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E DO DESENVOLVIMENTO

Anderson Padilha da Rocha

Secretoma de células estromais mesenquimais para o tratamento de alopecia: uma revisão sistemática de estudos pré-clínicos e clínicos.

Florianópolis
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Orientadora: Profa. Dra. Talita da Silva Jeremias

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O presente trabalho em nível de Mestrado foi avaliado e aprovado, em 30 de agosto de 2023, pela banca examinadora composta pelos seguintes membros:

Profa. Dra. Débora Cristina Olsson
Instituto Federal Catarinense

Profa. Dra. Evelise Maria Nazari
Universidade Federal de Santa Catarina

Profa. Dra. Norma Machado da Silva
Universidade Federal de Santa Catarina

Dra. Bianca Luíse Teixeira

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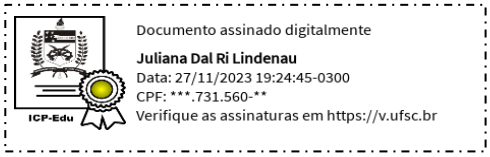
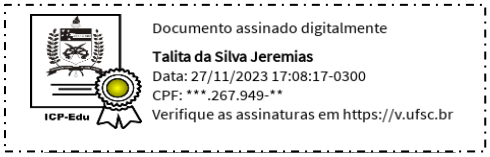


Profa. Dra. Juliana Dal Ri Lindenau
Coordenadora do Programa de Pós-Graduação em Biologia Celular e do Desenvolvimento



Profa. Dra. Talita da Silva Jeremias
Orientadora

Florianópolis, 2023

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Após pouco tempo do início do mestrado turma 2020/I desencadeou-se a pandemia devido ao COVID-19. Ocorreram mudanças na rotina acadêmica e surgiram as atividades por ensino a distância (EaD). Pensei em desistir, mas cheguei até este momento de conclusão de uma etapa. Por consequência da pandemia decidi por mudar o projeto inicial para um trabalho de revisão, sendo possível a realização à distância. Isso reduziu muito a ansiedade gerada pelo processo pandêmico que estávamos passando.

Este trabalho com a temática de alopecia e utilização de recursos celulares para seu tratamento foi muito satisfatório. Isso vem ao encontro de meu interesse em estética em que há inovações a cada momento e o trabalho desta dissertação tem muito a contribuir. Ou seja, atualmente estou concluindo uma especialização em biomedicina estética e esta dissertação se tornou muito atrativa aos meus interesses pessoais. Sendo assim, eu não mudaria em nada este trabalho se tivesse que recomeçá-lo, e futuramente pretendo continuar o meu aperfeiçoamento profissional realizando o Doutorado no PPGBCD.

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Por fim, agradeço a Universidade Federal de Santa Catarina por fomentar a formação científica e dar apoio para o aperfeiçoamento dos programas de pós-graduação desta Universidade. Que se não fosse a sua missão não teríamos a formação de profissionais na área da ciência e tecnologia.

RESUMO

A alopecia afeta os folículos pilosos, causa a perda dos pelos e cabelos, sendo responsável por complicações psicossociais. Apesar dos extensos esforços de pesquisa, encontrar tratamentos eficazes para a alopecia continua sendo um desafio. Uma possível alternativa no cenário da medicina regenerativa é a utilização do secretoma derivado de células estromais mesenquimais (CEM), evitando, assim, complicações como rejeição e formação de tumores. O secretoma de CEM engloba uma gama diversificada de moléculas bioativas com propriedades regenerativas e imunomoduladoras, oferecendo uma abordagem promissora para o tratamento da alopecia. No entanto, ainda faltam evidências para resumir a eficácia do secretoma das CEM no tratamento da queda de cabelo. Portanto, nosso objetivo é realizar uma revisão sistemática para identificar a evidência científica da eficácia do tratamento baseado no secretoma derivado de CEM para o tratamento de alopecia, analisando estudos pré-clínicos e clínicos. Uma pesquisa bibliográfica foi realizada em sete bases de dados e literatura cinza desde o início até outubro de 2021 (Medline/Pubmed, Embase, Lilacs, Scopus, Web of Science, CabAbstract, Livivo, Google Scholar, OpenGray e ProQuest), resultando em um total de 5.398 registros. Após a triagem, 10 estudos pré-clínicos e 6 estudos clínicos preencheram os critérios de inclusão para análise. Apenas estudos que compararam grupos de tratamento com secretoma de CEM com grupos de controle não tratados ou com veículo ou minoxidil foram incluídos. Os resultados revelaram que todos os estudos incluídos foram conduzidos em países asiáticos, em que a publicação mais antiga foi de 2010. A heterogeneidade entre os estudos foi observada em termos de modelos animais, tipos de distúrbios de perda de cabelo, fontes de CEM para produção de secretoma, métodos, doses e frequência de administração. Em relação aos resultados avaliados, a maioria dos estudos pré-clínicos e clínicos indicou que o tratamento com secretoma de CEM promove o crescimento do cabelo e aumenta a densidade do cabelo, respectivamente. Além disso, estudos pré-clínicos concluíram que o tratamento induz a angiogênese e promove a transição para a fase anágena do folículo piloso. Em conclusão, embora haja necessidade de relatórios claros em estudos futuros e um tamanho de amostra maior em estudos pré-clínicos e clínicos, os resultados sugerem um papel terapêutico para CEM no tratamento de distúrbios de perda de cabelo. Esses achados fornecem suporte para o uso de CEM em futuros ensaios clínicos para pacientes com alopecia.

Palavras-chave: célula estromal mesenquimal, célula-tronco mesenquimal, ensaios clínicos, perda de cabelo, secretoma.

ABSTRACT

Alopecia affects the hair follicles, causes hair loss and is responsible for psychosocial complications. Despite extensive research efforts, finding effective treatments for alopecia remains a challenge. An alternative in the scenario of regenerative medicine is the use of secretome derived from mesenchymal stromal cells (MSC), thus avoiding complications such as rejection and tumor formation. The MSC secretome encompasses a diverse range of bioactive molecules with regenerative and immunomodulatory properties, offering a promising approach for the treatment of alopecia. However, evidence is still lacking to summarize the effectiveness of MSC secretome in treating hair loss. Therefore, this review aims to provide a comprehensive overview of the current evidence regarding the therapeutic potential of MSC secretome-based interventions for hair growth, by analyzing preclinical (rodent) and clinical studies. A literature search was conducted in 7 databases and gray literature from inception to October 2021 (Medline/Pubmed, Embase, Lilacs, Scopus, Web of Science, CabAbstract, Livivo, Google Scholar, OpenGray e ProQuest), resulting in a total of 5,398 records. After screening, 10 preclinical studies and 6 clinical studies met the inclusion criteria for analysis. Only studies that compared MSC secretome treatment groups with untreated or vehicle or minoxidil control groups were included. The results revealed that all included studies were conducted in Asian countries, since 2010. Heterogeneity among the studies was observed in terms of animal models, types of hair loss disorders, MSC sources for secretome production, methods, doses, and frequency of administration. Regarding the evaluated outcomes, the majority of preclinical and clinical studies indicated that treatment with MSC secretome promotes hair growth and increases hair density, respectively. Furthermore, preclinical studies showed that the treatment induces angiogenesis and promotes the transition to the anagen phase of the hair follicle. In conclusion, although there is a need for transparent reporting in future studies and a larger sample size in both preclinical and clinical studies, the results suggest a therapeutic role for MSC in the treatment of hair loss disorders. These findings provide support for the use of MSC in future clinical trials for patients with alopecia

Keywords: Alopecia; Hair Loss; Mesenchymal stem cells; Secretome; Preclinical studies; clinical studies

LISTA DE ABREVIATURAS E SIGLAS

ADSC	Adipose stem cell
AGA	Androgenic alopecia
CEM	Células estromais mesenquimais
CM	Conditioned media
DMEM	Dulbecco's Modified Eagle Medium
EVs	Extracellular vesicles
FGF	Fibroblast Growth Factor
FPHL	Female pattern hair loss
IGF	Insulin-like Growth Factor
MC	Meio condicionado
MC-CEM	Meio condicionado de células estromais mesenquimais
MeSH	Medical Subject Heading
MNX	Minoxidil
MPB	Male pattern baldness
MSC	Mesenchymal stromal cells
NI	Not identified
PDGF	Platelet-Derived Growth Factor
PRISMA-p	Systematic Reviews and Meta-Analyses Protocols
RevMan	Review Manager
ROBINS-I	Risk of Bias In Non-randomized Studies of Interventions
SHED	Dental pulp stem cells obtained from human deciduous teeth
SYRCLE	Systematic Review Centre for Laboratory Animal Experimentation
TGF-β	Transforming Growth Factor-beta

LISTA DE FIGURAS

LISTA DE TABELAS

SUMÁRIO

1 INTRODUÇÃO

1.1 ALOPECIA

A alopecia é uma doença dermatológica que afeta os folículos pilosos e está relacionada com a perda parcial ou total dos pelos e cabelos, independentemente da causa (AL ABOUD & ZITO, 2020; MONSELISE *et al.*, 2013). Essa ausência de pelos provocada pela doença apresenta poucos efeitos físicos nocivos (REBELO & REIS, 2015), no entanto, é responsável por complicações psicossociais, representada por altos níveis de ansiedade, depressão e problemas de autoestima (HUNT & MCHALE, 2005; HUNT & MCHALE, 2007; REBELO & REIS, 2015).

Fatores genéticos e ambientais estão relacionados com a etiologia da doença (REBELO & REIS, 2015), que pode ser classificada em: alopecia cicatricial e não-cicatricial (WEIDE & MILÃO, 2009). A primeira é resultado de uma inflamação que leva a destruição dos folículos pilosos e, consequentemente, a perda irreversível de cabelo. Neste caso, apresenta-se de duas formas, a inflamação primária que ataca diretamente o folículo piloso; e a inflamação secundária que é de causa sistêmica como doenças neoplásicas, sarcoidose e inflamação granulomatosa (GORDON & TOSTI, 2011).

Em relação à alopecia não-cicatricial, esta pode ser classificada em quatro tipos principais: tricotilomania, eflúvio telógeno, alopecia areata e alopecia androgenética (MITEVA & TOSTI, 2012; RIVERA & GERRA-TAPIA, 2008). A tricotilomania é caracterizada por ser um transtorno crônico em que o indivíduo tem impulsos de puxar seu próprio cabelo, o que gera irritação cutânea, infecções, lesões e a perda de cabelo, sendo mais frequente em adolescentes e jovens adultos (FRANKLIN & ZAGRABBE & BENAVIDES, 2011). O eflúvio telógeno resulta da entrada precoce dos folículos na fase telógena (de queda) do ciclo capilar, o que ocasiona uma queda excessiva de cabelo (GORDON & TOSTI, 2011). Pode ser causado por doenças sistêmicas, estresse emocional, perda de peso e deficiência de vitamina D ou de ferro. Já a alopecia areata, está relacionada a fatores autoimunes e genéticos de etiologia desconhecida (GORDON & TOSTI, 2011; RIVITTI, 2005).

A alopecia androgenética, popularmente conhecida como calvície, destaca-se como a forma mais comum de perda de cabelo em homens e mulheres e é ocasionada por distúrbios hormonais e fatores genéticos (RATHNAYAKE & SINCLAIR, 2010).

Esse tipo de alopecia caracteriza-se pela perda progressiva do diâmetro, comprimento e pigmentação do cabelo (GORDON & TOSTI, 2011). A sua prevalência está diretamente relacionada à idade, quanto mais velho é o indivíduo, maior será a prevalência: em homens de 50 anos a prevalência é de 50%; homens com mais de 70 anos a prevalência é de 80%, nas mulheres é de 42% (BLUME-PEYTAVI *et al.*, 2008; BLUMEYER *et al.*, 2011; PERERA & SINCLAIR, 2014; RAFI & KATZ, 2011). Além disso, existem diferenças étnicas na sua prevalência, sendo que a população caucasiana é mais afetada que a população africana (BLUMEYER, A. *et al.*, 2011; ELLIS & SINCLAIR & HARRAP, 2002).

O tratamento é realizado de acordo com a etiologia e o tipo da alopecia. Na alopecia androgenética, os medicamentos utilizados comercialmente e aprovados pela Agência Nacional de Vigilância Sanitária (ANVISA) do Brasil e pelo *Food and Drug Administration* (FDA) dos Estados Unidos são o Minoxidil® e a Finasterida® (antiandrogênico) (BOISVERT *et al.*, 2017). Já na alopecia areata, corticosteroides de média potência e imunomoduladores tópicos podem beneficiar o paciente (ZHANG *et al.*, 2016). No entanto, apesar de eficazes há muitos efeitos colaterais graves relacionados a estes medicamentos, além da eficácia ser imprevisível (ZHANG *et al.*, 2016).

Adicionalmente, vem crescendo a realização de transplante de folículos pilosos, que é o único tratamento que pode aumentar substancialmente o número de cabelos. O transplante capilar é um procedimento cirúrgico invasivo que consiste em retirar os folículos pilosos de regiões específicos do couro cabeludo ou barba e transplantá-los na região onde há falta de cabelo (SANTOS & SHAPIRO, 2014). Outras técnicas também têm sido exploradas como alternativa para quem não quer passar por procedimentos cirúrgicos ou apresentam efeitos colaterais ao Minoxidil® e a Finasterida®. Por exemplo, a terapia com laser de baixa potência, microagulhamento, aplicação de fatores de crescimento e de plasma rico em plaquetas. Os resultados de estudos com estas abordagens terapêuticas ainda possuem controvérsias nos resultados, sendo necessários maiores comprovações científicas sobre as eficácias (SANTOS & SHAPIRO, 2014).

Tendo em vista este cenário, a medicina regenerativa tem utilizado estratégias terapêuticas focadas na terapia celular com células-tronco para o tratamento de alopecia, com a finalidade de regenerar e ativar o folículo piloso e restabelecer o crescimento do cabelo. Já é descrito na literatura, que a manutenção do folículo piloso é dependente de

células-tronco presentes em sua estrutura e da interação com as células mesenquimais da papila dérmica (GUASCH, 2017). As células mesenquimais da papila dérmica são importantes para a ativação do crescimento do cabelo e para a transmissão de sinais durante o ciclo do folículo piloso (ROMPOLAS & GRECO, 2014; ROMPOLAS & MESA & GRECO, 2013).

