MSCs-WJ	3	Alizarin Red Staining	>50 nodules	<20 nodules	NR	NR	NS: P2, S: P20. 21 days. Early passage showed robust osteogenic capacity assessed by calcium deposition, and CFU-ALP assays, whereas late passage (P20) cells exhibited reduced osteogenic outcomes.
(Govarthanan et al., 2025)	Serial Passaging	UC-MSCs-WJ	3	RT-qPCR - *OPN*	~8.09 ± 0.57	~2.03 ± 0.11	$p<0.0001$	Senescent	NS: P3, S: P15. 21 days. *COL1A1, RUNX2* expression were assessed and

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
									showed reduced levels in late passage, along with fewer mineralized nodules observed in the Alizarin Red S staining assay.
(Scheers et al., 2013)	Serial Passaging	UC-MSCs-WJ	5	Alizarin Red Staining (bone matrix deposition area - %)	~91.36 ± 1.36	~1.36 ± 0.45	NR	NR	NS: P3, S: P21. A significant difference was observed between P9 and P21 ($p < 0.001$). The percentage of area with bone matrix deposition was similar at passages 3, 9, and 18.
(Jin et al., 2016)	Serial Passaging	UC-MSCs-CB	NR	ALP staining	positive	reduced	NR	NR	NS: P5, S: P13. 14 days.

Supplementary Table 5. Individual results for the osteogenic differentiation outcome. This table summarizes individual study results evaluating osteogenic differentiation capacity in non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (inflammatory stimuli, Chronological aging, serial passaging) and sub-grouped by cell type (AD-MSCs, BM-MSCs, DPSCs, PDLSCs, UC-MSCs-WJ, UC-MSCs-CB). Data are presented as mean ± SD or SEM, as reported in the original studies. A variety of osteogenic assays were used, including Alizarin Red staining (area, optical density), von Kossa staining, alkaline phosphatase (ALP)

activity, calcium content, RT-qPCR of osteogenic markers (e.g., *OCN, OPN, RUNX2*), and others. When multiple assays were reported for the same outcome, only one representative assay was included. NR indicates “not reported”. Values preceded by a tilde (~) indicate data extracted manually from figures when numerical results were not reported in the text. An asterisk (*) denotes that SD or SEM was not provided in the original article.

SUPPLEMENTARY TABLE 6 Individual results for the adipogenesis differentiation outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Alt et al., 2012)	Chronological Aging	AD-MSCs	5	Oil Red O Staining (% positive cells)	~33.29 ± 2.6	~9.78 ± 2.21	NR	NR	NS: group 1 (<20 years), S: group 3 (>50 years). 12 days. Reduced *LPL* and *CEBPA* expression and increased *LEP* expression were observed in the elderly group.
(Choudhery et al., 2014)	Chronological Aging	AD-MSCs	3	Oil Red O staining (% positive cells)	67.4% ± 7.1%	62.2% ± 13.0%	Not significant	not significant	NS: group 1 (<30 years), S: group 3 (>60 years). 21 days. *PPAR-γ, LPL* expression showed no difference between groups.
(Kornicka et al., 2015)	Chronological Aging	AD-MSCs	≥2	Oil Red O positive area (%)	~21.53 ± 1.95	~39.27 ± 2.93	$p<0.01$	Non-Senescent	NS: >20 years, S: >70 years. 14 days. *LPL* and *CEBPA* expression were reduced and *LEP* expression was increased in the senescent group. Lipid droplet formation was higher at early time points and similar between groups by day 14.
(Legzdina et al., 2016b)	Chronological Aging	AD-MSCs	NR	Oil Red O staining	positive	positive	NR	NR	NS: CS-8 (27 years), S: CS-8 (63 years). 16 days.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Wu et al., 2022)	Chronological Aging	AD-MSCs	NR	RT-qPCR - *LPL*	~42.25 ± 3.59	~75.56 ± 9.35	p<0.01	Non-Senescent	NS: Infant, S: Adult. 14 days. *PPARγ* expression was increased in adult group.
(Ye et al., 2016)	Chronological Aging	AD-MSCs	6	Oil Red O Staining (% positive cells)	~78.7% ± 9.8	~59.4% ± 12.7	p<0.05	Senescent	14 days. *PPARγ2* and *LPL* expression were significantly reduced in old group.
(Stolzing et al., 2008)	Chronological Aging	BM-MSCs	NR	Oil Red O Staining (% positive cells)	66% ± 12	59% ± 9.5	Not significant	not significant	NS: young (7-18 years), S: aged (>40 years).
(Feng et al., 2014)b	Chronological Aging	DPSCs	3	Oil Red O staining (OD)	~0.76 ± 0.08	~0.16 ± 0.04	p<0.05	Senescent	3 weeks.
(Horibe et al., 2014)	Chronological Aging	DPSCs	6	Oil red O staining	positive	positive	Not significant	not significant	*PPARγ* and *aP2* expression were similar in both groups.
(Iezzi et al., 2019)	Chronological Aging	DPSCs	NR	Oil Red O staining	positive	reduced	NR	NR	NS: group A (21 years), S: group C (64 years). 14 days.
(Legzdina et al., 2016)	Serial Passaging	AD-MSCs	NR	Oil Red O staining	positive	reduced	NR	NR	NS: P3, S: P9. CS-8 donor (27 years). 16 days.
(Truong, et al., 2019)	Serial Passaging	AD-MSCs	NR	Oil Red O staining	positive	positive	NR	NR	NS: P5, S: P15. 7 days. Qualitative analyses showed almost no difference.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Wang et al., 2025)a	Serial Passaging	AD-MSCs	3	Oil red staining (450 nm)	positive	reduced	NR	NR	NS: P4, S: P8. 2 weeks. Qualitative analyses showed dramatically decayed in late passage.
(Bertolo et al., 2016)	Serial Passaging	BM-MSCs	7	RT-qPCR - *PPAR-ɣ*	~0.98 ± 0.08	~6.44 ± 1.66	$p<0.05$	Non-Senescent	NS: P3, S: P11. 3 weeks. Qualitative analysis of Oil Red O staining showed increased lipid accumulation at late passages.
(Bonab et al., 2006)	Serial Passaging	BM-MSCs	NR	Staining with Oil red O (lipid vacuoles – %)	100	40	NR	NR	NS: PQ-P4, S: P9. 2-3 weeks.
(Cheng et al., 2011)a	Serial Passaging	BM-MSCs	3	Oil Red-O staining (490 nm)	~0.65 ± 0.06	~0.2 ± 0.05	$p<0.01$	Senescent	NS: P4, S: P12. 21 days. *CEBPα, PPARRɣ* expression was significantly higher at early passages.
(Kim et al., 2021)	Serial Passaging	BM-MSCs	NR	Oil Red O (500 nm)	LD: ~0.98 ± 0.05 MD: ~1.05 ± 0.14	LD: ~0.55 ± 0.03 MD: ~0.48 ± 0.05	NR	NR	NS: P5, S: P15. Oil Red O staining decreased in a passage-dependent manner; however, no direct statistical test between P5 and P15 was performed (described in the text). *PPARɣ* and *FABP4* expression appeared similar

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
					HD: ~1.05 ± 0.14	HD: ~0.53 ± 0.05			across passages. LD, MD, and HD denote low-, medium-, and high-density cultures. Induction time was not reported.
(Wagner et al., 2008)	Serial Passaging	BM-MSCs	NR	Oil Red (595 nm)	~0.48 ± 0.02	~0.17 ± 0.14	NR	NR	NS: P3, S: P11. 21 days.
(Wenjing et al., 2024)	Serial Passaging	BM-MSCs	3	Oil Red O Staining (Positive Cell Rate - %)	40.8 ± 2.0	34.2 ± 1.8	Not significant	not significant	NS: P5, S: P15. Induction time was not reported.
(Diomede et al., 2017)	Serial Passaging	DPSCs	2	RT-qPCR - *PPAR-γ*	~1 ± 0.06	~1.06 ± 0.08	Not significant	not significant	NS: P2, S: P15. 28 days. Oil red solution staining demonstrates the presence of lipid vacuoles at cytoplasmic level in both passages. *FABP4* expression showed no difference.
(Diomede et al., 2017)	Serial Passaging	PDLSCs	2	RT-qPCR - *PPAR-γ*	~1.31 ± 0.1	~1.32 ± 0.12	Not significant	not significant	NS: P2, S: P15. 28 days. Oil red solution staining demonstrates the presence of lipid vacuoles at cytoplasmic level in both passages. *FABP4* expression showed no difference.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Cheng et al., 2011)a	Serial Passaging	UC-MSCs-WJ	3	Oil Red-O staining (490 nm)	~0.27 ± 0.06	~0.11 ± 0.04	p<0.01	Senescent	NS: P4, S: P12. 21 days. *CEBPα* increased at late passages, while *PPARγ* was higher at early passages.
(Scheers et al., 2013)	Serial Passaging	UC-MSCs-WJ	5	Oil Red O staining (% of cells containing lipid droplets)	~69.23 ± 2.41	~7.9 ± 2.64	NR	NR	NS: P3, S: P21. A significant difference was observed between P9 and P21 ($p < 0.001$). The percentage of area with lipid droplets was similar at passages 3, 9, and 18.
(Jin et al., 2016)	Serial Passaging	UC-MSCs-CB	NR	Oil Red O staining	positive	reduced	NR	NR	NS: P5, S: P13. Induction time was not reported.

Supplementary Table 6. Individual results for the adipogenic differentiation outcome. This table summarizes individual study results evaluating adipogenic differentiation capacity in non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (Chronological aging, serial passaging) and sub-grouped by cell type (AD-MSCs, BM-MSCs, DPSCs, PDLSCs, UC-MSCs-WJ, UC-MSCs-CB). Data are presented as mean ± SD or SEM, as reported in the original studies. Adipogenic assays included Oil Red O staining (positive area, optical density, percentage of positive cells) and RT-qPCR of adipogenic markers (e.g., *PPARγ, LPL, CEBPA*). When multiple assays were reported for the same outcome, only one representative assay was included. NR indicates "not reported". Values preceded by a tilde (~) indicate data extracted manually from figures when numerical results were not reported in the text. An asterisk (*) denotes that SD or SEM was not provided in the original article.

SUPPLEMENTARY TABLE 7 Individual results for the chondrogenic differentiation outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Differentiation Potential	Notes
(Alt et al., 2012)	Chronological Aging	AD-MSCs	5	RT-PCR – *COL2A*	baseline	-3±0.4	NR	NR	S: group 3 (>50 years). 21 days. *BMP-6* and *COL10A* expression were reduced in aged group.
(Choudhery et al., 2014)	Chronological Aging	AD-MSCs	3	Alcian Blue staining (620 nm)	0.51 ± 0.06	0.17 ± 0.02	p<0.001	Senescent	S: group 3 (>60 years). 3 weeks. *AGGRECAN* and *COL2* expression were significantly reduced in aged group.
(Wu et al., 2022)	Chronological Aging	AD-MSCs	NR	Alcian Blue – diameter of pellets (mm)	~2.01 ± 0.06	~2.47 ± 0.13	p<0.05	Non-Senescent	NS: infant, S: adult. Day 21. *SOX9, COL2* and *COL10* expression were significantly reduced in adult group.
(Ye et al., 2016)	Chronological Aging	AD-MSCs	6	Alcian Blue staining (620 nm)	0.76 ± 0.29	0.25 ± 0.16	p<0.05	Senescent	3 weeks. AGGRECAN and *COL2* were significantly reduced in aged group.
(Stolzing et al., 2008)	Chronological Aging	BM-MSCs	5	Alcian blue binding assay (630 nm)	3.2 ± 3	1.6 ± 1.9	Not significant	not significant	S: aged. 21 days.
(Iezzi et al., 2019)	Chronological Aging	DPSCs	NR	Alcian Blue staining	positive	positive	Not significant	not significant	S: group C (64 years). 14 days.

(Gruber et al., 2012)	Serial Passaging	AD-MSCs	NR	Alcian Blue staining	positive	positive	Not significant	not significant	NS: P1, S: P12. No quantitative analysis was performed; however, both passages analyzed tested positive for chondrogenic potential by forming micromass formation *in vitro*. Induction time was not reported.
(Truong, et al., 2019)	Serial Passaging	AD-MSCs	NR	Safranin O staining	positive	reduced	NR	NR	NS: P5, S: P15. 21 days.
(Wang et al., 2025)a	Serial Passaging	AD-MSCs	NR	Alcian Blue staining	positive	reduced	NR	NR	NS: P4, S: P8. 14 days
(Bertolo et al., 2016)	Serial Passaging	BM-MSCs	3	GAG/DNA (µg/µg)	~66.01 ± 9.91	~5.82 ± 4.47	$p<0.05$	Senescent	NS: P3, S: P11. 28 days. *COL2* were significantly reduced in late passage.
(Wenjing et al., 2024)	Serial Passaging	BM-MSCs	3	Safranin O Staining (Positive Cell Rate)	94.3 ± 6.5	84.5 ± 8.5	Not significant	not significant	NS: P5, S: P15. Induction time was not reported.
(Govarthanan et al., 2025)	Serial Passaging	UC-MSCs-WJ	3	RT-qPCR – *COL2A1*	~10.13 ± 0.39	~2.62 ± 0.22	$p<0.0001$	Senescent	NS: P3, S: P15. 28 days. *SOX9* expression was significantly reduced at late passage, whereas *COL1A1* expression was similar in both passages. Toluidine Blue staining was reduced in the late passage.

Supplementary Table 7. Individual results for the chondrogenic differentiation outcome. This table summarizes individual study results evaluating chondrogenic differentiation capacity in non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (Chronological aging,

serial passaging) and sub-grouped by cell type (AD-MSCs, BM-MSCs, DPSCs, UC-MSCs-WJ). Data are presented as mean ± SD or SEM, as reported in the original studies. Chondrogenic assays included Alcian Blue staining, Safranin O staining, glycosaminoglycan/DNA (GAG/DNA) quantification, pellet diameter measurement, and RT-qPCR of chondrogenic markers (e.g., *COL2A1, SOX9, AGGRECAN*). When multiple assays were reported for the same outcome, only one representative assay was included. Values preceded by a tilde (~) indicate data manually extracted from figures. NR indicates “not reported”.

SUPPLEMENTARY TABLE 8 Individual results for the migration outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Reduced Migration Potential	Notes
(Chen et al., 2024)	Chronological Aging	DPSCs	3	Transwell	~173.04 ± 18.64	~42.54 ± 4.3	p<0.001	Senescent	S: old group. 24 hours.
(Wenjing et al., 2024a)	Serial Passaging	BM-MSCs	3	Transwell	182.3 ± 18.3	19.2 ± 5.0	p<0.05	Senescent	NS: P5, S: P15. 24 hours. Scratch Healing Rate was assessed and showed reduced migration in late passage.
(Yang et al., 2021)	Serial Passaging	PDLSCs	3	Transwell (relative number of migrated cells)	~1 ± 0.06	~0.39 ± 0.03	p<0.01	Senescent	NS: P3, S: P12. 48 hours.

