UNIVERSIDADE FEDERAL DE SANTA CATARINA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA
ÁREA DE CONCENTRAÇÃO – IMPLANTODONTIA

LARA STEINER BACK

EFEITOS *IN VITRO* DA SINVASTATINA NA OSTEOINDUÇÃO DE CÉLULAS INDIFERENCIADAS: UMA REVISÃO SISTEMÁTICA

FLORIANÓPOLIS
2021

Lara Steiner Back

EFEITOS *IN VITRO* DA SINVASTATINA NA OSTEOINDUÇÃO DE CÉLULAS INDIFERENCIADAS: UMA REVISÃO SISTEMÁTICA

Dissertação submetida ao Programa de Pós-Graduação em Odontologia do Centro de Ciências da Saúde da Universidade Federal de Santa Catarina para a obtenção do Título de Mestre em Odontologia, Área de Concentração de Implantodontia.

Orientador: Prof. Dr. Ricardo de Souza Magini

Florianópolis
2021

Ficha de identificação da obra elaborada pelo autor,
através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Back, Lara
Efeitos in vitro da sinvastatina na osteoindução de células indiferenciadas: uma revisão sistemática / Lara Back ; orientador, Ricardo Magini, 2021.
74 p.

Dissertação (mestrado) - Universidade Federal de Santa Catarina, , Programa de Pós-Graduação em , Florianópolis, 2021.

Inclui referências.

1. . 2. Sinvastatina. 3. Diferenciação osteogênica. 4. Células indiferenciadas. 5. Engenharia tecidual. I. Magini, Ricardo. II. Universidade Federal de Santa Catarina. Programa de Pós-Graduação em . III. Título.

Lara Steiner Back

Efeitos *in vitro* da sinvastatina na diferenciação de células indiferenciadas: uma revisão sistemática

O presente trabalho em nível de mestrado foi avaliado e aprovado por banca examinadora composta pelos seguintes membros:

____

Prof.Dr. Cesar Augusto Mgalhães Benfatti
Universidade Federal de Santa Catarina

____

Prof Dr. Felipe Perozzo Daltoé
Universidade Federal de Santa Catarina

Certificamos que esta é a **versão original e final** do trabalho de conclusão que foi julgado adequado para obtenção do titulo de mestre em Odontologia área de concentração Implantodontia.

____

Coordenação do Programa de Pós-Graduação

____

Prof., Dr.Ricardo de Souza Magini Orientador(a)

Florianópolis, 2021

Dedico este trabalho aos meus pais Anivaldo e Sigrid.

AGRADECIMENTOS

Primeiramente, gostaria de agradecer à minha **Família.**

Aos meus pais, **Anivaldo e Sigrid**. Não existem palavras para expressar a imensa gratidão pelo amor, incentivo, apoio e todo o conhecimento e educação que me proporcionaram durante toda a minha vida. Obigada por acreditarem em mim. Meus verdadeiros mestres!

À minha irmã **Sofia**, obrigada pelo amor e amizade e companheirismo.

Ao meu namorado **Frederico**, obrigada por todo amor incentivo e compreensão.

Aos meus avós **Lucina** e **Donato**, por cada oração, incentivo e pela imensa fé que possuem em Deus e que transborda para mim.

Agradeço ao meu orientador, **Prof. Dr. Ricardo Magini**, por ser muito além de um professor, um ser humano excepcional, sempre com sábias palavras que ultrapassam o aprendizado profissional. Obrigada por acreditar no meu potencial e me incentivar a ser melhor.

À **Prof. Ariadne Cristiane Cabral da Cruz,** pela sua grande contribuição intelectual e todo o esforço para a elaboração desta pesquisa, pela disposição em ajudar a qualquer momento e pelo seu comprometimento, por todo aprendizado que me proporcionou principalmente nas últimas semanas de elaboração do artigo e por toda paciência comigo dignas de uma excelente docente.

Ao **Prof. Dr. César Augusto Magalhães Benfatti**, por se doar inteiramente ao CEPID e fazer com que seja um dos melhores programas de

ensino na área e por ser inspirador com todo seu esforço e dedicação à profissão.

À minha colega, doutoranda **Isabella Manso**, segunda revisora deste trabalho, por todo o companheirismo, comprometimento, aprendizado, ajuda e conhecimento que me proporcionou durante a minha jornada no mestrado. Por trabalhar lado a lado comigo neste trabalho que é tanto meu quanto seu.

Ao Dr. **Gabriel Magrin**, por ser um exemplo de profissionalismo e por todo o conhecimento transmitido durante às reuniões de Revisão Sistemática que foram imprescindíveis para que este trabalho acontecesse. Sempre disposto a esclarecer todas às dúvidas.

À doutoranda **Mariane Sordi,** terceira revisora deste trabalho, por transmitir seu conhecimento aprofundado e toda ajuda proporcionada durante a execução desta Revisão Sistemática. És inspiradora.

Ao **Prof. Dr. Águedo Aragonês**, por todo o conhecimento transmitido na área de engenharia tecidual, e por estar sempre disposto a ajudar.

Ao Prof. **Dr. Carlos Flores-Mir**, aceitar fazer parte e por auxiliar no desenvolvimento do protocolo e da execução desta Revisão Sistemática, transmitindo seu grande conhecimento.

Agradeço aos Professores **Felipe Perozz Daltoé, Elena Riet Correa Rivera e Luismar Porto** por aceitarem avaliar meu trabalho sendo banca da minha defesa de dissertação de mestrado e por contribuírem com seu conhecimento neste trabalho.

Agradeço também a todos os outros professores que tive a honra de ser aluna, **Prof. Dr. Claudia Ângela Maziero Volpato, Prof. Dr. Marco Aurélio Bianchini.**

Ao Prof. Dr. Antônio Carlos Cardoso por ser um dos principais responsáveis por aprimorar meu senso crítico e ensinar tanto sobre responsabilidade e postura.

Aos meus professores especialização em implantodontia e prótese dentária, **Prof. Dr. Bernardo Passoni, Prof. Dra. Carolina Morsch, Prof. MSc. Rodrigo Melim Ferreirada, Prof. MSC. Mário Homem**, por todo o conhecimento transmitido que refletiram diretamente no meu desempenho do mestrado.

Ao **Centro de Ensino e Pesquisa em Implantes Dentários (CEPID)**, e todos os profissionais que fazem parte dele.

Ao cirurgião dentista **Felipe Vieira Bez,** primeiro profissional em que tive oportunidade de trabalhar junto e que me ensinou tanto!

Agradeço às minhas amigas que tive o prazer de tê-las como colegas de mestrado **Luiza de Oliveira Matos, Roberta Michels, Eduarda Blasi Magini e Lais Duarte**. Vocês foram essenciais para que toda essa jornada de mestrado fosse possível, me proporcionando companheirismo, cumplicidade, leveza,e amizade.

Agradeço aos meus amigos e colegas do CEPID **Giovanni Riva, Mario Escobar, Lenin Israel, Francesco Esparrano**. Foi um prazer imenso ter conhecido todos vocês, por estarem sempre prontos para ajudar, por serem profissionais e pessoas admiráveis e por toda a amizade proporcionada.

Aos demais **colegas da pós-graduação do CEPID**, Marcos Cadó, Murilo Barreto, Antônio neves, Madalena Engler, Edwin Ruales, Karin Apaza, Valentina Rega, Renata Brum, Raissa Cutarelli, Joseane Silva, Fernanda Haverroth, Bruna Correa, Mariá Abrahan, Rodrigo Barbosa, Miguel Noronha e Jorge Caviccioni.

Obrigada pelas trocas de conhecimento, é um prazer ter conhecido a cada um de vocês.

Aos funcionários do Departamento de Odontologia pela atenção e eficiência em nos servir. Agradeço em especial a funcionária **Silvane Costa**, por não medir esforços para que o CEPID funcione da melhor maneira, sempre disposta a nos atender, tanto no âmbito profissional quanto no pessoal.

Aos demais professores do Departamento de Odontologia, bem como alunos da graduação e funcionários, muito obrigada por fazerem parte da minha trajetória.

*“Os que se encantam com a **prática** sem a **ciência** são como os timoneiros que entram no navio sem timão nem bússola, nunca tendo certeza do seu destino. Não há coisa que mais nos engane do que o nosso juízo”*
(Leonardo Da Vinci)

RESUMO

Esta revisão sistemática teve como objetivo avaliar criticamente a literatura disponível sobre o potencial do SIN para induzir a diferenciação osteogênica de células indiferenciadas in vitro. Os estudos foram selecionados em um processo de duas fases. Bancos de dados eletrônicos (Embase, Latin American and Caribbean Health Sciences, PubMed, SCOPUS, Web of Science) foram consultados, além de uma consulta adicional nas bases de dados da literatura cinza (Google Scholar, Open Gray e ProQuest) até 30 de novembro de 2020. O risco de viés (RoB) entre os estudos incluídos foi avaliado por meio da ferramenta Office of Health Assessment and Translation (OHAT) adaptada para estudos in vitro que avaliaram os artigos em risco de viés "Definitivamente baixo", "Provavelmente baixo", "Provavelmente alto" ou "Definitivamente alto". Quatorze estudos que avaliaram pelo ao menos a atividade da fosfatase alcalina e a mineralização da matriz extracelular após a osteoindução foram incluídos Altas concentrações de SIM demonstraram potencial citotóxico, enquanto concentrações variando de 0,1 nM a 1 μM estimularam a diferenciaç o osteogênica quando adicionadas ao meio de cultura não osteogênico ou osteogênico. Uma meta-análise não foi possível devido à heterogeneidade entre os estudos. Em conclusão, a suplementação com SIM foi capaz de promover a diferenciação osteogênica de células indiferenciadas em meios de cultura não osteogênicos e osteogênicos. Além disso, a diferenciação osteogênica foi considerada altamente dependente da concentração de SIM.

Palavras-chave:
Estatinas; Sinvastatina, Metabolismo ósseo; Células mesenquimais; Revisão Sistemática

.

ABSTRACT

This systematic review aimed to assess the potential of simvastatin (SIM) to induce the osteogenesis of undifferentiated cells in vitro. Studies were selected in a two-phase process. Electronic databases (Embase, Latin American and Caribbean Health Sciences, PubMed, SCOPUS, and Web of Science) and grey literature (Google Scholar, Open Grey, and ProQuest) were assessed up to November 30, 2020. The risk of bias in individual studies was assessed through the Office of Health Assessment and Translation (OHAT) tool adapted for in vitro studies which evaluated the articles in "Definitely Low", "Probably Low", "Probably High", or "Definitely High" risk of bias. Fourteen studies that evaluated at least alkaline phosphatase activity and extracellular matrix mineralization after osteoinduction were included. The risk of bias was considered as “probably low” or “definitely low” for most of the questions assessed. High SIM concentrations demonstrated cytotoxic potential while concentrations ranging from 0.1 nM to 1 μM stimulated osteogenic differentiation when added to the non-osteogenic or osteogenic culture medium. A meta-analysis was not possible due to the heterogeneity among the studies. In conclusion, SIM supplementation was able to promote osteogenic differentiation of undifferentiated cells in both non-osteogenic and osteogenic culture media. Also, osteogenic differentiation was highly dependent on the SIM concentration.

Keywords:
Statins; Simvastatin; Bone metabolism; Mesenchymal cells; Systematic Review

LISTA DE FIGURAS E TABELAS

TABELA 1 --53
TABELA 2 --50
FIGURA 1 --62
APÊNDICE 1 ---63
APÊNDICE 2 ---68

LISTA DE SÍMBOLOS E SIGLAS

SIN – Sinvastatina (*Simvastatin* (SIM))

% - Percentagem

< Menor

µg - Micrograma

U - Unidades de massa atômica

mM – Milimolar (es)

nM – Nanomolar (es)

µM – Micromolar(es)

ALP – Fosfatase alcalina (Alkaline Phosphatase)

BMP – Proteína óssea morfogenética (*Bone Morphogenetic Protein)*

BMP2 – Proteína óssea morfogenética 2 (*Bone Morphogenetic Protein 2*)

BMP9 – Proteína óssea morfogenética 9 (*Bone Morphogenetic Protein 9*)

CEPID – Centro de Ensino e Pesquisa em Implantes Dentários

Et al. – E outros (*Et alii*)

FDA – *Food and Drug Administration*

HMG-CoA – hidroxi-metilglutaril coenzima A

L – Litro

mg – Miligramas

mL – Mililitros

v/v – relação entre volumes (*volume/volume)*

nm – Nanômetro

O/W – Oil-in-Water

OP – Osteopontina

OC – Osteocalcina

PLGA – Ácido polilático-co-glicólico

rhBMP2 – Proteína óssea morfogenética recombinante humana 2 (*recombinant human Bone Morphogenetic Protein 2*)

SIN ou SIM – Sinvastatina (*Simvastatin*)

TNFα – Fator de necrose tumoral alfa (*Tumor Necrosis Factor alfa*)

OSX – Osterix

OSN – Osteonectina *(osteonectin)*

ALP – Fosfatase Alcalina (*Alkaline Phosphatase*)

Cbfa – Core-binding factor subunit alpha-1

BFS - Soro fetal bovino (*Bovine fetal sérum)*

Mol/L - Mol por litro

DMEM - Dulbecco's modified Eagle's medium

α-MEM - Minimum Essential Medium Alpha

RPMI1640 – Meio de cultura celular Roswell Park Memorial Institute 1640 (Roswell *Park Memorial Institute 1640 Medium)*

OM – Meio osteogenico (*Osteogenic médium*)

NOM – Meio não osteogênica (*Non Osteogenic médium)*

SUMÁRIO

1 INTRODUÇÃO E REVISÃO DE LITERATURA---------------------------17

2 OBJETIVOS --21

2.1 Objetivo geral---21

2.2 Objetivos específicos---21

3 ARTIGO – VERSÃO EM INGLÊS ---22

4 METODOLOGIA EXPANDIDA ---46

4.1 Desenho do Estudo---46

4.2 Critérios de elegibilidade --46

4.2.1 Critério de inclusão--46

4.2.2 Critério de exclusão---46

4.3 Estratégia de Busca --47

4.4 Seleção dos Estudos ---47

4.5 Processo de coleta de dados--48

4.6 Avaliação de risco de viés---48

4.7 Síntese dos resultados---48

5 CONSIDERAÇOES FINAIS --49

6 TABELAS --50

6.1 TABELA 1 - Resumo das características descritivas dos artigos incluídos (n = 14). --51

6.2 TABELA 2 – Risco de viés dos estudos individuais pela ferramenta OHAT *risk-of-bias* adaptada. ---58

7 FIGURAS --60

7.1 FIGURA 1 – Fluxograma de pesquisa e critérios de seleção. ----60

8 APÊNDICES --61

8.1 APENDICE 1 – Estratégia de busca------------------------------------61

8.2 APÊNDICE 2 – Estudos excluídos e razão para exclusão.--------66

1 INTRODUÇÃO E REVISÃO DE LITERATURA

Reparar grandes defeitos ósseos causados por traumas, tumores, perda dentária precoce e doenças periodontais progressivas ainda é um desafio. Além do mais, estudos mostram que a preservação do periodonto, da função mastigatória, e da estética facial é essencial para que não haja déficit na saúde e na qualidade de vida dos pacientes (BUSET et al., 2016; LIU; KERNS, 2014; NYMAN, 1991).