O ciclo de crescimento dos folículos pilosos é um processo essencial para a renovação e integridade dos mesmos (FUCHS, 2007). Ele consiste em três estágios distintos: crescimento (anágena), regressão (catágena) e repouso (telógena), sendo controlado por fatores complexos, tais como a alteação do número de células da matriz, bainha da raiz, papila dérmica e fatores de crescimento presentes em momentos específicos do desenvolvimento capilar (ALONSO & FUCHS, 2006). A fase de crescimento, anágena, é marcada pela intensa proliferação e diferenciação das células na base do folículo, estimuladas por fatores da papila dérmica: Wnt, Shh e TGFβ. Essa fase, mais longa e predominante, determina o comprimento do cabelo e envolve a queratinização celular, conferindo resistência e flexibilidade ao cabelo. À medida que a atividade mitótica das células-tronco diminui, a fase anágena cede lugar à fase de regressão, catágena, caracterizada pela apoptose das células epiteliais e da bainha reticular externa (ALONSO & FUCHS, 2006). Nesse processo, a região inferior do folículo regride e a papila dérmica move-se para cima, próxima às células-tronco do bulge. Durante a fase telógena, o folículo piloso entra em repouso e após a ativação pelas células da papila dérmica ocorre a indução da transição para a fase anágena e, consequentemente, crescimento do pelo (ALONSO & FUCHS, 2006).

Além das sinalizações intercelulares que ocorrem dentro do folículo piloso (CK19, Gli1, Sox9, LHX2, Hopx, Tcf3 e Nfatc1), sinais provenientes dos tecidos adjacentes, como o tecido adiposo e derme (BMP2, MHC-I, FGF-9, VEGF, IGF-I, PGE-2) também estão envolvidos na manutenção do folículo piloso e, consequentemente, no crescimento do cabelo (GENTILE & GARCOVICH, 2019). Assim, estudos recentes vêm sendo realizados baseados na terapia com células-tronco derivadas do folículo piloso (epidermais e da papila dérmica) e células estromais mesenquimais de diferentes tecidos, além da aplicação do secretoma (meio condicionado) destas células, a fim de avaliar o potencial biotecnológico para o tratamento de alopecia (GENTILE & GARCOVICH, 2019).

1.2 CÉLULA ESTROMAL MESENQUIMAL E SECRETOMA

Em 1976 foram isoladas e identificadas as células estromais mesenquimais por Friedenstein e colaboradores. Inicialmente estas células foram chamadas de unidade de colônia formadora de fibroblastos (*colony forming unit-fibroblast* – CFU-F), e posteriormente Arnold I. Caplan cunhou a nomenclatura células-tronco mesenquimais (CAPLAN, 1991). No entanto, somente em meados da década dos anos 2000 que a Sociedade Internacional para Terapia Celular (*International Society for Cellular Therapy*) padronizou a nomenclatura e a identificação da célula estromal mesenquimal baseados em propriedades semelhantes observadas em diversos trabalhos. Com esta padronização é possível que pesquisadores isolem e identifiquem este mesmo tipo celular, sendo possível alavancar os avanços científicos e o desenvolvimento de terapias celulares (DOMINICI *et al.*, 2006).

Para a caracterização destas células foram definidos três aspectos: 1) em cultivo *ex vivo* as células devem aderir em plástico em meio de cultura padrão; 2) multipotencialidade: induzir diferenciação destas células em cartilagem, osso ou gordura de acordo com o meio utilizado; 3) células extraídas de seres humanos as células estromais mesenquimais (CEM) devem apresentar padrão fenotípico positivo maior ou igual a 95% para CD105, CD73 e CD90, e negativo menor ou igual a 2% para CD45, CD34, CD14 ou CD11b, CD79α ou CD19, e HLA-DR (DOMINICI *et al.*, 2006).

Por conseguinte, estas CEM já possuem protocolos bem definidos para identificação e, pela abrangência de sua localização, há muitos órgãos e tecidos que podem ser utilizados para sua extração, como: cordão umbilical, fluido amniótico, tecido adiposo, pâncreas, pulmão, polpa de dente, derme (GRONTHOS *et al.*, 2000; IN'T ANKER *et al.*, 2003; JEREMIAS *et al.*, 2014; LIMBERT *et al.*, 2010; POPOVA *et al.*, 2010; SECCO *et al.*, 2008; ZAMINY *et al.*, 2008; ZUK *et al.*, 2001). As células isoladas destes locais não são totalmente idênticas, a idade e o local do órgão ou tecido podem influenciar na plasticidade e capacidade proliferativa destas células (GRONTHOS *et al.*, 2000; IN'T ANKER *et al.*, 2003; JEREMIAS *et al.*, 2014; LIMBERT *et al.*, 2010; POPOVA *et al.*, 2010; SECCO *et al.*, 2008; ZAMINY *et al.*, 2008; ZUK *et al.*, 2001). Ou seja, as CEM, de acordo com sua localização, apresentam pequenas variações fenotípicas e de expressão molecular da matriz extracelular (CORSELLI *et al.*, 2010).

Como características principais, as CEM migram e implantam-se em diversos tecidos do corpo após serem administradas via sistêmica ou local, além de apresentarem preferência a se implantarem em áreas lesionadas atraídas por quimiotaxia (MCBRIDE *et al.*, 2003; ORTIZ *et al.*, 2003; PEREIRA *et al.*, 1995; PEREIRA *et al.*, 1998). No local da lesão, a sua capacidade de transdiferenciação para outros fenótipos não é amplamente aceita (ALVAREZ-DOLADO *et al.*, 2003; PEREIRA LOPES *et al.*, 2010; RODIC *et al.*, 2004; TERADA *et al.*, 2002; VASSILOPOULOS *et al.*, 2003; WANG *et al.*, 2003; YING *et al.*, 2002). Ou seja, apesar destas células se implantarem em regiões próximas as lesões, não é evidente o efeito positivo pelo repovoamento celular a partir das células implantadas ou sua diferenciação e transdiferenciação. Além disso, estudos revelaram que as células implantadas não ficam viáveis durante muito tempo (VIZOSO *et al.*, 2017), o que restringe a eficácia terapêutica do uso de células em terapias (BALDARI *et al.*, 2017; LI *et al.*, 2017; LIU *et al.*, 2016; TANTO *et al.*, 2016).

Portanto, acredita-se que o maior efeito das CEM na regeneração tecidual ocorra pelo efeito parácrino, por meio do seu secretoma. Com isso, a hipótese para seus efeitos benéficos é pela secreção de fatores solúveis e vesículas extracelulares que atuam na manutenção da homeostasia de tecidos lesionados e promovem o reparo tecidual (CHAMBERLAIN *et al.*, 2007; HERRERO & PÉREZ-SIMÓN, 2010; KINNAIRD *et al.*, 2004).

O meio de cultura no qual as CEM são cultivadas apresentam as vesículas e fatores solúveis secretados por essas células, sendo chamado de meio condionado (MC) (VIZOSO *et al.*, 2017). Análises do MC-CEM mostram a presença de diversos bioativos, como citocinas, quimiocinas, fatores de crescimento, moléculas de matriz extracelular e metaloproteínases (CHEN *et al.*, 2008). Além desses fatores solúveis, recentemente, o papel das vesículas extracelulares na comunicação celular tem sido efetivamente comprovado e relacionado com diversas alterações teciduais (PHINNEY & PITTENGER, 2017; RAJENDRAN *et al.*, 2017). Em geral, essas vesículas são carregadas com proteínas, lipídeos e ácidos nucleicos, incluindo mRNA, microRNA, que chegam ativos à célula alvo (RAPOSO & STOORVOGEL, 2013), podendo induzir uma sinalização via interação receptor-ligante ou, ainda, podem ser internalizadas liberando seu conteúdo no citoplasma e alterando respostas fisiológicas da célula receptora (RAPOSO & STOORVOGEL, 2013; TKACH & THÉRY, 2016). Sendo assim, o MC-CEM já é sugerido como um novo tratamento “cell free” capaz de replicar os efeitos benéficos das células, com a vantagem de não ter riscos associados à terapia

baseada em células, como reações imunológicas e tumorigenicidade (GUNAWARDENA *et al.*, 2019; LEE & HONG, 2017; SAGARADZE *et al.*, 2018).

Dentre as pesquisas que utilizam o MC-CEM estão incluídos vários ensaios clínicos e pré-clinicos para inúmeras condições patológicas, entre elas a alopecia. De fato, as moléculas secretadas por essas células podem promover um microambiente tecidual favorável à proliferação e manutenção das células foliculares (GUNAWARDENA *et al.*, 2019). Estudos mostram que o MC pode aumentar a vascularização, prolongar a fase anágena (de crescimento) e promover o crescimento do pelo, tanto *in vitro*, *ex vivo*, como *in vivo* (GENTILE & GARCOVICH, 2019). Nesse sentido, o secretoma das CEM tem sido avaliado na área de medicina regenerativa como um produto biotecnológico promissor para o tratamento de alopecias.

Assim, o presente estudo apresenta evidências científicas disponíveis na literatura em relação a eficácia do secretoma das CEM para o tratamento de alopecia, em modelos pré-clínicos e clínicos, através da realização de uma revisão sistemática. Esse conhecimento é relevante para encontrar lacunas no conhecimento, o que irá contribuir por meio da sistematização da informação da literatura com subsídios para estabelecer ensaios clínicos seguros e o desenvolvimento de novas estratégias terapêuticas na área de medicina regenerativa.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Revisar de forma sistemática os estudos pré-clínicos e os clínicos que avaliem a eficácia do secretoma de células estromais mesenquimais no tratamento de alopecia.

2.2 OBJETIVOS ESPECÍFICOS

- Identificar a evidência científica disponível em estudos primários (artigos científicos que relatam os resultados de pesquisa iniciais) que associe a administração do secretoma das CEM (MC e vesículas extracelulares) e o crescimento de pelos em modelo pré-clínico e em estudos clínicos;

- Descrever as características dos estudos que aplicam o secretoma das CEM em modelos animais (roedores) e em pacientes diagnosticados com alopecia;
- Identificar os efeitos da administração do MC-CEM no crescimento do pelo e no folículo piloso (proliferação, transição da fase telógena para a fase anágena, neovascularização) em modelos animais (roedores) e em pacientes diagnosticados com alopecia.

3 APRESENTAÇÃO DO ARTIGO CIENTÍFICO

A seguir estão apresentados os dados obtidos nessa revisão sistemática no formato de artigo científico a ser submetido. A questão central da revisão é se o secretoma das CEM pode promover o crescimento do pelo em modelos pré-clínicos (camundongos e ratos) de perda de pelo e em pacientes com diferentes formas de alopecia.

Para responder nossa pergunta, foi realizada uma busca na literatura em 7 bancos de dados e na literatura cinzenta (Medline/Pubmed, Embase, Lilacs, Scopus, Web of Science, CabAbstract, Livivo, Google Scholar, OpenGray e ProQuest), desde o início das publicações até outubro de 2021. Foram obtidos um total de 5398 artigos, dos quais 10 estudos pré-clínicos e 6 estudos clínicos apresentaram todos os critérios de inclusão estabelecidos. A análise dos resultados foi realizada tendo em vista os desfechos encontrados em ambos os estudos, clínicos e pré-clínicos, de forma conjunta.

3.1 ARTIGO CIENTÍFICO

Mesenchymal Stromal Cell Secretome for Hair Loss Treatment: A Systematic Review of Preclinical and Clinical Studies

Anderson Padilha da Rocha[1], Maiara Marques da Silva[2], Andréa Gonçalves Trentin[3], Talita da Silva Jeremias[3]

1 University Hospital of Santa Catarina; 2 Federal University of Santa Catarina, Graduate Program in Biosciences and Biotechnology; 3 Federal University of Santa Catarina, Graduate Program in Cellular and Development Biology

Abstract

Alopecia, a common hair disorder characterized by hair loss, represents a significant psychological and emotional factor for affected individuals. Despite extensive research efforts, finding effective treatments for alopecia remains a challenge. Recently, attention has turned to the therapeutic potential of secretome derived from mesenchymal stromal cell (MSC). The MSC secretome have a diverse range of bioactive molecules with regenerative and immunomodulatory properties, offering a promising approach for the treatment of alopecia. However, there is still a lack of evidence to summarize the effectiveness of MSC secretome in the treatment of hair loss. Therefore, this systematic review aims to provide a comprehensive overview of the current evidence regarding the therapeutic potential of MSC secretome-based interventions for hair growth, by analyzing preclinical and clinical studies. A literature search was conducted in 7 databases and gray literature from inception to October 2021 (Medline/Pubmed, Embase, Lilacs, Scopus, Web of Science, CabAbstract, Livivo, Google Scholar, OpenGray e ProQuest), resulting in a total of 5,398 records. After screening, 10 preclinical studies and 6 clinical studies met the inclusion criteria for analysis. Only studies that compared MSC secretome treatment groups with untreated or vehicle or standard drugs control groups were included. The results revealed that all included studies were conducted in Asian countries, since 2010 (oldest article that was included). Heterogeneity among the studies was observed in terms of animal models, hair loss disorders classification, gender, MSC sources for secretome production, methods, doses, and frequency of administration. Regarding the evaluated outcomes, the majority of preclinical and clinical studies indicated that treatment with MSC secretome promotes hair growth and increases hair density, respectively. Furthermore, preclinical studies showed that the treatment induces angiogenesis and promotes the transition to the anagen phase of the hair follicle. In conclusion, although there is a need for transparent reporting in future studies and a larger sample size in both preclinical and clinical studies, the results suggest a therapeutic role for MSC secretome in the treatment of hair loss disorders. These findings provide support for the use of MSC in future clinical trials for patients with alopecia.

Keywords: Alopecia; Hair Loss; Mesenchymal stem cells; Secretome; Preclinical studies; clinical studies

Introduction

Alopecia is a pathological condition characterized by abnormal hair loss, that significantly impacts physical appearance and psychological well-being, affecting individuals life's quality (1). Various factors, such as genetic, hormonal, environmental, and immunological can influence the hair follicle cycle, thereby affecting hair growth (2). This condition comprises a spectrum of hair loss disorders, categorized into two subtypes: cicatricial alopecia and non-cicatricial alopecia (2). In cases of cicatricial alopecia, hair follicles are destroyed, leading to permanent hair loss. Non-cicatricial alopecia includes common conditions such as androgenetic alopecia, alopecia areata, and telogen effluvium, among others, in which the hair follicle cycle is altered, allowing hair regrowth.

To generate new hairs, existing follicles go through cycles of growth (anagen), regression (catagen), and rest (telogen). In each anagen phase, follicles produce a complete hair shaft from the tip to the root. The catagen phase is characterized by apoptosis of epithelial cells and external reticular sheath. Within this intricate process, the lower region of the hair follicle regresses while the dermal papilla migrates upwards, establishing close proximity to the bulge stem cells. Subsequently, the hair follicle transitions into the telogen phase, a period of quiescence. During this phase, stem cells receive the signal to initiate the subsequent growth phase and generate a new hair shaft (3).

