



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

EDUARDA BLASI MAGINI

**CONCENTRAÇÃO DE SINVASTATINA INCORPORADA EM  
ARCABOUÇOS DE ÁCIDO POLI(LÁCTICO-CO-GLICÓLICO) PARA PROMOVER  
A FORMAÇÃO ÓSSEA EM DEFEITOS ÓSSEOS CRÍTICOS *IN VIVO*: UMA  
REVISÃO SISTEMÁTICA**

Dissertação de Mestrado

Florianópolis  
2021

Eduarda Blasi Magini

**CONCENTRAÇÃO DE SINVASTATINA INCORPORADA EM  
ARCABOUÇOS DE ÁCIDO POLI (ÁCIDO LÁTICO-CO-GLICÓLICO) PARA  
PROMOVER A FORMAÇÃO ÓSSEA EM DEFEITOS ÓSSEOS CRÍTICOS *IN VIVO*:  
UMA REVISÃO SISTEMÁTICA**

Dissertação de Mestrado

Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, do Centro de Ciências da Saúde, da Universidade Federal de Santa Catarina, como parte dos requisitos para obtenção do título de Mestre em Odontologia - Área de Concentração: Implantodontia.

Professora Orientadora: Dra. Ariadne Cristiane Cabral da Cruz

Florianópolis

2021

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

Blasi Magini, Eduarda  
CONCENTRAÇÃO DE SINVASTATINA INCORPORADA EM ARCABOUÇOS  
DE ÁCIDO POLI(LÁCTICO-CO-GLICÓLICO) PARA PROMOVER A  
FORMAÇÃO ÓSSEA EM DEFEITOS ÓSSEOS CRÍTICOS IN VIVO: UMA  
REVISÃO SISTEMÁTICA / Eduarda Blasi Magini ; orientador,  
Ariadne Cristiane Cabral da Cruz, 2021.  
62 p.

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

Inclui referências.

1. . 2. Sistemase de liberação de fármacos. 3.  
Regeneração óssea. 4. Ácido Polilático-co-glicólico. 5.  
Revisão Sistemática. I. Cabral da Cruz, Ariadne Cristiane.  
II. Universidade Federal de Santa Catarina. Programa de Pós  
Graduação em . III. Título.

Eduarda Blasi Magini

**CONCENTRAÇÃO DE SINVASTATINA INCORPORADA EM  
ARCABOUÇOS DE ÁCIDO POLI (ÁCIDO LÁTICO-CO-GLICÓLICO) PARA  
PROMOVER A FORMAÇÃO ÓSSEA EM DEFEITOS ÓSSEOS CRÍTICOS *IN VIVO*:  
UMA REVISÃO SISTEMÁTICA**

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

Prof. Dr. Thalisson Saymo de Oliveira Silva  
Universidade Federal de Santa Catarina

Prof. Dr. Cesar Augusto Magalhães Benfatti  
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 título de mestre em Odontologia – Área de concentração em Implantodontia, e aprovada pelo Programa de Pós-Graduação em Odontologia.

---

Coordenação do Programa de Pós-Graduação

---

Profª. Dra. Ariadne Cristiane Cabral da Cruz  
Orientadora

Florianópolis  
2021

Este trabalho é dedicado aos meus pais, irmãos e a toda minha família pelo incessável apoio e carinho durante esta trajetória. Gostaria de homenagear especialmente aos meus queridos avôs que hoje vivem em meu coração. Ao Vovô Aluizio, que sempre me ensinou a ter disciplina e agir com virtude e ao Vovô Ney, que me enche de orgulho poder estar seguindo os seus passos nesta linda profissão.

## AGRADECIMENTOS

Agradeço primeiramente à minha mãe, Helena, que representa um papel fundamental em todas as minhas conquistas, sempre me dando muito amor e sendo a minha maior inspiração. Espero um dia poder me tornar metade da pessoa que ela representa pra mim.

Agradeço ao meu pai, Ricardo, por ser uma pessoa tão especial que me transmite grandes ensinamentos, aprendizados, amor e muito orgulho. Sou muito grata por poder aprender todos os dias com o meu maior exemplo dentro da Odontologia.

Ao meu padrasto, Rubens, que sempre esteve ao meu lado, me apoiando, me dando segurança e muito carinho. Sinto-me privilegiada por tê-lo em minha vida.

À minha irmã, Ana Leticia, que mesmo morando tão longe se faz sempre presente em minha vida. Ela é meu modelo de determinação e sucesso.

Ao Bernardo, meu noivo, pelo apoio incondicional e irrestrito. Por sempre me incentivar e acreditar em mim. Além de tudo, é um exemplo de profissional que eu levo para a minha vida. Agradeço por todos os ensinamentos e por todo carinho recebido.

À toda minha família, que está sempre me apoiando nos momentos de dificuldade e comemorando as minhas conquistas. Sou muito grata por todos os ensinamentos e todo o amor recebido de cada um.

À Prof<sup>a</sup>. Dr<sup>a</sup>. Ariadne Cristiane Cabral da Cruz, minha orientadora, a qual eu tenho enorme gratidão e admiração. Agradeço pela oportunidade de trabalhar com esta profissional tão dedicada, competente e inspiradora. Carregarei seus ensinamentos por toda a minha trajetória na Odontologia e na vida, pois seus ensinamentos vão além da área acadêmica, sendo um verdadeiro exemplo para mim.

À MSc. Raissa Borges Curtarelli, a qual eu tenho enorme admiração. Agradeço por todos os conhecimentos transmitidos, por toda a ajuda, dedicação, amizade e paciência durante esta jornada.

Aos meus colegas de turma no mestrado, em especial agradeço as colegas Lara Steiner Back e Luiza de Oliveira Matos, que foram tão importantes durante esta etapa de minha vida. Agradeço por todo apoio, troca de conhecimento e carinho. Sou extremamente grata pela amizade que construímos.

Aos funcionários, colegas e Professores do Centro de Estudo e Pesquisa em Implantes Dentários (CEPID), agradeço pelas oportunidades e pelos grandes ensinamentos transmitidos a mim. Em especial agradeço ao Prof. Dr. Cesar Augusto Magalhães Benfatti, membro desta banca, pela sua grande contribuição intelectual e disponibilidade em ajudar a qualquer momento.

Agradeço aos colegas e Professoras do Laboratório de Virologia Aplicada da UFSC, pelos enriquecedores ensinamentos passados.

Aos demais professores da Pós-Graduação em Odontologia da UFSC, que contribuíram para meu crescimento profissional e pessoal.