O osso autógeno foi considerado por muito tempo o padrão de referência entre os materiais para enxertia, no entanto, apresenta uma série de limitações, incluindo morbidade da área doadora e reduzida disponibilidade óssea nos locais de coleta. Ao longo dos anos surgiram inúmeras técnicas e estratégias terapêuticas visando a reparo do tecido ósseo, como por exemplo, regeneração tecidual guiada (RTG) associada à utilização de biomateriais como arcabouços, osso autógeno ou a combinação destes (GENTILE et al., 2011; LIU; KERNS, 2014; RETZEPI; DONOS, 2010; SCULEAN et al., 2015; WHANG et al., 2005).

A Engenharia Tecidual vem ampliando a indicação dos procedimentos reconstrutivos por meio do aprimoramento da capacidade de caracterizar com precisão os eventos biológicos a partir da interação de três elementos chaves: células viáveis, arcabouços e moléculas sinalizadoras (fatores de transcrição). Além destes, é conhecido que tempo adequado e fatores ambientais também influenciam diretamente no resultado da regenerativos ou reparativos (BARTOLD et al., 2000; HAN et al., 2014) .

As moléculas sinalizadoras orquestram o processo de formação óssea (BARTOLD et al., 2000; NEVINS et al., 2013). Com o objetivo de mimetizar o mesmo e induzir a reparação, diversos fatores de crescimento, como fator de crescimento derivado de plaquetas (PDGF) fator de crescimento de fibroblasto (FGF) e fator de crescimento transformante β (TGF- β) têm sido estudados quanto aos seus efeitos no metabolismo ósseo (COCHRAN et al., 2016; MISHRA et al., 2013; NEVINS et al., 2003, 2013).

Estudos mostram que o TGF-β é capaz de estimular a produção de matriz extracelular e agir como quimiotático para células ósseas, fibroblastos gengivais e fibroblastos do ligamento periodontal (DEREKA; MARKOPOULOU; VROTSOS, 2006; NISHIMURA; TERRANOVA, 1996); Ainda dentro da

superfamília do TGF-β, as proteínas ósseas morfogenéticas (BMPs) têm recebido especial atenção, devido ao seu potencial em estimular células progenitoras a se diferenciarem em células de linhagem osteoblástica (KING; COCHRANT, 2002; URIST, 1965; URIST; STRATES, 1971), tendo papel essencial na formação, manutenção e reparo do tecido ósseo, além de estimular a formação de novo cemento (LEE et al., 2010).

Disponível no mercado para comercialização, a rhBMP-2 (recombinante humana, produzida em larga escala a partir da tecnologia do DNA recombinante), é utilizada atualmente como auxiliar em procedimentos regenerativos (cicatriciais). No entanto sua utilização em dosagem única tem sido questionada por não induzir o estímulo adequado à osteoindução por ser uma modalidade de tratamento que apresenta alto custo e riscos (DRAGOO et al., 2003; JUNG; THOMA; HAMMERLE, 2008; SERVICE, ROBERT, 2000). Dentre eles destacam-se a falta de controle na atuação desses fatores (devido à cinética de liberação complexa), o uso de dosagens não fisiológicas (por ser requerida uma quantidade seis vezes maior do que a BMP endógena), imunogenicidade e segurança a longo prazo, exigindo cautela na sua utilização (BODEN, 1999; JUNG; THOMA; HAMMERLE, 2008; SERVICE, ROBERT, 2000)

Devido a estas dificuldades no uso das BMPs exógenas, têm-se procurado alternativas com menores riscos e custos, que sejam capazes de regular favoravelmente os fatores de crescimento intrínsecos e que possam ser associados a arcabouços e que promovam a diferenciação celular. Como alternativa, iniciou-se o estudo das estatinas como substâncias osteoindutoras, dentre elas destaca-se a Sinvastatina (SIN). Aprovada pela Food and Drug Administration (FDA), possui custo 16.000 vezes menor frente à rhBMP-2, e é de fácil sintetização (MUNDY et al., 1999).

As estatinas são comumente utilizadas no tratamento da hipercolesterolemia, pois seu mecanismo de ação consiste na sua capacidade em inibir a redutase da 3-hidroxi-3-metilglutaril coenzima da HMG-CoA, bloqueando a conversão do HMG- CoA em mevalonato e impedindo, a síntese do colesterol endógeno. São moléculas estáveis, não susceptíveis à degradação proteolítica e podem ser sintéticas ou originárias de culturas de fungos (AMERONGEN et al., 2000; NIU; DING; ZHANG, 2015; SHAH et al., 2015; STAAL et al., 2003) . Mundy *et al.* (1999) foram os primeiros a citar a SIN como

estimulador da formação óssea *in vivo*. Fora observado aumento na largura óssea a partir da injeção subcutânea de SIN na calvária de ratos, além de ter-se obtido aumento do volume de osso esponjoso na tíbia quando administrada oralmente (MUNDY et al., 1999).

Estudos demonstram que a SIN promove diferenciação osteoblástica por elevar o nível de expressão de proteínas osseas morfogenéticas 2 (BMP2), osteopontina, colágeno tipo I, sialoproteína e osteocalcina. Além disso, pela indução da diferenciação dos osteoblastos, antagoniza a ativação da proteína quinase pelo fator de necrose tumoral alpha (TNF-α), revertendo seu efeito supressor e prevenindo a inibição de BMP2 o que permite a expressão de marcadores osteogênicos como Runx2, osteopontina e fosfatase alcalina. Um estudo *in vitro*, mostrou também que as estatinas são capazes de inibir a reabsorção óssea por meio da supressão da cascata do mevalonato, bloqueando moléculas essenciais a atividade osteoclástica (STAAL et al., 2003; YAMASHITA et al., 2008).

A SIN pode ser administrada de formas variadas como, por exemplo, por via oral, injeções múltiplas em lesões e aplicação local em materiais específicos. Quando administrada por via oral, a grande parte da sua degradação ocorre a nível hepático, podendo resultar em sérios efeitos colaterais sistêmicos, além de disponibilizar pouco fármaco para atuação no tecido ósseo (JADHAV; JAIN, 2006; KHEIRALLAH; ALMESHALY, 2015). Outra opção é injetar a SIN diretamente no local de ação, em fraturas ou defeitos, no entanto, devido ao seu baixo peso molecular e difusão acelerada requer injeções repetidas para se obter o efeito desejado o que causa uma drástica redução da produção de colesterol local, substância importante para manter a integridade das membranas celulares (WHANG *et al*., 2005).

Dessa maneira estudos já mostraram que para se utilizar a SIN localmente é necessário um meio condutor adequado que controle a sua liberação, concentração local e efetividade permitindo a neoformação tecidual sem danos ao mesmo (ENCARNAÇÃO et al., 2016; QING et al., 2019; WHANG et al., 2005). A incorporação de SIN por diferentes tipos de arcabouços tem sido relatada na literatura, tanto em estudos *in vitro* quanto *in vivo*. Estes biomateriais se mostram promissores quanto sua capacidade de bioestimular a neoformação óssea, no entanto ainda não existe uma previsibilidade na resposta e o conhecimento dos

seus efeitos a longo prazo (BAO; FRANCO; LEE, 2011; NAITO et al., 2014; TAI et al., 2013).

Estudos também mostraram que as células-tronco multipotentes adultas podem substituir o enxerto ósseo no tratamento de defeitos ósseos, superando o obstáculo bioético da utilização de células-tronco embrionárias (DONZELLI et al., 2007; KIMELMAN et al., 2007; THESLEFF; TUMMERS, 2003). A formação óssea envolve a diferenciação das células indiferenciadas, primeiro em pré-osteoblastos e depois em osteoblastos maduros com capacidade de sintetizar matriz óssea extracelular, resultado da expressão de genes fenotípicos que levam à aquisição do fenótipo dos osteoblastos. Com a potencial capacidade da SIN na osteoindução de células indiferenciadas, a principal vantagem seria fornecer de forma ilimitada osteoblastos diferenciados e células osteoprogenitoras para transplante. (FRANCESCHI; XIAO, 2003; GREEN; TODD; HEALTH, 1990; MARIE, 2013; MARIE; KASSEM, 2011; RIMANDO et al., 2016).

Dessa maneira, considerando o contexto atual da engenharia de tecidos, buscam-se substâncias ativas capazes de atuar como fatores de osteoindução para promover a osteogênese de células indiferenciadas (KIMELMAN et al., 2007; MARIE, 2013; MARIE; KASSEM, 2011). Apesar do crescente número de estudos avaliando a sinvastatina como estimulador do metabolismo ósseo, sua concentração ideal em meio de cultura e o efeito em meio osteogênico e não osteogênico não estão completamente elucidados (KUPCSIK et al., 2009; PAGKALOS et al., 2010; ZHOU et al., 2010). Portanto, devido à inconsistência quanto à capacidade do SIM em induzir a osteogênese de células indiferenciadas como suplementação de meios de cultura não osteogênicos e osteogênicos, esta revisão sistemática (RS) teve como objetivo analisar estudos que propuseram o SIM como substância osteoindutora em células indiferenciadas in vitro.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Realizar uma revisão sistemática da literatura objetivando responder à pergunta de pesquisa “A sinvastatina tem potencial osteoindutor em células indiferenciadas *in vitro*?”

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar a expressão genética e quantificação do RNAm de proteínas específicas da osteogênese na presença da SIN;
- Avaliar a Atividade de Fosfatase Alcalina após a osteoindução com SIN;
- Avaliar a deposição e mineralização da matriz extracelular após a osteoindução com SIN.
- Avaliar a proliferação celular na presença da SIN.
- Avaliar a concentração ideal de SIN para a osteoindução.

3 ARTIGO – VERSÃO EM INGLÊS

CAN SIMVASTATIN HAVE OSTEOINDUCTIVE POTENTIAL ON UNDIFFERENTIATED CELLS? A SYSTEMATIC REVIEW OF *IN VITRO* STUDIES

This article is formatted on standards of the *Acta Biomateralia* Journal

Can simvastatin have osteoinductive potential on undifferentiated cells? A systematic review of *in vitro* studies

Lara Steiner Back[1] (https://orcid.org/0000-0003-4887-026X, lara_back@msn.com), Isabella Schönhofen Manso[1] (https://orcid.org/0000-0001-9785-6689, isabellamanso@yahoo.com.br) Mariane Beatriz Sordi[1] (https://orcid.org/0000-0001-7873-0765, marianesordi@hotmail.com), Gabriel Leonardo Magrin[1] (https://orcid.org/0000-0003-0724-0560, gabriel.magrin@posgrad.ufsc.br), Ariadne Cristiane Cabral Cruz[1] (http://orcid.org/0000-0001-7306-4708, ariadne.cruz@ufsc.br), Águedo Aragonês[2] (https://orcid.org/0000-0003-2243-1832, aguedo@terra.com.br), Carlos Flores Mir[4] (https://orcid.org/0000-0002-0887-9385, cf1@ualberta.ca), Ricardo de Souza Magini[1] (https://orcid.org/0000-0003-0760-5611, ricardo.magini@gmail.com).

[1] Department of Dentistry, Center for Education and Research on Dental Implants, Federal University of Santa Catarina, Delfino Conti street, 88040-900, Florianopolis, SC, Brazil.

[2] Department of Mechanical Engineering, Ceramic and Composite Materials Center (CERMAT), Federal University of Santa Catarina, Delfino Conti street, 88040-900, Florianopolis, SC, Brazil.

[3] Laboratory of Applied Virology, Federal University of Santa Catarina, Henrique da Silva Fontes avenue, 88040-900, Florianópolis, SC, Brazil.

[4] Faculty of Medicine and Dentistry, Department of Dentistry, University of Alberta, Edmonton,112 St. NW, Edmonton, Alberta, Canada.

Corresponding author:

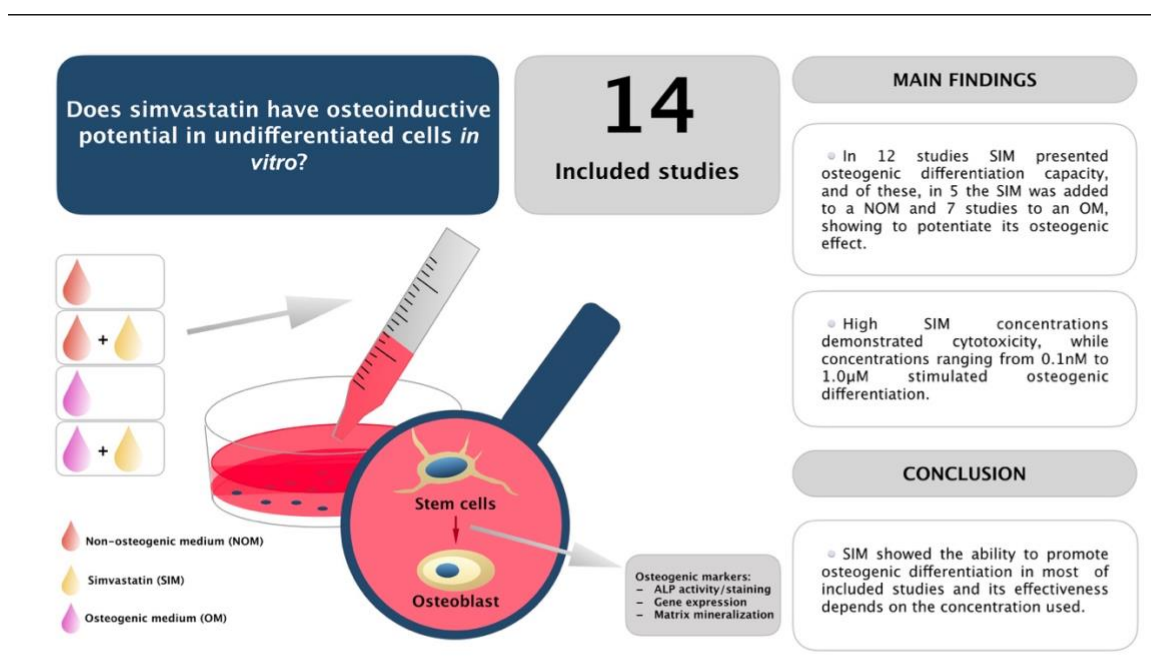
Ariadne Cristiane Cabral Cruz, Centro de Ensino e Pesquisa em Implantes Dentários (CEPID), Departamento de Odontologia, Universidade Federal de Santa Catarina (UFSC), 88040-900, Florianópolis, SC, Brasil.