Supplementary Table 8. Individual results for the migration outcome. This table summarizes individual study results evaluating migratory capacity in non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (Chronological aging, serial passaging) and sub-grouped by cell type (DPSCs, BM-MSCs, PDLSCs). Data are presented as mean ± SD or SEM, as reported in the original studies. Migration was assessed using Transwell assays (number of migrated cells) and scratch-wound healing assays. Values preceded by a tilde (~) indicate data manually extracted from figures. NR indicates “not reported”.

SUPPLEMENTARY TABLE 9 Individual results for the immune response outcome.

Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Notes
(Huang et al., 2023)	Chronological Aging	BM-MSCs	3	Elisa (pg/mL)	TNF-α: ~29.26 ± 4.18 IL-6: ~30.31 ± 2.61 IL-10: ~107.14 ± 4.7	TNF-α: ~105.1 ± 1.59 IL-6: ~116.19 ± 7.92 IL-10: ~63.90 ± 4.76	NR	NS: Lenti-Control (YMSCs), S: Control-siRNA (AMSCs). Groups were not directly compared.
(Horibe et al., 2014a)	Serial Passaging	DPSCs	6	RT-PCR	*IL-1β*: ~1.5 *IL-6*: ~2.2 *IL-8*: ~2.5 *Groα*: ~2.1	*IL-1β*: ~3.2 *IL-6*: ~5.2 *IL-8*: ~6.1 *Groα*: ~4.8	NR	NS: P6, S: P20.
(Zhuang et al., 2015)	Serial Passaging	UC-MSCs[1]	3	RT-qPCR	baseline: 1	*IDO-1*: ~1.02 ± 0.12 *HMOX-1*: ~1.59 ± 0.14 *IL-10*: ~1.56 ± 0.11 *IL-6*: ~1.52 ± 0.12 *iNOS*: ~1.42 ± 0.13 *IL-1a*: ~0.59 ± 0.1 *TGF-β1*: ~0.98 ± 0.08 *IL-1β*: ~0.53 ± 0.14 *IFN-γ*: ~0.44 ± 0.08	$p<0.05$ except: *IDO-1* and *TGF-β1*	NS: P3, S: P15.
(Yu et al., 2014)	Serial Passaging	UC-MSCs[2]	3	Elisa – PGE_2 (pg/mL)	1. 400.48 ± 109.22 2. 436.89 ± 109.22	1. 364.07 ± 109.23 2. 327.66 ± 109.23	NR	NS: P5-10, S: P15-P20. 1 and 2 represent independent donor-derived hMSC lines (#1 and #2).

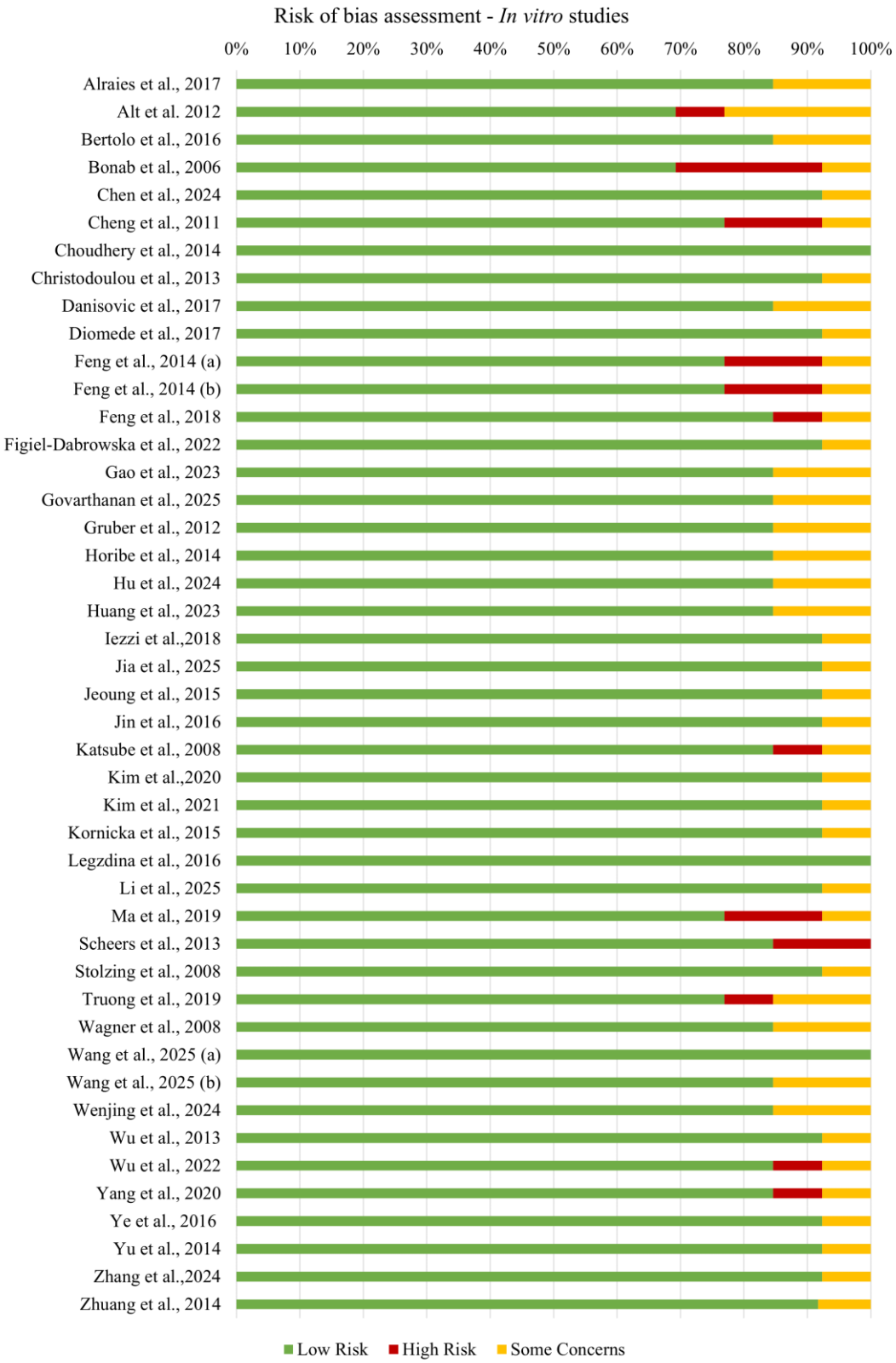
Supplementary Table 9. Individual results for the immune response outcome. This table summarizes individual study results evaluating immune-modulatory marker expression in non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (Chronological aging, serial passaging) and sub-grouped by cell type (BM-MSCs, DPSCs, UC-MSCs-WJ, UC-MSCs-CB). Data are presented as mean ± SD or SEM, as reported in the original studies. Immune-related markers were assessed by ELISA (cytokine secretion) or RT-qPCR (gene expression) for factors including *TNF-α, IL-6, IL-10, IL-1β, IL-8, Groat, IDO-1, HMOX-1, iNOS, TGF-β1, IFN-γ*, and PGE_2. Values preceded by a tilde (~) indicate data manually extracted from figures. NR indicates “not reported.”

SUPPLEMENTARY TABLE 10 Individual results for the tissue repair (*in vivo)* outcome.

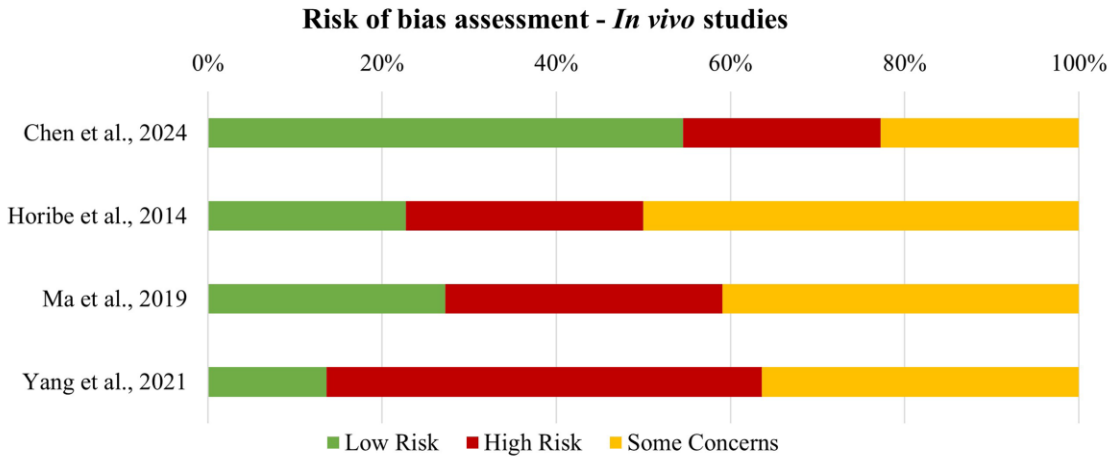
Author	Senescence Induction Method	Stem Cell Type	Nº of replicates (n)	Assay	NS (mean ± SD or SEM)	S (mean ± SD or SEM)	Significance (p-value)	Lower Tissue Repair	Notes
(Ma *et al.*, 2019)	Inflammatory Stimuli – LPS and TNF- α	BM-MSCs	5	HE staining (mineralization area - %)	~0.93 ± 0.16	~0.53 ± 0.49	NR	NR	Mineralization in periodontal tissue. Nude mice. LPS: 1µg/mL TNF-α: 100ng/mL
(Ma *et al.*, 2019)	Inflammatory Stimuli – LPS and TNF- α	DPSCs	5	HE staining (mineralization area - %)	~0.68 ± 0.11	~0.59 ± 0.08	NR	NR	Mineralization in periodontal tissue. Nude mice. LPS: 1µg/mL TNF-α: 100ng/mL
(Chen *et al.*, 2024)	Chronological Aging	DPSCs	6	Micro-CT analysis (bone mineral density g/cc)	~0.56 ± 0.05	~0.44 ± 0.04	NR	NR	Bone regeneration (rat calvarial bone defects). Sprague-Dawley rats.
(Horibe *et al.*, 2014)	Chronological Aging	DPSCs	4	Vascularization area/regenerated area	~9.17 ± 0.98	~7.21 ± 0.41	$p<0.05$	Senescent	Aged DPSCs exhibited reduced pulp regeneration, with smaller regenerated pulp tissue and fewer odontoblast-like cells compared with young DPSCs.
(Yang *et al.*, 2021)	Serial Passaging	PDLSCs	3	HE staining (mineralization)	positive	reduced	NR	NR	NS: P3, S: P12. Nude mice.

Supplementary Table 10. Individual results for the tissue repair (*in vivo*) outcome. This table summarizes individual study results evaluating *in vivo* tissue repair capacity of non-senescent (NS) and senescent (S) mesenchymal stem cell populations. Studies are grouped by senescence induction method (inflammatory stimuli, Chronological aging,

serial passaging) and sub-grouped by cell type (BM-MSCs, DPSCs, PDLSCs). Data are presented as mean ± SD or SEM, as reported in the original studies. *In vivo* repair outcomes were assessed using micro-CT analysis (bone mineral density) and histomorphometry (HE staining; mineralization area). Values preceded by a tilde (~) indicate data manually extracted from figures. NR indicates “not reported”.



SUPPLEMENTARY FIGURE 1 Risk of bias assessment of *in vitro* studies. Studies were appraised using the PETRICCS-based tool. Bars represent the percentage of items rated as “Yes” (low risk), “No” (high risk), or “Unclear” (some concerns).



SUPPLEMENTARY FIGURE 2 Risk of bias assessment of *in vivo* studies. Studies were appraised using the SYRCLE'S tool. Bars represent the percentage of items rated as "Yes" (low risk), "No" (high risk), or "Unclear" (some concerns).

CAPÍTULO 2 (Submitted: International Endodontic Journal; IF: 7.1)

Inflammation-Driven Senescence Reduces the Regenerative Capacity of apical papilla stem cell *in vitro*: Implications for regenerative endodontic therapy.

ABSTRACT

Aim: Senescence of stem cells from the apical papilla (SCAPs) may compromise their regenerative potential under the inflammatory conditions typical of regenerative endodontic procedures, but its impact on key cellular functions remains unclear. This study evaluated how senescence affects cellular metabolism, morphology, functional behavior, and the immunoinflammatory response of SCAPs under stimuli relevant to regenerative endodontic therapy (RET).

Methodology: Human SCAPs were assigned to non-senescent (NS) or senescent (S) groups. Senescence was induced by doxorubicin treatment and stimulated with control medium, LPS, or LPS plus IFN-γ. Senescence confirmation (SA-β-Gal, *p53*), mitochondrial activity (MTT), morphology (SEM), migration (scratch assay), proliferation (cell count) and cytokine expression (*TNF-α, IL-6, IL-10, TGF-β*) were assessed. Data were analyzed using appropriate statistical tests.

Results: Senescence induction was confirmed by increased SA-β-Gal ($p = 0.000041$) activity and *p53* expression ($p = 0.0368$). Mitochondrial metabolic activity was increased at baseline in senescent cells but remained unchanged between groups under inflammatory stimulation.; however, senescent SCAPs showed enlarged morphology (control: $p = 0.0006$; LPS: $p = 0.0008$; LPS plus IFN-γ: $p = 0.0017$) with reduced extensions (control: $p = 0.002$; LPS: $p = 0.0028$; LPS plus IFN-γ: $p = 0.0085$). Migration (control: $p = 0.0000000036$; LPS: $p = 0.00000001$; LPS plus IFN-γ: $p = 0.0000000021$) and proliferation (control: $p = 0.0026$; LPS: $p = 0.0042$; LPS plus IFN-γ: $p = 0.0018$) were significantly impaired across all conditions. Senescent cells exhibited higher *IL-6* (LPS $p = 0.002$; LPS plus IFN-γ $p = 0.000008$) and lower *TGF-β* expression ($p = 0.0003$; LPS $p = 0.0004$; LPS plus IFN-γ $p = 0.0005$), while *TNF-α* remained unchanged. *IL-10* expression increased only under LPS stimulation ($p = 0.002$), but this isolated response did not counteract the overall pro-inflammatory and dysregulated immunomodulatory profile.

Conclusions: Senescence markedly compromises critical SCAP functions and alters their immunoinflammatory behavior, which may negatively influence biological events essential for successful regenerative endodontic outcomes.