The current therapeutic approaches for alopecia include topical medications (4), oral drugs (5), and hair transplantation (6;7). Although some individuals may experience partial hair regrowth with these treatments, they are not universally effective, and their outcomes vary according to the type and severity of alopecia (8;9). Moreover, treatments can have adverse effects, and none provides a definitive cure for the condition (10). Therefore, the development of novel and more efficient therapeutic strategies to effectively manage alopecia is necessary.

In recent years, mesenchymal stem/stromal cell (MSC) therapy has emerged as a promising area of research for alopecia treatment (11;12;13;14). MSC have significant attention in regenerative medicine due to their unique properties, such as self-renewal, multilineage differentiation potential, and modulator effects (15). These multipotent stromal cells can be isolated from various tissues (16), including bone marrow, adipose

tissue, umbilical cord, dermal skin, and dental pulp, making them readily accessible for therapeutic purposes. In the alopecia context, MSC hold promising potential for promoting hair regrowth through their regenerative and paracrine effects (17).

Evidence suggests that paracrine secretion of bioactive mediators is the primary mechanism of therapeutic effects derived from MSC by playing essential roles in modulating cellular behavior, promoting tissue repair, and regulating the immune response (18;19). The MSC secretome (obtained from conditioned media, CM) represents a repertoire of bioactive molecules, including growth factors, cytokines, and extracellular vesicles (EVs), which are actively secreted by these cells (17;20). EVs (exosomes and microvesicles) contain proteins, lipids, carbohydrates, and nucleic acids (e.g., DNA, mRNA, miRNA, and lncRNA) that mediate intercellular communication and modulate the microenvironment (21).

In this sense, the therapeutic potential of MSC secretome provide an innovative and safer alternative to traditional cell-based therapies, as it eliminates the risks associated with direct cell transplantation while retaining the regenerative benefits (22). Previous studies have suggested that MSC secretome (CM and EVs) stimulates hair follicle proliferation, prolongs the anagen phase of the hair cycle, creating a conducive environment for hair regrowth (23;24;25;26). Several pre-clinical studies using animal models, particularly in mice, have demonstrated encouraging results regarding the hair growth-promoting effects of MSC secretome (25;27;28;29). These findings support the assessment of the therapeutic potential of the secretome in clinical trials involving human patients with alopecia (30;31).

However, despite the expanding interest in MSC secretome as an innovative therapeutic approach for alopecia, the existing evidence is still limited and requires comprehensive evaluation. In this systematic review, we aim to provide valuable insights into the therapeutic potential of MSC secretome in hair loss disorder treatment. The central question of this systematic review is whether the MSC secretome can effectively promote hair growth in pre-clinical models of hair loss and patients with different classification of alopecia. The results from this review can inform future research directions, optimize treatment protocols, and guide the development of novel and effective therapies for individuals with alopecia.

Materials and Methods

Protocol

This systematic review followed the Systematic Reviews and Meta-Analyses Protocols (PRISMA-p) guidelines (32). The PRISMA checklist (33) was used to prepare our study report.

Eligibility Criteria

The eligible preclinical studies had to meet all of the following criteria: 1) rodent animals (rats and mice) with model of hair loss; 2) received intervention by application of MSC-derived secretome; 3) compared to vehicle or placebo (negative control) or standard drugs (positive control); 4) reported primary outcome (effects on hair growth); 5) trials design by animal intervention studies (randomized control trials or not randomized control trials) and 6) published in the Latin (Roman) alphabet.

For clinical studies, the eligibility criteria were: 1) adults diagnosed with loss excessive hair (pattern hair loss) at any stage or alopecia at any etiology; 2) received intervention by application of MSC-derived secretome on scalp; 3) compared to vehicle or placebo (negative control) or standard drugs (positive control) or before of application or half-head with control; 4) reported primary outcome (effects on hair growth); 5) clinical trials (before/after; half-head; control-treated) and 6) published in the Latin (Roman) alphabet. The detailed of inclusion and exclusion criteria for preclinical and clinical studies are listed in Supplementary Table 1 and 2, respectively.

Information Sources and search strategy

The literature was electronically searched using keyword sets and MeSH terms (Medical Subject Heading), detailed in Supplementary Table 3. Search strategies were applied in the following databases: EMBASE, LILACS, PubMed, Scopus, Web of Science, LIVIVO, CABAbstract. Gray literature was searched by Google Scholar, OpenGrey, and ProQuest Dissertation & Theses Global. The search strategy used was the same for clinical and preclinical studies and was conducted on October 24, 2021.

Selection Process

Citations retrieved by the searches were imported into EndNote and duplicates were automatically removed. After, references were managed in Rayyan QCRI software, and duplicates were removed manually. Studies were independently selected by 2 reviewers (M.M.S., A.P.R) in 2 phases: (1) screening of titles and abstracts and (2) reading the full-text of the relevant studies to determine their final eligibility. Divergences between reviewers were consensually solved by consultation of a third reviewer (T.S.J).

Data extraction

To ensure accuracy and completeness of the data collected, a data extraction form was first developed to systematically collect relevant information from each eligible study. Two independent reviewers (A.P.R and T.S.J) extracted data from each study, and any discrepancies between the reviewers were resolved through discussion. The following data were collected: study characteristics (authors, year of publication, country and study design), study population (sample size, animal model or hair loss diseases type, age, sex), intervention (tissue source of MSC, secretome component, dose, route, frequency of administration, control and application in skin), evaluation (methods of evaluation and follow-up), and outcome (hair growth - hair density, hair thickness, hair diameter, growth time, hair coverage, hair follicle number, hair anagen/telogen number; angiogenesis; proliferation).

Risk of Bias

The quality of the included studies was assessed by the reviewers (T.S.J) using the risk of bias tool for animal studies provided by the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) (34). The assessment included the following items: (1) Selection bias, which considered random sequence generation, adequate allocation concealment, and baseline characteristics; (2) Detection bias, which assessed blinding of trial caregivers and researchers, as well as random housing; (3) Reporting bias, which examined random outcome reporting and blinded outcome assessment; (4) Attrition bias, which evaluated the completeness of outcome data; (5) Reporting bias, which assessed selective outcome reporting by assessors; and (6) Other bias from additional sources. Each domain was evaluated as "Yes" for low risk of bias,

"No" for high risk of bias, or "Unclear" for questions with an unclear risk of bias. The risk of bias figure was generated using the Review Manager (RevMan v.5.4) software. The effectiveness of treatment, specifically hair growth, was the main point evaluated across the included studies.

The ROBINS-I (Risk of Bias In Non-randomized Studies of Interventions) tool (35) was employed for assessing the quality of included clinical studies and estimating their risk of bias. This tool proves valuable for evaluating non-randomized intervention studies. The assessment encompassed seven domains, each probing potential sources of bias: confounding, selection of participants, classification of interventions, deviations from intended interventions, missing data, measurement of outcomes, and selection of reported results. For each domain, the risk of bias was categorized as low, moderate, serious, critical, or lacking information. An overarching evaluation of each study's bias risk was derived from the collective categorizations across the seven domains.

Synthesis of Results

The primary outcome assessed in the included studies was the effect of MSC secretome treatment on hair growth. Hair growth was predominantly evaluated in preclinical studies by analyzing skin darkening and hair coverage in animals, while clinical studies focused on hair density. Additionally, various secondary parameters were explored to understand the mechanisms underlying the effect of secretome on hair growth, including hair follicle count, hair weight, hair thickness, transition between hair growth phases, proliferation and angiogenesis.

Results

Study Selection

Figure 1 presents a flowchart outlining the study selection process. Initially, a total of 8,514 records were identified through systematic database searches. After removing duplicates, 5,398 records remained for screening based on titles and abstracts. Following the initial screening, 49 articles were submitted to a comprehensive review of the full text, according the predetermined inclusion and exclusion criteria. Among these, 33 articles did not meet the eligibility criteria and were subsequently excluded

(Supplementary Table 4). Ultimately, 16 articles were considered eligible for qualitative analysis, with 10 classified as preclinical studies and 6 as clinical studies.

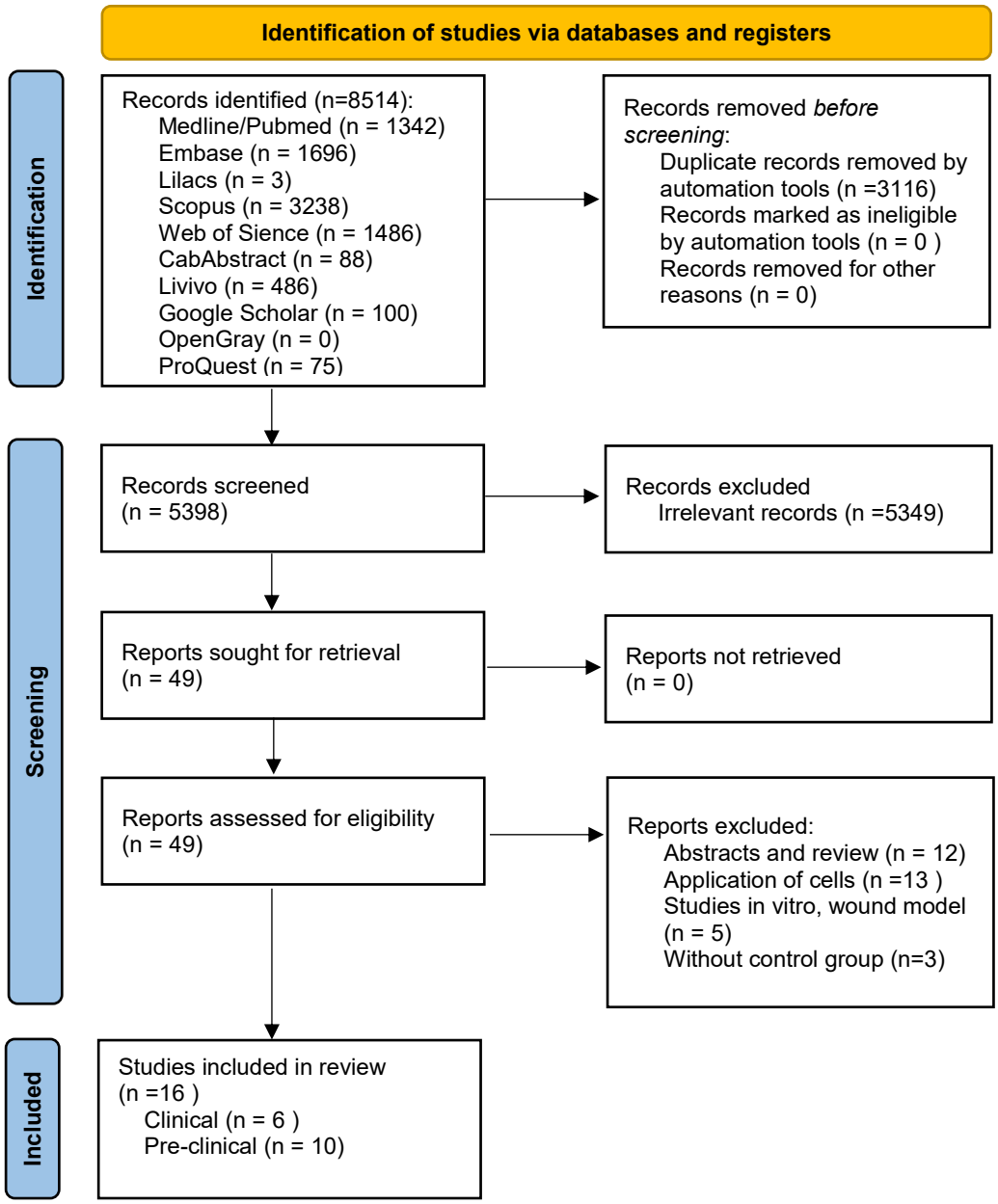

Figure. 1. Flow diagram of literature search and selection criteria. Adapted from Page et al., 2021; The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372:n71. DOI: 10.1136/bmj.n71 (http://www.prisma-statement.org/).

Study Characteristics

Preclinical studies

The characteristics of preclinical studies included are described in Table 1 and Figure 2 and 3. Articles were published from 2010 to 2021 and conducted in 4 countries: China (n=3), South Korea (n=5), Taiwan (n=1), and Malaysia (n=1). Among them, 3 corresponded to randomized interventional controlled studies, and 7 did not provide information about randomization. The studies were conducted in mice of 2 different strains: C57BL/6 at 7 weeks old (n=8) and C3H/HeN at 7 weeks old (n=2) (Figure 2). One of the studies did not report the age of the animals. The gender of animals in the studies was exclusively male (n=1) and exclusively female (n=3), with six studies not providing this information. The sample size ranged from 3 to 20 animals per group. The hair loss model used was hair shaving to induce the telogen phase, and in 2 studies, depilatory cream was also used.

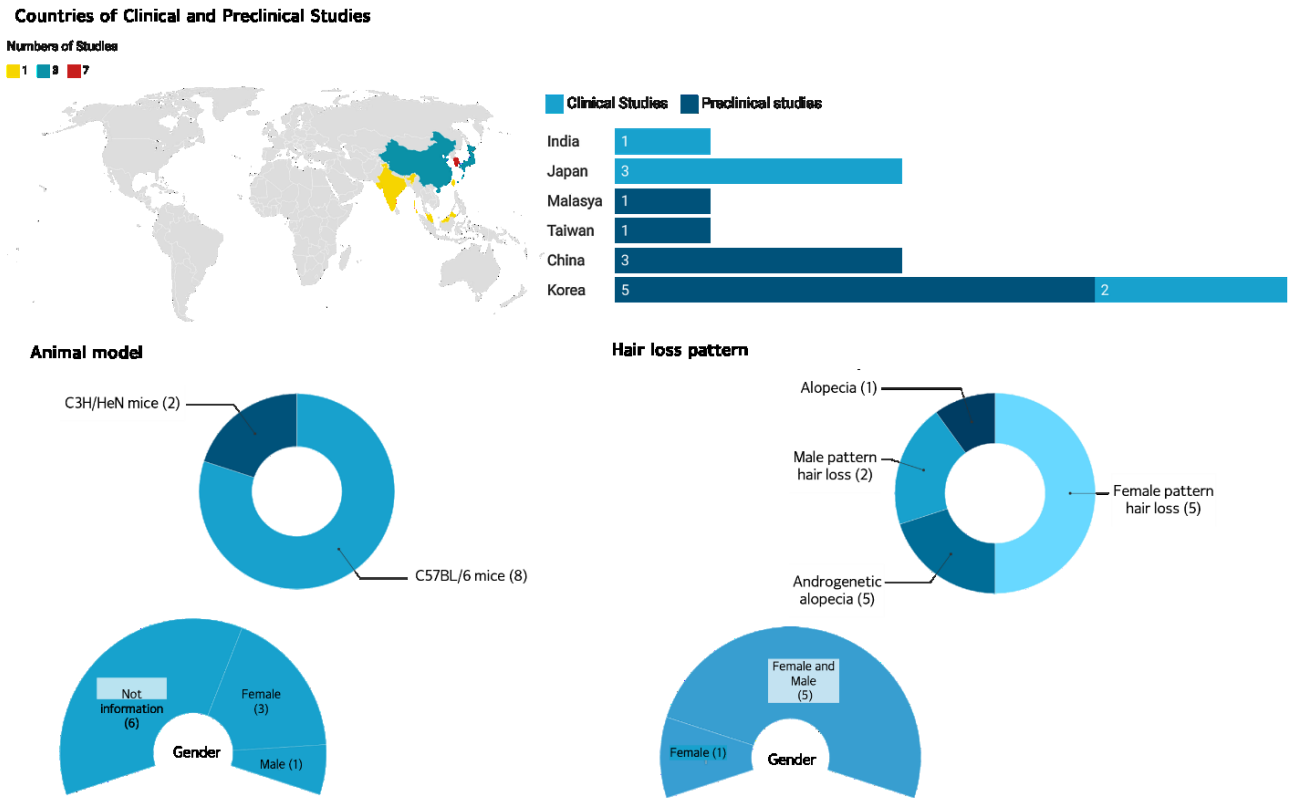

Figure 2. Characteristics of countries and population (animal model and patients) of preclinical and clinical studies included. Created with Datawrapper.