Email: ariadne.cruz@ufsc.br / Telefone: +55 48 37213407

ABSTRACT

The ability of bone substitutes to stimulate bone formation can be enhanced by the addition of osteoinductive substances. This systematic review aimed to assess the potential of simvastatin (SIM) to induce the osteogenesis of undifferentiated cells *in vitro*. Studies were selected in a two-phase process. Electronic databases (Embase, Latin American and Caribbean Health Sciences, PubMed, SCOPUS, and Web of Science) and grey literature (Google Scholar, Open Grey, and ProQuest) were assessed up to November 30, 2020. The risk of bias in individual studies was assessed through the Office of Health Assessment and Translation (OHAT) tool adapted for *in vitro* studies which evaluated the articles in "Definitely Low", "Probably Low", "Probably High", or "Definitely High" risk of bias. Fourteen studies that evaluated at least alkaline phosphatase activity and extracellular matrix mineralization after osteoinduction were included. The risk of bias was considered as “probably low” or “definitely low” for most of the questions assessed. High SIM concentrations demonstrated cytotoxic potential while concentrations ranging from 0.1 nM to 1 μM stimulated osteogenic differentiation when added to the non-osteogenic or osteogenic culture medium. A meta-analysis was not possible due to the heterogeneity among the studies. In conclusion, SIM supplementation was able to promote osteogenic differentiation of undifferentiated cells in both non-osteogenic and osteogenic culture media. Also, osteogenic differentiation was highly dependent on the SIM concentration.

Graphical abstract



Statement of significance

Considering the current context of tissue engineering, active substances capable of acting as osteoinduction factors to promote the osteogenesis of undifferentiated cells are sought. Despite the increasing number of studies evaluating simvastatin as a bone metabolism stimulator, its ideal concentration in culture medium and the effect in the osteogenic and non-osteogenic medium are not completed elucidate. Therefore, due to the inconsistency regarding the SIM capacity to induce the osteogenesis of undifferentiated cells as supplementation of non-osteogenic and osteogenic culture media, this systematic review aimed to analyze studies that proposed SIM as an osteoinductive substance in undifferentiated cells *in vitro*.

Keywords: Statins; Simvastatin; Bone metabolism; Mesenchymal cells; Systematic Review.

1. Introduction

Large bone defects caused by trauma, tumors, tooth loss, or periodontal disease are challenge situations for clinicians. The use of autologous bone grafts has limitations such as morbidity of the donor area and reduced bone availability in harvesting sites. For this reason, bone tissue engineering seeks to expand the indications of regenerative procedures based on the interaction of three key elements: cells, scaffolds, and signaling molecules with osteogenic potential [1–4].

The ability of bone substitutes to stimulate bone formation can be enhanced when associated with osteoinductive substances. Simvastatin (SIM), a member of the statin family [5], is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor and has been employed to treat hyperlipidemia, reduce cholesterol biosynthesis, and prevent cardiovascular diseases [6]. Moreover, studies have demonstrated that SIM has diverse cholesterol-independent functions, including cell differentiation, anti-inflammatory effect, and angiogenesis induction [7–9].

In calvaria defect models, Mundy et al. (1999) [5] suggested that SIM is a potential therapeutic product with an anabolic effect on bone metabolism. *In vitro* data showed an anabolic effect of statins on various cell lines, including human immortalized cells [10–12], human and murine osteoblast-like cells [12,13], and stem cells, such as murine bone marrow mesenchymal cells (BMSC) [14–17], human mesenchymal stem cells [18–21], and murine embryonic stem cells [22]. Additionally, studies have been demonstrated that SIM promotes osteoblastic differentiation by increasing the expression of type 2 bone morphogenetic protein (BMP2), osteopontin, collagen type I, sialoprotein, and osteocalcin. Furthermore, SIM activates tumor necrosis factor-alpha (TNF-α) molecules, activating protein kinase and reverses SIM suppressive effect, preventing BMP2 inhibition. Consequently, the expression of osteogenic markers such as Runx2, osteopontin, and alkaline phosphatase may occur [23,24].

Considering the need for biomaterials capable of inducing bone neoformation with osteoinductive properties, there is a demand for a systematic approach to synthesize the current evidence on SIM to induce osteogenesis of undifferentiated cells *in vitro* was the present systematic review aim.

2. Methods

2.1 Study design

The systematic review protocol was based on the Cochrane Handbook for Systematic Reviews of Interventions [25] and Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA 2020) checklist guidelines [26]. The protocol was registered on the Open Science Framework registration platform (10.17605/OSF.IO/GBK5S) on August 26, 2020.

2.2 Eligibility criteria

2.2.1 Inclusion criteria

The PICOS acronym (population, intervention, comparison, outcome, and type of studies) was used to create the focused question for this SR: “Does simvastatin have osteoinductive potential in undifferentiated cells *in vitro*?” [25], where:
Population (P): Undifferentiated cells.
Intervention (I): SIM treatment.
Comparison (C): No SIM treatment.
Outcome (O): Osteogenic differentiation.
Type of studies (S): *In vitro* studies

Studies were considered eligible when they evaluate the osteogenic differentiation of undifferentiated cells treated with SIM. No publication period or language restrictions were applied.

2.2.2 Exclusion Criteria

The following exclusion criteria were considered:
1) Studies investigating other cell types, such as differentiated cells; 2) Studies assessing drug or biomaterial associations that do not present separate data for SIM alone; 3) Studies that do not present an untreated control group with the same cell type; 4) Studies that do not present a control group without SIM treatment; 5) Studies that do not evaluate osteoinduction or osteogenic differentiation capacity; 6) Studies that are not *in vitro* (clinical studies, animal studies, conference abstracts, letters, pilot studies, review articles, case reports,

protocols, short communications, personal opinions, posters, or book chapters); 7) Studies that do not specify the culture medium; 8) Studies that do not assess at least the alkaline phosphatase (ALP) activity and extracellular matrix (ECM) mineralization tests; 9) Full text not available; 10) Duplicated data (e.g. dissertations/thesis in which correspondent published articles were available).

2.3 Search strategy

A search strategy was developed based on PICOS structure using MeSH terms and keywords. The following electronic databases were used: Embase, Literature of Latin American and Caribbean Health Sciences (LILACS), PubMed, SCOPUS, and Web of Science. Additionally, a search strategy was developed for Google Scholar web search (first 100 references), Open Grey, and ProQuest. The electronic search was performed until July 22, 2021. A manual search on the reference list of the identified records was also performed. All records were exported to a reference manager software (Mendeley Desktop, Elsevier, London, UK), and duplicates were removed. (Appendix 1)

2.4 Study selection

A two-phase selection process was performed. Two independent reviewers (LSB and ISM) selected the references using an online software [27] (Rayyan, Qatar Computing Research Institute, Qatar). In phase one, both reviewers read titles and abstracts independently while applying the eligibility criteria. In phase two, the reviewers performed a full-text reading while applying the eligibility criteria. In both phases, all the retrieved information was cross-checked by a third review (MBS). The final selection was always based on the full-text publication. Articles that met the eligibility criteria proceeded for data extraction.

2.5 Data collection process

Two independent reviewers (LSB and ISM) collected data from the selected articles. Once selected, they crosschecked the retrieved information with the third reviewer (MBS). Any disagreement was discussed among them. The following data were extracted for each included study: author, year of publication, country, cell type, the origin of cell lines, cell treatment, treatment concentration, methods

applied, results, and main conclusions. If necessary, corresponding authors of the included studies were contacted via e-mail to clarify any missing information and/or clarification of methodology and results.

2.6 Risk of Bias Assessment in Individual Studies

The Office of Health Assessment and Translation (OHAT) risk of bias tool was and was used for evaluating individual studies in this systematic review (CLARITY Group at McMaster University 2013) [28]. Included studies were independently assessed for risk of bias by two reviewers (LSB and ISM). Discrepancies between the reviewers were resolved by discussion until agreement. The possible answers for OHAT tool questions were “Definitely Low”, “Probably Low”, “Probably High”, or “Definitely High” risk of bias, following specific criteria detailed in the protocol.

2.7 Summary Measures and Synthesis of Results

Information concerning gene expression analysis (levels of osteogenic markers), bone proteins expression, quantitative staining of ECM mineralization, ALP activity or staining, and cell proliferation were considered during the evaluation of outcomes. A meta-analysis was planned if the data from included studies were deemed to be homogeneous.

3. Results

3.1 Study selection

In phase one, the electronic search on databases and grey literature retrieved 2464 records after duplicates removal. A comprehensive evaluation of titles and abstracts was performed, resulting in 69 potentially suitable references. After that, full-text articles were screened according to the inclusion and exclusion criteria. Finally, only 14 articles were included in this systematic review. More details are shown in Figure 1.

3.2 Study Characteristics

Included studies were published from 2009 to 2019, thirteen in English language [8,15,16,19–21,27,29–34] and one in Chinese language [35]. The studies were performed in six countries: China [19,20,30,35–37], Korea [21,32], South Korea [38], Switzerland [39], United Kingdom [31], and Taiwan [29,33,34]. The investigations used undifferentiated cell lines from different origins: human adipose tissue [19,20], human sinus membrane [21], human bone marrow [36,39], murine bone marrow [29,30,33–35,37], murine embryonic cells [31,32], and human gingival tissue [38]. Two-dimensional cell cultures were performed in 13 studies, while one study evaluated three-dimensional cell culture (cell spheroids) [38]. Cells received six different treatments: T1 – Non-Osteogenic Medium (NOM) [19–21,31,36,39]; T2 - NOM supplemented with SIM [19–21,31,36,39]; T3 - Osteogenic Medium (OM) [19–21,30–32,34–36,38–40]; T4 - OM supplemented with SIM [30,32,34,35,38,40,41]; T5 - NOM changing to OM [33,41]; and T6 - NOM changing to OM supplemented with SIM [33,41]. The osteogenic differentiation was evaluated by ALP activity or staining assay, gene expression by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR), ECM mineralization assay (Von Kossa or Alizarin Red staining), protein expression detection by western blot, calcium in ECM, and cell proliferation. According to the data provided, the concentration of SIM in Molar measurement unit (M) was determined. More details in Table 1.

3.3 Risk of Bias in Individual Studies

Most of the questions were considered as having a “Probably Low” or a “Definitely Low” risk of bias for all included studies. All included studies suggested a “Probably High” risk of bias for the questions regarding the randomization of administered SIM dose among groups or exposure level, allocation to study groups, and blinding of researchers during the study [19–21,30–36,38–41]. Two studies [20,21] did not report the complete outcome data without attrition or exclusion from analysis, and confidence in the outcome assessment was considered having a “Probably High” risk of bias. Additionally, the same studies had potential threats to the internal validity since the statistical analysis was not described. More details are in Table 2.

3.4 Synthesis of Results

It was impossible to carry out a meta-analysis due to the lack of statistical analysis and the heterogeneity among the studies concerning the intervention protocol, applied assays, culture medium, and SIM concentration.

Two studies compared NOM with NOM supplemented with 1 μM SIM [8,21] regarding the cell treatment. SIM supplementation promoted better results concerning the osteogenic assessment evaluated. Some studies compared the effect of NOM, NOM supplemented with SIM, and OM cell treatments, with SIM concentrations ranging from 0.1 nM to 10 μM [16,19,20,22]. Concentrations of 0.1 nM, 0.01 μM, and 0.1 μM promoted the best results regarding the osteogenic differentiation [16,19,22], while higher concentrations of SIM, such as 5 μM and 10 μM, demonstrated a cytotoxic effect [19]. Moreover, cell treatment with 5 μM SIM promoted less ALP activity than OM [16], while 1 μM SIM demonstrated less ALP activity and ECM mineralization than OM [16,22].

Studies comparing cell treatment with OM and OM supplemented with SIM used SIM concentrations of 1 nM [42], 0.1 μM[35], 0.25 μM [30] 0.3 μM [15] 0.5 μM, 1 μM [43], and 10 μM [44]. SIM concentrations of 0.25 μM [30] and 0.3 μM [15] showed more ALP activity, and ECM mineralization compared to OM and OM supplemented with 10 μM SIM [44]. In studies comparing cell treatments of NOM changing to OM and NOM changing to OM supplemented with SIM (0.25 μM and 0.5 μM) [33], the SIM supplementation [33] promoted more ALP activity and ECM mineralization. Also, 1 μM SIM promoted more ECM mineralization than NOM changing to OM cell treatment, despite that the ALP activity did not increase [29].

4. Discussion

Considering the current context of tissue engineering, active substances capable of acting as osteoinduction factors to promote the osteogenesis of undifferentiated cells are sought. Despite the increasing number of studies evaluating SIM as a bone metabolism stimulator, its ideal concentration in culture medium and the effect in the non-osteogenic and osteogenic culture medium are not entirely elucidated. Therefore, due to these inconsistencies regarding the SIM application as supplementation of non-osteogenic and osteogenic media, this

systematic review aimed to analyze studies that proposed SIM as an osteoinductive substance to promote osteogenesis undifferentiated cells *in vitro*. In summary, SIM promoted osteogenic differentiation by expressing osteogenic markers and ECM mineralization in 12 of the 14 included studies. Also, SIM supplemented NOM in five studies and OM in seven studies, potentiating the osteogenic effect [19–21,30–36,40,41].

Many studies have suggested that SIM promotes osteogenic differentiation in stem cells by increasing the expression of osteogenic markers [5,12,45,46]. When cells receive signals to differentiate, they become involved and gradually reduce their proliferation while expressing early markers, such as Runt-related transcription factor 2 (Runx2), ALP, and osterix (OSX). Late osteogenic markers, such as collagen type I, osteopontin, osteonectin, and osteocalcin, are expressed during the differentiation process. This sequential upregulation leads to the differentiation of stem cells into bone-like cells capable of depositing minerals in the ECM [47–49].

Two studies included in this systematic review showed that SIM had no potential for osteoinduction [38,39]. However, for one of these studies, three-dimensional cell culture of OM supplemented with SIM was used, with the limitation of applying tests addressed for two-dimensional cultures [38]. The other study used high SIM concentrations (1 µM and 5 µM) in NOM [39]. Considering the findings of other studies that tested a large range of concentrations, the ability of SIM to promote osteogenic differentiation seems to be dependent on the SIM concentration used and the type of culture medium [19,31]. When cells were treated with NOM supplemented with SIM, greater osteocalcin gene expression, ALP activity, and ECM mineralization were compared to NOM cells [21,36]. However, when compared to cells treated with OM, cells treated with NOM supplemented with SIM showed the expression of different osteogenic markers (osteocalcin, BMP-2, Cbfa1, osteonectin, collagen type I [20], FGEF [19], and OSX [31]). Conversely, cells treated with NOM supplemented with SIM showcased less ECM mineralization and ALP activity compared to OM treatment. Also, these findings were related to the SIM concentration used [31,39]. According to the studies that treated cells with OM supplemented with SIM, SIM enhanced the differentiation capacity of OM by expressing genes such as osteocalcin, BMP-2, ALP [30,34,40],

OSX, and RUNX 2 [32,34,38,40]. However, the ALP activity and the ECM mineralization were dependent of the SIM concentration used [30,32,34,35,38,40]. We observed that high SIM concentrations, such as 5 µM and 10 µM, were cytotoxic, while concentrations ranging from 0.1 nM to 1 µM stimulated osteogenic differentiation, mainly as supplementation of OM [19,31,38,39].