INTRODUCTION

Treatment of immature teeth with incomplete root development, whether resulting from pulp necrosis, irreversible pulpitis or dental trauma, poses a substantial clinical challenge due to their inherent structural fragility and open apical foramen (Jamshidi et al., 2018). Among the available therapeutic approaches, Regenerative Endodontic Therapy (RET) is particularly notable because it aims not only to disinfect the canal system but also to promote conti(Adel & Asgari, 2025; Jena et al., 2023) (Adel & Asgari, 2025; Jena et al., 2023). The success of RET fundamentally relies on tissue engineering principles, which require the presence of a mesenchymal stem cell (MSC) population capable of supporting regeneration (Ma et al., 2019; Salem et al., 2025). This population is primarily located within the apical papilla, a tissue rich in stem cells from the apical papilla (SCAPs) that serve as key effectors of the regenerative process (Q. Cheng et al., 2023; Smeda et al., 2022). Under favorable conditions, SCAPs are recruited into the canal space, where they proliferate and differentiate into similar dental tissues (Tawfik et al., 2013; Temmerman et al., 2012). Understanding the factors that modulate SCAP function is therefore essential for advancing the predictability and biological foundations of RET.

Despite its promise, RET exhibits variable success rates, and the biological factors underlying treatment failures remain insufficiently elucidated. It has been proposed that the persistence of an inflammatory microenvironment within the canal may play a critical role by inducing cellular senescence in SCAPs (Teawcharoensopa & Srisuwan, 2024b). Senescence is characterized as a stable and irreversible growth arrest triggered by cellular stresses such as DNA damage or chronic inflammation (Rodier et al., 2009). This state is mediated by activation of cell cycle regulators, including p16^INK4a^ and the p21/p53 pathway (Feng et al., 2018, 2014; Insinga et al., 2013). A hallmark of senescence is the development of the senescence-associated secretory phenotype (SASP), defined by sustained secretion of pro-inflammatory cytokines, such as IL-6, chemokines and proteolytic enzymes (Coppé et al., 2008). The SASP not only impairs local tissue regeneration but also perpetuates a self-reinforcing inflammatory milieu, comparable to inflammaging, by degrading the extracellular matrix and propagating senescence in neighboring cells (Rodier et al., 2009). These effects may critically undermine the regenerative potential of SCAPs (Z. Li et al., 2019a; Su et al., 2020). However, direct experimental evidence that senescence and SASP compromise SCAP functions that are

essential for RET remains limited, representing a knowledge gap that the present study aims to address.

Although several senescence-induction models are commonly used, including hydrogen peroxide, UV radiation and genotoxic agents, there is no consensus regarding which model best reflects the senescence processes occurring clinically (Cho et al., 2019b; Höfig et al., 2016b; Ma et al., 2019a). Within the endodontic setting, various pathological conditions are capable of generating a microenvironment conducive to SCAP senescence (G. Feng et al., 2018a). In addition to necrotic pulp, irreversible pulpitis and dental trauma can also elicit a highly inflammatory environment characterized by bacterial products, tissue damage, hypoxia and excessive release of immune mediators. As depicted, macrophage activation by LPS and related stimuli promotes the production of pro-inflammatory cytokines, including IL-6 and TNF-α, and reactive oxygen species (Hsu & Wen, 2002). T cells also modulate the immune response through signals such as IFN-γ (Zhu et al., 2024). Although regulatory mediators, such as IL-10 and TGF-β, attempt to limit inflammation, they may fail to prevent the establishment of persistent cellular stress (Putra et al., 2018). This constellation of factors fosters DNA damage and favors the onset of a senescent phenotype in stem cells residing in the apical papilla.

Despite these conceptual advances, it remains unclear whether existing *in vitro* models sufficiently replicate the functional consequences of this microenvironment. Among the available experimental approaches, senescence induction using genotoxic agents such as doxorubicin has emerged as a robust and reproducible method (Yaghoobi et al., 2020a). Although this model does not fully mimic the chronic inflammatory conditions observed clinically, it provides a controlled platform for investigating the mechanisms and functional outcomes of senescence in SCAPs, which justifies its use in mechanistic studies. In this study, doxorubicin was used to induce a senescent phenotype, which was subsequently challenged with immunoinflammatory stimuli, specifically LPS and IFN-γ, to more closely approximate the target clinical scenario. Then, the aim was to determine how induced senescence affects key SCAP functions, including cellular metabolism, proliferation, morphology, expression of senescence markers and immunoinflammatory responses.

MATERIALS AND METHODS

The manuscript of this laboratory study has been written following the Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines (Nagendrababu et al., 2021). The flowchart is presented in Figure 1.

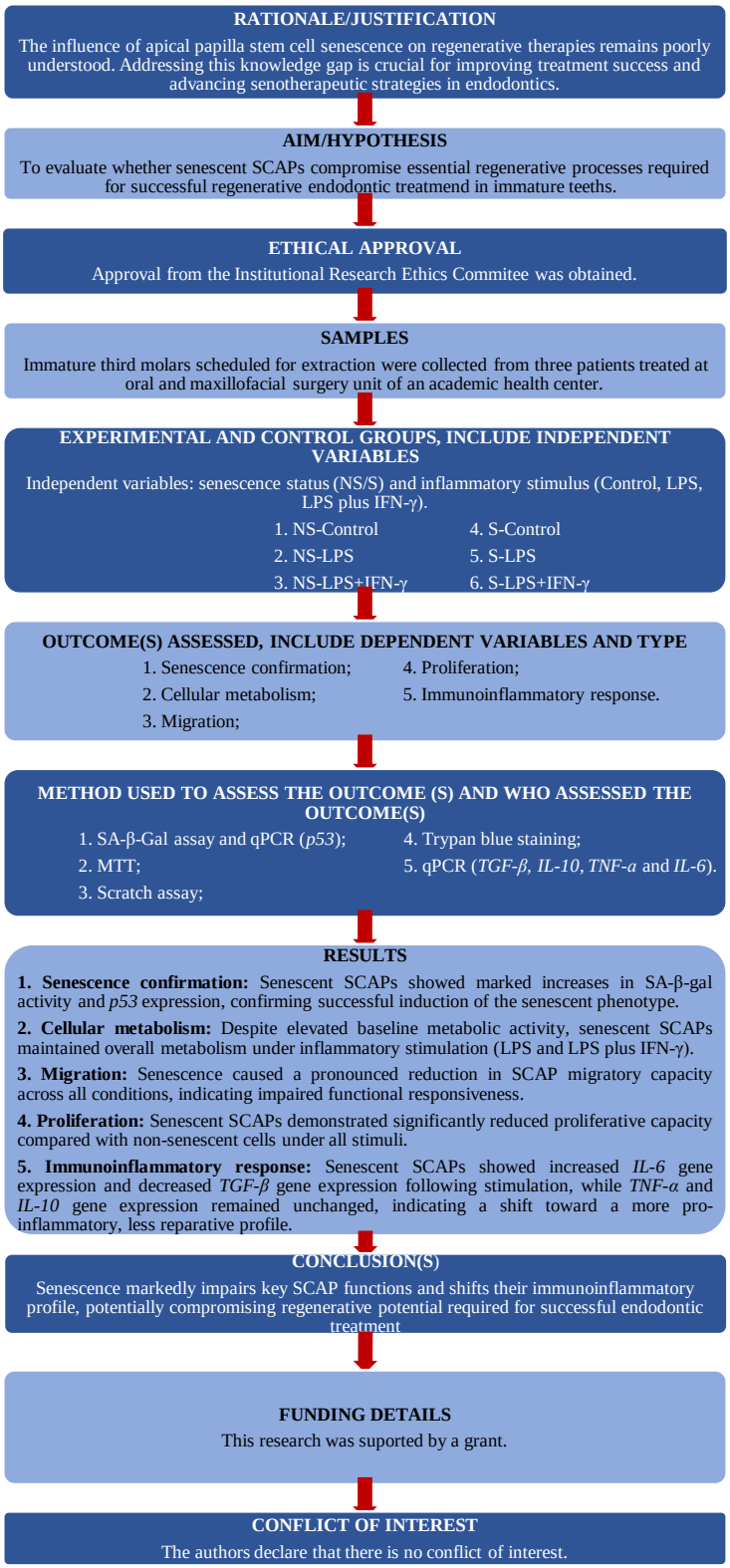


FIGURE 1 Flowchart for Preferred Reporting Items for Laboratory Studies in Endodontology (PRILE) 2021 guidelines.

Isolation and culture of SCAPs

Immature third molars indicated for extraction were obtained from three patients (two males, both aged 18 years, and one female, aged 15 years) undergoing dental treatment at the oral and maxillofacial surgery unit of an academic health center. Written informed consent was obtained from all participants or their legal guardians. This study was approved by the institutional Research Ethics Committees.

Apical papilla tissues were gently removed from the apical third of the roots using a sterile scalpel blade, fragmented, and transferred to six-well plates (Costar Corp., Cambridge, MA, USA) for explant culture. Tissue was cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Invitrogen, Carlsbad, CA, USA) supplemented with 20% (v/v) fetal bovine serum (Bionutrientes, São Paulo, Brazil), 100 $U.mL^{-1}$ penicillin, 100 $\mu g.mL^{-1}$ streptomycin, and 1% amphotericin (Invitrogen). Cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2, changing the medium every two days until 90% confluence was reached. Cells were then subcultured for expansion. To prevent replicative senescence, all experiments were performed using cells between passages 2 and 5.

Induction of cellular senescence by doxorubicin and immunoinflammatory stimulation using LPS and LPS plus IFN-γ

For senescence induction, cells were treated with $1\mu L.mL^{-1}$ doxorubicin (doxorubicin hydrochloride; Sigma-Aldrich, St. Louis, MO, USA) in supplemented DMEM for 24 hours. After treatment, the medium was replaced every two days for seven days (da Silva et al., 2025; de Farias et al., 2024). Non-senescent (NS) cells were cultured in complete DMEM under standard conditions. At the end of the induction period, both non-senescent and senescent (S) cells were stimulated with lipopolysaccharide (LPS; *Escherichia coli*, 1 $\mu g.mL^{-1}$; Sigma) and interferon-gamma (IFN-γ, 1 $\mu g.mL^{-1}$; Sigma) for 24 hours (Hong et al., 2021). Twenty-four hours after inflammatory stimulation, all experimental assays were performed.

Senescence-associated β-galactosidase assay

The β-galactosidase assay was performed in both senescent (S) and non-senescent (NS) groups to evaluate the effectiveness of senescence induction. SCAPs (2×10^5 cells/well) were seeded in 6-well plates (Costar Corp., Cambridge, MA, USA). The wells were aspirated and washed twice with phosphate-buffered saline (PBS), then fixed with 2% formaldehyde (Sigma-Aldrich, St. Louis, MO, USA) for 5 minutes at room temperature. After fixation, cells were washed again and incubated with 1 mL of X-Gal staining solution (40 mM citric acid/sodium phosphate buffer, 5 mM potassium ferrocyanide, 150 mM sodium chloride, 2 mM magnesium chloride, and 1 $mg.mL^{-1}$ X-Gal). The plates were wrapped in aluminum foil to protect from light and incubated at 37°C in a humidified chamber, without CO_2, for 12 hours. After incubation, the staining solution was aspirated, and the wells were washed twice with PBS for 30 seconds and once with methanol (Sigma-Aldrich) (Yaghoobi et al., 2020). Stained cells were quantified from five randomly selected microscopic fields per well (five images/well), captured using a phase-contrast microscope (Axio Observer D1, Zeiss, Oberkochen, Germany) at 5× magnification. Image analysis and cell counting were performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

MTT metabolic activity assay

To evaluate cellular metabolism, NS and S groups were seeded in 96-well plates (1×10^4 cells/well; Costar Corp., Cambridge, MA, USA) and treated with the previously described immunoinflammatory stimuli. After the incubation period, the medium was removed, and 100 µL of DMEM (Sigma-Aldrich, St. Louis, MO, USA) containing 10 µL of MTT solution (5 $mg.mL^{-1}$; Sigma-Aldrich) was added to each well. Plates were wrapped in aluminum foil and incubated for 4 hours at 37°C in 5% CO_2 and 95% humidity. The medium was then aspirated, and 100 µL of dimethyl sulfoxide (DMSO; Sigma-Aldrich) was added to each well to solubilize the formazan crystals, followed by gentle shaking for 30 minutes. Absorbance was measured at 570 nm using a microplate reader (SpectraMax Plus 384, Molecular Devices, San Jose, CA, USA) (Van De Loosdrecht et al., 1994). Negative control consisted of cells treated with lysis buffer (10 mM Tris, pH 7.4, 1 mM EDTA, and 0.1% Triton X-100).

Scanning electron microscopy (SEM)

To evaluate morphological alterations, round glass coverslips (13 mm × 13 mm; Fisher Scientific, Suwanee, GA, USA) were placed in 12-well plates, and 5×10^4 cells were seeded per well. After exposure to the previously described stimuli, cells were fixed with 0.1 M Karnovsky's solution (2% glutaraldehyde and paraformaldehyde) overnight, followed by washing with 0.1 M sodium cacodylate buffer. Subsequently, cells were post-fixed with 1% osmium tetroxide for 30 minutes and rinsed twice with distilled water. Dehydration was performed through a graded acetone series (50%, 70%, 90%, and 100%). The samples were dried in critical point equipment, model CPD 030 (Balzers, Liechtenstein) and metalized with gold with EM SCD 500 equipment (Leica, Austria). After drying, the coverslips were sputter-coated with a conductive metal layer and examined under a scanning electron microscope (JSM-7001F, JEOL Ltd., Tokyo, Japan) (dos Santos et al., 2019). Images were acquired at magnifications of 1500×, 3000×, and 5000×. Quantification of cell size and the number of cellular extensions was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA), analyzing approximately 15 cells per coverslip.

Cell migration assay

Cell migration was evaluated using the scratch assay, which simulates an artificial wound in a confluent monolayer, allowing cells at the wound edge to migrate toward the center in order to close the gap *in vitro*. For this assay, SCAPs were seeded in 6-well plates (2×10^5 cells/well; Prolab, São Paulo, Brazil). After reaching confluence, a linear scratch was made using a sterile 1000 µL pipette tip. Detached cells were removed by washing three times with PBS, and serum-free culture medium was then added to avoid cell proliferation during the migration process. Images were captured immediately after the scratch (0 h) and at 24 h and 48 h using a phase-contrast microscope (Axio Observer D1, Zeiss, Oberkochen, Germany) at 5× magnification (Martinotti & Ranzato, 2020). Wound closure was analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Cells located within the wound area were counted to provide a quantitative assessment of migration.