Ao Prof. Dr. Thalisson Saymo, por aceitar ser membro da banca e contribuir para a melhor finalização deste trabalho.

À bibliotecária Karyn Munyk Lehmkuhl, pela grande troca de conhecimentos e contribuição nesta pesquisa.

À Coordenação de Aperfeiçoamento de Pessoal de Ensino Superior (CAPES), pelo apoio financeiro à pesquisa, através da bolsa de estudos.

À Universidade Federal de Santa Catarina, por me proporcionar uma formação enriquecedora e de muito crescimento.

E por fim, desejo exprimir os meus agradecimentos a todos aqueles que contribuíram nesta etapa tão importante de minha vida.

## RESUMO

As estatinas são originalmente utilizadas para a redução da síntese do colesterol. Além de suas propriedades de reduzir as concentrações séricas de colesterol no sangue, estudos anteriores mostraram que as estatinas estimulam a formação óssea. No entanto, a concentração de simvastatina (SIM) necessária para promover a formação óssea por meio de sistemas locais de distribuição de fármacos não está completamente elucidada na literatura. A aplicação de arcabouços tridimensionais é o método mais utilizado de liberação de fármacos para regeneração óssea. Assim, esta revisão sistemática teve como objetivo analisar as doses de SIM incorporada em arcabouços à base de poli(ácido lático-co-glicólico) (PLGA) para promover a formação óssea em defeitos ósseos críticos em estudos pré-clínicos. Os estudos foram selecionados em um processo de duas fases. Bancos de dados eletrônicos (Embase, Latin American and Caribbean Health Sciences, LIVIVO, PubMed, SCOPUS e Web of Science) e bancos de dados da literatura cinzenta (Google Scholar, Open Gray e ProQuest) foram pesquisados até abril de 2021. O risco de viés foi avaliado por meio da ferramenta SYRCLE. Sete estudos foram incluídos avaliando modelos de ratos, coelhos e camundongos. SIM incorporada em arcabouços à base de PLGA promoveu a maior formação óssea em defeitos ósseos críticos em seis estudos pré-clínicos. A dose de SIM variou de 8 a 200 µg/arcabouço. Em um estudo, a SIM incorporada nos arcabouços não levou à neoformação óssea. Não foi possível realizar meta-análise devido à heterogeneidade entre os estudos. O risco de viés foi considerado baixo para a maioria dos itens avaliados nos estudos incluídos. Em conclusão, a concentração de SIM incorporada em arcabouços à base de PLGA necessária para promover a formação óssea variou de 8-50 µg/arcabouço para modelos de rato e camundongo e 100 µg/arcabouço para defeitos ósseos em modelos de coelho.

**Palavras-chave:** Sistema de liberação de fármacos. Regeneração óssea. Ácido Polilático-co-glicólico. Revisão Sistemática.



## ABSTRACT

Statins have been used clinically to reduce cholesterol synthesis. Besides their lipid-lowering properties, previous preclinical studies have shown that some statins stimulate bone formation, with promising results focused on simvastatin (SIM). However, the SIM concentration required to promote bone formation through local delivery systems is not completely clear. The application of three-dimensional scaffolds is the most widely used method of drug delivery for bone regeneration. Thus, this systematic review aimed to analyze the SIM doses embedded into poly(lactic-co-glycolic acid) (PLGA)-based scaffolds to promote bone formation in critical bone defects in preclinical studies. References were selected in a two-phase process. Electronic databases (Embase, Literature of Latin American and Caribbean Health Sciences, LIVIVO, PubMed, SCOPUS and Web of Science) and grey literature databases (Google Scholar, Open Grey, and ProQuest) were searched up to April 2021. The risk of bias in individual studies was assessed through SYRCLE tool. Seven studies were included evaluating rat, rabbit, and mouse models. SIM loaded into PLGA-based scaffolds promoted the highest bone formation in critical bone defects in six preclinical studies. The SIM dose ranged from 8 to 200 µg/scaffold. In one study, the SIM loaded into the scaffolds did not lead to bone neoformation. A meta-analysis was not possible due to the heterogeneity among the studies. The risk of bias was considered low for most items evaluated in the included studies. In conclusion, the SIM concentration embedded into PLGA-based scaffolds required to promote bone formation ranged from 8-50 µg/scaffold for rat and mouse model and 100 µg/scaffold for rabbit model bone defects.

**Keywords:** Drug delivery systems. Simvastatin. Bone regeneration. Polylactic Acid-Polyglycolic Acid Copolymer. Systematic Review

## LISTA DE FIGURAS

Figure 1 - Flowchart of the literature search and selection criteria. Adapted from PRISMA.....	54
Figure 2 - Scheme of characteristics of included studies (n=7) .....	55
Appendix 1 - Data search strategy.....	56
Appendix 2 - Articles excluded and the reasons for exclusion (n=11).....	56

## **LISTA DE TABELAS**

Table 1 - Summary of descriptive characteristics of included studies (n=7).....	57
Table 2 Risk of bias summary, assessed by Systematic Review Centre for Laboratory Animal Experiments (SYRCLE) tool. ....	60

## LISTA DE ABREVIATURAS E SIGLAS

BMPs – Proteínas ósseas morfogenéticas

USA –do inglês, *United States of America*

BPS – do inglês, *Bone Sialoprotein*

BTCP – do inglês, *Beta-tricalcium phosphate*

CPC – do inglês, *Calcium Phosphate Composite*

EMA - Agência Europeia de Medicina

FDA – do inglês, *Food and Drugs Administration*

H&E – hematoxilina e eosina

HA – do inglês, *Hydroxyapatite*

HMG-CoA - 3-hidroxi-3-metilglutaril-coenzima A

LILACS – do inglês, *Latin American and Caribbean Health Sciences*

LIVIVO – do inglês, *Leibniz Information Centre for Life Sciences*

MSC – do inglês, *Mesenchymal stem cells*

OPN – do inglês, *Osteopontin*

OSAD – do inglês, *Osteoadherin*

PBS – do inglês, *Phosphatase buffered saline*

PGA – Ácido Poliglicólico

PLA – Ácido Polilático

PLGA – do inglês, *Ppoly(lactic-co-glycolic) acid*

PLGA – Poli(ácido láctico-co-glicólico)

PRISMA – do inglês, *Preferred Reporting Items for Systematic Reviews and Meta-Analyses*