Regarding the risk of bias, most questions of the OHAT tool were considered as “Probably Low” or “Definitely Low” risk of bias for all included studies. It is important to mention that low RoB judgments denote that none or minor methodological flaws occurred in the assessed studies. Consequently, no or small deviations from the true effect estimation befallen, providing confidence in interpreting the results[50]. However, in an attempt to improve future studies, it is worth mentioning that some studies failed to report the randomization or exposure level, allocation concealment to study groups, and blinding of the research group during the investigation, being considered with “Probably High” risk of bias [19–21,30–36,38–41]. Furthermore, two studies presented a “Definitely High” risk of bias for not reporting if the outcome data was complete without attrition or exclusion from analysis, as well as if there was confidence in the outcome assessment, and threats to the internal validity due to the lack of statistical analysis [20,21]. Therefore, future experiments concerning *in vivo* osteogenic differentiation of stem cells should be more careful regarding these methodological details. It is also primordial not to assume that responses from *in vitro* studies will automatically translate to animal or human model responses. Properly design animal studies seem to be the next necessary step.

Since this systematic review aimed to evaluate the SIM capacity to induce the osteogenesis of undifferentiated cells *in vitro*, further systematic reviews assessing this capacity *in vivo* and clinical studies are indicated.

5. Conclusion

Based on the findings, SIM supplementation was able to promote osteogenic differentiation of undifferentiated cells in both non-osteogenic and osteogenic culture media. Also, osteogenic differentiation is highly dependent on the SIM concentration.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Lara Steiner Back is supported by a scholarship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES - Coordination for the Improvement of Higher Education Personnel), Brazil. Isabella Schönhofen Manso is supported by a scholarship from Fundação de Amparo à Pesquisa e Inovação do Estado de Santa Catarina (FAPESC - Research and Innovation Support Foundation of Santa Catarina State), Brazil.

ETHICAL APPROVAL

This article is not a study with human participants or animals.

INFORMED CONSENT

Formal consent is not required for this type of study.

ACKNOWLEDGMENTS

The authors thank Mrs. Karyn Munik Lehmkuhl for the instructions regarding the search strategy.

AUTHORS CONTRIBUITION

Lara Steiner Back, Isabella Schönhofen Manso, Mariane Beatriz Sordi, Gabriel Leonardo Magrin, Ariadne Cristiane Cabral Cruz, Aguedo Aragonês, Carlos Flores Mir, and Ricardo de Souza Magini contributed to the conception and the design of the study; Lara Steiner back, Isabella Schönhofen Manso, Mariane Beatriz Sordi, and Ariadne Cristiane Cabral Cruz collected the data; Lara Steiner Back, Isabella Schönhofen Manso, Mariane Beatriz Sordi, and Ariadne Cristiane Cabral Cruz analyzed the data; and Lara Steiner Back, Isabella Schönhofen Manso, Mariane Beatriz Sordi, Gabriel Leonardo Magrin, Aguedo Aragonês,

Ariadne Cristiane Cabral Cruz, Carlos Flores Mir, and Ricardo de Souza Magini drafted and critically revised the manuscript.

References

[1] M. Retzepi, N. Donos, Guided Bone Regeneration: Biological principle and therapeutic applications, Clinical Oral Implants Research. 21 (2010) 567–576. https://doi.org/10.1111/j.1600-0501.2010.01922.x.

[2] S. Nyman, Bone regeneration using the principle of guided tissue regeneration, Journal of Clinical Periodontology. 18 (1991) 494–498. https://doi.org/10.1111/j.1600-051X.1991.tb02322.x.

[3] J. Han, D. Menicanin, S. Gronthos, P.M. Bartold, Stem cells, tissue engineering and periodontal regeneration, Australian Dental Journal. 59 (2014) 117–130. https://doi.org/10.1111/adj.12100.

[4] P.M. Bartold, C.A.G. Mcculloch, A. Sampath Narayanan, S. Pitaru, Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology, Periodontology 2000. 24 (2000) 253–269. https://doi.org/10.1034/j.1600-0757.2000.2240113.x.

[5] G. Mundy, R. Garrett, S. Harris, J. Chan, D. Chen, G. Rossini, B. Boyce, M. Zhao, G. Gutierrez, Stimulation of Bone Formation in Vitro and in Rodents by Statins, Science. 286 (1999) 1946–1949. https://doi.org/10.1126/SCIENCE.286.5446.1946.

[6] L. Goldstein, M. Brown, Regulation of the mevalonate pathway, Nature. 343 (1990) 425–430. https://doi.org/https://doi.org/10.1038/343425a0.

[7] G.P. van N. Amerongen, M.A. Vermeer, P. Nègre-Aminou, J. Lankelma, J.J. Emeis, V.W.M. van Hinsbergh, Simvastatin Improves Disturbed Endothelial Barrier Function, Circulation. 102 (2000) 2803–2809. https://doi.org/10.1161/01.CIR.102.23.2803.

[8] J. Niu, G. Ding, L. Zhang, Effects of simvastatin on the osteogenic differentiation and immunomodulation of bone marrow mesenchymal stem cells, Molecular Medicine Reports. 12 (2015) 8237–8240. https://doi.org/10.3892/mmr.2015.4476.

[9] S.R. Shah, C.A. Werlang, F.K. Kasper, A.G. Mikos, Novel applications of statins for bone regeneration, National Science Review. 2 (2015) 85–99. https://doi.org/10.1093/nsr/nwu028.

[10] M. Sugiyama, T. Kodama, K. Konishi, K. Abe, S. Asami, S. Oikawa, Compactin and simvastatin, but not pravastatin, induce sone morphogenetic protein-2 in human osteosarcoma cells, Biochemical and Biophysical Research Communications. 271 (2000) 688–692. https://doi.org/10.1006/bbrc.2000.2697.

[11] P.D. Thunyakitpisal, R. Chaisuparat, Simvastatin, an HMG-CoA Reductase Inhibitor, Reduced the Expression of Matrix Metalloproteinase-9 (Gelatinase B) in Osteoblastic Cells and HT1080 Fibrosarcoma Cells, Journal of Pharmacological Sciences. 94 (2004) 403–409. https://doi.org/10.1254/jphs.94.403.

[12] S. Ruiz-Gaspa, X. Nogues, A. Enjuanes, J.C. Monllau, J. Blanch, R. Carreras, L. Mellibovsky, D. Grinberg, S. Balcells, A. Díez-Perez, J. Pedro-Botet, Simvastatin and atorvastatin enhance gene expression of collagen type 1 and osteocalcin in primary human osteoblasts and MG-63 cultures, Journal of Cellular Biochemistry. 101 (2007) 1430–1438. https://doi.org/10.1002/jcb.21259.

[13] T. Maeda, A. Matsunuma, T. Kawane, N. Horiuchi, Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells, Biochemical and Biophysical Research Communications. 280 (2001) 874–877. https://doi.org/10.1006/bbrc.2000.4232.

[14] Y. Huang, Y. Lin, M. Rong, W. Liu, J. He, L. Zhou, 20(S)-hydroxycholesterol and simvastatin synergistically enhance osteogenic differentiation of marrow stromal cells and bone regeneration by initiation of Raf/MEK/ERK signaling., Journal of Materials Science. Materials in Medicine. 30 (2019) 87. https://doi.org/10.1007/s10856-019-6284-0.

[15] M. Zhang, Y.-Q. Bian, H.-M. Tao, X.-F. Yang, W.-D. Mu, Simvastatin induces osteogenic differentiation of MSCs via Wnt/β-catenin pathway to promote fracture healing., European Review for Medical and Pharmacological Sciences. 22 (2018) 2896–2905. https://doi.org/10.26355/eurrev_201805_14992.

[16] L. Kupcsik, T. Meurya, M. Flury, M. Stoddart, M. Alini, Statin-induced calcification in human mesenchymal stem cells is cell death related, Journal of Cellular and Molecular Medicine. 13 (2009) 4465–4473. https://doi.org/10.1111/j.1582-4934.2008.00545.x.

[17] Y.-Q. Jin, L. Zhang, F.-M. Tian, L. Zhang, T. Cheng, X.-N. Liu, Effects of simvastatin on Wnt and bone morphogenetic protein 2 signaling pathway during osteoblast differentiation of bone marrow stromal cells, Journal of Clinical Rehabilitative Tissue Engineering Research. 15 (2011) 6732–6736. https://doi.org/10.3969/j.issn.1673-8225.2011.36.019.

[18] H. Lee, H. Lee, C. bin Na, J.B. Park, The effects of simvastatin on cellular viability, stemness and osteogenic differentiation using 3-dimensional cultures of stem cells and osteoblast-like cells, Advances in Clinical and Experimental Medicine. 28 (2019) 699–706. https://doi.org/10.17219/acem/94162.

[19] Y. Zhou, Y. Ni, Y. Liu, B. Zeng, Y. Xu, W. Ge, The role of simvastatin in the osteogenesis of injectable tissue-engineered bone based on human adipose-

derived stromal cells and platelet-rich plasma, Biomaterials. 31 (2010) 5325–5335. https://doi.org/10.1016/j.biomaterials.2010.03.037.

[20] S.H. Sun, I.K. Lee, J. Lee, I. Shim, S.H. Kim, K.S. Kim, Simvastatin induces osteogenic differentiation and suppresses adipogenic differentiation in primarily cultured human adipose-derived stem cells, Biomolecules and Therapeutics. 17 (2009) 353–361. https://doi.org/10.4062/biomolther.2009.17.4.353.

[21] K.-I. Yun, D.-J. Kim, J.-U. Park, Osteogenic potential of adult stem cells from human maxillary sinus membrane by Simvastatin in vitro: preliminary report., Journal of the Korean Association of Oral and Maxillofacial Surgeons. 39 (2013) 150–155. https://doi.org/10.5125/jkaoms.2013.39.4.150.

[22] J. Pagkalos, J.M. Cha, Y. Kang, M. Heliotis, E. Tsiridis, A. Mantalaris, Simvastatin induces osteogenic differentiation of murine embryonic stem cells, Journal of Bone and Mineral Research. 25 (2010) 2470–2478. https://doi.org/10.1002/jbmr.163.

[23] M. Yamashita, F. Otsuka, T. Mukai, H. Otani, K. Inagaki, T. Miyoshi, J. Goto, M. Yamamura, H. Makino, Simvastatin antagonizes tumor necrosis factor-alpha inhibition of bone morphogenetic proteins-2-induced osteoblast differentiation by regulating Smad signaling and Ras/Rho-mitogen-activated protein kinase pathway., The Journal of Endocrinology. 196 (2008) 601–613. https://doi.org/10.1677/JOE-07-0532.

[24] I.C. Encarnação, C.C. Ferreira Xavier, F. Bobinski, A.R.S. dos Santos, M. Corrêa, S.F.T. de Freitas, A. Aragonez, E.M. Goldfeder, M.M.R. Cordeiro, Analysis of Bone Repair and Inflammatory Process Caused by Simvastatin Combined With PLGA+HA+βTCP Scaffold, Implant Dentistry. 25 (2016) 140–148. https://doi.org/10.1097/ID.0000000000000359.

[25] I.G. Needleman, A guide to systematic reviews, Journal of Clinical Periodontology. 29 (2002) 6–9. https://doi.org/10.1034/j.1600-051x.29.s3.15.x.

[26] M.J. Page, J.E. McKenzie, P.M. Bossuyt, I. Boutron, T.C. Hoffmann, C.D. Mulrow, L. Shamseer, J.M. Tetzlaff, E.A. Akl, S.E. Brennan, R. Chou, J. Glanville, J.M. Grimshaw, A. Hróbjartsson, M.M. Lalu, T. Li, E.W. Loder, E. Mayo-Wilson, S. McDonald, L.A. McGuinness, L.A. Stewart, J. Thomas, A.C. Tricco, V.A. Welch, P. Whiting, D. Moher, The PRISMA 2020 statement: An updated guideline for reporting systematic reviews, The BMJ. 372 (2021). https://doi.org/10.1136/bmj.n71.

[27] H. Lee, H. Lee, C.-B. Na, J.-B. Park, Effects of simvastatin on the viability and secretion of vascular endothelial growth factor of cell spheroids cultured in growth media, Implant Dentistry. 27 (2018) 480–487. https://doi.org/10.1097/ID.0000000000000774.

[28] National Institute of Environmental Health Sciences, OHAT Risk of Bias Rating Tool for Human and Animal Studies Organization of This Document Indirectness , Timing , and Other Factors Related to Risk of Bias, 2015.

[29] S.-C. Chuang, C.-H. Chen, Y.-C. Fu, I.-C. Tai, C.-J. Li, L.-F. Chang, M.-L. Ho, J.-K. Chang, Estrogen receptor mediates simvastatin-stimulated osteogenic effects in bone marrow mesenchymal stem cells, Biochemical Pharmacology. 98 (2015) 453–464. https://doi.org/10.1016/j.bcp.2015.09.018.

[30] Y. Huang, Y. Lin, M. Rong, W. Liu, J. He, L. Zhou, 20(S)-hydroxycholesterol and simvastatin synergistically enhance osteogenic differentiation of marrow stromal cells and bone regeneration by initiation of Raf/MEK/ERK signaling, Journal of Materials Science: Materials in Medicine. 30 (2019). https://doi.org/10.1007/s10856-019-6284-0.

[31] J. Pagkalos, J.M. Cha, Y. Kang, M. Heliotis, E. Tsiridis, A. Mantalaris, Simvastatin induces osteogenic differentiation of murine embryonic stem cells, Journal of Bone and Mineral Research. 25 (2010) 2470–2478. https://doi.org/10.1002/jbmr.163.

[32] L.J. Qiao, K.L. Kang, J.S. Heo, Simvastatin promotes osteogenic differentiation of mouse embryonic stem cells via canonical Wnt/β-catenin signaling, Molecules and Cells. 32 (2011) 437–444. https://doi.org/10.1007/s10059-011-0107-6.