Cell proliferation assay

Cell proliferation was evaluated using Trypan Blue staining (Sigma-Aldrich, St. Louis, MO, USA). Cells (5×10^4 per well) were seeded in 24-well plates. Cells were then exposed to the experimental conditions in serum-free medium, to avoid interference from growth factors present in fetal bovine serum. At 24 and 48 h, cells were detached with 0.25% trypsin, resuspended, and mixed with 0.4% Trypan Blue solution (1:1, v/v) (Crowley et al., 2016). Viable cells were counted using a Neubauer chamber under a light microscope, excluding stained (non-viable) cells.

Gene expression analysis of inflammatory, anti-inflammatory, and senescence marker

Gene expression was quantified by real-time PCR using the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific). Transcripts associated with inflammatory, anti-inflammatory, and senescence-related processes were analyzed, including *TNF-α, IL-6, IL-10, TGF-β1*, and *p53*, with *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* serving as the endogenous reference gene control. PCR amplifications were performed using the SYBR™ Green PCR Master Mix (Thermo Fisher Scientific), and the specific primer sequences for each target gene are listed in Table 1. Reactions were run on the StepOnePlus™ platform, with fluorescent data acquired at the end of each cycle. Relative gene expression was calculated using the 2^-ΔΔCT method, with the control group used as the calibrator (Livak and Schmittgen, 2001).

TABLE 1 Primer sequences for each gene used in the qPCR assay.

GENE	*FOWARD*	*REVERSE*
GAPDH	TCAACGACCACTTTGTCAAGCTCAGCT	GGTGGTCCAGGGGTCTTAC
TGF-β1	GCTGTATTTAAGGACACCGTGC	TGACACAGAGATCCGCAGTC
TNF-α	CACAGTGAAGTGCTGGCAAC	GATCAAAGCTGTAGGCCCCA
IL-10	GGTGGTCCAGGGGTCTTAC	ACTCTGCTGAAGGCATCTCG
IL-6	TCAATATTAGAGTCTCAACCCCCA	TTCTCTTTCGTTCCCGGTGG
p53	AGAAAACCTACCAGGGCAGC	ACATCTTGTTGAGGGCAGGG

Statistical Analysis

Student's *t*-test (for parametric data) or the Mann-Whitney test (for non-parametric data) were used. Comparisons involving multiple groups were analyzed by one-way or two-way ANOVA, followed by appropriate post-hoc tests, as indicated in the figure legends. Data are presented as mean ± standard deviation (SD). A $p < 0.05$ was considered statistically significant. All analyses were performed using GraphPad Prism version 8 (GraphPad Software, CA, USA).

RESULTS

Senescence induction and metabolic activity assessment in SCAPs

Metabolic alterations associated with SCAP senescence are illustrated in Figure 2A. Senescent SCAPs displayed a marked increase in SA-β-Gal activity compared with

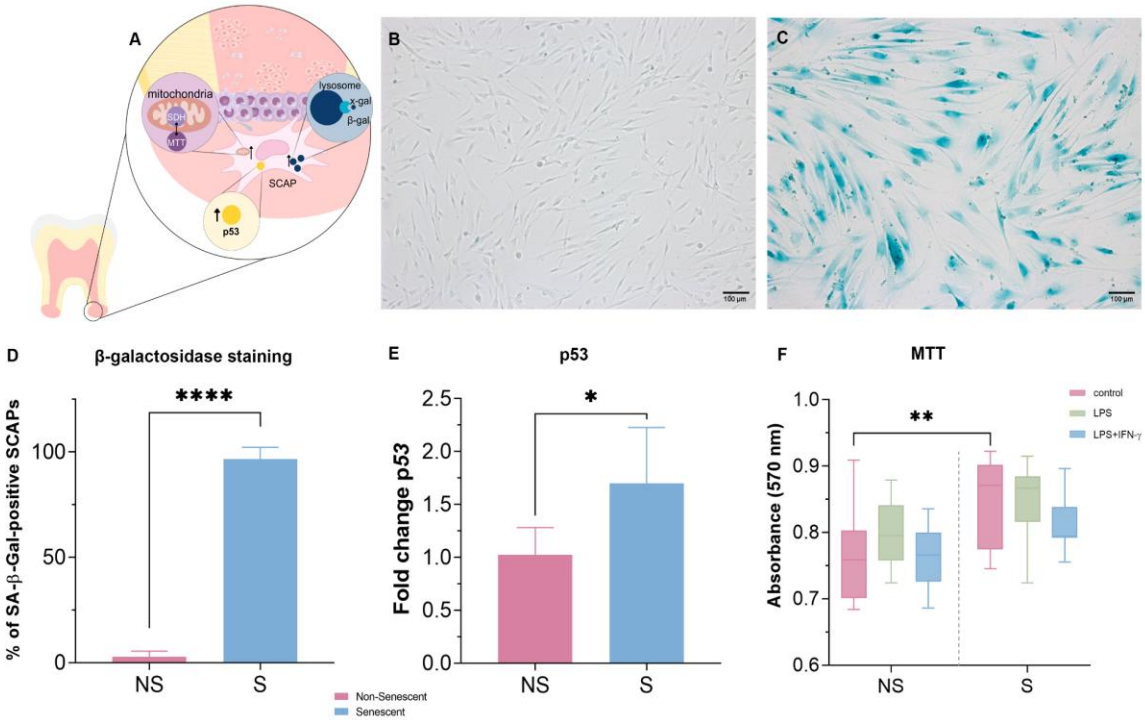


FIGURE 2 Schematic illustration of metabolic alterations in senescent SCAPs (A). Representative images of non-senescent control cells (B; NS) and doxorubicin-induced senescent cells (S; 500 µM) showing SA-β-Gal-positive cells (blue). Scale bar: 100 µm (C). Quantification of SA-β-Gal-positive SCAPs revealed significant increase in senescent cells ($p = 0.000041^{****}$, Mann–Whitney test; D). *P53* expression, represented as fold change, was significantly higher in senescent SCAPs compared with non-senescent controls ($p = 0.0368^{*}$, Student's t-test; E). MTT assay after 24-hour immunomodulatory stimulation with LPS (1 µg.mL^{-1}) or LPS plus IFN-γ (1 µg.mL^{-1}) showed higher metabolic activity in senescent cells under control conditions ($p = 0.0082$), whereas no significant differences were observed for LPS ($p = 0.2064^{**}$) or LPS plus IFN-γ ($p = 0.2282$) (F).

non-senescent cells (NS vs S; 2.82 ± 2.59 vs 96.64 ± 5.57; p = 0.000041) (Figure 2B–D), confirming successful senescence induction. Consistently, *p53* expression was significantly upregulated in senescent cells compared with non-senescent controls (1.02 ± 0.26 vs 1.7 ± 0.53; p = 0.0368) (Figure 2E). Mitochondrial metabolic activity was also higher under baseline conditions in senescent cells (0.76 ± 0.05 vs 0.84 ± 0.05; p = 0.0082); however, no significant differences were detected between groups after LPS (0.79 ± 0.03 vs 0.84 ± 0.03; p = 0.2064) or LPS plus IFN-γ stimulation (0.76 ± 0.03 vs 0.81 ± 0.03; p = 0.2282), indicating preserved cellular metabolism under inflammatory challenge (Figure 2F).

Morphological analysis of SCAPs by scanning electron microscopy

To evaluate the impact of SCAP senescence on cellular morphology, scanning electron microscopy (SEM) was performed at 1,500×, 3,000×, and 5,000× magnifications. SEM analysis revealed that senescent SCAPs exhibited an enlarged and morphologically altered phenotype with markedly fewer cellular extensions compared with non-senescent cells across all conditions (Figure 3A–B). Cell size increased significantly in senescent cells in control (NS vs S; 59.6 ± 7.39 vs 129.4 ± 9.48; p = 0.0006), LPS (87.06 ± 12.99 vs 156.5 ± 2.84; p = 0.0008) and LPS plus IFN-γ conditions (82.06 ± 5.7 vs 132.2 ± 9.99; p = 0.0017) (Figure 3C). Likewise, the number of cellular extensions was significantly reduced in senescent SCAPs under control (12.3 ± 1.4 vs 6.16 ± 0.4; p = 0.002), LPS (20.33 ± 3.6 vs 6.43 ± 0.11; p = 0.0028) and LPS plus IFN-γ conditions (15.6 ± 2.7 vs 6.4 ± 1.8; p = 0.0085) (Figure 3D), indicating compromised structural features that may be critical for regenerative endodontic processes, suggesting that cellular senescence may adversely affect tissue repair capacity and overall clinical outcomes.

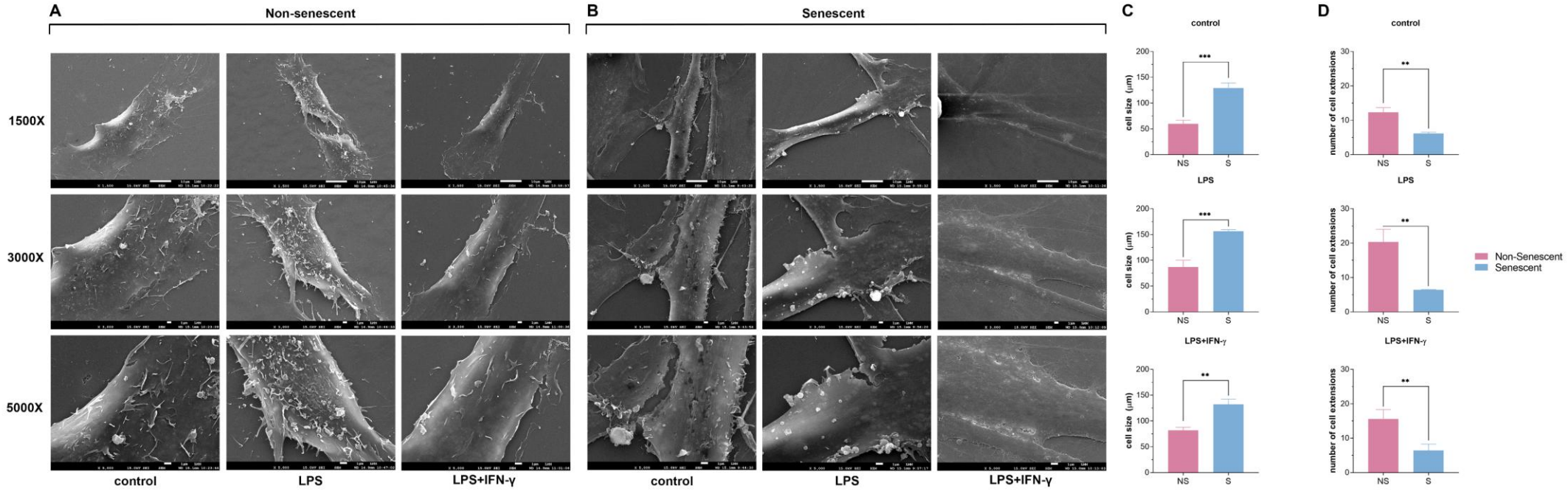


Figure 3 SEM of SCAPs. Representative images of non-senescent (NS, A) and senescent (S, B) cells after immunomodulatory stimulation with LPS or LPS plus IFN-γ, shown at 1,500×, 3,000×, and 5,000× magnifications. Scale bar: 10 μm. Graphs **show** cell size (C) and number of cellular extensions (D) under each condition. Cell size was significantly increased in senescent cells across all conditions (control: $p = 0.0006^{***}$; LPS: $p = 0.0008^{***}$; LPS plus IFN-γ: $p = 0.0017^{**}$), while senescent cells showed a significant reduction in cellular extensions compared with NS cells (control: $p = 0.002^{**}$; LPS: $p = 0.0028^{**}$; LPS plus IFN-γ: $p = 0.0085^{**}$). Statistical analyses were performed using Student's *t*-test.

Assessment of SCAP migration and proliferation

A schematic illustration shows the processes of migration and proliferation, which are essential for the success of RET (Figure 4A). Migration and proliferation assays demonstrated a marked functional impairment in senescent SCAPs. Senescent cells exhibited significantly reduced migratory capacity at both 24 and 48 hours under control (NS vs S; 223.7 ± 67.4 vs 45.17 ± 13.2; p = 0.0000000036), LPS (205.8 ± 51.15 vs 42.83 ± 17.91; p = 0.00000001), and LPS plus IFN-γ conditions (200.7 ± 52.56 vs 62.83 ±12.26; p = 0.0000000021), compared with non-senescent cells (Figure 4B–D). Similarly, senescent SCAPs showed significantly lower proliferative capacity at 24 hours across all stimuli, with only a modest increase at 48 hours in the control and LPS plus IFN-γ groups, while non-senescent cells maintained exponential growth under all conditions These differences were statistically significant (NS vs S; control 70.56 ± 21.75 vs 49 ± 10.54; p=0.0026), (LPS 69.89 ±19.36 vs 43 ± 6.69; p = 0.0042), (LPS plus IFN-γ 59 ± 10.59 vs 42.22 ± 7.37; p = 0.0018) (Figure 4E). However, because cellular migration and proliferation are essential for regenerative endodontic procedures, these findings indicate that senescence may markedly impair tissue repair capacity and compromise clinical outcomes.