RoB – do inglês, *Risk of Bias*

SDF-1a - do inglês, *Stromal Cell-derived Factor-1a*

SIM - do inglês, *Simvastatin*

SIN – Sinvastatina

SYRCLE – do inglês, *Systematic Review Centre for Laboratory Animal Experiments*

μCT – do inglês, *Micro-computed tomography*

## SUMÁRIO

<b>CAPÍTULO I.....</b>	<b>14</b>
<b>1 INTRODUÇÃO.....</b>	<b>15</b>
<b>2 REVISÃO DE LITERATURA.....</b>	<b>17</b>
<b>3 OBJETIVOS.....</b>	<b>20</b>
3.1 OBJETIVO GERAL.....	20
3.2 OBJETIVOS ESPECÍFICOS.....	20
4 METODOLOGIA EXPANDIDA.....	21
4.1 PROTOCOLO E REGISTRO.....	21
4.2 CRITÉRIOS DE ELEGIBILIDADE.....	21
<b>4.2.1 Critérios de Inclusão.....</b>	<b>21</b>
<b>4.2.2 Critérios de Exclusão.....</b>	<b>21</b>
4.3 FONTES DE DADOS.....	22
4.4 SELEÇÃO DOS ESTUDOS.....	22
4.5 PROCESSO DE COLETA DE DADOS .....	23
4.6 QUALIDADE E AVALIAÇÃO DO RISCO DE VIÉS.....	23
4.7 MEDIDAS RESUMIDAS.....	23
4.8 SÍNTESE DE RESULTADOS.....	24
<b>CAPÍTULO II.....</b>	<b>25</b>
<b>ARTICLE – ENGLISH VERSION.....</b>	<b>26</b>
<b>CAPÍTULO III.....</b>	<b>47</b>
<b>CONSIDERAÇÕES FINAIS.....</b>	<b>48</b>
<b>CAPÍTULO IV.....</b>	<b>49</b>
<b>REFERÊNCIAS.....</b>	<b>50</b>
<b>CAPÍTULO V.....</b>	<b>53</b>
Figure 1 .....	54
Figure 2 .....	55
Appendix 1.....	56
Appendix 2.....	57
Table 1.....	60
Table 2.....	62

# CAPÍTULO I

## 1 INTRODUÇÃO

As extrações dentais representam procedimentos comuns devido à doença periodontal avançada e às lesões cáries cujas restaurações são inexecutáveis (TAN et al., 2012). Após a exodontia, o osso alveolar sofre um processo de atrofia, levando a alterações dimensionais e remodelação dos tecidos moles (ARAÚJO et al., 2015). Diante da perda dentária, ocorre a reabsorção de osso alveolar por atividade osteoclástica contínua, provocando primeiramente a diminuição da espessura do rebordo por meio de reabsorção da tábua óssea vestibular e posteriormente em altura, com diferentes padrões nas regiões de maxila e mandíbula (ARAÚJO; LINDHE, 2005).

As consequências clínicas das alterações fisiológicas dos tecidos ósseos e moles podem afetar o resultado da terapia reabilitadora e dificultar, ou mesmo inviabilizar, o adequado posicionamento do implante dentário (MEZZOMO et al., 2011). Portanto, à procura de biomateriais e/ou técnicas que impulsionem o processo de regeneração para maximizar a previsibilidade, bem como o volume de osso regenerado é constante (WANG; YEUNG, 2017). Assim, diferentes estratégias vêm sendo desenvolvidas para a estimulação da regeneração do tecido ósseo e recuperação da função do tecido lesionado. Essas estratégias variam entre o uso dos biomateriais até a aplicação da engenharia tecidual (ANITUA et al., 2015). Entretanto, encontrar um substituto ósseo ideal tem sido o objetivo dos pesquisadores há muitos anos. Idealmente, esse biomaterial deve apresentar capacidade de osteodiferenciação, osteoindução e osteocondução (PRECHEUR, 2007; RUHAIMI, 2001).

Apesar da utilização de enxertos autógenos e alógenos melhorarem o reparo de defeitos ósseos em diversos aspectos, nenhum desses materiais apresenta a excelência biológica aliada à baixa morbidade, ausência de restrição da quantidade e custo razoável. Recentemente, visando melhorar a regeneração óssea, os biomateriais vêm recebendo células e/ou substâncias bioativas (OLIVEIRA et al., 2009). A escolha do arcabouço é fundamental para o sucesso da regeneração óssea, pois por meio dele é possível obter um ambiente adequado para as células osteogênicas migrarem, proliferarem, diferenciarem e promoverem a formação de novo osso, além de proporcionar função mecânica durante a regeneração óssea. Assim, o arcabouço deve apresentar biocompatibilidade, ser poroso e permeável para as relações celulares e o transporte de nutrientes, crescimento de tecido e vascularização. Além de que, um substituto ósseo ideal também deve ser osteocondutor (para recrutar células ósseas do receptor), osteoindutor (para

diferenciar células-tronco em células formadoras de osso) e osteointegrativo (para fornecer fixação permanente e funcional ao osso nativo) (FRÖHLICH et al., 2008).

Diversas pesquisas foram realizadas sobre a administração de medicamentos por polímeros biodegradáveis. O poli (ácido lático-co-glicólico) (PLGA) é um dos polímeros biodegradáveis mais utilizados e apresentam grande taxa de sucesso. O PLGA é aprovado pela *Food and Drug Administration* dos EUA e pela Agência Europeia de Medicina (EMA) em vários sistemas de administração (DANHIER et al., 2012). A biocompatibilidade, biodegradabilidade, flexibilidade e mínimos efeitos colaterais são as principais vantagens ao usar este polímero para aplicações biomédicas (VIRLAN et al., 2015). Para aprimorar a utilização do PLGA como carreador de medicamentos, foram desenvolvidos microesferas ou camadas de revestimento no arcabouço (SADIASA; KIM; LEE, 2013a).

As estatinas, fármacos inibidores da 3-hidroxi-3-metilglutaril-coenzima A (HMG-CoA) redutase, são originalmente utilizadas clinicamente a fim de reduzir o nível de colesterol no sangue (LITTUMA et al., 2020; TAI et al., 2013a). Adicionalmente, Mundy et al. (MUNDY et al., 1999; TAI et al., 2013a) verificaram que além de reduzir as concentrações séricas de colesterol, as estatinas estimulam a expressão do gene BMP-2 em osteoblastos e promovem a formação óssea. Descobrimos assim o seu potencial como estimuladores da formação óssea (LEE et al., 2008; MENDES et al., 2017). Logo, além de promover a diferenciação dos osteoblastos e estimular as proteínas morfogenéticas ósseas (BMPs), há evidências de que a sinvastatina (SIN) mostrou aumento da atividade da fosfatase alcalina, dos níveis de expressão da osteocalcina em células-tronco da medula óssea humana e também aumento na densidade mineral óssea (LEE et al., 2008).