[33] P.-L. Shao, S.-C. Wu, Z.-Y. Lin, M.-L. Ho, C.-H. Chen, C.-Z. Wang, Alpha-5 integrin mediates simvastatin-induced osteogenesis of bone marrow mesenchymal stem cells, International Journal of Molecular Sciences. 20 (2019). https://doi.org/10.3390/ijms20030506.

[34] I.-C. Tai, Y.-H. Wang, C.-H. Chen, S.-C. Chuang, J.-K. Chang, M.-L. Ho, Simvastatin enhances Rho/actin/cell rigidity pathway contributing to mesenchymal stem cells' osteogenic differentiation, International Journal of Nanomedicine. 10 (2015) 5881–5894. https://doi.org/10.2147/IJN.S84273.

[35] Y. qiao Jin, L. Zhang, F. ming Tian, L. Zhang, T. Cheng, X. ning Liu, Effects of simvastatin on Wnt and bone morphogenetic protein 2 signaling pathway during osteoblast differentiation of bone marrow stromal cells, Journal of Clinical Rehabilitative Tissue Engineering Research. 15 (2011) 6732–6736. https://doi.org/10.3969/j.issn.1673-8225.2011.36.019.

[36] J. Niu, G. Ding, L. Zhang, Effects of simvastatin on the osteogenic differentiation and immunomodulation of bone marrow mesenchymal stem cells, Molecular Medicine Reports. 12 (2015) 8237–8240. https://doi.org/10.3892/mmr.2015.4476.

[37] M. Zhang, Y.-Q. Bian, H.-M. Tao, X.-F. Yang, W.-D. Mu, Simvastatin induces osteogenic differentiation of MSCs via Wnt/β-catenin pathway to promote fracture healing, European Review for Medical and Pharmacological Sciences. 22 (2018) 2896–2905. https://doi.org/10.26355/eurrev_201805_14992.

[38] H. Lee, H. Lee, C.-B. Na, J.-B. Park, The effects of simvastatin on cellular viability, stemness and osteogenic differentiation using 3-dimensional cultures of stem cells and osteoblast-like cells, Advances in Clinical and Experimental Medicine. 28 (2019) 699–706. https://doi.org/10.17219/acem/94162.

[39] L. Kupcsik, T. Meurya, M. Flury, M. Stoddart, M. Alini, Statin-induced calcification in human mesenchymal stem cells is cell death related, Journal of Cellular and Molecular Medicine. 13 (2009) 4465–4473. https://doi.org/10.1111/j.1582-4934.2008.00545.x.

[40] M. Zhang, Y.-Q. Bian, H.-M. Tao, X.-F. Yang, W.-D. Mu, Simvastatin induces osteogenic differentiation of MSCs via Wnt/β-catenin pathway to promote fracture healing, European Review for Medical and Pharmacological Sciences. 22 (2018) 2896–2905. https://doi.org/10.26355/eurrev_201805_14992.

[41] S.-C. Chuang, C.-H. Chen, Y.-C. Fu, I.-C. Tai, C.-J. Li, L.-F. Chang, M.-L. Ho, J.-K. Chang, Estrogen receptor mediates simvastatin-stimulated osteogenic effects in bone marrow mesenchymal stem cells, Biochemical Pharmacology. 98 (2015) 453–464. https://doi.org/10.1016/j.bcp.2015.09.018.

[42] L.J. Qiao, K.L. Kang, J.S. Heo, Simvastatin promotes osteogenic differentiation of mouse embryonic stem cells via canonical Wnt/β-catenin signaling, Molecules and Cells. 32 (2011) 437–444. https://doi.org/10.1007/s10059-011-0107-6.

[43] I.-C. Tai, Y.-H. Wang, C.-H. Chen, S.-C. Chuang, J.-K. Chang, M.-L. Ho, Simvastatin enhances Rho/actin/cell rigidity pathway contributing to

mesenchymal stem cells' osteogenic differentiation, International Journal of Nanomedicine. 10 (2015) 5881–5894. https://doi.org/10.2147/IJN.S84273.

[44] H. Lee, H. Lee, C.-B. Na, J.-B. Park, The effects of simvastatin on cellular viability, stemness and osteogenic differentiation using 3-dimensional cultures of stem cells and osteoblast-like cells., Advances in Clinical and Experimental Medicine : Official Organ Wroclaw Medical University. 28 (2019) 699–706. https://doi.org/10.17219/acem/94162.

[45] K.H. Baek, W.Y. Lee, K.W. Oh, H.J. Tae, J.M. Lee, E.J. Lee, J.H. Han, M. il Kang, B.Y. Cha, K.W. Lee, H.Y. Son, S.K. Kang, The effect of simvastatin on the proliferation and differentiation of human bone marrow stromal cells., Journal of Korean Medical Science. 20 (2005) 438–444. https://doi.org/10.3346/jkms.2005.20.3.438.

[46] T. Maeda, T. Kawane, N. Horiuchi, Statins Augment Vascular Endothelial Growth Factor Expression in Osteoblastic Cells via Inhibition of Protein Prenylation, Endocrinology. 144 (2003) 681–692. https://doi.org/10.1210/en.2002-220682.

[47] M.G. Rimando, H.H. Wu, Y.A. Liu, C.W. Lee, S.W. Kuo, Y.P. Lo, K.F. Tseng, Y.S. Liu, O.K.S. Lee, Glucocorticoid receptor and Histone deacetylase 6 mediate the differential effect of dexamethasone during osteogenesis of mesenchymal stromal cells (MSCs), Scientific Reports. 6 (2016) 1–13. https://doi.org/10.1038/srep37371.

[48] R.T. Franceschi, G. Xiao, Regulation of the osteoblast-specific transcription factor, Runx2: Responsiveness to multiple signal transduction pathways, Journal of Cellular Biochemistry. 88 (2003) 446–454. https://doi.org/10.1002/jcb.10369.

[49] E. GREEN, B. TODD, D. HEALTH, Mechanism of glucocorticoid regulation of alkaline phosphatase gene expression in osteoblast-like cells, European Journal

of Biochemistry. 188 (1990) 147–153. https://doi.org/10.1111/j.1432-1033.1990.tb15382.x.

[50] G.H. Guyatt, A.D. Oxman, G.E. Vist, R. Kunz, Y. Falck-Ytter, P. Alonso-Coello, H.J. Schünemann, GRADE: An emerging consensus on rating quality of evidence and strength of recommendations, Chinese Journal of Evidence-Based Medicine. 9 (2009) 8–11.

FIGURE LEGENDS

Table 1: Summary of descriptive characteristics of included articles (n=14)

Table 2: Risk of Bias in individual studies assessed by the adapted OHAT risk-of-bias tool

Figure 1: Flow diagram of literature search and selection criteria

Appendix 1: Data search strategy

Appendix 2: Articles excluded and the reasons for exclusion (n=56)

4 METODOLOGIA EXPANDIDA

4.1 DESENHO DO ESTUDO

O protocolo para a confecção desta SR foi baseado no Cochrane Handbook for Systematic Reviews of Interventions (JOHN WILEY & SONS LTD, 2011) e nas diretrizes da lista de verificação de itens de relatório obrigatórios para revisão sistemática e meta-análise (PRISMA-P) (KAMIOKA, 2019). O protocolo foi registrado na plataforma de registro *Open Science Framework* (Doi:10.17605/OSF.IO/GBK5S) em 26 de agosto de 2020.

4.2 CRITÉRIOS DE ELEGIBILIDADE

4.2.1 Critério de inclusão

O acrônimo PICOS (NEEDLEMAN, 2002) (população, intervenção, comparação, resultado e tipo de estudos) foi utilizado para criar a pergunta de pesquisa desta RS: “A sinvastatina tem potencial osteoindutor em células indiferenciadas *in vitro*?”, onde:

População (P): Células indiferenciadas;

Intervenção (I): tratamento SIN;

Comparação (C): células indiferenciadas tratadas sem SIN;

Resultado (O): Diferenciação osteogênica;

Tipo de estudos (S): estudos in vitro.

Os estudos foram considerados elegíveis quando avaliam a diferenciação osteogênica de células indiferenciadas tratadas com SIN. Nenhum período de publicação ou restrições de idioma foram aplicadas.

4.2.2 Critério de exclusão

Foram considerados os seguintes critérios de exclusão: 1) Estudos que investigassem outros tipos de células, como células diferenciadas; 2) Estudos avaliando associações de fármacos ou biomateriais que não apresentam dados separados apenas para a SIN; 3) Estudos que não apresentam grupo controle não tratado com o mesmo tipo de célula; 4) Estudos que não apresentam grupo controle sem tratamento com SIN; 5) Estudos que não avaliam as capacidades de osteoindução / diferenciação osteogênica da SIN; 6) Estudos que não sejam in vitro (estudos clínicos, estudos em animais, resumos de conferências, cartas,

estudos pilotos, artigos de revisão, relatos de casos, protocolos, relatos breves, opiniões pessoais, pôsteres ou capítulos de livros); 7) Estudos que não especificam o meio de cultura; 8) Estudos que não realizem pelo menos os testes de atividade da fosfatase alcalina (ALP) e mineralização da matriz extracelular; 9) Texto completo não disponível; 10) Dados duplicados (por exemplo, dissertações / teses em que os correspondentes artigos publicados estavam disponíveis).

4.3 ESTRATÉGIA DE BUSCA

Uma estratégia de busca foi desenvolvida com base na estrutura PICOS usando palavras chaves e *Mesh Terms* (MeSH). Foram utilizadas as seguintes bases de dados eletrônicas: Embase, Literatura Latino-Americana e do Caribe em Ciências da Saúde (LILACS), PubMed, SCOPUS e Web of Science. Além disso, uma estratégia de busca foi desenvolvida para bases de dados da literatura cinzenta como: Google Scholar (primeiras 100 referências), Open Gray e ProQuest. A busca eletrônica foi realizada até 30 de novembro de 2020. Uma busca manual nas referências dos estudos incluídos também foi realizada. Todos os registros encontrados foram exportados para um software gerenciador de referências (Mendeley Desktop, Elsevier, London, UK) e as duplicatas foram removidas. As estratégias de busca detalhadas estão disponíveis no Apêndice 1.

4.4 SELEÇÃO DOS ESTUDOS

Um processo de seleção de duas fases foi realizado. Dois revisores independentes (LSB e ISM) selecionaram as referências. Primeiro, na fase um, ambos os revisores leram os títulos e resumos de forma independente enquanto aplicavam os critérios de elegibilidade utilizando um software online (Rayyan, Qatar Computing Research Institute, Qatar). Posteriormente, na fase dois, os mesmos dois revisores realizaram a leitura do texto completo também aplicando os critérios de elegibilidade. Em ambas as fases, todas as informações recuperadas foram cruzadas e conferidas por um terceiro revisor (MBS). A seleção final sempre foi feita com base no texto completo do estudo. Os artigos

que atenderam aos critérios de elegibilidade tiveram seus dados e resultados extraídos.

4.5 PROCESSO DE COLETA DE DADOS

Dois revisores independentes (LSB e ISM) coletaram os dados dos artigos selecionados e as informações obtidas foram conferidas pelo terceiro revisor (MBS). Para qualquer desacordo entre os dois primeiros revisores, o terceiro revisor tomou a decisão final. Os seguintes dados foram extraídos para cada estudo incluído: autor, ano de publicação, país, tipos de células, origem das linhagens celulares, tratamento celular, concentração do tratamento, métodos aplicados, resultados e principais conclusões. Se necessário, os autores correspondentes dos estudos incluídos foram contatados via e-mail para esclarecimento de qualquer informação faltante e / ou esclarecimento da metodologia e resultados.

4.6 AVALIAÇÃO DE RISCO DE VIÉS

A ferramenta de risco de viés Office of Health Assessment and Translation (OHAT) adaptada para estudos *in vitro* foi utilizada para avaliar os estudos de forma individual nesta revisão sistemática (CLARITY Group at McMaster University 2013) [28]. Os estudos incluídos foram avaliados de forma independente quanto ao risco de viés por dois revisores (LSB e ISM). Discrepâncias entre os revisores foram resolvidas por meio de um acordo com o terceiro revisor. As respostas possíveis para as perguntas da ferramenta OHAT eram “Definitivamente baixo”, “Provavelmente baixo”, “Provavelmente alto” ou “Definitivamente alto” risco de viés, seguindo critérios específicos detalhados no protocolo.

4.7 SÍNTESE DOS RESULTADOS

Informações como: análise da expressão gênica (nível de expressão de marcadores osteogênicos), expressão de proteínas ósseas, mineralização da matriz extracelular, atividade de fosfatase alcalina e proliferação celular, foram consideradas durante a avaliação dos resultados.

5 CONSIDERAÇOES FINAIS

Com base nos achados, a suplementação com SIM foi capaz de promover a diferenciação osteogênica de células indiferenciadas em meios de cultura não osteogênicos e osteogênicos, se tornando um agente promissor para utilização em Engenharia Tecidual. Além disso, a diferenciação osteogênica foi altamente dependente da concentração de SIM.

6 TABELAS

6.1 TABELA 1 - Resumo das características descritivas dos artigos incluídos (n = 14).

Table 1: Summary of descriptive characteristics of included articles (n=14)

Author (year); Country	Cell Type; (origin)	Treatment Type	Methods (time)	Results	Main Conclusions
Chuang et al. (2015) Taiwan	mBMSC (Murine bone marrow – D1 Cells)	T5a / T6a	RT-PCR (days 2, 3, and 5) ALP Staining (day 5) Alizarin Red Staining (5 until 10 days after OM induction)	T6 (0.5μM SIM) induced the highest BMP-2, ALP, and OC gene expression (P<0.01) Positive ALP staining for T6 (0,25μM and 0.5μM SIM) (no statistical analyses) T6 (0.25μM to 1.0μM SIM) promoted the highest Alizarin Red Staining (P<0.01)	SIM stimulated osteogenic differentiation mediated by ERα.
Huang et al. (2019) China	mBMSC (Murine bone marrow)	T3a / T4a	Proliferation (days 3 and 7) RT-PCR (days 3 and 7) ALP Activity (days 3 and 7) Alizarin Red Staining (days 3, 7, and 14) Western Blot (day 7)	Similar cell proliferation between groups on day 3; Inhibition of cell proliferation from day 7 (T4 – 0,25 μM SIM). (P<0.05) T4 (0.10 μM, 0.025μM SIM) promoted the highest OC and BMP-2 gene expression on day 7 (P<0.05). T4 (0.25μM SIM) promoted the highest ALP, OC, BMP-2, and BMP-9 gene expression on day 7 (P<0.01). T4 (1.0 μM SIM) induced the highest ALP, OC, and BMP-9 gene expression on day 7 (P<0.01). T4 (0.25μM SIM) induced the highest ALP, BMP-2, and BMP-9 gene expression on day 3 (P<0.05) T4 (0,25 μM SIM) promoted the highest ALP activity on days 3 and 7 (P<0.05) T4 (0.25 μM SIM) and T3 demonstrated negative Alizarin Red Staining on day 3. T4 (0.25 μM SIM) promoted positive Alizarin Red Staining, while was negative for T3 on days 7 and 14 (P<0.05 and P<0.01, respectively) T4 (0.25 μM SIM) promoted more phospho-MEK and phospho-ERK1/2 proteins expression compared to T3 (P<0.05)	SIM induced osteogenic differentiation and increased the expression levels of phospho-MEK and phospho-ERK1/2 proteins of the Raf/MEK/ERK signaling pathway.