Treatments were performed using conditioned medium (n=9) or EVs (n=1) derived from MSC obtained from tooth pulp (n=1), adipose tissue (n=3), bone marrow (n=3), hair follicle (n=1), dermal progenitor cells (n=1) or vascular fraction of adipose tissue (n=1) (Figure 3). MSC derived from bone marrow were obtained from mice (n=2) and rats (n=1), while the other tissue sources were derived from humans. The mode of application of MSC secretome was subcutaneous (n=4), topical gel (n=1), topical (n=2), or intradermal (n=3). Application consisted of a single dose (n=1) or multiple doses (n=9), and frequency was daily or at intervals of different days or weeks. The controls used were culture medium (n=7), saline solution (PBS) (n=2), without treatment (n=1), and minoxidil as a positive control (n=3) (Figure 3). Some studies used more than one type of control.

The outcomes analyzed were hair growth (n=10), hair follicle number (n=2), hair follicle phase transition (n=4), angiogenesis (n=2), and cell proliferation (n=2) (Figure 3). Hair growth was analyzed by the rate of skin darkening (macroscopic images) (n=10) and hair weight (n=1). The number of follicles and phase transition were analyzed by histology. Angiogenesis was evaluated by the number of vessels (histology) (n=1), labeling with CD31 (immunohistochemistry) (n=2) or presence of vessels in the inner portion of the skin (macroscopic image) (n=1). Proliferation was evaluated by immunohistochemistry for Ki67 (n=1).

Clinical Studies

Table 2 and Figure 2 and 3 describe the characteristics of each clinical study included. Articles were published from 2010 to 2021 and conducted in 3 countries: Japan (n=3), South Korea (n=2), and India (n=1) (Figure 2). None of the studies provided information on randomization. The studies were conducted with groups of both male and female patients (n=5) and with female patients only (n=1). The age of the patients ranged from 20 to 74 years and the sample size ranged from 6 to 58 patients. The hair loss classification of the patients included alopecia (n=1), androgenetic alopecia (n=2), female pattern hair loss (n=5) and male pattern hair loss/baldness (n=2). Some studies had population subgroups with different classifications of hair loss patterns.

Treatments were performed using conditioned medium (n=6), five of them lyophilized, obtained from MSC derived from adipose tissue (n=5) and umbilical cord

(n=1) (Figure 3). The mode of application of conditioned medium was topical with microneedling or mesotherapy (n=1), topical with microneedling (n=2), or intradermal (n=3). Doses, frequency and duration of treatment varied, as described in Table 2. The controls used were without treatment (n=5), in the case of before/after studies, and saline (n=1) or culture medium (n=1) in half-side studies. Some studies used more than one type of application and control. The outcomes analyzed were hair density (n=5), hair thickness (n=2), increase in hair anagen phase number (n=2), and hair growth (n=1) assessed through trichograms analysis (n=5) and using clinical grading (n=1).

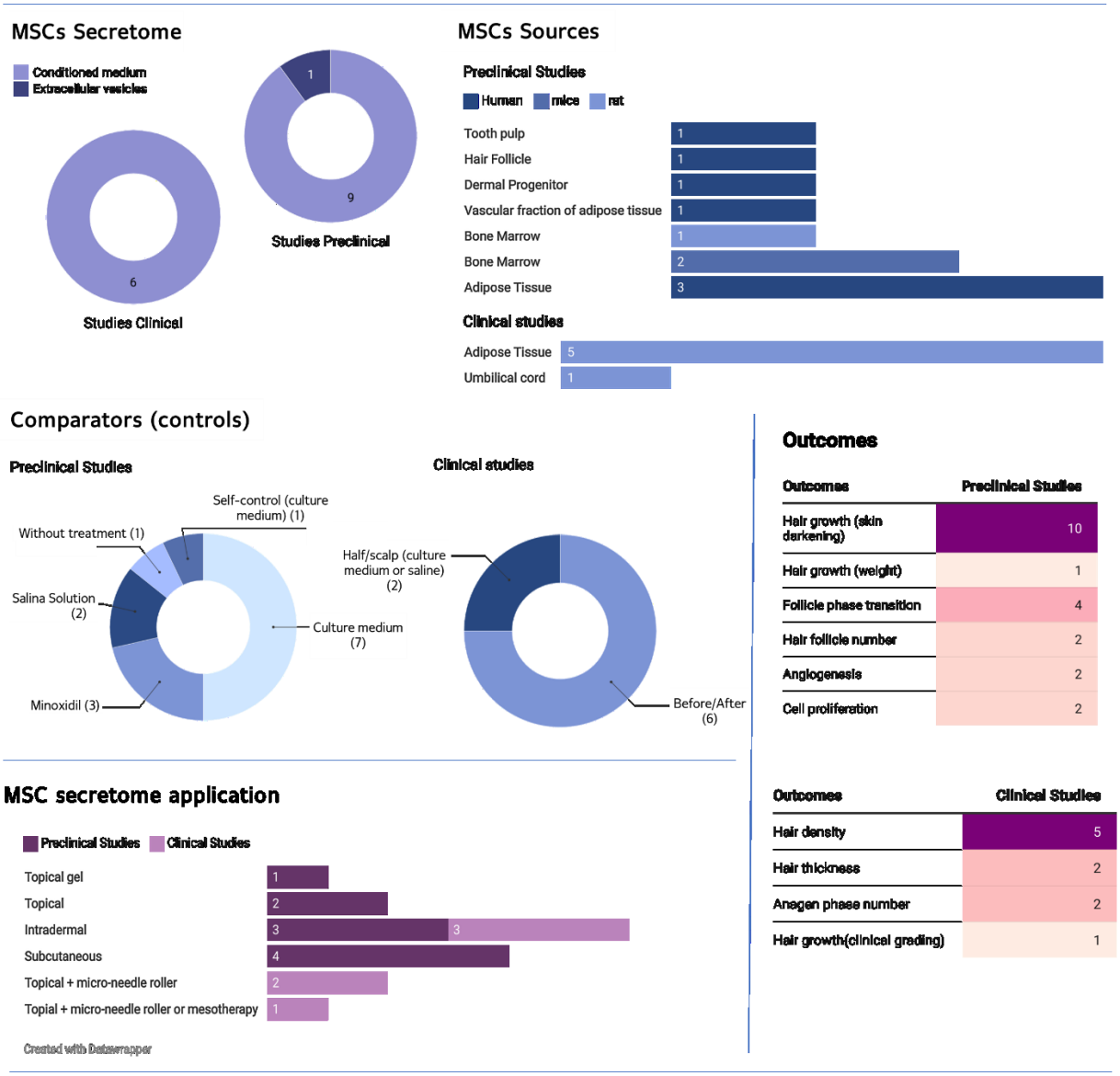

Figure 3. Characteristics of intervention and analyzed the outcomes of preclinical and clinical studies included. Created with Datawrapper.

Table 1. Characteristics of each preclinical study included.

PRECLINICAL STUDIES												
STUDY DESIGN			**ANIMAL CHARACTERISTICS**		**INTERVENTION CHARACTERISTICS**				**METHODOLOGY**		**OUTCOME**	
Author Year Country	**Kind of study**	**Sample size,n**	**Animal model Gender Age**	**Hair loss model**	**Comparator (controls)**	**Type of MSC derivative Cell source Origin**	**Application mode**	**Dose, frequency and treatment time**	**Evaluation days**	**Evaluation method**	**Results**	**Major Findings**
Gunawardena et al. 2019 Malasya (63)	NI (not rando mized)	n=9 (CM) n=3 (culture medium STK-2) n=2 (without treatment)	C3H/HeN mice Female 7 weeks	Hair shaving	1.Culture medium (STK2) 2. Without treatment	Conditioned medium Tooth pulp Human (SHED)	Subcutaneous	100µL 3 times Interval of 3 days	Up to 51 days (on alternate days) - Hair growth	1. Skin darkening (macroscopic images) 2. Full hair coverage	The visualization of hair spots in the CM-group ranged from 8 to 12 days (mean of 11 days), while in the culture medium group it was on day 15 and in the untreated group on day 14. 2. Almost complete coverage of hair CM-SHED - supplementary figure data	↑ Accelerates hair growth ↑ Promotes the transition from the telogen to anagen phase.
Jung et al. 2015 Korea (64)	NI (not rando mized)	n=8 per group	C57BL/6 mice NI 7 weeks	Hair shaving and depilatory cream	Culture medium (DMEM)	Conditioned medium Adipose tissue Human	Subcutaneous	100µL 3 times Interval of 1 week	Up to 3 weeks (every 3 days) - Hair growth (2 and 3 week) - Histology and immunohistochemistry for CD31	1. Skin darkening (macroscopic images) - score 2. Histology - vessels and follicle phase 3. Immunohistochemistry for CD31	Animals in the CM-treated group had a hair growth score of 8, while the control group had a score of 4 after 3 weeks ($p<0.05$). Increase in the number of mature vessels and in the number of CD31-labeled vessels was observed.	↑ Hair growth rate. ↑ Angiogenesis
Ou et al. 2020 Taiwan (36)	Self-control Rando mized	n=4 per group	C57BL/6 mice NI Adults	Hair shaving	Culture medium (DMEM-F12)	Conditioned medium Hair follicle Human	Topical gel	NI 1 time NI	Up to 15 days (every 5 days) - Hair growth (15 days) - Histology	1. Skin darkening (macroscopic images) 2. Histology - hair follicle number	No statistical difference in the hair growth was observed between the CM-treated group and control group. Histological analysis, there was also no significant difference in the number of hair follicles.	= Hair growth = Hair follicle number
Park et al 2010 Korea (28)	NI (not rando mized)	n=5 per group	C3H/HeN mice Female 7 weeks	Hair shaving	Culture medium (DMEM-F12)	Conditioned medium Adipose tissue Human	Subcutaneous	100µL 3 times Interval of 3 days	Up to 12 weeks (NI days) - Hair growth	1. Skin darkening (macroscopic images)	Dark spots were observed earlier and in more animals in the CM-treated group compared to the control group, but no statistical difference in the hair growth was observed.	= Hair growth

Shim et al. 2015 Korea (38)	Rando mized	n=6 per group	C57BL/6 mice NI 7 weeks	Hair shaving	1.Culture medium (DMEM) 2. Minoxidil	Conditioned medium Dermal progenitor Human	Topical	100µL Daily For 5 weeks	Up to 5 weeks (NI days) - Hair growth (5 weeks) - Hair weight	1. Skin darkening (macroscopic images) - score 2. Hair weight	Dark spots were observed earlier and in more animals in the CM- treated group compared to the control group (culture medium. The CM-treated group showed a statistically greater hair growth different from the control after 3rd week (p,0.01) and the Minoxidil group from the 4th week onwards. Both the CM and MNX groups showed greater hair weight on the 35th day when compared to the control (p,0.01).	↑ Hair growth rate ↑ Hair weight
Xiao et al. 2020 China (27)	NI (not rando mized)	n=10 per group	C57BL/6 mice NI 7 weeks	Hair shaving and depilatory cream	Saline (PBS)	Conditioned medium Vascular fraction of adipose tissue Human	Subcutaneous	NI 3 times Interval of 1 week	Up to 3 weeks (every week) - Hair growth (2 week) - Histology and immunohistochemistr y for CD31 and Ki67 (2 week) - presence of vessels in the inner portion of the skin	1. Skin darkening (macroscopic images) - score 2. Histology - vessels 3. Immunohistochemis try for CD31 and Ki67 4. Vessels in the inner portion of the skin (macroscopic images)	CM-treated group had higher scores on the skin darkening scale when compared to the control group (p<0.05), after 1 week. The treated group had more number of mature vessels and CD31-labeled blood vessels than the control group (p<0.05). Furthermore, Ki67 (proliferation marker) positive cells in the bulge increased in CM-treated group (p<0.05)	↑ Hair growth rate ↑ Angiogenesis ↑ Proliferation of bulge cells
Rajendran et al. 2017 Korea (29)	NI (not rando mized)	n=5 (C) n=5 (saline) n=6 (minoxidil 3%)	C57BL/6 mice NI 7 weeks	Hair shaving	1. Saline (PBS) 2. Minoxidil	Extracellular vesicles Bone marrow Mice	Intradermal	100µL Every 2 days For 28 days	Up to 28 days (0, 4, 7, 11, 15, 18, 21, 24 and 28 day) - Hair growth)NI- Histology	1. Skin darkening (macroscopic images) 2. Histology - follicle phase	Hair growth in the group treated with VE and Minoxidil presents a statistically significant difference compared to the control group from day 11 (p<0.05) and progressively the hair grows until its totality on day 27 (p<0.001). Histologycal analisys showed that VE promotes he transition from the telogen to anagen phase.	↑ Hair growth ↑ Promotes the transition from the telogen to anagen phase.
Dong et al. 2014 China (40)	NI (not rando mized))	n=3 per group	C57BL/6 mice Male 7 weeks	Hair shaving	Culture medium (DMEM)	Conditioned medium Bone marrow Rat	Intradermal	100µL Daily For 7 days	Up to 21 days (3, 7 and 14 day) - Hair growth and histology (21 day) - Hair density	1. Skin darkening (macroscopic images) 2. Histology - follicle phase and hair follcle number	CM-treated group showed visual differences in skin darkening compareted to contorl, on day 14 (increased). Histologycal and Immunohistochemistry analisys showed higher number of hair follicle (p<0.05) and Ki67 positive cells in CM-treated group compareted to control .Furthermore, it was possible to identify that the hair follicles in the CM-MSC group were more advanced in the anagen phase than in the control group on the evaluated days (3, 7 and 14)	↑ Hair growth ↑ Promotes the transition from the telogen to anagen phase. ↑ Proliferation of cells

Yang et al. 2016 Korea (39)	NI (not rando mized)	14 (total)	C57BL/6 mice NI 7 weeks	Hair shaving	1.Culture medium (NI) 2. Minoxidil	Conditioned medium Adipose tissue Human	Topical	NI Daily For 18 days	Up to 2 weeks (every 3 days) - Hair growth	1. Skin darkening (macroscopic images) - score	Hair growth score was higher in the CM-group than in the control, treated only with culture medium. Respectively, hair regenerationgrowth around 50% and 30%, in tretated group compared to control (p<0,05). Minoxil group showed 95-100% of hair growth.	↑ Hair growth (compared to negative control group) ↓ Hair growth (compared to minoxidil group)
Zhang et al. 2021 China (37)	Rando mized	n=20 per group	C57BL/6 mice Female 7 weeks	Hair shaving	1.Culture medium (NI)	Conditioned medium Bone marrow Mice	Intradermal	250 uL Every 2 days For 2 weeks	Up to 15 days (0, 7, 10 and 15 day) - Hair growth and histology	1. Skin darkening (macroscopic images) - score 2. Histology - follicle phase	Hair growth were higher in CM-treated group compared to control, but no statistically significant difference was observed. Follicle length at 7, 10, and 15 days was greater in the treated group (p.0.001/p<0.05/p<0.01 respectively). Also, a higher number of Ki67 positive cells in the bulbe was observed qualitatively in the treated group.	= Hair growth ↑ Hair follicle length ↑ Promotes the transition from the telogen to anagen phase. ↑ Proliferation of bulbe cells

CM: conditioned medium; ↑: increase; ↓: decrease; = unchanged; NI: not identified; STK2: serum-free medium developed for Human Mesenchymal Stromal Cells; DMEM: Dulbecco's Modified Eagle Medium; MNX: minoxidil; SHED; dental pulp stem cells obtained from human deciduous teeth.