Assim diversos estudos tem sido feitos mostrando a eficácia da sinvastatina incorporada nos polímeros biodegradáveis, utilizados para transportar e administrar medicamentos para a regeneração de defeitos teciduais, porém a dose de sinvastatina necessária para promover esta formação óssea por sistemas de liberação de fármacos ainda não está elucidada. De acordo com isso, esta revisão sistemática tem o objetivo de coletar informações fundamentais na literatura sobre a dose de sinvastatina necessária para promover a formação óssea em defeitos críticos *in vivo*.



## 2 REVISÃO DE LITERATURA

A alteração dimensional que ocorre na crista óssea alveolar após a extração dentária já está elucidada na literatura, por meio de estudos experimentais e clínicos (SANZ et al., 2017). Deste modo, a insuficiência óssea continua sendo um grande desafio (ELGALI et al., 2017), tendo em vista que para um bom prognóstico da terapia com implantes e para obtenção de uma reconstrução protética ideal, estética e funcional, é necessário um volume ósseo suficiente e uma favorável arquitetura da crista óssea alveolar (SCHROPP et al., 2003). Assim, diversas estratégias regenerativas vêm sendo desenvolvidas visando a preservação do processo alveolar após a extração dental (MONTERO; MANZANO; ALBALADEJO, 2014).

Para procedimentos de reconstrução óssea, em muitas situações, o material eleito como referência, é o osso autógeno (MCALLISTER; HAGHIGHAT, 2007; OLIVEIRA et al., 2009), pois ele é capaz de promover osteogênese, osteoindução e osteocondução (MONTERO; MANZANO; ALBALADEJO, 2014). Porém, o osso autógeno apresenta desvantagens, como disponibilidade óssea limitada, morbidade adicional no local doador e impossibilidade de armazenamento (MONTERO; MANZANO; ALBALADEJO, 2014). Decorrente dessas desvantagens, é fundamental a busca por alternativas ao enxerto autógeno. Como seu substituto, tem-se os enxertos alógenos e xenógenos, que são muito utilizados, porém também podem apresentar limitações, como alta variabilidade nas propriedades osteoindutoras e transmissão de doenças (OLIVEIRA et al., 2009). Desse modo, a busca por biomateriais eficazes nos procedimentos de regeneração óssea se faz pertinente (LANDSBERG et al., 2021; MONTERO; MANZANO; ALBALADEJO, 2014).

Há cerca de três décadas, diversas pesquisas vêm sendo conduzidas sobre a administração de medicamentos por polímeros biodegradáveis. Entre todos os biomateriais, o PLGA apresentou um enorme potencial para atuar como veículo para a liberação de medicamentos (HIRENKUMAR K. MAKADIA; SIEGEL, 2011). O PLGA é um copolímero linear, composto de ácido poli-láctico (PLA), que apresenta um carbono  $\alpha$  assimétrico, usualmente descrito na forma D ou L em termos esteroquímicos clássicos, e de ácido poliglicólico (PGA) (HIRENKUMAR K. MAKADIA; SIEGEL, 2011). O PLGA apresenta inúmeras vantagens, como a alta biocompatibilidade, biodegradabilidade, flexibilidade, mínimos efeitos colaterais (VIRLAN et al., 2015), características de degradação favoráveis, possibilidades de administração controlada de substâncias bioativas (MENDES et al., 2017), além da possibilidade de ser facilmente processado e fabricado em diversas formas e tamanhos,

se tornando o mais popular entre os vários polímeros biodegradáveis disponíveis (HIRENKUMAR K. MAKADIA; SIEGEL, 2011). Durante a biodegradação do polímero, pode ocorrer mudanças nas propriedades do PLGA, podendo influenciar a taxa de liberação e degradação das moléculas bioativas incorporadas. Desse modo, é possível realizar ajustes nas propriedades físicas gerais da matriz polímero-substância bioativa para controlar parâmetros relevantes, como a proporção de lactídeo para glicolídeo, peso molecular do polímero e concentração da substância bioativa para alcançar a dosagem e o intervalo de liberação desejado (HIRENKUMAR K. MAKADIA; SIEGEL, 2011; TAI et al., 2013b). A degradação completa do PLGA ocorre em um curto período de tempo, resultando na redução de efeitos adversos comumente acontecidos em polímeros de degradação longa pela liberação de fragmentos cristalinos (SILVA et al., 2015).

Contudo, o PLGA não apresenta bioatividade intrínseca nas células, necessitando à associação de outras substâncias para aumentar sua bioatividade (SADIASA et al., 2013). É importante destacar que este sistema de administração de substâncias bioativas deve ser capaz de transportar agentes bioativos em locais específicos e liberá-los em doses e taxas controláveis. Para aumentar as vantagens terapêuticas e diminuir os danos ao paciente, a dosagem e a duração do efeito da substância bioativa devem ser regulados (SADIASA; KIM; LEE, 2013b).

As estatinas, inibidores da 3-hidroxi 3-metil glutaril coenzima A (HMG-CoA) redutase, foram desenvolvidas para o tratamento da hipercolesterolemia, pois impede a síntese de colesterol (MONTERO; MANZANO; ALBALADEJO, 2014; ORYAN; KAMALI; MOSHIRI, 2015). Em 1999, Mundy et al, relataram que as estatinas, além de serem inibidores da biossíntese do colesterol, tinham a capacidade de estimular a formação e regeneração óssea (MONTERO; MANZANO; ALBALADEJO, 2014; YAMASHITA et al., 2008). A partir disso, diversas pesquisas têm sido desenvolvidas para a aplicação clínica da SIN nas mais diversas áreas, como a traumatologia, cirurgia oral e periodontia (MONTERO; MANZANO; ALBALADEJO, 2014). Com base nessas pesquisas, foi relatado que as estatinas têm capacidade de modular a inflamação, melhorar a osteoindução, osteogênese e angiogênese, além de inibir a apoptose osteoblástica e a osteoclastogênese. Logo, as estatinas apresentam características benéficas no reparo ósseo por diferentes mecanismos (ORYAN; KAMALI; MOSHIRI, 2015). Adicionalmente, foi relatado na literatura que a SIN atua diretamente na ativação dos osteoblastos, aumentando a expressão de BMP-2, e na inibição dos osteoclastos. Do mesmo modo, atua indiretamente estimulando a neovascularização e levando à um aumento

na liberação do fator de crescimento endotelial vascular (MONTERO; MANZANO; ALBALADEJO, 2014). A SIN aumenta também a atividade da fosfatase alcalina, além de aumentar a expressão de marcadores osteogênicos, como colágeno tipo I, sialoproteína óssea e osteocalcina (PARK, 2009).