Jin et al. (2011) China	mBMSC (Murine bone marrow)	T3b / T4b	Gene Expression (days 14 to 21) ALP Staining (day 14) Von kossa Staining (day 28) Observation of cell morphology (days 14 to 21)	T4 (0.1 μM SIM) promoted more Wnt signaling pathway related gene expression (b-catenin and Cbfa1) than T3 on days 14 and 21 (P<0.05) T4 promoted positive ALP staining, while for T3 was negative on day 14 (P<0.05) T4 demonstrated positive Von Kossa staining, while the result was negative for T3 on day 28 (P<0.05) T3 and T4 induced osteoblastic-shaped cells. However, T4 promoted more cell growth and extracellular matrix secretion.	SIM promoted osteogenic differentiation and expression of genes related to the Wnt signaling pathway.
Kupcsik et al. (2015) Switzerland	hBMSC (Human bone marrow)	T1b / T2b / T3c	RT-PCR (days 4, 11, and 18) ALP activity (day 11) Von kossa Staining (day 25) ^{45}Ca Incorporation (days 18 and 25)	T2 (1μM and 5μM SIM) promoted the highest BMP-2 gene expression compared to T1 and T3 on day 4 (P<0.05). BMP-2 gene expression increased in T1 and decreased in T3 on days 11 and 18 (P<0.05). T2 promoted the ALP gene expression suppression on days 4, 11, and 18. T3 increased the ALP gene expression on day 4 (P<0.05) T3 demonstrated the highest ALP activity compared to T1 and T2 (1μM and 5μM SIM) (P<0.05) T2 (1μM and 5μM SIM) and T3 demonstrated positive Von Kossa staining (no statistical analyses) T2 (1μM and 5μM SIM) and T3 demonstrated positive ^{45}Ca incorporation in extracellular matrix on days 18 and 25 (no statistical analyses)	SIM was not considered suitable for bone tissue engineering applications as osteogenic factor due to its long-term cytotoxicity effect.

Lee et al. (2019) South Korea	GMSC Spheroids (Human gengiva)	T3d / T4d	RT-PCR (day 7) ALP Activity (day 14) Alizarin Red Staining (days 7 and 14)	T4 (10.0μM SIM) promoted the highest Collagen I (P<0.05) and RUNX2 (P<0.01) gene expression T4 (1μM and 10μM SIM) did not demonstrate ALP activity on day 14. T3 showed ALP activity that decreased with the SIM addition (P<0.05) T3 demonstrated positive Alizarin Red staining, while was negative for T4 (1.0μM and 10.0μM SIM) on days 7 and 14 (no statistical analyses)	SIM enhanced Collagen I and RUNX2 expression but did not lead to increase ALP activity and Alizarin Red Staining, not promoting osteogenic differentiation compared to osteogenic medium.
Niu et al. (2015) China	hBMSC (Human bone marrow)	T1a / T2a	RT-PCR (day 14) ALP activity (days 1 and 7) Von kossa Staining (day 21)	T2 (1.0μM SIM) promoted the OC and Bone Sialoprotein gene expression (no statistical analyses) *No ALP activity for T1 and T2 on day 1; T2 (1.0μM SIM) promoted the highest ALP activity on day 7 (P<0.05) T1 and T2 demosntrated positive Von Kossa Staining on day 21 (no statistical analyses)	Treatment with 1μM of SIM induced osteogenic differentiation of BMSCs without altering their immunomodulatory properties.
Pagkalos et al. (2010) United Kingdom	mESC (Murine embryonic cells - E14Tg2α)	T1c / T2c / T3e	RT-PCR (day 12) ALP activity (days 4, 8, 12, 16, and 21) Alizarin Red Staining (days 12, 16, and 21)	T2 (0.1nM and 0.1μM SIM) promoted the highest OC gene expression (P<0.05), while T2 (0.1nM and 0.1μM SIM) and T3 induced the highest OSX gene expression (P<0.05) T2 (0.1nM SIM) and T3 promoted the highest ALP activity on day 8 (P<0.05) Alizarin Red staining was positive for T2 (0.1μM and 1.0μM SIM) on day 12; for T2 (0.1nM and 0.1μM SIM) and T3 on days 16 and 21. T2 (0.1nM and 0.1μM SIM) promoted higher staining than T2 (1.0μM SIM) and T3 on days 16 and 21 (P<0.05)	High concentrations of SIM (0.1μM and 1.0μM) and an optimally effective concentration of 0.1 nM promotes osteogenic differentiation of ESCs.

Qiao et al. (2011) Korea	mESC (Murine embryonic cells, D3 line (ATCC))	T3f / T4f (Cells are pre-induced for 1 day with OM prior to treatment)	RT-PCR (day 4) ALP Activity (days 4 and 7) Alizarin Red Staining (days 7 and 14) Western Blot (day 7)	T4 (1.0nM SIM) promoted the highest RUNX2 gene expression, T4 (0.01µM and 0.01µM SIM) promoted the highest RUNX2, OSX, OC genes and T4 (0.2µM) promote the highest OSX gene. (P<0.05) There was no ALP activity on day 4. T4 (1.0nM, 0.01µM, 0.1µM, and 0.2µM SIM) promoted the highest ALP activity on day 7 (P<0.05) T4 (0.1µM and 0.2µM SIM) induced the highest Alizarin Red staining on day 7 (P<0.05). T4 (1.0nM, 0.01µM, 0.1µM, and 0.2µM SIM) promoted the highest Alizarin Red staining on day 14 (P<0.01) T4 (1.0nM, 0.01µM, 0.1µM, and 0.2µM SIM) promoted the highest OC, OPN, and Collagen I protein expression (P<0.05)	SIM stimulated the osteogenic differentiation and the Wnt signaling pathway contributed to this process.
Shao et al. (2019) Taiwan	mBMSC (Murine bone marrow – D1 Cells)	T5b / T6b	RT-PCR (day 3) ALP Activity (1 day after OM induction – day 4) Alizarin Red Staining (5 days after OM induction – day 7) Calcium Deposition (5 days after OM induction – day 7)	T6 (0.25µM SIM) promoted the highest BMP-2, ALP, RUNX2, OC (P<0.05), and Collagen I (P<0.01) gene expression. T5 (0.5µM SIM) – ALP, OC (P<0.05) and BMP-2, RUNX2, Collagen I (P<0.01). T6 (0.25µM and 0.5µM SIM) demonstrated the highest ALP activity (P<0.05) T6 (0.25µM and 0.5µM SIM) promoted the highest Alizarin Red staining (P<0.05) T6 (0.25µM SIM) (P<0.05) and T6 (0.5µM SIM) (P<0.01) promoted the highest calcium deposition on extracellular matrix	SIM promoted osteogenic differentiation.
Sun et al. (2009) China	ADSC (Human adipose tissue)	T1d / T2d / T3g	RT-PCR (days 1, 3, 6, 7, 9, and 12) ALP Staining (days 3, 6, 7, 9, 12, 14, 21, and 28) Von kossa Staining (days 3, 6, 7, 9, 12, 14, 21, and 28)	T2 (1.0µM SIM) promoted the OC, cbfa1, Collagen I, OSN, and BMP-2 gene expression on days 3, 6, 7, 9, and 12 (no statistical analyses) T1 (1.0µM SIM) and T3 showed positive ALP staining from the day 6 and 7, respectively (no statistical analyses) T1 (1.0µM SIM) and T3 showed positive Von Kossa staining from the day 6 and 7, respectively (no statistical analyses)	SIM increased the osteogenic differentiation.

Tai et al. (2015) Taiwan	mBMSC (Murine bone marrow – D1 Cells)	T3j / T4j	RT-PCR (12, 24, and 48 hours) ALP Staining (day 3) Alizarin Red Staining (day 5)	T4 (1.0μM SIM) promoted the highest OC gene expression on 12 and 24 hours, while T4 (0.5μM and 1.0μM SIM) promoted the highest expression on 48 hours. T4 (1.0μM SIM) promoted the highest RUNX2 gene expression on 12, 24, and 48 hours, while T4 (0.5μM SIM) induced the highest gene expression on 24 and 48 hours and T4 (0.1μM SIM) on 24 hours. T4 (1.0μM SIM) promoted the highest BMP-2 gene expression on 12, 24, and 48 hours, while T4 (0.1μM and 0.5μM SIM) promoted the highest expression on 12 hours ($P<0.05$). T4 (0.5μM and 1.0μM SIM) induced the highest ALP stanning ($P<0.05$) T4 (0.5μM and 1.0μM SIM) induced the highest Alizarin Red stanning ($P<0.05$)	SIM enhanced the osteogenic differentiation by activating the RhoA/actin/cell rigidity signaling pathway.
Yun et al. (2013) Korea	SMSC (Human sinus maxillary membrane)	T1d / T2d	RT-PCR (days 3, 6, 9, and 12) Western Blot (day 3) ALP Staining (days 3, 6, 9, and 12) Von kossa Staining (days 3, 6, 9, and 12)	T2 (1.0μM SIM) promoted the highest OC gene expression from day 3 (no statistical analyses). T2 (0.1, 0.2, 0.5, 1.0 and 2.0μM SIM) promoted the highest BMP-2 protein expression (no statistical analyses). T2 (1.0μM SIM) demonstrated positive ALP staining from day 3 (no statistical analyses) T2 (1.0μM SIM) showed positive Von Kossa staining from day 6 (no statistical analyses)	SIM promoted osteogenic differentiation of SMSC and can be used on tissue engineer.

Zhang et al. (2018) China	mBMSC (Murine bone marrow)	T3h / T4h	Proliferation (day 1) RT-PCR (day 7) ALP Activity (day 7) ALP Staining (day 7) Alizarin Red Staining (day 7) Western Blot (day 7)	Cell proliferation was observed at 1.0 nM and 3 nM SIM, with no proliferation at 0.3 nM SIM (P<0.01) T4 (0.3 nM SIM) promoted the highest OC, RUNX2, OSX, and ALP gene expression (P<0.001) T4 (0.3 nM SIM) showed the highest ALP activity (P<0.01) T4 (0.3 nM SIM) promoted the highest ALP staining (P<0.05) T4 (0.3 nM SIM) demonstrated the highest Alizarin Red staining (P<0.05) T4 (0.3 nM SIM) promoted the highest ALP, RUNX2, OC, and OSP protein expression (P<0.05)	SIM promoted the osteogenic differentiation, and the mechanism may be related of Wnt/β-catenin pathway.
Zhou et al. (2010) China	ADSC (Human adipose tissue)	T1e / T2e / T3i	Proliferation (day 1 to 9) RT-PCR (day 3) ALP Activity (days 6 and 14) Alizarin Red Staining (day 14)	T2 (10μM and 5μM SIM) inhibited the cell proliferation from day 3 T2 (0,01 μM, 0,1 μM, and 1 μM SIM) promoted the OC, Cbfa1, BMP-2, VEGF, and FGF-2 gene expression, while T3 promoted the Cbfa1 and OC gene expression (no statistical analyses) No ALP activity was observed on day 6. T2 (1μM, 0.1μM, and 0.01μM SIM) and T3 promoted more ALP activity than T1 (P<0.05) on day 14 T2 (1μM, 0.1μM, and 0.01μM SIM) and T3 promoted more Alizarin Red staining than T1 (P<0.05) on day 14	SIM induced the osteogenic differentiation, depending on its concentration. Concentrations of 0.01μM, 0.1μM, and especially 1μM promoted the highest differentiation.

Treatment type composition:
T1 – Non-Osteogenic Medium (NOM).
T2 – SIM + NOM.
a.) Dulbecco's modified essential medium (DMEM) +10% Bovine Fetal Serum (FBS) + 2 mol/L glutamine + 100 U/mL penicillin + 100 U/mL streptomycin.
b.) Minimum Essential Medium alfa (α-MEM) + 10% FBS + non-essential amino acids + 10 mmol/L β-glycerophosphate + 0.1 mmol/L ascorbic acid.
c.) α-MEM + 15% FBS + 1% penicillin/streptomycin + 10 mmol/L β-glycerophosphate.
d.) α-MEM + 10% FBS + 1% penicillin/streptomycin.
e.) DMEM +10% FBS + 100 U/mL penicillin + 100/mL streptomycin.
f.) α-MEM + 10% FBS + 1% penicillin/streptomycin.

T3 – Osteogenic Medium (OM).
T4 – SIM + OM.
a.) Roswell Park Memorial Institute 1640 Medium (RPMI1640) + 10% BFS + 0.2 mmol/L ascorbic acid + 10 nmol/L dexamethasone + 10 mM β-glycerophosphate.
b.) DMEM + 10% FBS + 100U/mL penicillin + 100mg/mL streptomycin + 50 mg/L vitamin C + 10^{-8mol}/L dexamethasone.
c.) α-MEM + 10% FBS + non-essential amino acids + 10 mmol/L β-glycerophosphate + 0.1 mmol/L ascorbic acid + 10 nmol/L dexamethasone;
d.) α-MEM + 15%FBS + 100 U/mL penicillin + 100 μg/mL streptomycin + 200 mmol/L L-glutamine +10 mmol/L ascorbic acid + 38 μg/mL dexamethasone.
e.) α-MEM + 15%FBS + 1% penicillin/streptomycin + 10 mmol/L β-glycerophosphate + 50mg/mL ascorbic acid + 1 mmol/L dexamethasone.
f.) α-MEM +5% FBS + 50 μg/mL ascorbic acid + 1 μmol/L dexamethasone + 3 mmol/L β-glycerophosphate.
g.) α-MEM + 10% FBS + 1% penicillin/streptomycin + 0.1 μmol/L dexamethasone + 10μmol/L β-glycerophosphate + 50 μmol/L ascorbic acid.
h.) DMEM + 10% FBS + 1% penicillin/streptomycin + 10μmol/L β-glycerophosphate + 50 μmol/L ascorbic acid.
i.) DMEM +10% FBS + 100 U/mL of penicillin + 100 U/mL of streptomycin + 1 μmol/L dexamethasone
j.) DMEM + 10% FBS +100 μg/mL ascorbic acid + 100 μg/mL non-essential amino acids + 100 μg/mL penicillin/streptomycin + 0.2 mmol/L ascorbic acid + 100 nmol/L dexamethasone.
T5 – NOM changing to OM.
T6 – SIM + NOM changing to OM.
a.) MEM + 10% SFB + 100 U/mL of penicillin + 100 mg/mL non-essential amino acids + 50 μg/mL ascorbic acid / DMEM + 10% SFB + 100 U/mL of penicillin + 100 mg/mL non-essential amino acids + 50 μg/mL ascorbic acid + 10 mmol/L β-glycerophosphate + 0.1 μmol/L dexamethasone (NOM changes to OM on day 5. Alizarin Red staining performed 5 days after medium changes).
b.) DMEM + 10% SFB + 100 U/mL of penicillin/ streptomycin + 100 μg/mL ascorbic acid / DMEM + 10% SFB + 100 U/mL of penicillin + 100 mg/mL non-essential amino acids + 50 μg/mL ascorbic acid + 10 mmol/L β-glycerophosphate + 0.1 μmol/L dexamethasone (NOM changes to OM on day 3. ALP assay was performed 1 day after medium changes. Alizarin Red staining and calcium deposition analysis were performed 5 days after medium changes).