Table 2. Characteristics of each clinical study included.

CLINICAL STUDIES												
STUDY DESIGN			**POPULATION CHARACTERISTICS**		**INTERVENTION CHARACTERISTICS**				**METHODOLOGY**		**OUTCOME**	
Author Year Country	**Kind of study**	**Sample size,n**	**Gender Age**	**Hair loss model**	**Comparator (controls)**	**Type of MSC derivative Cell source Origin**	**Application mode**	**Dose, frequency and treatment time**	**Evaluation days**	**Evaluation method**	**Results**	**Major Findings**
Fukuoka & Suga 2015 Japan (31)	non-randomized before/after and half-scalp	total=32 n=22 (before/after) n=10 (half-side)	19 men and 13 women; 20-73 years. 1) 11 men (6 with and 5 without finasteride administration) and 11 women treat alopecia; 2) 8 men and 2 women treat half side hair.	Alopecia	1. Without treatment (before/after) 2. Saline (half-side)	Conditioned medium (lyophilized) Adipose tissue Human	Intradermal injections	0.02 mL/cm² of solution, 3-4mL to the scalp; 6 sessions; Interval of 3 to 5 weeks each	Before starting treatment and at 1 to 3 months after the final treatment	Trichograms Density hair	In the before/after study the number of hair increased significantly after treatment ($p<0.01$), no significant difference was observed between men and women, like this men with and without finasteride. In the half-side comparasion study, both treated ($p<0.01$) and placebo ($p<0.05$) sides had an increase in the number of hairs, being greater on the treated side ($p<0.01$).	↑ hair number
Fukuoka et al. 2017 Japan (65)	non-randomized before/after and half-scalp	Total=31 n=21 (before/after) n=10 (half-side)	Men and women; 20-73 years. 1) 27-69 years 2) 20 – 73 years	1) 16 androgenectic alopecia + female pattern hair loss (FPHL) 2) 8 androgenetic alopecia + 2 FPHL	1. Without treatment (before/after) 2. Saline (half-side)	Conditioned medium (lyophilized) Adipose tissue Human	Intradermal injections	1) 0.02 mL/cm² of solution, 3-4mL to the entire scalp. Injections was given once a month and was repeated 6-8 times, in some patientes ≥ 10 times 2) 6 times on one side, and the same volume of placebo (saline) was injected on the opposite side	Before starting treatment and at 3 months after the final treatment	Trichograms Density hair and anagen hair phase	1) The number of hairs at 3 months after the first treatment increased significantly in comparison with the number of hairs before treatment (141.3±31.4, 109.8±43.5, respectively; ($P < 0.01$). and the number of hairs in the anagen phase increases. 2) Although the total number of hairs increased on both the treatment and placebo sides after 6 months of treatment, the increase over the treatment period was significantly different between the treatment and placebo sides (increase in hair count; 18.4±9.4, 6.5±11.7, respectively; $P <0.01$).	↑ hair number ↑ hair anagen phase number

Mathen & Dsouza 2021 India (25)	non-randomized Prospective, open, self-controlled, proof-of-concept, single-center study	Total=15	13 Male) and 2 female; 20-58 years	Males (n=13) showing male pattern baldness (MPB) grade of II to IV on Hamilton Norwood scale, and females (n=2) showing female pattern hair loss (FPHL) grade of II to IV on Sinclair scale,	Without treatment	Conditioned medium Umbilical cord Human	Topical applied using a 1.5 mm derma roller	12 treatments at weekly intervals	3 months	improvements in hair fall control using clinical grading, macro photographic evaluation,	HN/Sinclair scores were 86.6% and a reduction in grades by 1 point was reported over 3 months.	↑ Hair growth
12. Narita et al. 2019 Japan (30)	non-randomized before/after	Total=40	21 men and 19 women 23-74 years 1) 21 men (2 without finasteride administration) and 19 women (16 without finasteride administration	Androgenetic alopecia or female patter hair loss of varying severity	Without treatment	Conditioned medium (lyophilized) Adipose tissue Human	Intradermal	4mL every month for 6 sessions	Before starting treatment and at 2, 4 to 6 months	Trichograms Density hair and anagen hair phase	hair density and anagen hair rate improved significantly, . Hair density increased from T0 to T6 ($p < .001$). The anagen hair rate increased from T0 to T6 ($p = .022$) Subgroup analysis was performed on some parameters across the subpopulations with different sex and finasteride administration: Hair density increased significantly in all groups, but significant increases in the anagen hair rate were limited to the male or finasteride groups.	↑ hair number ↑ hair anagen phase number
Shin et al 2015 Korea (41)	non-randomized before/after	Total=27	Women 22-69 years (SD=41.9 +-13.4)	FPHL - Ludwig type I.	Without treatment	Conditioned medium (lyophilized) Adipose tissue Human	Topical applied using micro-needle roller (0.5mm)	4 mL once per week for 12 consecutive weeks	12 weeks	Trichograms Hair density and hair thickness	Mean hair density increased from 105.4 to 122.7 hairs/ cm2 over the 12 weeks of treatment ($P < 0.001$) (Fig. 2a, c), representing an increase of 16.4%. Mean hair thickness increased from 57.5 lm to 64.0 lm ($P < 0.001$) (Fig. 2b, d), an increase of 11.3%. The application of ADSC-CM for 12 weeks induced statistically significant improvements in hair density and hair thickness	↑ hair number ↑ hair thickness

Shin et al 2017 Korea (42)	non-randomized before/after and half-scalp	Total=58 1) n=27 – ***data from the study by Shin et al 2015** 2) n=25 3) Half-side n=6	Women and Men 1) wonen – 22-69 years(SD) 41.9+-13.4 2: mens - 28 e 60 years SD 48,6+-8.5 3: mens 20-52 years, média 35.8+-11.8 (hamilton-norwood classification types II-IV	Male and female pattern hair loss (FPHL) Male - without any other treatments such as oral or topical agents.	Without treatment 3)Saline or	Conditioned medium (lyophilized) Adipose tissue Human	Topical applied using micro-needle roller (0.5mm) or mesotherapy gun	4 mL once per week for 12 consecutive weeks	12 weeks	Trichograms Hair density and hair thickness	2)hair density increased from 97.7 to 108.1 counts/cm2 (P < 0.001), and mean hair thickness increased from 65.4 to 71.8 m (P < 0.001) The hair density and thickness increased by 10.6% and 9.8%, respectively, after 12 weeks. 3) After 12 weeks of therapy, the total hair count was significantly higher on the AAPE®-treated side of the reference circle than on the vehicle-treated side (P = 0.002, paired ttest) (Table 1 and Fig. 6). The mean hair diameter, however, did not significantly differ between the sides.	↑ hair number ↑ hair thickness = hair thickness in study hal-scalp

CM: conditioned medium; ↑: increase; ↓: decrease; = unchanged; FPHL: female pattern hair loss; MPB: male pattern baldness; NI: not identified; ADSC: adipose derived stem cell.

Risk of Bias within Studies

Preclinal Studies

The risk of bias was assessed using the SYRCLE tool, and the percentages and categorizations represent the distribution of risk of bias across the included studies (Figure 4). Most of the questions were categorized as "Unclear Risk of Bias." Regarding selection bias (questions 1 to 3), 40% of the studies had a low risk of bias in terms of baseline characteristics, while 100% of the studies had an unclear risk of bias for allocation sequence and allocation concealment. In the analysis of Performance and detection bias (questions 4 to 7), 100% of the studies were classified as unclear risk of bias.

Attrition bias (question 8) varied between the studies, with 60% of them classified as low, 20% as uncertain and 20% as high risk of bias. Detection bias (question 9) also exhibited variation, with 70%, 10%, and 20% of the studies classified as low, unclear, and high risk, respectively. Moreover, all studies identified other parameters (question 10) that could result in a high risk of bias. Notably, a substantial proportion of the studies were categorized under "Unclear Risk of Bias", indicating a lack of comprehensive clarity in addressing critical methodological aspects.

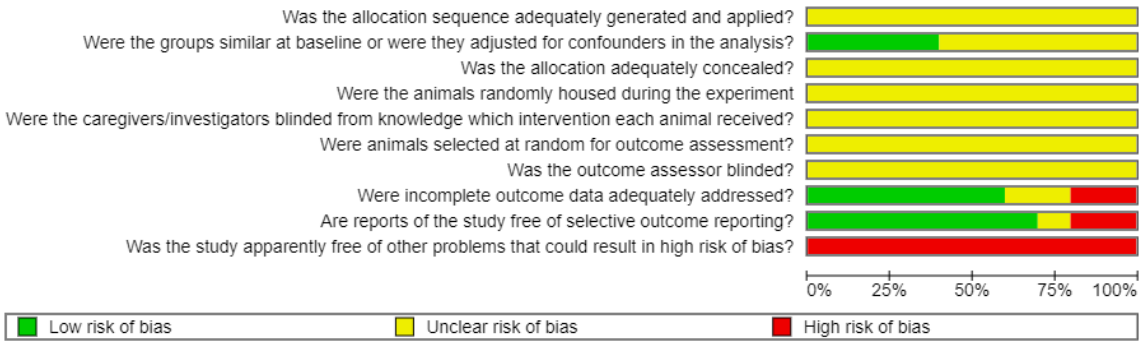

Figure 4. Analysis of bias risk by SYRCLE tool. Figure by RevMan web.

Clinical Studies

The risk of bias analysis, conducted using the ROBINS-I tool, provides an overarching assessment of the included studies' methodological quality. The distribution of risk across the seven evaluated domains reveals insights into potential sources of bias

(Figure 5). In domain of bias due to confounding, a critical risk of bias was identified across all studies, indicating a high potential for confounding variables to influence the study outcomes. Similarly, bias in classification of interventions exhibited a critical risk of bias in studies, suggesting potential misclassification of interventions that could impact the validity of results.

For domain bias due to deviations from intended interventions, studies demonstrated varied risk levels, with 33,3% classified as low risk and 66,7% as a serious risk. This highlights potential inconsistencies in implementing interventions as intended, which could introduce bias into the findings. Regarding domain bias in the measurement of outcomes, a significant risk was noted in 66.7% of the studies. Bias in selection of the reported result showed a moderate risk in all studies, suggesting potential selective reporting of results. For analysis of bias in selection of participants and bias due to missing data, all studies included had no information available, which underscores a need for improved reporting and transparency in these areas.

In summary, the risk of bias analysis indicates that studies included in this review share critical or serious risks of bias in domains related to confounding, classification of interventions, deviations from intended interventions, and measurement of outcomes. These findings underscore the importance of cautious interpretation when considering the potential impact of bias on the reported outcomes and conclusions of the included studies.

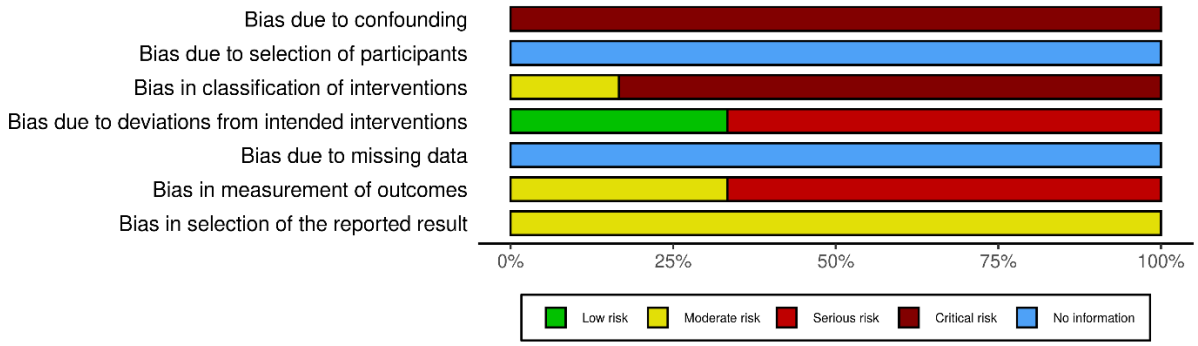

Figure 5. Analysis of bias risk by ROBINS-I tool. Figure by Robvis.

Results of individual studies

The main results of individual included studies are listed in Table 1 and 2. The table reports the analyzed outcomes, main results obtained in comparison to the

comparator group and statistical differences. Population and intervention details for each study are also described.

Synthesis of Results

Preclinical Studies

Hair growth

Hair growth on the shaved skin of animals treated with MSC secretome was macroscopically assessed in all studies by evaluating skin darkening over time. Most studies indicated that secretome promotes increased hair growth. Out of the 10 articles included, seven demonstrated that MSC secretome-treated group had a significant increase in hair growth compared to the control group. In those studies, demonstrating a positive treatment effect, it was observed that the animals exhibited earlier appearance of dark spots or achieved higher scores on the skin darkening scale, as compared to control group.