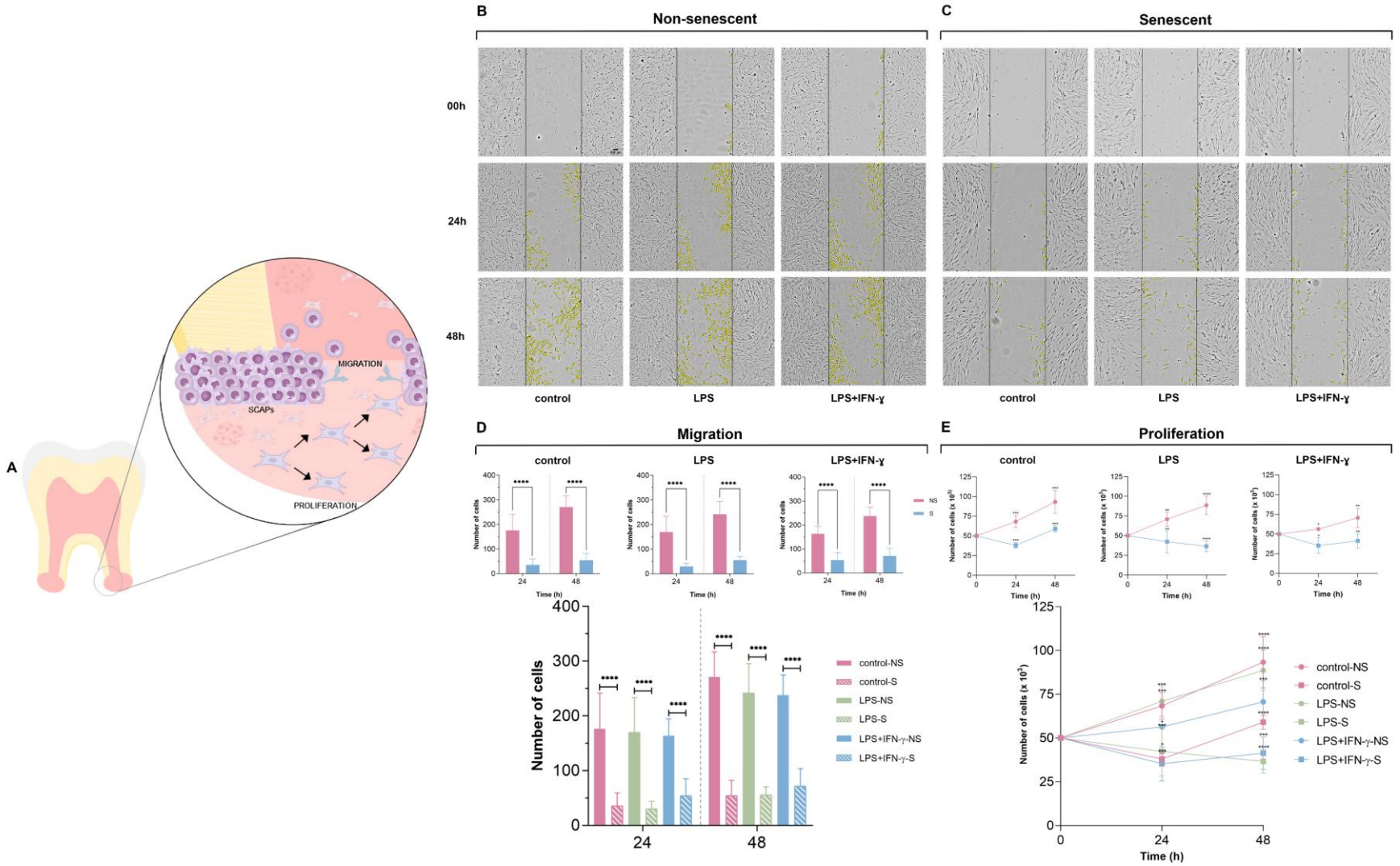


FIGURE 4 Schematic representation of SCAP functions related to regenerative endodontic therapy. SCAP migration and proliferation are shown as essential processes for tissue regeneration (A). Representative micrographs of NS and S SCAPs in the wound-healing migration assay at 0, 24, and 48 hours under control, LPS, or LPS plus IFN-γ stimulation (B–C). Quantification of migrated cells (control: $p = 0.0000000036^{****}$; LPS: $p = 0.00000001^{****}$; LPS plus IFN-γ: $p = 0.0000000021^{****}$) (D). Proliferative activity evaluated by Trypan Blue exclusion at 24 and 48 hours (NS vs. S; control: $p = 0.0026^{***}$; LPS: $p = 0.0042^{**}$; LPS plus IFN-γ: $p = 0.0018^{**}$). Statistical analysis was performed using two-way ANOVA.

Evaluation of immunomodulatory mediators

To evaluate the immunomodulatory profile of NS and S SCAPs, the expression of pro-inflammatory mediators (*TNF-α* and *IL-6*) and anti-inflammatory mediators (*TGF-β* and *IL-10*) was assessed. Overall, senescent SCAPs exhibited a distinct inflammatory phenotype compared with non-senescent cells. *TNF-α* expression showed no significant differences between groups under any condition (NS vs. S: control 1.014 ± 0.17 vs. 1.18 ± 0.35, p = 0.8; LPS 1.03 ± 0.26 vs. 1.31 ± 0.30, p = 0.4; LPS plus IFN-γ 1.06 ± 0.43 vs. 1.34 ± 0.88, p = 0.8) (Figure 5A). For *IL-10,* senescent cells displayed a significant increase only under LPS stimulation (control 0.94 ± 0.33 vs. 0.93 ± 0.17, p = 0.8; LPS 1.00 ± 0.09 vs. 2.60 ± 1.10, p = 0.002; LPS plus IFN-γ 1.16 ± 0.52 vs. 1.97 ± 1.22, p = 0.14) (Figure 5D). In contrast, *IL-6* expression was markedly elevated in senescent SCAPs following immunoinflammatory stimulation (control 1.01 ± 0.18 vs. 0.89 ± 0.16, p = 0.14; LPS 1.10 ± 0.46 vs. 9.02 ± 1.49, p = 0.002; LPS plus IFN-γ 1.04 ± 0.31 vs. 12.77 ± 3.46, p = 0.000008) (Figure 5B). Additionally, *TGF-β* expression was significantly reduced in senescent cells across all conditions (control 1.02 ± 0.23 vs. 0.46 ± 0.09, p = 0.0003; LPS 1.02 ± 0.21 vs. 0.36 ± 0.22, p = 0.0004; LPS plus IFN-γ 1.03 ± 0.31 vs. 0.32 ± 0.13, p = 0.0005), indicating impaired immunoregulatory potential (Figure 5C). Together, these findings demonstrate that senescent SCAPs shift toward a pro-inflammatory phenotype, characterized by heightened *IL-6* production and reduced *TGF-β* expression, while displaying limited capacity to sustain anti-inflammatory and reparative signaling

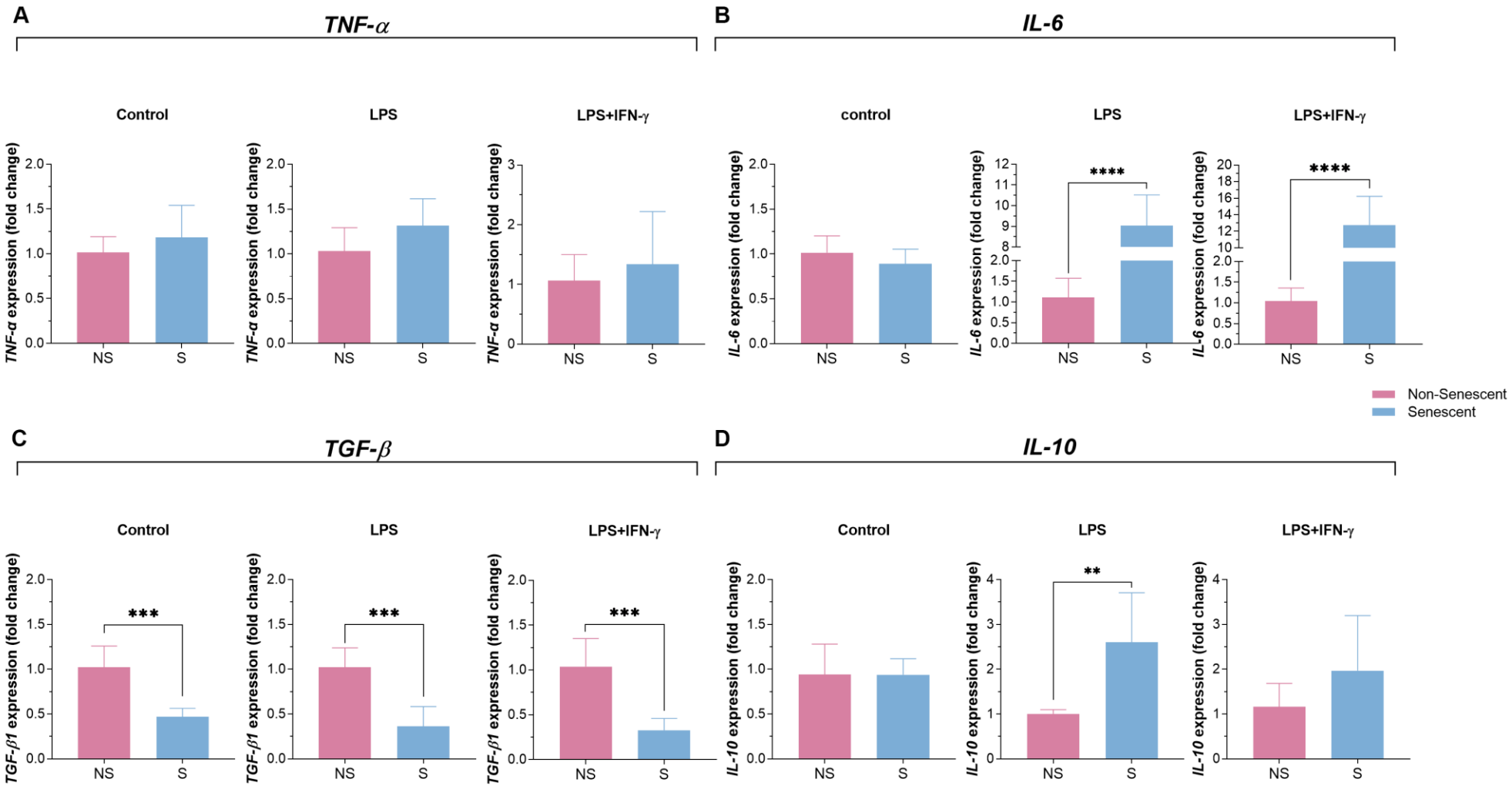


FIGURE 5 Gene expression of pro- and anti-inflammatory mediators in senescent and non-senescent SCAP cultures following stimulation with LPS or LPS plus IFN-γ. *GAPDH* was used as the constitutive gene for normalization. Expression levels of *TNF-α* conditions (control p = 0.8; LPS p = 0.4; LPS plus IFN-γ p = 0.8) (A), *IL-6* (LPS p = 0.002**; LPS plus IFN-γ p = 0.000008****) (B), *TGF-β* (control p = 0.0003***; LPS p = 0.0004***; LPS plus IFN-γ p = 0.0005***) (C), and *IL-10* (control p = 0.8; LPS p = 0.002**; LPS plus IFN-γ p = 0.14) (D). Statistical analysis was performed using the Student's *t*-test for *TGF-β* and *IL-6* (LPS plus IFN-ɣ); the remaining groups did not pass the Shapiro–Wilk normality test and were analyzed by Mann–Whitney test.

DISCUSSION

This study investigated how the senescence of SCAPs affects biological processes fundamental to RET. Although still an emerging topic, accumulating evidence indicates that stem cell senescence compromises functions directly related to tissue repair and regeneration (Siennicka et al., 2021; J. Wang et al., 2025b). In the clinical context of RET, SCAPs are indispensable for apical papilla survival, root maturation, and formation of vital tissue within the canal (Yoo et al., 2016). Consequently, understanding how senescence alters SCAP behavior is crucial for interpreting treatment outcomes, especially in immature teeth affected by persistent infection or chronic inflammation.

To this end, aiming to reproduce the senescent phenotype commonly observed in inflamed or necrotic immature teeth, we selected doxorubicin as the senescence-inducing agent. This compound has been validated in dental pulp cells and offers experimental advantages such as short induction time (Cheng et al., 2011; da Silva et al., 2025; de Farias et al., 2024). Previous studies in dental pulp cells have shown that this agent is capable of inducing cellular senescence, similarly to other commonly used stimuli such as H_2O_2 or ultraviolet light (Yaghoobi et al., 2020). These findings support its application in SCAPs under the conditions adopted in the present study.

The senescent phenotype in doxorubicin-induced SCAPs was validated with strong and consistent experimental evidence, confirming the robustness of this senescence induction model. More than 96% of SCAPs stained positive for SA-β-Gal, closely matching findings from other doxorubicin-based models (D. Wang et al., 2016). At the molecular level, senescent SCAPs expressed markedly higher *p53* levels, approximately 1.7-fold, than non-senescent cells. Since p53 orchestrates the DNA damage response and is a key regulator of senescence, this increase corroborates previous *in vivo* findings demonstrating the centrality of the p53 axis in MSC senescence (Höfig et al., 2016b). The combination of phenotypic and molecular evidence therefore reinforces that the model reliably replicates SCAP senescence, which is clinically relevant because increased p53 expression has been linked to aging, impaired proliferation, and reduced differentiation capacity, processes that may compromise the biological basis of RET (Z. Li et al., 2019b; Shi et al., 2024; Teawcharoensopa & Srisuwan, 2024b).

Interestingly, senescent SCAPs exhibited increased metabolic activity in the MTT assay, diverging from previous reports showing reduced mitochondrial function in senescent stem cells from other tissues (Cho et al., 2019b; Wenjing et al., 2024b). This finding may be explained by transient mitochondrial hyperfunction, previously described in early phases of senescence. During this state, increased mitochondrial mass and elevated ATP production enhance dehydrogenase activity, yielding higher MTT reduction without necessarily reflecting improved cellular metabolism (D. Wang et al., 2016). Additionally, discrepancies between our findings and previous studies may be attributable to differences in the senescence induction model and the specific stem cell source evaluated, as mitochondrial responses can vary according to both the triggering stimulus and the intrinsic biological properties of each cell type. When exposed to an inflammatory environment simulating clinical pulp infection (LPS or LPS plus IFN-γ), differences between senescent and non-senescent cells were no longer detected. Because LPS itself induces senescence, both groups may converge toward similar functional impairment under inflammatory stress (G. Feng et al., 2018b; Ma et al., 2019b). Clinically, this suggests that inflamed immature teeth might exhibit widespread cellular dysfunction, regardless of baseline SCAP status, thus affecting RET outcomes.

Supporting the observed functional alterations, SEM analysis revealed morphological changes typical of senescent MSCs, larger cell size and fewer cytoplasmic extensions (He et al., 2024). These structural characteristics correlate with reduced motility and proliferative potential, two processes indispensable for RET (Oja et al., 2018). In line with this, senescent SCAPs showed significantly diminished migration across all conditions. The same trend was observed for proliferation: non-senescent SCAPs displayed progressive growth, whereas senescent cells showed either reduced or stimulus-dependent responses. Chronic inflammatory stimulation with LPS further impaired senescent cell proliferation, consistent with evidence that persistent infection in the root canal system can accelerate senescence in dental stem cells (G. Feng et al., 2018b). Within the context of RET, where SCAP recruitment and expansion within the canal space are essential for tissue ingrowth and root maturation, these findings help explain why regenerative procedures may fail in cases of long-standing infection or untreated pulp necrosis (Razghonova et al., 2022).