Legend:
OC – Osteocalcin
OSX – Osterix
ONC – Osteonectin
BMP-2 – Bone Morphogenetic Protein-2
ALP – Alkaline Phosphatase
Cbfa – Core-binding factor subunit alpha-1
hBMSC – Human Bone Marrow Stem Cells
mBMSC – Murine Bone Marrow Stem Cells
GMSC – Gingiva-derived Stem Cells
mESC – Murine Embryonic Stem Cells
ADSC – Adipose-derived Stem Cells
SMSC – Sinus Maxillary Stem Cells

6.2 TABELA 2 – Risco de viés dos estudos individuais pela ferramenta OHAT *risk-of-bias* adaptada.

Table 2 - Risk of Bias in individual studies assessed by the adapted OHAT risk-of-bias tool.

Bias Domains and Questions	Chuang et al. (2015)	Huang et al. (2019)	Jin et al. (2011)	Kupcsik et al. (2009)	Lee et al. (2019)	Niu et al. (2015)	Pagkalos et al. (2010)	Qiao et al. (2011)	Shao et al. (2019)	Sun et al. (2009)	Tai et al., 2015	Yun et al. (2013)	Zhang et al. (2018)	Zhou et al. (2010)
Selection Bias														
1. Was administered dose or exposure level adequately randomized?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2. Was allocation to study groups adequately concealed?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Performance Bias														
5. Were experimental conditions identical across study groups?	++	++	++	++	++	++	++	++	++	++	++	++	++	++
6. Were the research personnel and human subjects blinded to the study group during the study?	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Attrition/Exclusion Bias														
7. Were outcome data complete without attrition or exclusion from analysis?	++	++	++	++	++	+	++	++	++	-	++	-	++	+
8. Can we be confident in the exposure characterization?	++	++	++	++	++	++	++	+	++	++	++	++	++	++
9. Can we be confident in the outcome assessment?	++	++	++	++	++	+	++	++	++	-	++	-	++	+
Selective Reporting Bias														
10. Were all measured outcomes reported?	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Other Soucers of Bias														
11. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate, and researchers adhered to the study protocol)?	++	++	++	++	++	+	++	++	++	-	++	-	++	+

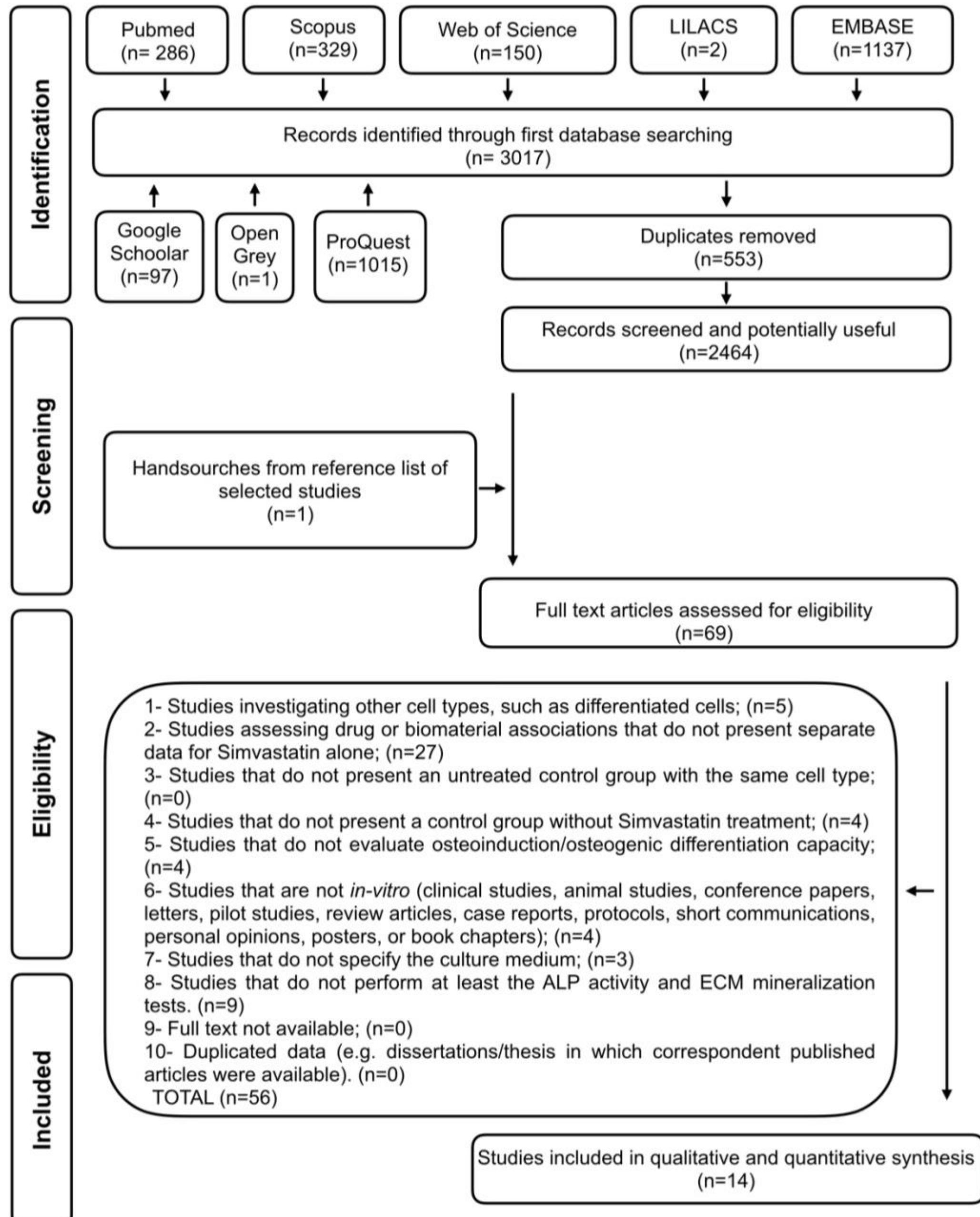
Answer Format

++	**Definitely Low** risk of bias: There is direct evidence of low risk of bias practices. (May include specific example of low risk of bias practices).
+	**Probably Low** risk of bias: There is indirect evidence of low risk of bias practices OR it is deemed that deviations from low risk of bias practices for these criteria during de study would not appreciably bias results, including consideration of direction and magnitude of bias.
-	**Probably High** risk of bias: There is indirectly evidence of high risk of bias practices OR there is insufficient information (e.g., not reported or “NR”) provided about relevant risk of bias practices.
--	**Definitely High** risk of bias: There is direct evidence of high risk of bias practices. (May include specific example of high risk of bias practices).

7 FIGURAS

7.1 FIGURA 1 – Fluxograma de pesquisa e critérios de seleção.

Figure 1 – Flow Diagram of literature search and selection criteria



Adapted from PRISMA

8 APÊNDICES

8.1 APENDICE 1 – Estratégia de busca

Appendix 1 - Data search strategy.

Database	Search query, 2020, November 30th
PubMed	"Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony Forming Units" OR "undifferentiated cells" OR Pericytes OR Pericyte OR "Rouget Cells" AND "Simvastatin" OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl CoA Inhibitors" OR Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors" AND "Osteogenesis" OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein" AND "Osteogenesis" OR "bone formation" OR Ossification OR Ossifications OR Osteoclastogenesis OR osteoinduction OR osteoinducting OR osteoinductive OR osteoinductivity OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein"
LILACS (English, Portuguese and Spanish)	("Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony Forming Units" OR "Undifferentiated Cells" OR Pericytes OR Pericyte OR "Rouget Cells" OR "Células-Tronco" OR "Células Básicas" OR "Células Mãe" OR "Células Precursoras" OR "Células Primitivas" OR "Células Primordiais" OR "Células Progenitoras" OR "Células Tronco" OR "Unidades Formadoras de Colônias" OR "Pericitos" OR "Células de Rouget" OR "Células Madre" OR "Células Básicas" OR "Células Primitivas" OR "Células Primordiales" OR "Células Progenitoras" OR "Células Troncales" OR "Unidades que Forman Colonias") AND ("Simvastatin" OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl

	CoA Inhibitors" OR Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors" OR "Sinvastatina" OR "Inibidores da Hidroximetilglutaril-CoA Redutases" OR "Estatinas" OR "Estatinas de HMG-CoA" OR "Inibidor de Hidroximetilglutaril-CoA Redutases" OR "Inibidores de HMG-CoA Redutases" OR "Simvastatina" OR "Inhibidores de Hidroximetilglutaril-CoA Reductasas" OR "Estatinas HMG-CoA" OR "Inhibidor de Hidroximetilglutaril-CoA Reductasas" OR "Inhibidor de Hidroximetilglutaril-CoA-Reductasa" OR "Inhibidor de las Hidroximetilglutaril-CoA Reductasas" OR "Inhibidores de HMG CoA Reductasa") AND (Osteogenesis OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein" OR Osteogênese OR "Formação dos Ossos" OR "Formação Óssea" OR Ossificação OR "Ossificação Endocondral" OR "Ossificação Fisiológica" OR Osteoclastogênese OR Osteoindução OR "Diferenciação Celular" OR "Processos de Crescimento Celular" OR "Processos de Crescimento da Célula" OR "Proteínas Morfogenéticas Ósseas" OR "Proteínas Morfogenéticas do Osso" OR "Proteínas Morfogênicas do Osso" OR "Proteínas Morfogênicas Ósseas" OR "Proteínas Ósseas Morfogenéticas" OR "Fosfatase Alcalina" OR Osteocalcina OR "Gla-Proteína Óssea" OR "Proteína de Ligação a Cálcio Dependente de Vitamina K" OR "Proteína de Ligação a Cálcio Vitamina K-Dependente" OR "Proteína Óssea Dependente de Vitamina K" OR "Proteína Óssea Vitamina K-Dependente" OR "Subunidade alfa 1 de Fator de Ligação ao Core" OR Osteogénesis OR "Formación del Hueso" OR Osificación OR "Osificación Endocondral" OR "Osificación Fisiológica" OR Osteoclastogénesis OR Osteoinducción OR "Diferenciación Celular" OR "Procesos de Crecimiento Celular" OR "Proteínas Morfogenéticas Óseas" OR "Proteínas Morfogenéticas de Hueso" OR "Proteínas Óseas Morfogenéticas" OR "Fosfatasa Alcalina" OR "Gla-Proteína Ósea" OR "Proteína Gla del Hueso" OR "Proteína de Unión a Calcio Dependiente de Vitamina K" OR "Proteína de Unión a Calcio Vitamina K-Dependiente" OR "Proteína Ósea Dependiente de Vitamina K" OR "Proteína Ósea Vitamina K-Dependiente" OR "Subunidad alfa 1 del Factor de Unión al Sitio Principal" OR "Subunidad alfa del CBF")
SCOPUS	(TITLE-ABS-KEY("Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony

	Forming Units" OR "undifferentiated cells" OR Pericytes OR Pericyte OR "Rouget Cells") AND TITLE-ABS-KEY(Simvastatin OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl CoA Inhibitors" OR Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors") AND TITLE-ABS-KEY(Osteogenesis OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein")
EMBASE	('stem cells'/exp OR 'stem cells' OR 'stem cell'/exp OR 'stem cell' OR 'progenitor cells' OR 'progenitor cell'/exp OR 'progenitor cell' OR 'mother cells' OR 'mother cell'/exp OR 'mother cell' OR 'colony forming unit'/exp OR 'colony forming unit' OR 'colony forming units' OR 'undifferentiated cells' OR 'pericytes'/exp OR pericytes OR 'pericyte'/exp OR pericyte OR 'rouget cells') AND ('simvastatin'/exp OR simvastatin OR 'zocor'/exp OR zocor OR 'mk 733'/exp OR 'mk 733' OR 'mk733'/exp OR 'mk733' OR 'synvinolin'/exp OR synvinolin OR 'hydroxymethylglutaryl coa reductase inhibitors'/exp OR 'hydroxymethylglutaryl coa reductase inhibitors' OR 'hmg coa reductase inhibitors'/exp OR 'hmg coa reductase inhibitors' OR 'hmg coa statins' OR 'hydroxymethylglutaryl coa inhibitors' OR 'statins'/exp OR statins OR 'hydroxymethylglutaryl coenzyme a inhibitors') AND ('osteogenesis'/exp OR osteogenesis OR 'bone formation'/exp OR 'bone formation' OR 'ossification'/exp OR ossification OR ossifications OR 'osteoclastogenesis'/exp OR osteoclastogenesis OR 'osteoinduction'/exp OR osteoinduction OR osteoinducting OR osteoinductive OR 'osteoinductivity'/exp OR osteoinductivity OR 'cell differentiation'/exp OR 'cell differentiation' OR 'cell differentiations' OR 'cell growth processes'/exp OR 'cell growth processes' OR 'bone morphogenetic proteins'/exp OR 'bone morphogenetic proteins' OR 'bone morphogenetic protein'/exp OR 'bone morphogenetic protein' OR 'alkaline phosphatase'/exp OR 'alkaline phosphatase' OR 'osteocalcin'/exp

	OR osteocalcin OR 'core binding factor alpha 1 subunit'/exp OR 'core binding factor alpha 1 subunit' OR 'runx2 protein'/exp OR 'runx2 protein')
Web of Science	TS=("Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony Forming Units" OR "undifferentiated cells" OR Pericytes OR Pericyte OR "Rouget Cells") AND TS=(Simvastatin OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl CoA Inhibitors" OR Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors") AND TS=(Osteogenesis OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein")
Grey Literature	
Open Gray	("Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony Forming Units" OR "undifferentiated cells" OR Pericytes OR Pericyte OR "Rouget Cells") AND ("Simvastatin" OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl CoA Inhibitors" OR Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors") AND (Osteogenesis OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein")
ProQuest	noft("Stem Cells" OR "Stem Cell" OR "Progenitor Cells" OR "Progenitor Cell" OR "Mother Cells" OR "Mother Cell" OR "Colony Forming Unit" OR "Colony Forming Units" OR "undifferentiated cells" OR Pericytes OR Pericyte OR "Rouget Cells") AND ("Simvastatin" OR Zocor OR "MK 733" OR "MK733" OR Synvinolin OR "Hydroxymethylglutaryl CoA Reductase Inhibitors" OR "HMG CoA Reductase Inhibitors" OR "HMG CoA Statins" OR "Hydroxymethylglutaryl CoA Inhibitors" OR