Tradicionalmente, as estatinas eram administradas de forma sistêmica. Estudos clínicos e *in vivo* sugerem que a administração da sinvastatina por via oral degradada no fígado, resulta em pouco acúmulo do fármaco no tecido ósseo, necessitando de doses elevadas para superar a degradação hepática, o que pode levar à efeitos colaterais (TAI et al., 2013b). Deste modo, a administração tópica de sinvastatina apresenta ser vantajosa devido a sua capacidade de aplicação local com concentrações controladas e benéficas para a regeneração óssea. Desde a descoberta da relação da sinvastatina com o aumento da formação óssea (MUNDY et al., 1999), sabe-se que a dose das estatinas desempenham papel fundamental na formação óssea *in vivo*. Diversos estudos mostraram que a dose de sinvastatina modula a capacidade da droga de aumentar o volume ósseo (HO et al., 2009). Inclusive, foi relatado que a SIM levou ao aumento da atividade da fosfatase alcalina em células-tronco mesenquimais da medula óssea de camundongo de maneira dose-dependente (LIU et al., 2014).

A maior limitação para aplicação clínica da sinvastatina, visando a regeneração óssea, é encontrar a dose e o veículo de administração ideal, que permita uma apropriada liberação do fármaco. A liberação de altas doses de sinvastatina *in vivo* está associada à uma resposta inflamatória exacerbada, alta citotoxicidade e formação óssea prejudicada. Por outro lado, baixas doses de sinvastatina podem não apresentar relevância no processo de formação óssea.

Frente aos estudos que mostram a eficácia da sinvastatina incorporada em polímeros biodegradáveis para regeneração óssea em defeitos críticos, propõe-se neste trabalho avaliar a dose de sinvastatina necessária para estimular a formação óssea *in vivo*.

### 3 OBJETIVOS

#### 3.1 OBJETIVO GERAL

Esta revisão sistemática tem como objetivo analisar as doses de sinvastatina (SIM) incorporada em arcabouços à base de poli(ácido lático-co-glicólico) (PLGA) para promover a formação óssea em defeitos ósseos críticos em estudos pré-clínicos.

#### 3.2 OBJETIVOS ESPECÍFICOS

- Avaliar a capacidade da SIN em estimular a neoformação óssea associada a arcabouços à base de PLGA.
- Identificar a dose mínima necessária para estimular a formação óssea *in vivo*.
- Verificar a formação óssea em diferentes modelos animais, como rato, camundongo e coelho.
- Avaliar a regeneração tecidual em defeitos criados em diferentes localizações ósseas.

## 4 METODOLOGIA EXPANDIDA

### 4.1 PROTOCOLO E REGISTRO

Esta revisão sistemática seguiu a lista de Principais Itens para Relatar Revisões sistemáticas e Meta-análises (PRISMA) [19]. O protocolo está registrado no Registro Prospectivo Internacional de Revisões Sistemáticas (PROSPERO) sob o número de identificação CRD42021206667.

### 4.2 CRITÉRIOS DE ELEGIBILIDADE

#### 4.2.1 Critérios de inclusão:

A sigla PICOS (população, intervenção, comparação, desfecho e tipo de estudos) foi utilizada para criar a pergunta desta RS [20], onde:

População (P): Animais que receberam SIN incorporada em arcabouços à base de PLGA em defeitos ósseos críticos.

Intervenção (I): Liberação local de SIN incorporada em arcabouços à base de PLGA em defeitos ósseos críticos *in vivo*.

Comparação (C): Arcabouços à base de PLGA sem incorporação de SIN.

Resultado (O): Dose de SIN necessária para a formação óssea.

Os estudos foram considerados elegíveis quando atenderam aos seguintes critérios de inclusão: Avaliar a formação óssea por meio da liberação local de SIN incorporada em arcabouços a base de PLGA em defeitos ósseos críticos *in vivo*, em comparação com arcabouços a base de PLGA sem SIN. Não foram incluídas restrições referentes ao período de publicação.

#### 4.2.2 Critérios de Exclusão

Foram considerados os seguintes critérios de exclusão: 1) Estudos *in vitro*; 2) Estudos que avaliam pacientes humanos (ensaios clínicos); 3) Estudos avaliando defeitos ósseos não críticos *in vivo*; 4) Estudos que avaliam a administração sistêmica de SIN; 5) Estudos avaliando arcabouços com composição diferente à PLGA; 6) Estudos com dados insuficientes quanto à

formação óssea ou efeito citotóxico; 7) Estudos com menos de quatro semanas de acompanhamento; 8) Estudos não publicados no alfabeto latino-romano; 9) Artigos de revisão, relatos de casos, protocolos, opiniões pessoais, cartas, pôsteres, resumos de conferências ou capítulos de livros; 10) Texto completo não disponível; 11) Dados duplicados (por exemplo, dissertações/teses em que os correspondentes artigos publicados estavam disponíveis).

#### 4.3 FONTES DE DADOS

Uma estratégia de busca detalhada foi desenvolvida para cada uma das seguintes bases de dados eletrônicas: Embase, Latin American and Caribbean Health Sciences (LILACS), Leibniz Information Centre for Life Sciences (LIVIVO), PubMed, SCOPUS, The Cochrane Library e Web of Science. Como literatura adicional, uma estratégia de busca foi desenvolvida para a Google Scholar (primeiras 100 referências), Open Grey e ProQuest (Dissertações e Teses). Além disso, listas de referências de artigos potencialmente relevantes foram pesquisadas manualmente para identificar quaisquer estudos que poderiam ter sido perdidos na etapa anterior. Não foram estabelecidas limitações quanto à data de publicação. As pesquisas em todas as bases de dados foram realizadas em 24 de abril de 2021. A estratégia de busca detalhada está disponível no Apêndice 1. Listas de referência dos artigos incluídos foram pesquisadas manualmente, conforme recomendado por Greenhalgh e Peacock [20]. Um software (EndNote X7, Thomson Reuters, Canadá) foi usado para gerenciar as referências.