Beyond proliferation and migration, the immunomodulatory behavior of senescent SCAPs also aligned with the SASP (Figure 6). Senescent cells exhibited elevated *IL-6* and reduced *TGF-β* expression, two cytokines with opposing roles in inflammation and repair. While IL-6 promotes chronic inflammation, TGF-β is essential for immune modulation and

tissue reconstruction. Notably, *TNF-α* remained unchanged between groups Interestingly, *IL-10* expression increased in senescent cells exclusively under LPS stimulation, suggesting a stimulus-dependent and potentially compensatory anti-inflammatory response rather than a consistent shift toward immune suppression. This selective upregulation indicates that not all immunomodulatory mediators are uniformly altered by senescence, reinforcing the concept of a partially dysregulated secretory phenotype. A similar IL-10 expression pattern was observed in senescent dental pulp stem cells using the same doxorubicin-induced model with LPS stimulation (de Farias, 2022). Additionally, increased IL-10 expression under baseline conditions has also been reported with this protocol, suggesting that senescence alone may modulate the immunoregulatory profile of these cells(da Silva et al., 2025). The imbalance observed here reflects an inflammaging profile previously described in dental MSCs (Horibe et al., 2014b; Josephson et al., 2019). This inflammatory microenvironment can significantly alter the cell differentiation pattern, potentially leading to disorganized mineralization or the deposition of poorly organized hard tissue, thereby deviating from the desired regenerative outcome (Park et al., 2019). Because RET success depends on shifting the microenvironment from pro-inflammatory to pro-regenerative, the predominance of SASP mediators in the apical papilla may reinforce persistent inflammation, impair SCAP functionality, and hinder the formation of new vital tissue. These findings, however, must be interpreted considering important methodological limitations. Although LPS/IFN-γ stimulation and doxorubicin-induced senescence offer valuable mechanistic insights, they simplify the complex and dynamic inflammatory milieu present *in vivo*. Moreover, chemically induced senescence may not fully capture the heterogeneous senescent states observed in clinically infected immature teeth. Thus, future studies incorporating animal models and ex vivo RET simulations will be essential to validate the functional impairments observed here.

Despite these limitations, the present findings highlight that SCAP senescence profoundly alters cell migration, proliferation, immunomodulation, and morphology, functions central to the biological success of RET. These results underscore the need to explore therapeutic strategies capable of preventing, reversing, or eliminating senescent phenotypes in apical papilla cells. Senolytic and senomorphic approaches may represent promising adjuncts to improve predictability and long-term outcomes of regenerative endodontic procedures.

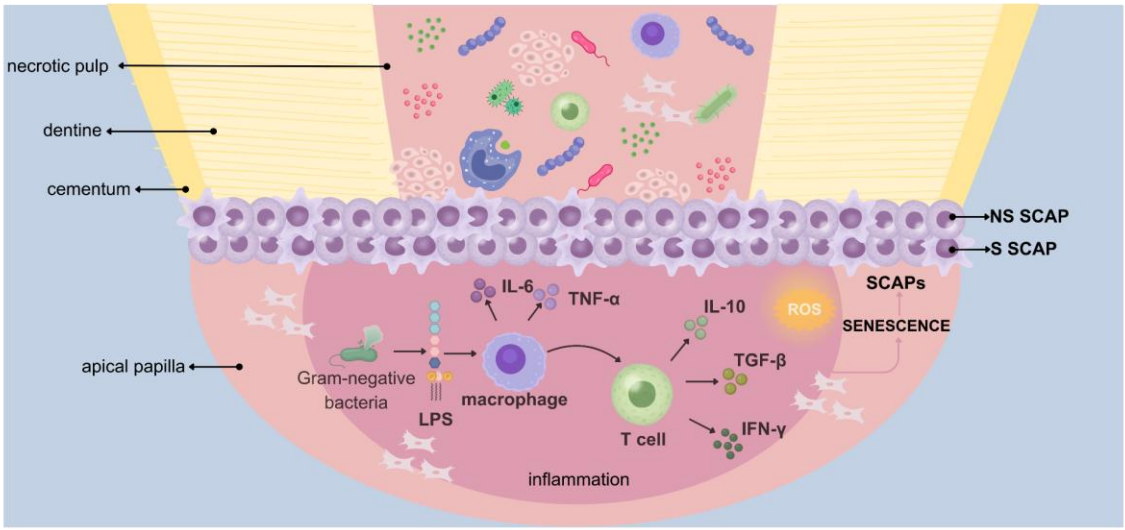


FIGURE 6 Schematic representation of the study framework. Inflammatory microenvironment of necrotic pulp, showing bacterial products, immune cell activation, cytokine release, and ROS generation, which contribute to SCAP senescence and potentially limiting pulp tissue regeneration and root maturation in immature teeth.

CONCLUSION

Senescence in SCAPs compromises essential functions such as migration, proliferation, and morphology, while promoting a pro-inflammatory profile, with increased *IL-6* and reduced *TGF-β* gene expression. Even when mitochondrial activity appears preserved, these cells may be functionally impaired, potentially limiting pulp tissue regeneration and root maturation in immature teeth. Clinically, these findings suggest that the presence of senescent cells in the apical papilla, particularly under chronic inflammatory conditions, may contribute to the failure of regenerative endodontic therapies, highlighting the need for strategies that prevent or reverse cellular senescence to improve clinical outcomes.

REFERENCES

1. Adel, M., Asgari, Z., 2025. Regenerative Endodontic Treatment in an Immature Permanent Tooth With Necrotic Pulp and Periradicular Lesion. Clin Case Rep 13. https://doi.org/10.1002/ccr3.70941
2. Cheng, H., Qiu, L., Ma, J., Zhang, H., Cheng, M., Li, W., Zhao, X., Liu, K., 2011. Replicative senescence of human bone marrow and umbilical cord derived mesenchymal stem cells and their differentiation to adipocytes and osteoblasts. Mol Biol Rep 38, 5161–5168. https://doi.org/10.1007/s11033-010-0665-2
3. Cheng, Q., Liu, C., Chen, Q., Luo, W., He, T.C., Yang, D., 2023. Establishing and characterizing human stem cells from the apical papilla immortalized by hTERT gene transfer. Front Cell Dev Biol 11. https://doi.org/10.3389/fcell.2023.1158936
4. Cho, A., Park, S.R., Kim, S.R., Nam, S., Lim, S., Park, C.H., Lee, H.Y., Hong, I.S., 2019. An Endogenous Anti-aging Factor, Sonic Hedgehog, Suppresses Endometrial Stem Cell Aging through SERPINB2. Molecular Therapy 27, 1286–1298. https://doi.org/10.1016/j.ymthe.2019.04.019
5. Coppé, J.P., Patil, C.K., Rodier, F., Sun, Y., Muñoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6. https://doi.org/10.1371/journal.pbio.0060301
6. Crowley, L.C., Marfell, B.J., Christensen, M.E., Waterhouse, N.J., 2016. Measuring cell death by trypan blue uptake and light microscopy. Cold Spring Harb Protoc 2016, 643–646. https://doi.org/10.1101/pdb.prot087155
7. da Silva, J.C., Ramos, R.F., da Silva, A.A.S.V., de Melo, M.E.F., de Oliveira, M.A., de Sousa, L.B., Bispo, E.C.I., da Silva, I.G.M., Sousa, M.G.C., Corrêa, J.R., Araujo, F.S., Rezende, T.M.B., 2025. Impact of Cellular Senescence on the Immune-Inflammatory Response and Regenerative Capacity of Human Dental Pulp Cells. https://doi.org/10.1016/j.joen.2025.07.009
8. de Farias, J.O., da Costa Sousa, M.G., Martins, D.C.M., de Oliveira, M.A., Takahashi, I., de Sousa, L.B., da Silva, I.G.M., Corrêa, J.R., Silva Carvalho, A.É., Saldanha-Araújo, F., Rezende, T.M.B., 2024. Senescence on Dental Pulp Cells: Effects on Morphology, Migration, Proliferation, and Immune Response. J Endod 50, 362–369. https://doi.org/10.1016/j.joen.2023.12.009

9. dos Santos, A.F., Pacheco, J.M., Silva, P.A.O., Bedran-Russo, A.K., Rezende, T.M.B., Pereira, P.N.R., Ribeiro, A.P.D., 2019. Direct and transdentinal biostimulatory effects of grape seed extract rich in proanthocyanidin on pulp cells. Int Endod J 52, 424–438. https://doi.org/10.1111/iej.13019
10. Feng, G., Zheng, K., Cao, T., Zhang, J., Lian, M., Huang, D., Wei, C., Gu, Z., Feng, X., 2018a. Repeated stimulation by LPS promotes the senescence of DPSCs via TLR4/MyD88-NF-κB-p53/p21 signaling. Cytotechnology 70, 1023–1035. https://doi.org/10.1007/s10616-017-0180-6
11. Feng, G., Zheng, K., Cao, T., Zhang, J., Lian, M., Huang, D., Wei, C., Gu, Z., Feng, X., 2018b. Repeated stimulation by LPS promotes the senescence of DPSCs via TLR4/MyD88-NF-κB-p53/p21 signaling. Cytotechnology 70, 1023–1035. https://doi.org/10.1007/s10616-017-0180-6
12. Feng, X., Xing, J., Feng, G., Huang, D., Lu, X., Liu, S., Tan, W., Li, L., Gu, Z., 2014. P16INK4A mediates age-related changes in mesenchymal stem cells derived from human dental pulp through the DNA damage and stress response. Mech Ageing Dev 141–142, 46–55. https://doi.org/10.1016/j.mad.2014.09.004
13. He, L., Li, M., Wang, X., Wu, X., Yue, G., Wang, T., Zhou, Y., Lei, B., Zhou, G., 2024. Morphology-based deep learning enables accurate detection of senescence in mesenchymal stem cell cultures. BMC Biol 22. https://doi.org/10.1186/s12915-023-01780-2
14. Höfig, I., Ingawale, Y., Atkinson, M.J., Hertlein, H., Nelson, P.J., Rosemann, M., 2016. P53-dependent senescence in mesenchymal stem cells under chronic normoxia is potentiated by low-dose γ -irradiation. Stem Cells Int 2016. https://doi.org/10.1155/2016/6429853
15. Hong, J.H., Kim, M.R., Lee, B.N., Oh, W.M., Min, K.S., Im, Y.G., Hwang, Y.C., 2021. Anti-inflammatory and mineralization effects of bromelain on lipopolysaccharide-induced inflammation of human dental pulp cells. Medicina (Lithuania) 57. https://doi.org/10.3390/medicina57060591
16. Horibe, H., Murakami, M., Iohara, K., Hayashi, Y., Takeuchi, N., Takei, Y., Kurita, K., Nakashima, M., 2014. Isolation of a stable subpopulation of Mobilized Dental Pulp Stem Cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. PLoS One 9. https://doi.org/10.1371/journal.pone.0098553
17. Hsu, H.Y., Wen, M.H., 2002. Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression. Journal of Biological Chemistry 277, 22131–22139. https://doi.org/10.1074/jbc.M111883200

18. Insinga, A., Cicalese, A., Faretta, M., Gallo, B., Albano, L., Ronzoni, S., Furia, L., Viale, A., Pelicci, P.G., 2013. DNA damage in stem cells activates p21, inhibits p53, and induces symmetric self-renewing divisions. Proc Natl Acad Sci U S A 110, 3931–3936. https://doi.org/10.1073/pnas.1213394110
19. Jamshidi, D., Homayouni, H., Moradi Majd, N., Shahabi, S., Arvin, A., Ranjbar Omidi, B., 2018. Impact and Fracture Strength of Simulated Immature Teeth Treated with Mineral Trioxide Aggregate Apical Plug and Fiber Post Versus Revascularization. J Endod 44, 1878–1882. https://doi.org/10.1016/j.joen.2018.09.008
20. Jena, D., Sabiha, P.B., Kumar, N.S., Ahmed, S.S., Bhagat, P., Singh, S.P., Mustafa, M., 2023. Regenerative therapy for the permanent immature teeth: A long term study. An original research. J Pharm Bioallied Sci 15, 127–131. https://doi.org/10.4103/jpbs.jpbs_435_22
21. Josephson, A.M., Bradaschia-Correa, V., Lee, S., Leclerc, K., Patel, K.S., Lopez, E.M., Litwa, H.P., Neibart, S.S., Kadiyala, M., Wong, M.Z., Mizrahi, M.M., Yim, N.L., Ramme, A.J., Egol, K.A., Leucht, P., 2019. Age-related inflammation triggers skeletal stem/ progenitor cell dysfunction. Proc Natl Acad Sci U S A 116, 6995–7004. https://doi.org/10.1073/pnas.1810692116
22. Li, Z., Ge, X., Lu, J., Bian, M., Li, N., Wu, X., Li, Y., Yan, M., Yu, J., 2019a. MiR-141-3p regulates proliferation and senescence of stem cells from apical papilla by targeting YAP. Exp Cell Res 383. https://doi.org/10.1016/j.yexcr.2019.111562
23. Li, Z., Ge, X., Lu, J., Bian, M., Li, N., Wu, X., Li, Y., Yan, M., Yu, J., 2019b. MiR-141-3p regulates proliferation and senescence of stem cells from apical papilla by targeting YAP. Exp Cell Res 383. https://doi.org/10.1016/j.yexcr.2019.111562
24. Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods 25, 402–408. https://doi.org/10.1006/meth.2001.1262
25. Ma, L., Hu, J., Cao, Y., Xie, Y., Wang, H., Fan, Z., Zhang, C., Wang, J., Wang, S., Wu, C.T., 2019a. Maintained properties of aged dental pulp stem cells for superior periodontal tissue regeneration. Aging Dis 10, 793–806. https://doi.org/10.14336/AD.2018.0729
26. Ma, L., Hu, J., Cao, Y., Xie, Y., Wang, H., Fan, Z., Zhang, C., Wang, J., Wang, S., Wu, C.T., 2019b. Maintained properties of aged dental pulp stem cells for superior periodontal tissue regeneration. Aging Dis 10, 793–806. https://doi.org/10.14336/AD.2018.0729
27. Martinotti, S., Ranzato, E., 2020. Scratch wound healing assay, in: Methods in Molecular Biology. Humana Press Inc., pp. 225–229. https://doi.org/10.1007/7651_2019_259