However, three studies did not observe a statistically significant difference between the groups, despite reporting qualitatively higher growth in the treated group. OU et al. (2020), reported that the sample group was limited, which could have affected the statistical analysis (36). Furthermore, it was the only study that used the self-control model (half the back). Park et al. (2010), observed the appearance of dark spots earlier in animals from the CM-treated group, but no statistical difference in hair growth was observed compared to the control group (28). Additionally, in the study performed by Zhang et al. (2021), no difference was observed in hair growth over time, but other analyses, such as hair length, transition between hair follicle phases, and cell proliferation, were greater in the CM-treated group relative to the control (37).

Some studies also used Minoxidil as a positive control, in addition to the control group (culture medium or saline). Shin et al. (2015) showed that treatment with CM was more effective in hair growth than conventional treatment with minoxidil, although the study did not indicate whether there was a statistical difference between these two groups (38). In the study performed by Rajendran et al. (2017), treatment with EVs was conducted, and there was no difference between the EVs-treated and minoxidil

groups regarding hair growth (29). In contrast, in the study by Yang et al. (2016), the minoxidil group was more efficient in hair growth than the CM-treated group (39).

Only one study evaluated hair growth by measuring the weight of shaved hairs after 5 weeks of starting treatment. The results showed greater hair weight in the CM-treated compared to the control group (culture medium) (38). This greater weight corroborates the observed higher growth rate, as analyzed through skin darkening.

Overall, these findings suggest a favorable effect of MSC secretome, particularly CM, on hair growth, though some variation in results was observed among the studies, possibly due to differences in study design and sample sizes.

Transition of the hair follicle phase and Number of hair follicles

In dark-coated mice, shaved skin typically appears pink during the telogen phase of the hair follicle cycle and darken at the beginning of the anagen phase, indicating the transition between phases and hair growth (hair tips begin to emerge from the epidermis). Thus, transition analysis between the phases of the hair follicle can be indirectly evaluated by skin darkening and directly through histological analysis.

Among the studies included in this review, four analyzed hair follicle phase transition, and the data revealed that treatment with CM was sufficient to induce the transition from the telogen to the anagen phase.

Regarding the number of hair follicles, only two studies conducted this analysis. Dong et al. (2014) showed a positive effect of treatment with CM resulting in an increase in the number of hair follicles compared to the control group (40). Differently, Ou et al. (2020) found that follicles number was similar between the groups (36). This analysis of hair follicles number can indicate the effect of treatments on hair density. In this context, the included studies showed divergent effects in relation to the number of hair follicles.

In summary, the assessment of hair follicle phase transition through skin darkening and histological analysis, as well as the analysis of the number of hair follicles, provide valuable insights into the regenerative effects of MSC secretome treatments on hair growth.

Angiogenesis

The angiogenesis process was evaluated in two studies by examining the presence of vessels in the skin using histological, immunohistochemical (CD31) and macroscopic analysis. An increase in angiogenesis was reported in the skin of animals treated with CM compared to the control groups. The studies observed that treated group had a higher number of mature vessels and CD31-labeled blood vessels in contrast to the control group. In addition, Xiao et al. (2020) also demonstrated that number of vessels in the inner dorsal skin (macroscopically) increased in the CM-treated group (27). It is important to note that enhancement of angiogenesis is usually accompanied by positive hair growth promotion, and both studies also showed increased hair growth in the treated animals.

Proliferation of hair follicle cells

Proliferation activation in different hair follicle cell populations is associated with the induction of the phase transition of the hair follicle and hair growth. Two studies evaluated Ki-67 expression in hair follicle cells using immunohistochemical staining to assess the effects of MSC-CM on cell proliferation. Xiao et al. (2020) demonstrated that cell proliferation in the bulge region was greater in CM-treated group compared to the control group (27), while Dong et al. (2014) showed an increase of proliferation in bulb region. Both regions play significant roles in modulating the hair follicle cycle (40).

Clinical Studies

Hair density

In order to evaluate hair growth in clinical studies, the main result analyzed in the studies was the density of hair in the scalp. All five included studies that examined hair density by trichograms showed an increase in the number of hairs after treatment with MC, with no distinction between cell origin and application form. One of the studies used clinical grading and scoring to assess hair growth, and also demonstrated a positive effect of MC in relation to these parameters (25). Notably, some of these studies included patients who had previously or during the study received other typical treatments for alopecia, such as finasteride.

Narita et al. (2019) additionally performed a subgroup analysis across subpopulations with different sex and finasteride administration. The results showed that hair density increased significantly in all groups (30).

Hair thickness and anagen hair follicle

Regarding hair thickness, two before-after studies observed that CM treatment promotes an increase in hair diameter (41;42). Shin et al. (2019) also evaluated the effect of CM through a half-scalp analysis, and the mean hair diameter, in this case, did not significantly differ between the sides (42). In this study, the authors report that treating half of the scalp may indirectly affect the untreated side and influence the outcome.

The number of hairs in the anagen phase increases after CM treatment in the two studies that evaluated this parameter. However, Narita et al. (2019) also performed a subgroup analysis and found that significant increase in the anagen hair rate were limited to the male or finasteride groups (30).

Taken together, these findings suggest that the use of MSC secretome may play a key role in stimulating hair growth, positively influencing hair density, thickness, and growth phase.

Discussion

In this systematic review, we explored the therapeutic efficacy of MSC secretome from different sources in promoting hair growth, both in preclinical models and patients with hair loss disorders. A total of 10 preclinical studies involving 132 animals and 6 clinical studies with 176 patients were included, providing a comprehensive assessment of the effects of MSC secretome in hair loss treatment. The results indicated that the application of MSC secretome led to significant improvements in hair growth and increased hair density, regardless of its origin. Furthermore, in some studies, the mechanisms underlying the hair growth-promoting effects of MSC-derived secretome were investigated and showed that the induction of follicle phase transition, hair follicle cell proliferation and angiogenesis are factors influenced by the action of the secretome.

Different tissue sources of MSC secretome were utilized in the preclinical and

clinical studies, including bone marrow, adipose tissue, umbilical cord, dermal, hair follicle, and dental pulp. The majority of MSC secretome applied to hair loss treatment were derived from adipose tissue. In fact, among tissue sources of MSC, research with cells derived from adipose tissue has been more robust in the field of regenerative medicine (43). This can be attributed to a variety of factors including its abundant availability, ease of access, minimally invasive harvesting techniques, and notable proliferative capabilities that facilitate successful expansion in culture while preserving its characteristics (44). Moreover, adipose-derived MSC have been extensively studied and employed in various clinical trials and treatments, contributing to their credibility as a reliable and effective cellular source.

Although different MSC populations are known to share phenotypic characteristics, their secretome is likely to vary and show differences in therapeutic potential according to MSC origin (45). Comparative proteomic analysis of MSC secretome from bone marrow, adipose tissue, umbilical cord and placenta by mass spectrometry showed that secretome of fetal-derived MSC, such as placenta and umbilical cord, had a more diverse composition than that of adipose and bone marrow-derived MSC (46). Furthermore, another study found that MSC from umbilical cord preferentially expresses secreted factors related to neuroprotection, neurogenesis and angiogenesis compared to bone marrow-derived MSC (47).

In this context, it is important to highlight that since MSC actively respond to the environment, different culture conditions can also modify the secretion profile of cells (48). In the clinical studies included in the review, the secretome samples from adipose tissue were obtained commercially (lyophilized), while in only one study involving umbilical cord MSC, the CM was obtained from cells amplified by the researchers themselves (25). The lyophilized secretome used in the studies was obtained from MSC maintained under hypoxic conditions. Hypoxia preconditioning has been proposed as an engineering approach to improve the therapeutic potential of MSC secretome, by stimulating the paracrine activities of MSC and increasing the production of secretome both in terms of soluble factors as well as EVs (49).

In preclinical studies, although no secretome was obtained from MSC subjected to preconditioning, either by hypoxia or gene overexpression or growth factor stimulation, the basal cell culture conditions are highly heterogeneous. These alterations in the basal culture conditions, such as medium type, supplements and culture time, can modify the biological properties of secretome and, consequently, its therapeutic

potential (50). Therefore, MSC tissue source and culture conditions should be points of attention when comparatively analyzing the results of these studies and developing future research, as there is no standardization of the secretome production and its composition can be different.

The effectiveness of MSC secretome treatment depends on several factors, including the administration protocol. In view of the great variability in the secretome application protocols in the studies, which include differences in the concentration, frequency, interval and method of administration, it was not possible to correlate the different protocols with better outcomes. However, in the preclinical study that applied only a single dose of the secretome, topically in a gel, no statistical difference in the hair growth was observed between the CM-treated group and control group (36). The application protocol is relevant to clinical translation by diminishing the time of therapy and patient invasiveness, and can be better explored in studies.

Regarding the outcomes of the studies, our aim was to comprehensively evaluate the effects of MSC secretome on hair growth in animal models (rodents) and in patients diagnosed with hair loss. Analysis of ten preclinical selected articles revealed a remarkable tendency to promote increased hair growth after MSC secretome treatment. Macroscopic evaluation of rodent skin darkening, considered a parameter for hair growth, indicated a positive impact of the secretome treatment. Seven out of ten studies demonstrated a statistically significant increase in hair growth in the treated groups compared to controls. These findings in animal models underscore the potential of the MSC secretome as a hair growth stimulating agent.

Interestingly, despite the overall positive trend, it is important to acknowledge that three studies did not observe a statistically significant difference in hair growth between the treated and control groups, even though qualitative assessments suggested greater hair growth in the treated animals. One possible explanation for the lack of statistical significance in these cases could be attributed to limitations in sample size, as indicated by Ou et al (2020) (36). Such limitations can impact the statistical power of the analysis and potentially obscure true differences between the groups.

Although several studies have primarily relied on skin darkening as an indicator of hair growth, others have delved into more complex assessments such as hair follicle phase transitions, cell proliferation and angiogenesis, reporting favorable results in the group treated with secretome. This suggests that the MSC secretome may influence several aspects of the hair growth process, underscoring their pivotal roles in

modulating cellular and tissue processes.

The growth factors found within the MSC secretome, including Platelet-Derived Growth Factor (PDGF)(51), Transforming Growth Factor-beta (TGF-β)(52), Fibroblast Growth Factor (FGF), and Insulin-like Growth Factor (IGF)(53,54), possess significant potential for stimulating hair follicle stem cells. Through the modulation of cell proliferation and differentiation, these growth factors exert a subtle influence on initiating and sustaining the anagen phase—the active growth stage—of the hair cycle. Preclinical studies have revealed the induction of the anagen phase and an increase in hair follicle cell proliferation, specifically in the bulge and bulb regions, after treatment with conditioned medium and EVs. The bulge region is distinguished by the presence of stem cells, while bulb region plays a role in the hair follicle cycle, regulating the regrowth process through the dermal papilla signaling (54). The impact of secretome on dermal papilla cells and on organotypic cultures of the hair follicle has been explored *in vitro*, yielding results that underscore the secretome capacity to enhance cell proliferation among diverse hair follicle cell populations (55;56;57;58).

The findings regarding cell proliferation provide valuable insights into the mechanisms underlying the regenerative effects of MSC secretome treatments on hair growth. The increased proliferation of hair follicle cells suggests an active and dynamic cellular response to the treatment, potentially contributing to the promotion of hair growth and maintenance.

One particular effect of MSC secretome that can influence on hair growth is its potential to modulate angiogenesis. The secretion of factors such as VEGF suggests a role in promoting the formation of new blood vessels. This molecule can interact with the endothelial cells of blood vessels, promoting the proliferation and formation of new capillaries in the region of the hair follicle. By increasing vascularity in the scalp, the MSC secretome can improve blood perfusion at the site, increasing the supply of oxygen and nutrients to the hair follicle cells. This increase in vascularity can therefore support more robust hair growth. The effect of MSC and their secretome on increasing angiogenesis has been reported in different pathologies, such as cutaneous wounds (59), showing that the secretion of angiogenic factors stimulate better tissue repair.

Indeed, increasing angiogenesis is a therapeutic target for hair growth. It is believed that the action of minoxidil, the standard drug used in the treatment of alopecia, is related to vasodilation (60). Although minoxidil's exact mechanisms of action are still not fully understood (25), its use as a positive control in some studies

allows a further analysis of the findings. Shim et al. (2015) reported superior hair growth with MSC secretome treatment compared to minoxidil, although statistical significance was not explicitly stated (38). In contrast, Rajendran et al. (2017) found comparable results between EVs and minoxidil treatments (29), while Yang et al. (2016) noted that minoxidil outperformed adipose tissue-derived conditioned medium (39). These findings suggest that the efficacy of MSC secretome may rival or even surpass current established hair growth interventions.

Furthermore, certain components within the MSC secretome exhibit anti-inflammatory properties, such as cytokines and chemokines, contributing to immune modulation (45). Chronic inflammation can contribute to hair loss, and the immunomodulatory capacity of the MSC secretome can establish a more conducive environment for hair growth. Moreover, the antioxidant property of MSC secretome also confers a protective and reparative function against damage, cellular aging, and graying of hair (25). Lastly, the secretome components can influence the Wnt/β-catenin signaling pathway, which is essential for hair growth (61). It is recognized that conditioned media obtained from cells with WNT overexpression exhibit a superior effect on hair growth compared to non-preconditioned media (40).

Following our comprehensive evaluation of outcomes from preclinical studies and the elucidation of potential mechanisms through which the secretome of MSC elicits its hair growth-promoting effects, we proceeded to assess the translational implications of these findings in human hair growth via clinical studies. In the present systematic review, the study population consisted mostly of patients diagnosed with androgenetic alopecia. In the description, most studies used the term female or male pattern hair loss, which in the literature is synonymous with androgenetic alopecia. Only one study did not specify the type of alopecia. Androgenic alopecia (AGA) is a common form of hair loss both in men and women, and is likely due to an excessive response to androgens (62). AGA is characterized by the gradual miniaturization of hair follicles, shortening of the hair growth period, and reduced number of hairs.

All clinical studies consistently demonstrated an increase in hair growth after treatment with conditioned medium from MSC. The use of trichograms and clinical grading provides objective and reliable methods for assessing treatment outcomes, further supporting the positive effects of conditioned medium on hair growth. The increase in hair growth was consistent across studies, regardless of cell source and

protocol of application, suggesting the potential therapeutic efficacy of MSC-CM in promoting hair growth in patients with hair loss conditions.

However, it is essential to consider the potential confounding effects of concomitant treatments received by some patients, such as finasteride, on the observed outcomes. Furthermore, the micro-needling procedure itself, used to facilitate the topical application and enhance the absorption of MSC secretome, may contribute to improved hair growth. Currently, this tool is being assessed for its potential in alopecia treatment.