#### 4.4 SELEÇÃO DOS ESTUDOS

Um processo de seleção de duas fases foi realizado usando um *software online* (Rayyan, Qatar Computing Research Institute, Qatar). Na fase 1, dois revisores (E.B.M. e L.O.M) conduziram de forma independente a análise por título e resumo para identificar estudos potencialmente elegíveis. Na fase 2, os mesmos revisores realizaram a leitura do texto completo dos artigos selecionados. Em ambas as fases de seleção, as divergências foram resolvidas em uma discussão de consenso. Se o consenso não fosse alcançado, um terceiro revisor (R.B.C.) com experiência era envolvido para tomar a decisão final. Se dados importantes para a revisão estivessem faltando ou não fossem claros, seria feita a tentativa de entrar em contato com os autores do estudo correspondentes para resolver ou esclarecer o problema.

#### 4.5 PROCESSO DE COLETA DE DADOS E ITENS DE DADOS

Um autor (E.B.M) realizou a coleta de dados dos estudos incluídos e um segundo autor (L.O.M) verificou todas as informações coletadas. Discordâncias foram resolvidas com discussão. Se necessário, um terceiro autor (R.B.C.) foi envolvido para tomar a decisão final. Os seguintes dados referentes aos estudos incluídos foram registrados: características do estudo (autor, ano e país de publicação), características da população (total de animais/defeitos, grupo controle, grupo teste, espécie do animal, área do defeito ósseo e dimensão do defeito ósseo), propriedades do arcabouço (dose de SIM, sistema de liberação de droga utilizado e relação PLA/PGA) e avaliação dos resultados (métodos de análise, principais resultados e valor de p).

#### 4.6 QUALIDADE E AVALIAÇÃO DO RISCO DE VIÉS

O risco de viés (RoB) dos artigos incluídos foi avaliado independentemente por dois revisores (E.B.M. e L.O.M.) usando a ferramenta do Centro de Revisão Sistemática para Experimentos com Animais de Laboratório (SYRCLE) [21]. Esta ferramenta é baseada na *Cochrane Collaboration RoB Tool* e foi adaptada para avaliar os aspectos de viés em experimentos com animais com o objetivo de avaliar a qualidade metodológica dos estudos. As respostas possíveis para cada uma das perguntas do RoB eram "Sim", "Não" ou "Não claro". Resumidamente, os seguintes pontos e questões foram considerados: viés de seleção (geração de sequência, características de linha de base e ocultação de alocação), viés de desempenho (alojamento aleatório e cegamento), viés de detecção (aleatoriedade e cegamento dos avaliadores de resultados), viés de atrito (dados de resultados incompletos), viés de relatório e outras fontes de vieses.

#### 4.7 MEDIDAS RESUMIDAS

A análise qualitativa dos resultados foi realizada com base na formação óssea em defeitos críticos *in vivo* com a SIN incorporada em arcabouços à base de PLGA. A capacidade de formação óssea foi avaliada por meio de análises histológicas, microscopia eletrônica de varredura,  $\mu$ -tomografia computadorizada ( $\mu$ CT), imunohistoquímica e/ou análises de densitometria.

#### 4. 8 SÍNTESE DE RESULTADOS

Uma análise qualitativa dos resultados foi realizada com base na dose necessária de SIN incorporada em arcabouços a base de PLGA para promover a formação óssea em defeitos críticos *in vivo* (relatados ou calculados). O agrupamento estatístico de dados usando meta-análise era planejado se os estudos fossem considerados suficientemente homogêneos no que diz respeito à metodologia e disponibilidade de dados.



# CAPÍTULO II

ARTICLE – ENGLISH VERSION

**SIMVASTATIN CONCENTRATION EMBEDDED INTO POLY(LACTIC-CO-  
GLYCOLIC ACID)-BASED SCAFFOLDS ON PROMOTING BONE FORMATION  
IN CRITICAL *IN VIVO* BONE DEFECTS: A SYSTEMATIC REVIEW**

This article is formatted on standards of the Journal of Acta Biomaterialia - Elsevier

....

**SIMVASTATIN CONCENTRATION EMBEDDED INTO POLY(LACTIC-CO-GLYCOLIC ACID)-BASED SCAFFOLDS ON PROMOTING BONE FORMATION IN CRITICAL *IN VIVO* BONE DEFECTS: A SYSTEMATIC REVIEW**

Eduarda Blasi Magini<sup>a,b</sup>, Luiza de Oliveira Matos<sup>a,b</sup>, Raissa Borges Curtarelli<sup>a,b</sup>, Mariane Beatriz Sordi<sup>a,b</sup>, Gabriel Leonardo Magrin<sup>a</sup>), Carlos Flores-Mir<sup>c</sup>, Ariadne Cristiane Cabral da Cruz <sup>a,b</sup>.

<sup>a</sup> Center for Education and Research on Dental Implants, Federal University of Santa Catarina, Delfino Conti street, Florianópolis, 88040-900, Brazil

<sup>b</sup> Laboratory of Applied Virology, Federal University of Santa Catarina, Henrique da Silva Fontes avenue, Florianópolis, 88040-900, Brazil.

<sup>c</sup> Faculty of Medicine and Dentistry, Department of Dentistry, University of Alberta, Edmonton, 112 St. NW, Edmonton, Alberta, Canada.

Correspond to:

Ariadne Cristiane Cabral da Cruz

Departamento de Odontologia, Centro de Ciências da Saúde, Universidade Federal de Santa Catarina

Campus Universitário Caixa Postal 476 – Trindade, Florianópolis, Santa Catarina – Brasil.

CEP: 88040-900

E-mail: [ariadne.cruz@ufsc.br](mailto:ariadne.cruz@ufsc.br)

Telephone number: +55 48 3721-3407 / Fax: +55 48 3721-9523

## **SIMVASTATIN CONCENTRATION EMBEDDED INTO POLY(LACTIC-CO-GLYCOLIC ACID)-BASED SCAFFOLDS ON PROMOTING BONE FORMATION IN CRITICAL *IN VIVO* BONE DEFECTS: A SYSTEMATIC REVIEW**