28. Nagendrababu, V., Murray, P.E., Ordinola-Zapata, R., Peters, O.A., Rôças, I.N., Siqueira, J.F., Priya, E., Jayaraman, J., Pulikkotil, S.J., Suresh, N., Dummer, P.M.H., 2021. PRILE 2021 guidelines for reporting laboratory studies in Endodontology: explanation and elaboration. Int Endod J. https://doi.org/10.1111/iej.13565
29. Oja, S., Komulainen, P., Penttilä, A., Nystedt, J., Korhonen, M., 2018. Automated image analysis detects aging in clinical-grade mesenchymal stromal cell cultures. Stem Cell Res Ther 9. https://doi.org/10.1186/s13287-017-0740-x
30. Park, Y.T., Lee, S.M., Kou, X., Karabucak, B., 2019. The Role of Interleukin 6 in Osteogenic and Neurogenic Differentiation Potentials of Dental Pulp Stem Cells. J Endod 45, 1342–1348. https://doi.org/10.1016/j.joen.2019.08.002
31. Putra, A., Ridwan, F.B., Putridewi, A.I., Kustiyah, A.R., Wirastuti, K., Sadyah, N.A.C., Rosdiana, I., Munir, D., 2018. The role of tnf-α induced mscs on suppressive inflammation by increasing tgf-β and il-10. Open Access Maced J Med Sci 6, 1779–1783. https://doi.org/10.3889/oamjms.2018.404
32. Razghonova, Y., Zymovets, V., Wadelius, P., Rakhimova, O., Manoharan, L., Brundin, M., Kelk, P., Romani Vestman, N., 2022. Transcriptome Analysis Reveals Modulation of Human Stem Cells from the Apical Papilla by Species Associated with Dental Root Canal Infection. Int J Mol Sci 23. https://doi.org/10.3390/ijms232214420
33. Rodier, F., Coppé, J.P., Patil, C.K., Hoeijmakers, W.A.M., Muñoz, D.P., Raza, S.R., Freund, A., Campeau, E., Davalos, A.R., Campisi, J., 2009. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. Nat Cell Biol 11, 973–979. https://doi.org/10.1038/ncb1909
34. Salem, N.A., ElShebiney, S.A., Mabrouk, M., Kishta, M.S., Galal, A.F., Osama, L., Beherei, H.H., 2025. Enhanced bone regeneration using mesenchymal stem cell-loaded 3D-printed alginate-calcium Titanate scaffolds: A Calvarial defect model study. Int J Biol Macromol 302. https://doi.org/10.1016/j.ijbiomac.2025.140516
35. Shi, Y., Xiao, T., Weng, Y., Xiao, Y., Wu, J., Wang, J., Wang, W., Yan, Maoshen, Yan, Ming, Li, Z., Yu, J., 2024. 3D culture inhibits replicative senescence of SCAPs via UQCRC2-mediated mitochondrial oxidative phosphorylation. J Transl Med 22. https://doi.org/10.1186/s12967-024-05953-7
36. Siennicka, K., Zołocińska, A., Dębski, T., Pojda, Z., 2021. Comparison of the Donor Age-Dependent and *in Vitro* Culture-Dependent Mesenchymal Stem Cell Aging in Rat Model. Stem Cells Int 2021. https://doi.org/10.1155/2021/6665358

37. Smeda, M., Galler, K.M., Woelflick, M., Rosendahl, A., Moehle, C., Lenhardt, B., Buchalla, W., Widbiller, M., 2022. Molecular Biological Comparison of Dental Pulp-and Apical Papilla-Derived Stem Cells. Int J Mol Sci 23. https://doi.org/10.3390/ijms23052615
38. Su, X., Yang, H., Shi, R., Zhang, C., Liu, H., Fan, Z., Zhang, J., 2020. Depletion of SNRNP200 inhibits the osteo−/dentinogenic differentiation and cell proliferation potential of stem cells from the apical papilla. BMC Dev Biol 20. https://doi.org/10.1186/s12861-020-00228-y
39. Tawfik, H., Abu-Seida, A.M., Hashem, A.A., Nagy, M.M., 2013. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. Int Endod J 46, 910–922. https://doi.org/10.1111/iej.12079
40. Teawcharoensopa, C., Srisuwan, T., 2024. The potential use of ascorbic acid to recover the cellular senescence of lipopolysaccharide-induced human apical papilla cells: an *in vitro* study. Clin Oral Investig 28. https://doi.org/10.1007/s00784-023-05455-8
41. Temmerman, L., Vral, A., Meire, M., Verbeeck, R.M., Deschepper, E., Dermaut, L.R., De Pauw, G.A., 2012. Pulpal regeneration and root development after subcutaneous transplantation of cryopreserved immature teeth in rats. Cryobiology 64, 81–90. https://doi.org/10.1016/j.cryobiol.2011.11.004
42. Van De Loosdrecht, A.A., Beelen, R.H.J., Ossenkoppele, G.J., Broekhoven, M.G., Langenhuijsen, M.M.A.C., 1994. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia, Journal of Immunological Methods.
43. Wang, D., Liu, Y., Zhang, R., Zhang, F., Sui, W., Chen, L., Zheng, R., Chen, X., Wen, F., Ouyang, H.-W., Ji, J., Ouyang, H.W., 2016. Apoptotic transition of senescent cells accompanied with mitochondrial hyper-function.
44. Wang, J., Jian, K., Yang, Q., Gu, C., Sheng, J., Zhou, Y., Yin, H., Zhang, Z., Hua, K., Zhang, C., 2025. Retarding human adipose-derived MSCs senescence and promoting tendon repair using cell sheet engineering with a histone methyltransferase inhibitor. Sci Rep 15. https://doi.org/10.1038/s41598-025-89234-3
45. Wenjing, Z., Baikun, L., Qiulian, L., Xi, C., 2024. Effects of long-term subculture on biological characteristics of bone marrow mesenchymal stem cells. Chinese Journal of Tissue Engineering Research 28, 4926–4930. https://doi.org/10.12307/2024.702
46. Yaghoobi, M.M., Sheikoleslami, M., Ebrahimi, M., 2020a. Effects of hydrogen peroxide, doxorubicin and ultraviolet irradiation on senescence of human dental pulp stem cells. Arch Oral Biol 117. https://doi.org/10.1016/j.archoralbio.2020.104819

47. Yaghoobi, M.M., Sheikoleslami, M., Ebrahimi, M., 2020b. Effects of hydrogen peroxide, doxorubicin and ultraviolet irradiation on senescence of human dental pulp stem cells. Arch Oral Biol 117. https://doi.org/10.1016/j.archoralbio.2020.104819
48. Yaghoobi, M.M., Sheikoleslami, M., Ebrahimi, M., 2020c. Effects of hydrogen peroxide, doxorubicin and ultraviolet irradiation on senescence of human dental pulp stem cells. Arch Oral Biol 117. https://doi.org/10.1016/j.archoralbio.2020.104819
49. Yoo, Y.J., Oh, J.H., Lee, W.C., Woo, K.M., 2016. Regenerative Characteristics of Apical Papilla–derived Cells from Immature Teeth with Pulpal and Periapical Pathosis. J Endod 42, 1626–1632. https://doi.org/10.1016/j.joen.2016.08.004
50. Zhu, L., Wang, B., Gu, J., Zhou, J., Wu, Y., Xu, W., Yang, M., Cai, X., Shen, H., Lu, L., Wang, F., 2024. IFNγ-secreting T cells that highly express IL-2 potently inhibit the growth of intracellular M. tuberculosis in macrophages. Front Immunol 15. https://doi.org/10.3389/fimmu.2024.1469118

CONSIDERAÇÕES FINAIS

Esta dissertação, estruturada em uma revisão sistemática seguida de um estudo experimental *in vitro*, investigou o papel da senescência celular no reparo e regeneração tecidual. O primeiro capítulo sintetizou, de forma sistemática, as evidências sobre o impacto desse processo em células humanas. O segundo capítulo, derivado dessa base teórica, delimitou seu foco à análise dos efeitos da senescência em células da papila apical, propondo uma interpretação pouco explorada para o contexto das terapias endodônticas regenerativas em dentes com rizogênese incompleta. De forma integrada, os resultados obtidos permitem ampliar a compreensão dos mecanismos biológicos envolvidos na resposta tecidual, oferecendo subsídios para a reflexão crítica sobre o papel da senescência celular em estratégias regenerativas e para o delineamento de investigações futuras nessa área.

A revisão sistemática evidenciou que a senescência celular constitui um fator crítico capaz de comprometer funções essenciais das MSCs. A síntese dos quarenta e cinco estudos incluídos demonstrou que as capacidades proliferativa e migratória dessas células são negativamente afetadas pelo estado senescente, independentemente da origem tecidual e do método de indução da senescência. Em contraste, observou-se uma heterogeneidade significativa quanto ao potencial de diferenciação osteogênica, adipogênica e condrogênica, o que impossibilitou a identificação de um padrão consistente entre os diferentes estudos analisados. Outro desfecho de elevada relevância foi o perfil imunomodulador das MSCs senescentes. Embora esse aspecto tenha sido investigado em um número limitado de estudos, observou-se a predominância de um perfil secretor pró-inflamatório, com potencial para perpetuar um microambiente desfavorável à reparação tecidual e para induzir ou propagar a senescência em células e tecidos adjacentes. Por fim, os estudos *in vivo* que avaliaram o reparo tecidual indicaram que a presença de células senescentes está associada a prejuízos na organização, qualidade e funcionalidade do tecido reparado, reforçando o impacto negativo da senescência sobre processos regenerativos.

Além da investigação sobre os impactos da senescência MSCs nos processos de reparo e regeneração tecidual, o Capítulo 1 evidenciou uma lacuna relevante no conhecimento: a escassa caracterização da senescência celular na papila apical e de suas implicações para os protocolos clínicos de Terapia Endodôntica Regenerativa (TER) em dentes com rizogênese incompleta. Com o objetivo de abordar essa lacuna, foi delineado um estudo experimental *in*

vitro. Em consonância com os achados do primeiro capítulo — que incluiu MSCs derivadas da medula óssea, tecido adiposo, cordão umbilical, polpa dentária e ligamento periodontal — as SCAPs em estado senescente também apresentaram redução significativa das capacidades proliferativa e migratória, tanto em condições basais quanto após a exposição a estímulos inflamatórios com LPS isoladamente ou associado ao IFN-γ. De forma semelhante, essas células exibiram aumento significativo na expressão de *IL-6* e redução na expressão de *TGF-β*, confirmando a aquisição de um perfil secretor pró-inflamatório característico das células senescentes. A análise da atividade metabólica por meio do ensaio de MTT demonstrou que, apesar das alterações funcionais observadas, as SCAPs senescentes mantiveram atividade mitocondrial preservada, inclusive após os desafios inflamatórios. Adicionalmente, a caracterização morfológica evidenciou aumento do tamanho celular e redução no número de prolongamentos citoplasmáticos, alterações compatíveis com o comprometimento funcional observado, em especial no que se refere às menores taxas de migração e proliferação celular.

Diante dos achados integrados, conclui-se que a senescência celular representa uma barreira biológica relevante para as TER, particularmente no contexto da participação da papila apical como fonte de células-tronco. Esta dissertação contribui para o avanço do conhecimento ao traduzir e contextualizar, no microambiente apical, as consequências funcionais da senescência previamente descritas em outras fontes de MSCs, estabelecendo uma relação mecanística entre o estado senescente e o comprometimento de processos celulares essenciais à REP. A observação de que células senescentes mantêm atividade metabólica concomitantemente à disfunção morfofuncional indica um cenário biologicamente desafiador, no qual essas células podem persistir no nicho regenerativo e, por meio do SASP, influenciar de forma adversa o microambiente tecidual e a progressão adequada dos eventos regenerativos.

A interpretação dos achados desta dissertação deve considerar limitações inerentes aos métodos empregados em cada um de seus capítulos. Para a revisão sistemática, é importante destacar uma possível limitação na estratégia de busca. Embora o termo "células-tronco mesenquimais" (MSCs) seja historicamente predominante, a nomenclatura atual recomendada pela Sociedade Internacional de Terapia Celular e Gênica (ISCT) privilegia "células estromais mesenquimais". Dada a adoção progressiva deste termo na literatura mais recente, é possível que estudos que utilizaram exclusivamente "estromal" sem mencionar "stem" tenham sido excluídos, introduzindo um viés de seleção. Ademais, a síntese pode ter sido influenciada pelo viés de publicação e pela elevada heterogeneidade metodológica dos estudos primários, como a diversidade de modelos para induzir senescência, marcadores de confirmação e ensaios

funcionais. Essa heterogeneidade das variáveis impediu uma meta-análise e limitou a generalização de conclusões, especialmente sobre os efeitos na diferenciação celular. Quanto ao estudo experimental *in vitro*, embora controlado e direcionado, apresenta limitações translacionais. O modelo de senescência induzida por doxorubicina, ainda que robusto, não replica fielmente a senescência gradual e multifatorial que se estabelece no nicho da papila apical sob a influência crônica do microambiente inflamatório de um canal radicular necrótico. Além disso, o próprio modelo de cultura celular bidimensional e a ausência de interações com outros componentes do nicho tecidual, limitam a extrapolação direta dos mecanismos observados para a complexidade do cenário *in vivo*.

Nesse contexto, torna-se pertinente que investigações futuras avancem para a avaliação da senescência celular em modelos *in vivo* de RET em dentes com rizogênese incompleta, a fim de validar os achados observados *in vitro* e compreender sua relevância biológica em um ambiente tecidual mais complexo e dinâmico. Paralelamente, a investigação de abordagens senoterapêuticas, deve ser conduzida de forma criteriosa, com o objetivo de esclarecer seu potencial para modular o microambiente da papila apical e mitigar os efeitos deletérios associados ao acúmulo de células senescentes. A translação desses achados para estudos clínicos poderá contribuir para o refinamento dos protocolos de RET em dentes com rizogênese incompleta.

REFERÊNCIAS (INTRODUÇÃO / MATERIAIS E MÉTODOS)

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, *et al*. Multilineage Potential of Adult Human Mesenchymal Stem Cells. Science (1979) [Internet]. 1999 Apr 2;284(5411):143–7.

2. Kern S, Eichler H, Stoeve J, Klüter H, Bieback K. Comparative Analysis of Mesenchymal Stem Cells from Bone Marrow, Umbilical Cord Blood, or Adipose Tissue. Stem Cells. 2006 May;24(5):1294–301.

3. Heo JS, Choi Y, Kim HS, Kim HO. Comparison of molecular profiles of human mesenchymal stem cells derived from bone marrow, umbilical cord blood, placenta and adipose tissue. Int J Mol Med. 2016 Jan 1;37(1):115–25.

4. Dagnino APA, Chagastelles PC, Medeiros RP, Estrázulas M, Kist LW, Bogo MR, *et al*. Neural Regenerative Potential of Stem Cells Derived from the Tooth Apical Papilla. Stem Cells Dev. 2020 Dec 1;29(23):1479–96.

5. Kang J, Fan W, Deng Q, He H, Huang F. Stem Cells from the Apical Papilla: A Promising Source for Stem Cell-Based Therapy. Vol. 2019, BioMed Research International. Hindawi Limited; 2019.

6. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, *et al*. Multilineage Cells from Human Adipose Tissue: Implications for Cell-Based Therapies. Vol. 7, TISSUE ENGINEERING. Mary Ann Liebert, Inc; 2001.

7. Wagner W, Horn P, Castoldi M, Diehlmann A, Bork S, Saffrich R, *et al*. Replicative senescence of mesenchymal stem cells: A continuous and organized process. PLoS One. 2008 May 21;3(5).