While the concurrent use of other treatments might introduce confounding variables, the consistent increase in hair density across these studies, despite such interventions, suggests that MSC secretome could offer an additional or complementary therapeutic avenue. Future research could delve into potential synergistic effects between MSC secretome and established treatments, shedding light on potential combination therapies for enhanced hair growth outcomes. Additionally, variations in patient characteristics and treatment protocols among the studies may contribute to heterogeneity in the results. Despite these considerations, the collective evidence supports the potential of MSC-CM as a promising intervention for individuals with alopecia, warranting further investigations to optimize treatment approaches and establish its role in clinical practice.

Limitations of Studies

Several limitations were identified in the reviewed articles that could impact the accurate interpretation of clinical and preclinical data, affecting their potential application in future therapies. First, the heterogeneity across MSC tissue sources, culture conditions, animal models, classification of alopecia patients, experimental setups, interventions, and assessment protocols resulted in a significant risk of bias. Many studies lacked clear descriptions of their experimental designs, introducing potential bias in various categories assessed by the SYRCLE bias risk tool. Notably, issues included the absence or lack of clarity in addressing selection bias and random sequence generation methods for animal allocation. Furthermore, most studies exhibited performance and detection bias as they failed to specify random housing conditions for animals and whether caregivers and evaluators were blinded to the interventions.

Additionally, inadequate sample sizes in some studies contributed to statistical inaccuracies and reduced reproducibility.

Moreover, randomized controlled trials should be conducted in clinical studies. None of the studies included a control group of patients. The obtained results were compared with baseline analyses in each patient, conducted prior to the application of MSC secretome. Therefore, the interpretation of its findings may be limited. To address these limitations and attain more consistent results, future studies should implement and transparently report robust randomization, adequate sample sizes, allocation, and blinding protocols.

Review Limitations

The bias risk analysis was performed by a single author. Since this process was not conducted in a dual and independent manner, the potential for introducing errors exists. Additionally, the final date for searching articles in the databases was in 2021. Therefore, it is advisable to conduct a supplementary search spanning from that date until the present.

Conclusion

The findings synthesized in this systematic review highlight the potential of MSC secretome as a promising agent for promoting hair growth in animal models and patients with alopecia. The majority of studies demonstrated a significant enhancement in hair growth, while variations in experimental conditions did not consistently modify the positive trend. In this systematic review we can observe that the CEM secretome served as a treatment for hair loss. However, it is important to acknowledge the limitations posed by small sample sizes in some studies and the complex interplay of factors influencing hair growth assessment. Furthermore, although the hair shaving model in mice can be used in studies on hair growth to assess hair regeneration capacity, this model does not faithfully reproduce the complex mechanisms involved in the alopecia pathology. Future research should aim to address these limitations and elucidate the underlying mechanisms by which MSC secretome exerts its hair growth-promoting effects.

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Statement of Ethics

An ethics statement is not applicable because this study is based exclusively on published literature.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Supporting Information Additional

Supporting Information may be found attached to this article: Supplementary Table 1. Detailed of inclusion and exclusion criteria for preclinical studies, based in PICOS strategy. Supplementary Table 2. Detailed of inclusion and exclusion criteria for clinical studies, based in PICOS strategy. Supplementary Table 3. Database search strategy. Supplementary Table 4. Articles selected by analyzing the title and abstract (n = 48). Supplementary Table 5. Articles diverged of eligibility criterion and were excluded in the systematic review.

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Appendix - Supplementary Tables

Supplementary Table 1. Detailed of inclusion and exclusion criteria for preclinical studies, based in PICOS strategy.

PRECLINICAL STUDIES		
Element	**Inclusion criteria**	**Exclusion criteria**
Population	- rats and mice with model of hair loss, from any age, sex, strain and weight.	- other animal species other than rats and mice.
Intervantion	-Application of MSC-derived secretome (conditioned medium, exosomes or extracellular vesicles)in skin, from any MSC source, by intradermic, subcutaneous or topical via. - There is no restriction on the method of obtaining the conditioned medium, exosomes or extracellular vesicles and on the applied dose	- application of stem cells or stem cell extract or the secretome combined with other products (such as platelets rich plasm, growth factors). - Trials with secretomes obtained from other cell types (such as epidermal stem cells, dermal papilla cells) or which used systemic application of the secretome. - Studies using only preconditioned MSC-derived secretome by drug exposure, and genetic manipulation (e.g., gene transfection and protein or microRNA overexpression) or hypoxia.
Control	- vehicle or placebo (negative control) or standard drugs (positive control)	- Studies without a comparator group - secretome obtained of preconditioned MSC by hypoxia or drug exposure, and genetic manipulation (e.g., gene transfection and protein or microRNA overexpression) - Secretome of other cell types.
Outcomes	<u>Primary</u> Hair growth: hair density, hair thickness, hair diameter, growth time. Secondary: hair follicle number (histology analyses); anagen/telogen number hair;neovascularization;	Studies that do not report at least one of the predefined primary outcomes;
Study Type	-trials design by animal intervention studies (randomized control trials or not randomized control trials) -published in the Latin (Roman) alphabet.	Reviews, letters, books, conference abstracts, case report, case series, posters and guidelines; Studies with duplicated data from another included study; and full-text not available, even after trying to contact the corresponding authors (three attempts in a 3 week period).

Supplementary Table 2. Detailed of inclusion and exclusion criteria for clinical studies, based in PICOS strategy.

CLINICAL STUDIES		
Element	**Inclusion criteria**	**Exclusion criteria**
Population	- adult (>18 years) diagnosed with loss excessive hair (pattern hair loss) at any stage or alopecia (any etiology), without gender restriction, age and ethnicity.	Patients with hair loss submitted to chemotherapy.
Intervantion	-Application of MSC-derived secretome (conditioned medium, exosomes or extracellular vesicles)in skin, from any MSC source, by intradermic, subcutaneous or topical via. - There is no restriction on the method of obtaining the conditioned medium, exosomes or extracellular vesicles and on the applied dose	- application of stem cells or stem cell extract or the secretome combined with other products (such as platelets rich plasm, growth factors). - Trials with secretomes obtained from other cell types (such as epidermal stem cells, dermal papilla cells) or which used systemic application of the secretome. - Studies using only preconditioned MSC-derived secretome by drug exposure, and genetic manipulation (e.g., gene transfection and protein or microRNA overexpression)
Control	- vehicle or placebo (negative control) or standard drugs (positive control) - before/after - Half head controlled	- Studies without a comparator group - secretome obtained of preconditioned MSC by drug exposure, and genetic manipulation (e.g., gene transfection and protein or microRNA overexpression) - Secretome of other cell types.
Outcomes	<u>Primary</u> Hair growth: hair density, hair thickness, hair diameter, growth time. Secondary: hair follicle number (histology analyses); anagen/telogen number hair;neovascularization;	Studies that do not report at least one of the predefined primary outcomes;
Study Type	-intervention clinical trials - treated/control; after/before; half/head -published in the Latin (Roman) alphabet.	Reviews, letters, books, conference abstracts, case report, case series, posters and guidelines; Studies with duplicated data from another included study; and full-text not available, even after trying to contact the corresponding authors (three attempts in a 3 week period).

Supplementary Table 3. Database search strategy.

Database	Search	Total
Medline/ **PubMed** Oct 25th, 2021	("stem cells"[MeSH Terms] OR "stem cells"[All Fields] OR "stem cell"[All Fields] OR "mother cells"[All Fields] OR "mother cell"[All Fields] OR "adult stem cells"[MeSH Terms] OR "adult stem cells"[All Fields] OR "adult stem cell"[All Fields] OR "progenitors cells"[All Fields] OR "progenitor cell"[All Fields] OR "mesenchymal stem cells"[MeSH Terms] OR "mesenchymal stem cells"[All Fields] OR "mesenchymal stem cell"[All Fields] OR "mesenchymal stromal cell"[All Fields] OR "mesenchymal stromal cells"[All Fields] OR "multipotent mesenchymal stromal cell"[All Fields] OR "multipotent mesenchymal stromal cells"[All Fields] OR "multipotent stem cells"[MeSH Terms] OR "multipotent stem cells"[All Fields] OR "multipotent stem cell"[All Fields] OR "derived stem cell"[All Fields] OR "stromal cells"[MeSH Terms] OR "stromal cells"[All Fields] OR "stromal cell"[All Fields] OR "stromal"[All Fields] OR "MSC"[All Fields] OR "MSC"[All Fields] OR "culture media"[MeSH Terms] OR "culture media"[All Fields] OR "culture media, conditioned"[MeSH Terms] OR "conditioned culture media"[All Fields] OR "conditioned culture medium"[All Fields] OR "conditioned media"[All Fields] OR "conditioned medium"[All Fields] OR "secretome"[All Fields] OR "extracellular vesicles"[MeSH Terms] OR "extracellular vesicles"[All Fields] OR "extracellular vesicle"[All Fields] OR "exovesicle"[All Fields] OR "exovesicles"[All Fields] OR "cell-derived microparticles"[MeSH Terms] OR "microvesicles"[All Fields] OR "microvesicle"[All Fields] OR "nanovesicles"[All Fields] OR "nanovesicle"[All Fields] OR "exosomes"[MeSH Terms] OR "exosomes"[All Fields] OR "exosome"[All Fields] OR "secretory vesicles"[MeSH Terms] OR "secretory vesicles"[All Fields] OR "secretory vesicle"[All Fields] OR "EVs"[All Fields] OR "MVs"[All Fields] OR "EV"[All Fields] OR "MV"[All Fields]) AND ("alopecia"[MeSH Terms] OR "alopecia"[All Fields] OR "baldness"[All Fields] OR "hair loss"[All Fields] OR "hair regrowth"[All Fields] OR "hair disease"[All Fields] OR "hair diseases"[All Fields] OR "hair follicle disease"[All Fields] OR "hair disorders"[All Fields] OR "hair regeneration"[All Fields] OR "hair follicle regeneration"[All Fields] OR "hair growth"[All Fields])	1342
EMBASE Oct 25th, 2021	('stem cells'/exp OR 'stem cells' OR 'stem cell'/exp OR 'stem cell' OR 'mother cells' OR 'mother cell'/exp OR 'mother cell' OR 'adult stem cells'/exp OR 'adult stem cells' OR 'adult stem cell'/exp OR 'adult stem cell' OR 'progenitors cells' OR 'progenitor cell'/exp OR 'progenitor cell' OR 'mesenchymal stem cells'/exp OR 'mesenchymal stem cells' OR 'mesenchymal stem cell'/exp OR 'mesenchymal stem cell' OR 'mesenchymal stromal cell'/exp OR 'mesenchymal stromal cell' OR 'mesenchymal stromal cells'/exp OR 'mesenchymal stromal cells' OR 'multipotent mesenchymal stromal cell'/exp OR 'multipotent mesenchymal stromal cell' OR 'multipotent mesenchymal stromal cells' OR 'multipotent stem cells'/exp OR 'multipotent stem cells' OR 'multipotent stem cell'/exp OR 'multipotent stem cell' OR 'derived stem cell' OR 'stromal cells'/exp OR 'stromal cells' OR 'stromal cell'/exp OR 'stromal cell' OR stromal OR MSC OR msc OR 'culture media'/exp OR 'culture media' OR 'culture media conditioned'/exp OR 'culture media conditioned' OR 'conditioned culture media'/exp OR 'conditioned culture media' OR 'conditioned culture medium'/exp OR 'conditioned culture medium' OR 'conditioned media'/exp OR 'conditioned media' OR 'conditioned medium'/exp OR 'conditioned medium' OR 'secretome'/exp OR secretome OR 'extracellular vesicles'/exp OR 'extracellular vesicles' OR 'extracellular vesicle'/exp OR 'extracellular vesicle' OR exovesicle OR exovesicles OR 'cell-derived microparticles'/exp OR 'cell-derived microparticles' OR microvesicles OR 'microvesicle'/exp OR	1696

	microvesicle OR nanovesicles OR 'nanovesicle'/exp OR nanovesicle OR 'exosomes'/exp OR exosomes OR 'exosome'/exp OR exosome OR 'secretory vesicles'/exp OR 'secretory vesicles' OR 'secretory vesicle'/exp OR 'secretory vesicle' OR evs OR mvs OR ev OR mv) AND ('alopecia'/exp OR alopecia OR 'baldness'/exp OR baldness OR 'hair loss'/exp OR 'hair loss' OR 'hair regrowth'/exp OR 'hair regrowth' OR 'hair disease'/exp OR 'hair disease' OR 'hair diseases'/exp OR 'hair diseases' OR 'hair follicle disease' OR 'hair disorders' OR 'hair regeneration'/exp OR 'hair regeneration' OR 'hair follicle regeneration' OR 'hair growth'/exp OR 'hair growth') AND [embase]/lim NOT ([embase]/lim AND [medline]/lim)	
LILACS Oct 25th, 2021	(alopecia OR calvície OR baldness OR "hair loss" OR “perda de cabelo” OR “queda de cabelo” OR "hair regrowth" OR “crescimento do cabelo” OR “crescimento do pelo” OR “crecimiento del cabello” OR “perdida de cabello” OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR “doenças do folículo piloso” OR “trastornos del cabello” OR “trastornos del folículo piloso” OR "hair regeneration" OR “regeneração do cabelo” OR “regeneração do pelo” OR “regeneración del cabello” OR "hair follicle regeneration" OR “regeneração do folículo piloso” OR “regeneracíon del folículo piloso OR "hair growth") AND ("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR células-tronco OR célula-tronco OR “células madre” OR “célula madre” OR "adult stem cells" OR "adult stem cell" OR “célula-tronco adulta” OR “célula madre adulta” OR "progenitors cells" OR "progenitor cell" OR “célula progenitora” OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR “célula-tronco mesenquimal” OR “células-tronco mesenquimais” OR “células madre mesenquimatosas” OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR “célula estromal mesenquimal” OR “células estromais mesenquimais” OR “célula mesenquimal estromal” OR “células mesenquimais estromais” OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR “célula estromal multipotente” OR “célula estromal mesenquimal multipotente” OR “célula multipotente” OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR “célula estromal” OR “células estromais” OR estromal OR MSC OR msc OR or ctms OR ctm OR "culture media" OR “meio de cultura” OR “medios de cultivo” OR "culture media conditioned" OR “meio condicionado” OR “medio condicionado” OR “medio acondicionado” OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR secretoma OR "extracellular vesicles" OR "extracellular vesicle" OR “vesículas extracelulares” OR “vesícula extracelular” OR exovesicle OR exovesicles OR "cell-derived microparticles" OR microvesicles OR microvesicle OR microvesículas OR nanovesicles OR nanovesicle OR nanovesículas OR exosomes OR exosome OR exossomo OR exossomos OR "secretory vesicles" OR "secretory vesicle" OR evs OR mvs OR ev OR mv)	3
Scopus Oct 25th, 2021	TITLE-ABS-KEY("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR "adult stem cells" OR "adult stem cell" OR "progenitors cells" OR "progenitor cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR MSC OR MSC OR "culture media" OR "culture media conditioned" OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR "extracellular vesicles" OR "extracellular vesicle" OR exovesicle OR exovesicles OR "cell-derived microparticles" OR microvesicles OR microvesicle OR nanovesicles OR nanovesicle OR exosomes OR exosome OR "secretory vesicles" OR "secretory	3238