### **ABSTRACT**

Statins have been used clinically to reduce cholesterol synthesis. Besides their lipid-lowering properties, previous preclinical studies have shown that some statins stimulate bone formation, with promising results focused on simvastatin (SIM). However, the SIM concentration required to promote bone formation through local delivery systems is not completely clear. The application of three-dimensional scaffolds is the most widely used method of drug delivery for bone regeneration. Thus, this systematic review aimed to analyze the SIM doses embedded into poly(lactic-co-glycolic acid) (PLGA)-based scaffolds to promote bone formation in critical bone defects in preclinical studies. References were selected in a two-phase process. Electronic databases (Embase, Literature of Latin American and Caribbean Health Sciences, LIVIVO, PubMed, SCOPUS, The Cochrane Library, and Web of Science) and grey literature databases (Google Scholar, Open Grey, and ProQuest) were searched up to April 2021. The risk of bias in individual studies was assessed through SYRACLE tool. Seven studies were included evaluating rat, rabbit, and mouse models. SIM loaded into PLGA-based scaffolds promoted the highest bone formation in critical bone defects in six preclinical studies. The SIM dose ranged from 8 to 200  $\mu\text{g/scaffold}$ . A meta-analysis was not possible due to the heterogeneity among the studies. In one study, the SIM loaded into the scaffolds did not lead to bone neoformation. The risk of bias was considered low for most items evaluated in the included studies. In conclusion, the SIM concentration embedded into PLGA-based scaffolds required to promote bone formation ranged from 8-50  $\mu\text{g/scaffold}$  for rat and mouse model and 100  $\mu\text{g/scaffold}$  for rabbit model bone defects.

**Statement of significance:** The release of high concentrations of simvastatin *in vivo* is associated with exacerbated inflammatory responses and impairment of bone formation. Conversely, simvastatin at lower concentrations may not reach a minimum dosage capable of stimulating bone formation *in vivo*. Furthermore, the simvastatin dosage to promote of bone formation *in vivo* is dependent on the animal model and anatomical region of implantation.

Therefore, determining the optimal simvastatin concentration embedded into PLGA-based scaffolds required to promote bone formation in critical bone defects will contribute to the experimental design of translational studies in bone tissue engineering.

**Keywords** Drug delivery systems. Simvastatin. Bone regeneration. Polylactic Acid-Polyglycolic Acid Copolymer. Systematic Review

## 1. Introduction

Since the alveolar process depends on tooth function, this bone will undergo atrophy following the tooth extraction [1]. Consequently, the loss of tissue dimension can lead to clinical aspects that difficult, or even prevent, prosthetic rehabilitation due to the esthetic impairment and/or the limitation to install the dental implant in the correct position [2]. Therefore, since vertical bone augmentation in craniomaxillofacial areas is challenging, biomaterials and predictable techniques that enhance bone regeneration are still lacking in clinical applications [3–5].

Among the biodegradable polymers for drug delivery, the poly(lactic-co-glycolic acid) (PLGA) has shown great potential due to the biocompatibility, biodegradability, flexibility, minimal side effects [6], favorable degradation characteristics, and the ability for sustained drug delivery [7,8]. Additionally, the US Food and Drugs Administration (FDA) and European Medicine Agency (EMA) approved the use of PLGA in various drug delivery systems [9,10]. Changes in PLGA proprieties also influence the release and degradation rate of the embedded substances. Therefore, it is possible to tune the overall physical properties of the polymer-substance matrix by controlling relevant parameters, such as the ratio of lactide to glycolide, the polymer molecular weight, and substance concentration to achieve the desired dosage and release interval [7,11].

Simvastatin (SIM), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is used initially to reduce blood cholesterol levels [11,12]. Moreover, this medicament has been extensively investigated due to its pleiotropic effects, such as angiogenic, immunomodulatory, and anti-inflammatory properties [12–14]. Also, the osteoinductive potential of SIM has been reported to promote osteogenic differentiation and increase bone formation [8,15,16]. Furthermore, it is relevant to emphasize that SIM in-site applications require a suitable carrier to allow its controlled release, thus preventing burst release, medicament degradation, and exacerbated inflammatory responses [14,17].

Despite several studies that have been reported the efficacy of SIM embedded into PLGA-based scaffolds for bone formation, the concentration of SIM required to promote bone formation through local drug delivery systems is not fully elucidated. Consequently, this systematic review (SR) aimed to critically discuss the available scientific evidence to answer the following focused question: “What is the appropriate concentration of SIM embedded into PLGA-based scaffolds required to promote bone formation in critical *in vivo* bone defects?”

## **2. Methods**

### **2.1 Protocol and registration**

This SR followed the checklist Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[18]. The protocol is registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the identification number CRD42021206667.

### **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 question of this SR [20], where:

Population (P): Animals who received SIM loaded into PLGA-based scaffolds in critical bone defects.

Intervention (I): Local delivery of SIM loaded into PLGA-based scaffolds in critical *in vivo* bone defects.

Comparison (C): PLGA-based scaffolds without SIM.

Outcome (O): SIM dose required for bone formation.

Studies were considered eligible when they met the following inclusion criteria: Evaluate the bone formation through local delivery of SIM embedded into PLGA-based scaffolds in critical *in vivo* bone defects, comparing to PLGA-based scaffolds without SIM. No publication period restrictions were applied.

#### *2.2.2 Exclusion Criteria*

The following exclusion criteria were considered: 1) In vitro studies; 2) Studies evaluating human patients (clinical trials); 3) Studies evaluating non-critical in vivo bone defects; 4) Studies evaluating SIM systemic administration; 5) Studies evaluating scaffolds with a different composition than PLGA; 6) Studies with insufficient data regarding bone formation or cytotoxic effect; 7) Studies with less than four weeks of follow-up; 8) Studies not published in the Roman Latin alphabet; 9) Review articles, case reports, protocols, short communications, personal opinions, letters, posters, conference abstracts, or book chapters; 10) Full text not

available; 11) Duplicate data (e.g. dissertations/thesis in which correspondent published articles were available).

### **2.3 Information sources**

A detailed research strategy was developed for each following electronic databases: Embase, Latin American and Caribbean Health Sciences (LILACS), Leibniz Information Centre for Life Sciences (LIVIVO), PubMed, SCOPUS, The Cochrane Library, and Web of Science. As additional literature, a search strategy was developed for Google Scholar web search (first 100 references), Open Grey, and ProQuest (Dissertations and Thesis). Besides that, reference lists of potentially relevant articles were hand-searched to identify any studies that could have been missed in the previous step. No limitations were established regarding the publication date. Searches in all databases were conducted on April 24, 2021. Detailed search strategies are available in Appendix 1. Reference lists of included studies were manually searched, as recommended by Greenhalgh and Peacock [19]. A software (EndNote X7, Thomson Reuters, Canada) was used to manage references.