8. Wenjing Z, Baikun L, Qiulian L, Xi C. Effects of long-term subculture on biological characteristics of bone marrow mesenchymal stem cells. Chinese Journal of Tissue Engineering Research. 2024 Nov 8;28(31):4926–30.

9. Coppé JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, *et al*. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol. 2008;6(12).

10. Su X, Yang H, Shi R, Zhang C, Liu H, Fan Z, *et al*. Depletion of SNRNP200 inhibits the osteo−/dentinogenic differentiation and cell proliferation potential of stem cells from the apical papilla. BMC Dev Biol. 2020 Dec 1;20(1).

11. Choudhery MS, Badowski M, Muise A, Pierce J, Harris DT. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med [Internet]. 2014 Jan 7;12:8. Available from: http://www.translational-medicine.com/content/12/1/8

12. Gruber HE, Somayaji S, Riley F, Hoelscher GL, Norton HJ, Ingram J, *et al*. Human adipose-derived mesenchymal stem cells: Serial passaging, doubling time and cell senescence. Biotechnic and Histochemistry. 2012;87(4):303–11.

13. Feng X, Feng G, Xing J, Shen B, Tan W, Huang D, *et al*. Repeated lipopolysaccharide stimulation promotes cellular senescence in human dental pulp stem cells (DPSCs). Cell Tissue Res. 2014;356(2):369–80.

14. Horibe H, Murakami M, Iohara K, Hayashi Y, Takeuchi N, Takei Y, *et al*. Isolation of a stable subpopulation of Mobilized Dental Pulp Stem Cells (MDPSCs) with high proliferation, migration, and regeneration potential is independent of age. PLoS One. 2014 May 28;9(5).

15. Zhang Y, Zhang J, Lesani P, Lu Z, Zreiqat H. Osteopontin Rejuvenates Senescent Adipose-Derived Stem Cells and Restores their Bone Tissue Regenerative Function. Stem Cell Rev Rep. 2024 May 1;20(4):1106–20.

16. Ma L, Hu J, Cao Y, Xie Y, Wang H, Fan Z, *et al*. Maintained properties of aged dental pulp stem cells for superior periodontal tissue regeneration. Aging Dis. 2019;10(4):793–806.

17. Jamshidi D, Homayouni H, Moradi Majd N, Shahabi S, Arvin A, Ranjbar Omidi B. Impact and Fracture Strength of Simulated Immature Teeth Treated with Mineral Trioxide Aggregate Apical Plug and Fiber Post Versus Revascularization. J Endod. 2018 Dec 1;44(12):1878–82.

18. Adel M, Asgari Z. Regenerative Endodontic Treatment in an Immature Permanent Tooth With Necrotic Pulp and Periradicular Lesion. Clin Case Rep. 2025 Oct;13(10).

19. Jena D, Sabiha PB, Kumar NS, Ahmed SS, Bhagat P, Singh SP, *et al*. Regenerative therapy for the permanent immature teeth: A long term study. An original research. J Pharm Bioallied Sci. 2023 Jul 1;15(5):127–31.

20. Salem NA, ElShebiney SA, Mabrouk M, Kishta MS, Galal AF, Osama L, *et al*. Enhanced bone regeneration using mesenchymal stem cell-loaded 3D-printed alginate-calcium Titanate scaffolds: A Calvarial defect model study. Int J Biol Macromol. 2025 Apr 1;302.

21. Cheng Q, Liu C, Chen Q, Luo W, He TC, Yang D. Establishing and characterizing human stem cells from the apical papilla immortalized by hTERT gene transfer. Front Cell Dev Biol. 2023;11.

22. Smeda M, Galler KM, Woelflick M, Rosendahl A, Moehle C, Lenhardt B, *et al*. Molecular Biological Comparison of Dental Pulp-and Apical Papilla-Derived Stem Cells. Int J Mol Sci. 2022 Mar 1;23(5).

23. Tawfik H, Abu-Seida AM, Hashem AA, Nagy MM. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. Int Endod J. 2013 Oct;46(10):910–22.

24. Temmerman L, Vral A, Meire M, Verbeeck RM, Deschepper E, Dermaut LR, *et al*. Pulpal regeneration and root development after subcutaneous transplantation of cryopreserved immature teeth in rats. Cryobiology. 2012 Apr;64(2):81–90.

25. Teawcharoensopa C, Srisuwan T. The potential use of ascorbic acid to recover the cellular senescence of lipopolysaccharide-induced human apical papilla cells: an in vitro study. Clin Oral Investig. 2024 Jan 1;28(1).

26. Hsu HY, Wen MH. Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression. Journal of Biological Chemistry. 2002 Jun 21;277(25):22131–9.

27. Zhu L, Wang B, Gu J, Zhou J, Wu Y, Xu W, *et al*. IFNγ-secreting T cells that highly express IL-2 potently inhibit the growth of intracellular M. tuberculosis in macrophages. Front Immunol. 2024;15.

28. Putra A, Ridwan FB, Putridewi AI, Kustiyah AR, Wirastuti K, Sadyah NAC, *et al*. The role of tnf-α induced mscs on suppressive inflammation by increasing tgf-β and il-10. Open Access Maced J Med Sci. 2018;6(10):1779–83.

29. Cho A, Park SR, Kim SR, Nam S, Lim S, Park CH, *et al*. An Endogenous Anti-aging Factor, Sonic Hedgehog, Suppresses Endometrial Stem Cell Aging through SERPINB2. Molecular Therapy. 2019 Jul 3;27(7):1286–98.

30. Höfig I, Ingawale Y, Atkinson MJ, Hertlein H, Nelson PJ, Rosemann M. P53-dependent senescence in mesenchymal stem cells under chronic normoxia is potentiated by low-dose γ -irradiation. Stem Cells Int. 2016;2016.

31. Yaghoobi MM, Sheikoleslami M, Ebrahimi M. Effects of hydrogen peroxide, doxorubicin and ultraviolet irradiation on senescence of human dental pulp stem cells. Arch Oral Biol. 2020 Sep 1;117.

32. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. Vol. 372, BMJ. BMJ Publishing Group; 2021.

33. Monteiro MM, dos Santos JA, Paiva Barbosa V, De Luca Canto G, Squarize CH, Coletta RD, *et al*. PETRICCS Guideline Protocol: A Call to Improve Reporting Standards in Cell Culture Research. Journal of Oral Pathology and Medicine. 2025 Feb 1;54(2):112–9.

34. Hooijmans CR, Rovers MM, De Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. BMC Med Res Methodol. 2014 Mar 26;14(1).

35. Guirado E, Zhang Y, George A. Establishment of stable cell lines from primary human dental pulp stem cells. In: Methods in Molecular Biology. Humana Press Inc.; 2019. p. 21–7.

36. de Farias JO, da Costa Sousa MG, Martins DCM, de Oliveira MA, Takahashi I, de Sousa LB, *et al*. Senescence on Dental Pulp Cells: Effects on Morphology, Migration, Proliferation, and Immune Response. J Endod. 2024 Mar 1;50(3):362–9.

37. da Silva JC, Ramos RF, da Silva AASV, de Melo MEF, de Oliveira MA, de Sousa LB, *et al*. Impact of Cellular Senescence on the Immune-Inflammatory Response and Regenerative Capacity of Human Dental Pulp Cells. 2025 Jul.

38. Hong JH, Kim MR, Lee BN, Oh WM, Min KS, Im YG, *et al*. Anti-inflammatory and mineralization effects of bromelain on lipopolysaccharide-induced inflammation of human dental pulp cells. Medicina (Lithuania). 2021 Jun 1;57(6).

39. Van De Loosdrecht AA, Beelen RHJ, Ossenkoppele GJ, Broekhoven MG, Langenhuijsen MMAC. A tetrazolium-based colorimetric MTT assay to quantitate human monocyte mediated cytotoxicity against leukemic cells from cell lines and patients with acute myeloid leukemia. Vol. 174, Journal of Immunological Methods. 1994.

40. dos Santos AF, Pacheco JM, Silva PAO, Bedran-Russo AK, Rezende TMB, Pereira PNR, *et al*. Direct and transdentinal biostimulatory effects of grape seed extract rich in proanthocyanidin on pulp cells. Int Endod J. 2019 Apr 1;52(4):424–38.

41. Martinotti S, Ranzato E. Scratch wound healing assay. In: Methods in Molecular Biology. Humana Press Inc.; 2020. p. 225–9.

42. Crowley LC, Marfell BJ, Christensen ME, Waterhouse NJ. Measuring cell death by trypan blue uptake and light microscopy. Cold Spring Harb Protoc. 2016 Jul 1;2016(7):643–6.

43. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. Methods. 2001;25(4):402–8.

ANEXOS

Anexo 1. Parecer consubstanciado de aprovação do projeto no CEP/UnB (primeira e última página do parecer).

FACULDADE DE CIÊNCIAS DA SAÚDE DA UNIVERSIDADE DE BRASÍLIA - UNB

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação in vitro da senescência em células da papila apical humana
Pesquisador: Letícia Odaguiri Watanabe
Área Temática:
Versão: 2
CAAE: 86052124.4.0000.0030
Instituição Proponente: FACULDADE DE SAÚDE - FS
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.586.748

Apresentação do Projeto:
Conforme documento "PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_2459965.pdf", postado em 23/04/2025:

"Desenho: A Endodontia Regenerativa, é uma área que tem ganhado destaque nos últimos anos e busca a substituição de estruturas dentárias danificadas por meio de princípios propostos pela engenharia de tecidos. O tratamento de dentes com rizogênese incompleta e necrose pulpar pode ser desafiador e tais dentes devem ser manejados com cuidado por apresentarem paredes dentinárias delgadas e ápice aberto. Para tal situação alguns tratamentos costumam ser realizados, dentre eles apicificação, confecção de plug apical e terapias endodônticas regenerativas (TER). Contudo, apenas esta última possibilita a continuação do desenvolvimento radicular. A papila apical é composta por células mesenquimais indiferenciadas, um dos pilares da engenharia de tecidos que auxilia no desenvolvimento radicular nas TER, inclusive em casos de necrose. Por ser uma área relativamente nova, suas limitações ainda estão sendo relatadas. Entretanto, quando células da papila apical humana (CPAH) entram em processo de senescência, falhas na migração para o interior dos condutos ou na diferenciação celular podem ocorrer, acarretando falha do tratamento. Assim, os objetivos do projeto são avaliar os efeitos morfológicos, proliferativos, imunológicos e migratórios de CPAH senescentes e não senescentes. Para isso, cultura primária

Endereço: Faculdade de Ciências da Saúde, Universidade de Brasília - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

Não

BRASILIA, 22 de Maio de 2025

Assinado por:
Janine Araki
(Coordenador(a))

Endereço: Faculdade de Ciências da Saúde, Universidade de Brasília - Campus Darcy Ribeiro
Bairro: Asa Norte **CEP:** 70.910-900
UF: DF **Município:** BRASILIA
Telefone: (61)3107-1947 **E-mail:** cepfsunb@gmail.com

Anexo 2. Parecer consubstanciado de aprovação do projeto no CEP/UCB (primeira e última página do parecer).

UNIVERSIDADE CATÓLICA DE BRASÍLIA - UCB

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação in vitro da senescência em células da papila apical humana
Pesquisador: Letícia Odaguiri Watanabe
Área Temática:
Versão: 1
CAAE: 86052124.4.3002.0029
Instituição Proponente: Stricto Sensu em Ciências Genômicas e Biotecnologia
Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 7.628.189

Apresentação do Projeto:
Trata-se de um projeto de pesquisa do Programa de Pós-graduação em Ciências da Saúde da Instituição Universidade de Brasília a ser desenvolvido pela estudante de Letícia Odaguiri Watanabe (mestrado) sob orientação da professora Taia Maria Berto Rezende.

RESUMO:
A Endodontia Regenerativa, é uma área que tem ganhado destaque nos últimos anos e busca a substituição de estruturas dentárias danificadas por meio de princípios propostos pela engenharia de tecidos. O tratamento de dentes com rizogênese incompleta e necrose pulpar pode ser desafiador e tais dentes devem ser manejados com cuidado por apresentarem paredes dentinárias delgadas e ápice aberto. Para tal situação alguns tratamentos costumam ser realizados, dentre eles apicificação, confecção de plug apical e terapias endodônticas regenerativas (TER). Contudo, apenas esta última possibilita a continuação do desenvolvimento radicular. A papila apical é composta por células mesenquimais indiferenciadas, um dos pilares da engenharia de tecidos que auxilia no desenvolvimento radicular nas TER, inclusive em casos de necrose. Por ser uma área relativamente nova, suas limitações ainda estão sendo relatadas. Entretanto, quando células da papila apical humana (CPAH) entram em processo de senescência, falhas na migração para o interior dos condutos ou na diferenciação celular podem ocorrer, acarretando falha do tratamento. Assim, os objetivos do projeto são avaliar os

Endereço: QS 07 ¿ Lote 01 ¿ EPCT ¿ Águas Claras, Bloco C, 2° Andar, Sala C204-A.
Bairro: Taguatinga **CEP:** 71.966-700
UF: DF **Município:** BRASILIA
Telefone: (61)3356-9784 **Fax:** (61)33563-9636 **E-mail:** cep@ucb.br

UNIVERSIDADE CATÓLICA DE BRASÍLIA - UCB

Continuação do Parecer: 7.628.189

Outros	Termo_de_concordancia_UCB.doc	21:30:14	Watanabe	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BRASILIA, 09 de Junho de 2025

Assinado por:
MARCELO HENRIQUE SOLLER RAMADA
(Coordenador(a))

Endereço: QS 07 ¿ Lote 01 ¿ EPCT ¿ Águas Claras, Bloco C, 2° Andar, Sala C204-A.
Bairro: Taguatinga **CEP:** 71.966-700
UF: DF **Município:** BRASILIA
Telefone: (61)3356-9784 **Fax:** (61)33563-9636 **E-mail:** cep@ucb.br

Anexo 3. Trabalho elaborado durante o período de mestrado que se encontra submetido para apreciação.

INTERNATIONAL ENDODONTIC JOURNAL
The official journal of the British Endodontic Society and the European Society of Endodontology

Original Article

INFLAMMATION-DRIVEN SENESCENCE REDUCES THE REGENERATIVE CAPACITY OF APICAL PAPILLA STEM CELLS IN VITRO: IMPLICATIONS FOR REGENERATIVE ENDODONTIC THERAPY

Submission ID	9e44a0bc-3625-483e-a7cc-e0dea6d4fb81
Manuscript ID	3563557
Submission Version	Initial Submission
PDF Generation	16 Dec 2025 06:19:03 EST by Atypon ReX