	Statins OR "Hydroxymethylglutaryl Coenzyme A Inhibitors") AND (Osteogenesis OR "bone formation" OR Ossification* OR Osteoclastogenesis OR "osteoinduction" OR osteoinduct* OR "Cell Differentiation" OR "Cell Differentiations" OR "Cell Growth Processes" OR "Bone Morphogenetic Proteins" OR "Bone Morphogenetic Protein" OR "Alkaline Phosphatase" OR Osteocalcin OR "Core Binding Factor Alpha 1 Subunit" OR "Runx2 Protein")
Google Schoolar	"Undifferentiated Cells" AND "Simvastatin" AND "Cell Differentiation" AND "Bone Formation"

8.2 APÊNDICE 2 – Estudos excluídos e razão para exclusão.

Aeppendix 2 - Articles excluded and the reasons for exclusion (n=56)

Author, Year	Reason for Exclusion*
Aly et al. (2018)	8
Arpornmaeklong et al. (2017)	2
Baek et al. (2005)	8
Biouki et al. (2019)	2
chi et al. (2019)	7
Cui et al. (2013)	5
Feng et al. (2020)	7
Huang et al. (2014)	2
Huang et al. (2019)	5
Hungaro et al. (2017)	2
Janz et al. (2014)	8
Kamada et al. (2017)	8
Kim et al. (2011)	1
Lai et al. (2018)	2
Lai et al. (2019)	1
Lee et al. (2016)	2
Lee et al. (2018)	2
Li et al. (2018)	2
Liu et al. (2009)	8
Liu et al. (2014)	2
Liu et al. (2014)	8
Liu et al. (2016)	8
Matos et al. (2015)	2
Park et al. (2012)	1
Pullisaar et al. (2014)	2
Qi et al. (2013)	2
Qing et al. (2019)	4
Rostami et al. (2020)	2
Samiei et al. (2016)	2

Simões et el. (2013)	2
Song et al. (2003)	8
Song et al. (2008)	8
Sukul et al. (2015)	2
Tai et al. (2010)	6
Tai et al. (2011)	6
Tai et al. (2013)	6
Wadagaki et al. (2011)	2
Wang et al. (2018)	2
Wang et al. (2020)	1
Wang et al. (2016)	6
Wang et al. (2014)	2
Wu et al. (2014)	4
Wu et al. (2020)	2
Xu et al. (2015)	4
Xu et al. (2018)	5
xue et al. (2019)	2
Yu et al. (2017)	2
Yuan et al. (2019)	2
Zanette et al. (2015)	5
Zhang et al. (2019)	4
Zhang et al. (2015)	2
Zhang et al. (2018)	2
Zhang et al. (2018)	2
Zhao e Liu (2014)	7
Zheng et al. (2010)	1
Zijah et al. (2016)	2

*1) Studies investigating other cell types, such as differentiated cells; 2) Studies assessing drug or biomaterial associations that do not present separate data for SIM alone; 3) Studies that do not present an untreated control group with the same cell type; 4) Studies that do not present a control group without SIM treatment; 5) Studies that do not evaluate osteoinduction or osteogenic differentiation capacity; 6) Studies that are not *in vitro* (clinical studies, animal studies, conference abstracts, letters, pilot studies, review articles, case reports, protocols, short communications, personal opinions, posters, or book chapters); 7) Studies that do not specify the culture medium; 8) Studies that do not assess at least the alkaline phosphatase (ALP) activity and extracellular matrix (ECM) mineralization tests; 9) Full text not available; 10) Duplicated data (e.g. dissertations/thesis in which correspondent published articles were available).

REFERÊNCIAS

AMERONGEN, G. P. VAN N. et al. Simvastatin Improves Disturbed Endothelial Barrier Function. **Circulation**, v. 102, n. 23, p. 2803–2809, 5 dez. 2000.

BAO, T. Q.; FRANCO, R. A.; LEE, B. T. Preparation and characterization of novel poly(ε-caprolactone)/ biphasic calcium phosphate hybrid composite microspheres. **Journal of Biomedical Materials Research - Part B Applied Biomaterials**, v. 98 B, n. 2, p. 272–279, 2011.

BARTOLD, P. M. et al. Tissue engineering: a new paradigm for periodontal regeneration based on molecular and cell biology. **Periodontology 2000**, v. 24, p. 253–269, 2000.

BODEN, S. D. Bioactive Factors for Bone Tissue Engineering. **Clinical Orthopaedics and Related Research**, v. 367, p. 84–94, 1999.

BUSET, S. L. et al. Are periodontal diseases really silent? A systematic review of their effect on quality of life. **Journal of Clinical Periodontology**, v. 43, n. 4, p. 333–344, 1 abr. 2016.

COCHRAN, D. L. et al. A Randomized Clinical Trial Evaluating rh-FGF-2/β-TCP in Periodontal Defects. **Journal of Dental Research**, v. 95, n. 5, p. 523–530, 2016.

DEREKA, X. E.; MARKOPOULOU, C. E.; VROTSOS, I. A. Role of growth factors on periodontal repair. **Growth Factors**, v. 24, n. 4, p. 260–267, dez. 2006.

DONZELLI, E. et al. Mesenchymal stem cells cultured on a collagen scaffold: In vitro osteogenic differentiation. **Archives of Oral Biology**, v. 52, n. 1, p. 64–73, 2007.

DRAGOO, J. L. et al. Bone induction by BMP-2 transduced stem cells derived from human fat. **Journal of Orthopaedic Research**, v. 21, p. 622–629, 2003.

ENCARNAÇÃO, I. C. et al. Analysis of Bone Repair and Inflammatory Process Caused by Simvastatin Combined With PLGA+HA+βTCP Scaffold. **Implant Dentistry**, v. 25, n. 1, p. 140–148, 1 fev. 2016.

FRANCESCHI, R. T.; XIAO, G. Regulation of the osteoblast-specific transcription factor, Runx2: Responsiveness to multiple signal transduction pathways. **Journal of Cellular Biochemistry**, v. 88, n. 3, p. 446–454, 2003.

GENTILE, P. et al. Polymeric membranes for guided bone regeneration. **Biotechnology Journal**, v. 6, n. 10, p. 1187–1197, out. 2011.

GREEN, E.; TODD, B.; HEALTH, D. Mechanism of glucocorticoid regulation of alkaline phosphatase gene expression in osteoblast-like cells. **European Journal of Biochemistry**, v. 188, n. 1, p. 147–153, 1990.

HAN, J. et al. Stem cells, tissue engineering and periodontal regeneration. **Australian Dental Journal**, v. 59, n. SUPPL. 1, p. 117–130, 2014.

JADHAV, S. B.; JAIN, G. K. Statins and osteoporosis: new role for old drugs. **Journal of Pharmacy and Pharmacology**, v. 58, n. 1, p. 3–18, jan. 2006.

JOHN WILEY & SONS LTD. **Cochrane Handbook for Systematic Reviews of Interventions version 5.1.0.** England, UK: [s.n.].

JUNG, R. E.; THOMA, D. S.; HAMMERLE, C. H. F. Assessment of the potential of growth factors for localized alveolar ridge augmentation: A systematic review. **Journal of Clinical Periodontology**, v. 35, n. SUPPL. 8, p. 255–281, set. 2008.

KAMIOKA, H. Preferred reporting items for systematic review and meta-analysis protocols (prisma-p) 2015 statement. **Japanese Pharmacology and Therapeutics**, v. 47, n. 8, p. 1177–1185, 2019.

KHEIRALLAH, M.; ALMESHALY, H. Simvastatin, dosage and delivery system for supporting bone regeneration, an update review. **Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology**, v. 28, n. 3, p. 205–209, 1 maio 2015.

KIMELMAN, N. et al. Review: Gene- and stem cell-based therapeutics for bone regeneration and repair. **Tissue Engineering**, v. 13, n. 6, p. 1135–1150, 2007.

KING, G. N.; COCHRANT, D. L. Factors That Modulate the Effects of Bone Morphogenetic Protein-Induced Periodontal Regeneration: A Critical Review. **Journal of Periodontology**, v. 73, n. 8, p. 925–936, ago. 2002.

KUPCSIK, L. et al. Statin-induced calcification in human mesenchymal stem cells is cell death related. **Journal of Cellular and Molecular Medicine**, v. 13, n. 11–12, p. 4465–4473, 2009.

LEE, J. et al. Periodontal Regeneration: Focus on Growth and Differentiation Factors. **Dental Clinics of North America**, v. 54, n. 1, p. 93–111, jan. 2010.

LIU, J.; KERNS, D. G. Mechanisms of Guided Bone Regeneration: A Review. **The Open Dentistry Journal**, v. 8, n. 1, p. 56–65, 2014.

MARIE, P. J. Targeting integrins to promote bone formation and repair. **Nature Reviews Endocrinology**, v. 9, n. 5, p. 288–295, 2013.

MARIE, P. J.; KASSEM, M. Osteoblasts in osteoporosis: Past, emerging, and future anabolic targets. **European Journal of Endocrinology**, v. 165, n. 1, p. 1–10, 2011.

MISHRA, A. et al. Efficacy of modified minimally invasive surgical technique in the treatment of human intrabony defects with or without use of rhPDGF-BB gel - A randomized controlled trial. **Journal of Clinical Periodontology**, v. 40, n. 2, p. 172–179, fev. 2013.

MUNDY, G. et al. Stimulation of Bone Formation in Vitro and in Rodents by Statins. **Science**, v. 286, n. 5446, p. 1946–1949, 3 dez. 1999.

NAITO, Y. et al. The effect of simvastatin-loaded polymeric microspheres in a critical size bone defect in the rabbit calvaria. **International Journal of Pharmaceutics**, v. 461, n. 1–2, p. 157–162, 30 jan. 2014.

NEEDLEMAN, I. G. A guide to systematic reviews. **Journal of Clinical Periodontology**, v. 29, n. SUPPL. 3, p. 6–9, 2002.

NEVINS, M. et al. Periodontal Regeneration in Humans Using Recombinant Human Platelet- Derived Growth Factor-BB (rhPDGF-BB) and Allogenic Bone. **Journal of Periodontology**, v. 74, n. 9, p. 1282–1292, set. 2003.

NEVINS, M. et al. Platelet-Derived Growth Factor Promotes Periodontal Regeneration in Localized Osseous Defects: 36-Month Extension Results From a Randomized, Controlled, Double-Masked Clinical Trial. **Journal of Periodontology**, v. 84, n. 4, p. 456–464, abr. 2013.

NISHIMURA, F.; TERRANOVA, V. P. Comparative Study of the Chemotactic Responses of Periodontal Ligament Cells and Gingival Fibroblasts to Polypeptide Growth Factors. **J Dent Res**, v. 75, n. 4, p. 986–992, 1996.

NIU, J.; DING, G.; ZHANG, L. Effects of simvastatin on the osteogenic differentiation and immunomodulation of bone marrow mesenchymal stem cells. **Molecular Medicine Reports**, v. 12, n. 6, p. 8237–8240, 2015.

NYMAN, S. Bone regeneration using the principle of guided tissue regeneration. **Journal of Clinical Periodontology**, v. 18, n. 6, p. 494–498, 1991.

PAGKALOS, J. et al. Simvastatin induces osteogenic differentiation of murine embryonic stem cells. **Journal of Bone and Mineral Research**, v. 25, n. 11, p. 2470–2478, 2010.

QING, W. et al. Simvastatin sustained-release microspheres promote osteogenesis of bone tissue engineering in vitro. **Chinese Journal of Tissue Engineering Research**, v. 23, n. 2, p. 239–244, 2019.

RETZEPI, M.; DONOS, N. Guided Bone Regeneration: Biological principle and therapeutic applications. **Clinical Oral Implants Research**, v. 21, n. 6, p. 567–576, 2010.

RIMANDO, M. G. et al. Glucocorticoid receptor and Histone deacetylase 6 mediate the differential effect of dexamethasone during osteogenesis of mesenchymal stromal cells (MSCs). **Scientific Reports**, v. 6, n. October, p. 1–13, 2016.

SCULEAN, A. et al. Biomaterials for promoting periodontal regeneration in human intrabony defects: A systematic review. **Periodontology 2000**, v. 68, n. 1, p. 182–216, 2015.

SERVICE, ROBERT, F. Tissue Engineers Build New Bone. **Science**, v. 289, p. 1498–1500, 2000.

SHAH, S. R. et al. Novel applications of statins for bone regeneration. **National Science Review**, v. 2, n. 1, p. 85–99, 2015.

STAAL, A. et al. The Ability of Statins to Inhibit Bone Resorption Is Directly Related to Their Inhibitory Effect on HMG-CoA Reductase Activity. **Journal of Bone and Mineral Research**, v. 18, p. 88–96, 2003.

TAI, I. C. et al. Local delivery of controlled-release simvastatin/PLGA /HA p microspheres enhances bone repair. **International Journal of Nanomedicine**, v. 8, p. 3895–3905, 2013.

THESLEFF, I.; TUMMERS, M. Stem cells and tissue engineering: Prospects for regenerating tissues in dental practice. **Medical Principles and Practice**, v. 12, n. SUPPL. 1, p. 43–50, 2003.

URIST, M. R. Bone: Formation by Autoinduction. **Science**, v. 150, p. 893–899, 1965.

URIST, M. R.; STRATES, B. S. Bone Morphogenetic Protein. **Journal of Dental Research**, v. 50, n. 6, p. 1392–1406, 1971.

WHANG, K. et al. A novel osteotropic biomaterial OG-PLG: Synthesis and in vitro release. **Journal of Biomedical Materials Research - Part A**, v. 74, n. 2, p. 237–246, 1 ago. 2005.

YAMASHITA, M. et al. Simvastatin antagonizes tumor necrosis factor-alpha inhibition of bone morphogenetic proteins-2-induced osteoblast differentiation by regulating Smad signaling and Ras/Rho-mitogen-activated protein kinase pathway. **The Journal of endocrinology**, v. 196, n. 3, p. 601–613, mar. 2008.

ZHOU, Y. et al. The role of simvastatin in the osteogenesis of injectable tissue-engineered bone based on human adipose-derived stromal cells and platelet-rich plasma. **Biomaterials**, v. 31, n. 20, p. 5325–5335, 2010.