	vesicle" OR EVs OR MVs OR EV OR MV) AND TITLE-ABS-KEY(alopecia OR baldness OR "hair loss" OR "hair regrowth" OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR "hair regeneration" OR "hair follicle regeneration" OR "hair growth")	
Web of Science Oct 25th, 2021	("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR "adult stem cells" OR "adult stem cell" OR "progenitors cells" OR "progenitor cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR MSC OR MSC OR "culture media" OR "culture media conditioned" OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR "extracellular vesicles" OR "extracellular vesicle" OR exovesicle OR exovesicle OR "cell-derived microparticles" OR microvesicles OR microvesicle OR nanovesicles OR nanovesicle OR exosomes OR exosome OR "secretory vesicles" OR "secretory vesicle" OR EVs OR MVs OR EV OR MV) (Tópico) and (alopecia OR baldness OR "hair loss" OR "hair regrowth" OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR "hair regeneration" OR "hair follicle regeneration" OR "hair growth") (Tópico)	1486
CAB Abstract Oct 25th, 2021	("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR "adult stem cells" OR "adult stem cell" OR "progenitors cells" OR "progenitor cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR MSC OR MSC OR "culture media" OR "culture media conditioned" OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR "extracellular vesicles" OR "extracellular vesicle" OR exovesicle OR exovesicles OR "cell-derived microparticles" OR microvesicles OR microvesicle OR nanovesicles OR nanovesicle OR exosomes OR exosome OR "secretory vesicles" OR "secretory vesicle" OR EVs OR MVs OR EV OR MV) AND (alopecia OR baldness OR "hair loss" OR "hair regrowth" OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR "hair regeneration" OR "hair follicle regeneration" OR "hair growth")	88
LIVIVO Oct 25th, 2021	("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR "adult stem cells" OR "adult stem cell" OR "progenitors cells" OR "progenitor cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR MSC OR MSC OR "culture media" OR "culture media conditioned" OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR "extracellular vesicles" OR "extracellular vesicle" OR exovesicle OR exovesicles OR "cell-derived microparticles" OR microvesicles OR microvesicle OR nanovesicles OR nanovesicle OR exosomes OR exosome OR "secretory vesicles" OR "secretory vesicle" OR EVs OR MVs OR EV OR MV) AND (alopecia OR baldness OR "hair loss" OR "hair regrowth" OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR "hair regeneration" OR "hair follicle regeneration" OR "hair growth")	486
Google Scholar Oct 25th, 2021	("stem cell" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR MSC OR "culture media" OR "conditioned media" OR secretome OR "extracellular vesicles" OR exosome) AND (alopecia OR "hair loss" OR "hair	100

	regrowth" OR "hair regeneration")	
OpenGrey Oct 25th, 2021	("stem cell" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR MSC OR "culture media" OR "conditioned media" OR secretome OR "extracellular vesicles" OR exosome) AND (alopecia OR "hair loss" OR "hair regrowth" OR "hair regeneration")	0
ProQuest Dissertations &Theses Global Oct 25th, 2021	NOFT(("stem cells" OR "stem cell" OR "mother cells" OR "mother cell" OR "adult stem cells" OR "adult stem cell" OR "progenitors cells" OR "progenitor cell" OR "mesenchymal stem cells" OR "mesenchymal stem cell" OR "mesenchymal stromal cell" OR "mesenchymal stromal cells" OR "multipotent mesenchymal stromal cell" OR "multipotent mesenchymal stromal cells" OR "multipotent stem cells" OR "multipotent stem cell" OR "derived stem cell" OR "stromal cells" OR "stromal cell" OR stromal OR MSC OR MSC OR "culture media" OR "culture media conditioned" OR "conditioned culture media" OR "conditioned culture medium" OR "conditioned media" OR "conditioned medium" OR secretome OR "extracellular vesicles" OR "extracellular vesicle" OR exovesicle OR exovesicles OR "cell-derived microparticles" OR microvesicles OR microvesicle OR nanovesicles OR nanovesicle OR exosomes OR exosome OR "secretory vesicles" OR "secretory vesicle" OR EVs OR MVs OR EV OR MV) AND (alopecia OR baldness OR "hair loss" OR "hair regrowth" OR "hair disease" OR "hair diseases" OR "hair follicle disease" OR "hair disorders" OR "hair regeneration" OR "hair follicle regeneration" OR "hair growth"))	75

Supplementary Table 4. Articles selected by analyzing the title and abstract (n = 48).

Author, year	Title
Anderi, R. et al 2018	Cellular therapy with human autologous adipose-derived adult cells of stromal vascular fraction for alopecia areata
Butt, G. et al. 2020	Stromal vascular fraction-enriched platelet-rich plasma therapy reverses the effects of androgenetic alopecia
Choi, H. I. et al 2019	Effect of exosomes from human adipose-derived stem cells on hair growth
Choi, N. et al. 2018	Generation of trichogenic adipose-derived stem cells by expression of three factors
Choi, N. et al. 2019	HB-EGF Improves the Hair Regenerative Potential of Adipose-Derived Stem Cells via ROS Generation and Hck Phosphorylation
Dong, L. et al. 2014	Treatment of MSC with Wnt1a-conditioned medium activates DP cells and promotes hair follicle regrowth
Dong, L. et al. 2017	A Conditioned Medium of Umbilical Cord Mesenchymal Stem Cells Overexpressing Wnt7a Promotes Wound Repair and Regeneration of Hair Follicles in Mice
Fukioka, H. et al. 2017	Hair Regeneration Therapy: Application of Adipose-Derived Stem Cells
Fukuoka, H. & Suga, H. 2012	Hair regeneration treatment using stem cell conditioned medium
Fukuoka, H. & Suga, H. 2015	Hair Regeneration Treatment Using Adipose-Derived Stem Cell Conditioned Medium: Follow-up With Trichograms
Fukuoka, H. et al 2010	Hair Regenerated therapy with growth factors in adipose-derived stem cells secreted protein
Gunawardena, T. N. et al 2019	Dental derived stem cell conditioned media for hair growth stimulation
Han, H. et al. 2019	Efficacy of a hair tonic containing human umbilical cord blood mesenchymal stem cell-derived conditioned media in patients with androgenetic alopecia
He, J. 2013	Participation of CD34-enriched mouse adipose cells in hair morphogenesis
Huh, C. H.; Kwon, S. H.; 2019	Exosome for hair regeneration: From bench to bedside
Huh, C. H.; Park, B. S.; 2020	Exosome for hair regeneration: From bench to bedside
Jeong, Y. M. et al 2013	Ultraviolet B Preconditioning Enhances the Hair Growth-Promoting Effects of Adipose-Derived Stem Cells Via Generation of Reactive Oxygen Species
Jin, S. E.; Sung, J. H.; 2016	Hair regeneration using adipose-derived stem cells
Jung, M. K. et al 2015	Hair-growth stimulation by conditioned medium from vitamin D3-activated preadipocytes in C57BL/6 mice
Kim, J. H. et al 2014	The Molecular Mechanism Underlying the Proliferating and Preconditioning Effect of Vitamin C on Adipose-Derived Stem Cells
Kim, S. J. et al 2021	Innovative method of alopecia treatment by autologous adipose-derived SVF
Lee, S. B. et al 2021	Clinical efficacy of adipocyte-derived stem cells conditioned media combined with micro-injury in refractory patch of alopecia areata
Lee, Y. I. et al. 2020	The Effect of Conditioned Media From Human Adipocyte-Derived Mesenchymal Stem Cells on Androgenetic Alopecia After Nonablative Fractional Laser Treatment
Lindenbaum, E. S. et al 2002	Cell culture media stimulate hair regrowth in chemotherapy-induced alopecia in mice
Liu, Y. R. et al. 2019	Stromal Vascular Fraction and Platelet-Rich Plasma Upregulate Vascular Endothelial Growth Factor Expression to Promote Hair Growth via the Wnt/beta-Catenin Signaling Pathway
Mansbridge, J. et al. 2012	Stimulation of hair growth in humans by cell-secreted proteins
Mathen, C.; Dsouza, W.; 2021	In vitro and clinical evaluation of umbilical cord-derived mesenchymal stromal cell-conditioned media for hair regeneration
Narita, K. et al 2020	Sequential Scalp Assessment in Hair Regeneration Therapy Using an Adipose-Derived Stem Cell-Conditioned Medium
Naughton, G. et al 2021	707 Hypoxia induced Multipotent Stem Cell-Secreted Proteins Induce Hair Growth in a Phase 1a/2b trial in Male Pattern Baldness
Naughton, G. K. et al 2012	Stimulation of hair growth in humans by cell-secreted proteins
Oh, H. A. et al 2020	Migration Inhibitory Factor in Conditioned Medium from Human Umbilical Cord Blood-Derived Mesenchymal Stromal Cells Stimulates Hair Growth
Oh, H. et al 2018	Hair growth stimulated by conditioned media of human umbilical cord blood-derived mesenchymal stem cells is enhanced by preconditioning with transforming growth factor-beta and lithium chloride
Ou, K. L. et al 2020	The Potential of a Hair Follicle Mesenchymal Stem Cell-Conditioned Medium for Wound Healing and Hair Follicle Regeneration

Park, B. S. et al 2010	Hair growth stimulated by conditioned medium of adipose-derived stem cells is enhanced by hypoxia: evidence of increased growth factor secretion
Perez-Meza, D. et al 2017	Hair follicle growth by stromal vascular fraction-enhanced adipose transplantation in baldness
Rajendran et al 2017	Extracellular vesicles derived from MSC activates dermal papilla cell and promotes hair follicle conversion from telogen to anagen in
Rajendran, R. L. 2017	Mesenchymal stem cell-derived extracellular vesicle promotes hair growth on human follicles in vitro and hair regrowth in mouse
Ramdasi, Sushilkumar; 2016	Growth factors and cytokines secreted in conditioned media by mesenchymal stem cells-promising possible therapeutic approach for hair regeneration
Seo, M et al 2018	A Novel Secretory Vesicle from Deer Antlerogenic Mesenchymal Stem Cell-Conditioned Media (DaMSC-CM) Promotes Tissue Regeneration
Shim, J. H. 2015	Hair Growth-Promoting Effect of Human Dermal Stem/Progenitor Cell-Derived Conditioned Medium
Shin, H. et al 2015	Clinical use of conditioned media of adipose tissue-derived stem cells in female pattern hair loss: a retrospective case series study
Shin, H. et al 2017	Up-to-date Clinical Trials of Hair Regeneration Using Conditioned Media of Adipose-Derived Stem Cells in Male and Female Pattern Hair Loss
Stevens, H. P. et al 2018	Introducing Platelet-Rich Stroma: Platelet-Rich Plasma (PRP) and Stromal Vascular Fraction (SVF) Combined for the Treatment of Androgenetic Alopecia
Tak, Y. J. et al 2020	A randomized, double-blind, vehicle-controlled clinical study of hair regeneration using adipose-derived stem cell constituent extract in androgenetic alopecia
Won, C. et al 2011	Hair growth stimulation by mesenchymal stem cell-derived proteins
Wu, J. Y. et al 2021	Adipose-Derived Stem Cell Exosomes Promoted Hair Regeneration
Xiao, S. N. et al 2020	Promotion of Hair Growth by Conditioned Medium from Extracellular Matrix/Stromal Vascular Fraction Gel in C57BL/6 Mice
Yang, Y. et al. 2016	LL-37 stimulates the functions of adipose-derived stromal/stem cells via early growth response 1 and the MAPK pathway
Zhang, C. et al. 2021	TMT-Based Quantitative Proteomic Analysis Reveals the Effect of Bone Marrow Derived Mesenchymal Stem Cell on Hair Follicle Regeneration

Supplementary Table 5. Articles diverged of eligibility criterious and were excluded in systematic review. (1) Studies that do not include at least one predefined intervention in the study. (2) Studies that do not include at least one predefined population in the study. (3) literature review, congress abstracts, editorials. (4) data repeated in another included study. (5) studies that do not include a control group, according to the inclusion criteria.

Author, year	Exclusion reason
Fukuoka, H. & Suga, H., 2012	3
Fukuoka, H. et al 2010	3
Han, H. et al 2019	1
Huh, C. H.; Kwon, S. H.; 2019	3
Lee, S. B. et al 2021	1
Lee, Y. I. et al. 2020	1
Oh, H. A. et al 2020	1
Oh, H. et al 2018	3
Tak, Y. J. et al 2020	1
Won, C. et al 2011	3
Wu, J. Y. et al 2021	2
Choi, H. I. et al 2019	2
Huh, C. H.; Park, B. S.; 2020	3
Naughton, G. et al 2021	3
Rajendran, R. L. 2017	3
Choi, N. et al. 2019	5
He, J. 2013	1
Ramdasi, Sushilkumar; 2016	3
Anderi, R. et al 2018	1
Butt, G. et al. 2020	1
Choi, N. et al. 2018	1
Dong, L. et al. 2017	2
Jeong, Y. M. et al 2013	5
Jin, S. E.; Sung, J. H.; 2016	3
Kim, J. H. et al 2014	5
Kim, S. J. et al 2021	1
Liu, Y. R. et al. 2019	1
Perez-Meza, D. et al 2017	1
Seo, M et al 2018	2
Stevens, H. P. et al 2018	1
Mansbridge, J. et al. 2012	3
Naughton, G. K. et al 2012	3
Lindenbaum, E. S. et al 2002	2

4 CONCLUSÃO DO ESTUDO

Os achados sintetizados nesta revisão sistemática destacam o potencial do secretoma das CEM como um agente promissor para promover o crescimento capilar em modelos animais e pacientes com alopecia. A maioria dos estudos demonstrou um aumento significativo no crescimento do cabelo, enquanto que as variações nas condições experimentais não alteraram consistentemente a tendência positiva dos achados. No entanto, é importante reconhecer as limitações impostas pelos pequenos tamanhos amostrais em alguns estudos e a complexa interação de fatores que influenciam a avaliação do crescimento do cabelo. Além disso, embora o modelo de depilação em camundongos possa ser utilizado em estudos de crescimento capilar para avaliar a capacidade de regeneração capilar, esse modelo não reproduz fielmente os complexos mecanismos envolvidos na patologia da alopecia. Pesquisas futuras devem ter como objetivo abordar essas limitações e elucidar os mecanismos subjacentes pelos quais o secretoma das CEM exerce seus efeitos ao promover o crescimento capilar.

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