### **2.4 Study Selection**

A two-phase selection process was performed using online software (Rayyan, Qatar Computing Research Institute, Qatar). In phase1, two reviewers (E.B.M. and L.O.M) independently conducted title and abstract reading to identify potentially eligible studies. The same reviewers performed the full-text reading of eligible articles in phase 2. In both selection phases, any disagreements were solved in a consensus discussion. A third reviewer (R.B.C.) with experience was involved in making the final decision if the consensus was not reached. If important data for the review are missing or unclear, an attempt will be made to contact the corresponding authors to resolve or clarify the issue.

### **2.5 Data collection process and data items**

One author (E.B.M) performed data collection from included studies and a second author (L.O.M) cross-checked all the collected information. Disagreements were resolved by discussion. If needed, a third author (R.B.C.) was involved in making the final decision. The following data regarding included studies were recorded: study characteristics (author, year, country of publication), population characteristics (total animals/defects, control group, test



group, animal species, bone defect area, bone defect dimension), scaffold properties (SIM dosage, PLA/PGA ratio), and outcome measures (analyses methods, main findings, p-value). In case of uncertainty concerning the methodological details of the included studies, the authors were contacted by email.

## **2.6 Quality and risk of bias assessment**

The risk of bias (RoB) of the included articles was assessed independently by two reviewers (E.B.M. and L.O.M.) using the Systematic Review Centre for Laboratory Animal Experiments (SYRCLE) tool [20]. This tool is based on the Cochrane Collaboration RoB Tool. It has been adapted to evaluate the bias aspects in animal experiments aiming to assess the methodological quality of the studies. The possible answers to each of the RoB questions were “Yes”, “No” or “Unclear”. Briefly, the following points and questions were considered: selection bias (sequence generation, baseline characteristics, and allocation concealment), performance bias (random housing and blinding of study personnel), detection bias (random and blinding of outcome assessors), attrition bias (incomplete outcome data), reporting bias, and other sources of biases.

## **2.6 Summary measures**

A qualitative analysis of results was performed based on bone formation in critical defects *in vivo* due to the SIM loaded into PLGA-based scaffolds. The bone formation capacity was measurement using histologic data, scanning electron microscopy,  $\mu$ -computed tomography ( $\mu$ CT), immunohistochemical analyses, and/or densitometry analyses.

## **2.7 Synthesis of results**

A qualitative analysis of results was performed based on the SIM dose loaded into PLGA-based scaffolds required to promote bone formation in critical defects *in vivo* (reported or calculated). Statistical pooling of data using meta-analysis was planned if studies were considered sufficiently homogeneous regarding methodology and data availability.

# **3. Results**

## **3.1 Study selection**

At phase 1, 715 references were electronically retrieved in the following databases: EMBASE (169), LILACS (03), LIVIVO (71), PubMed (125), SCOPUS (96), Web of Science (67), ProQuest (43), Google Scholar (140), and Open Grey (01). No additional references were identified manually. After the duplicated papers removal, 259 references remained. After evaluating the title and abstract, 18 articles were included in phase 2 for full-text reading. Finally, after full-text analyses, seven studies matched the inclusion criteria and were included for further analyses (see Appendix 2). Figure 1 shows a flowchart describing the complete process of identification, inclusion, and exclusion of studies.

### **3.2 Study characteristics**

The characteristics of the selected studies are shown in Table 1 and Figure 2. The included studies were published in the English language from 2013 up to 2017. The studies were conducted in China (3) and Brazil (4). Different experimental animal models were tested, including rats (5), rabbits (1), and mice (1). In total, 399 animals were analyzed. As expected, due to the selection criteria, all the included studies evaluated critical-size defects to assess the *in vivo* biocompatibility and osteogenic capacity of the implanted drug delivery systems. The bone defects were made in the parietal [21–23], calvaria [8,17,24], and femur [25] bones. PLGA-based scaffolds, PLGA-based scaffolds loading SIM, and no treatment were used in the bone defects.

Different strategies were used to analyze the bone formation capacity, such as histology, scanning electron microscopy,  $\mu$ CT, immunohistochemistry, and densitometry analyses. All included articles used different SIM concentrations.

### **3.3 Risk of bias (RoB) in individual studies**

The RoB was assessed using the SYRCLE tool [20]. In summary, the RoB was considered low for most items evaluated in the studies (Table 2). However, all the included studies failed to report if the allocation sequence was adequately generated and applied, as well as if the caregivers/investigators and outcome assessors were blinded to knowledge the received intervention of each animal during the experiment [8,17,21–25]. Also, the question related to the animals selected at random for outcome assessment was unclear for all the studies [8,17,21–25].

### 3.4 Results of individual studies

Assaf et al. (2013) [22] evaluated 32 male Wistar rats (250-300 g) divided in two groups (n = 16 each). In each rat, two critical-size defects of 5.25 mm in diameter were created in the dorsal part of the parietal bone. The defect on the right side was always the experimental group, while the left side was the control (no treatment). In the first group, the right-side defect was filled with PLGA scaffold and the second group received a PLGA-based scaffold with SIM (20 µg/scaffold). The ratio of lactide to glycolic used to produce the PLGA scaffold was 50:50. To evaluate the material biocompatibility and tissue regeneration, histological analysis was performed after 4 and 8 weeks. The results demonstrated more bone formation promoted by scaffolds loading SIM than scaffolds alone or control group (p <0.05).

Encarnação et al. (2015) [21] created two defects of 5 mm in diameter in the calvaria of 180 Wistar rats (180 g). Six groups were evaluated: naive (incision and detachment of the periosteum); sham (negative control); vehicle (ethyl alcohol + phosphate-buffered saline (PBS)); PLGA+HA+βTCP; PLGA+HA+βTCP+SIM (200 µg/scaffold); and 200 µg SIM only. The ratio of lactide to glycolic used to produce the PLGA scaffold was 82:18. To evaluate bone mineralization, tissue samples were collected at 1, 7, 15, 30, and 60 days after surgery. Radiographs were carried out, and bone densitometry was determined. SIM and PLGA+HA+βTCP scaffold, associated or not, led to similar bone formation compared to control groups on day 60 after surgery (p >0.05).

Ferreira et al. (2014) [23] evaluated the potential of SIM loaded into PLGA microspheres to heal critical-size defects on parietal bones of 66 male Wistar rats (280-300 g). For this, PLGA scaffolds were synthesized as pure membranes or as microspheres loaded with SIM (50 µg/scaffold). The created defects were filled by blood clot in the control (C) group, covered with PLGA membrane (M) group, filled with SIM-loaded PLGA microspheres and covered with PLGA membrane (MSI) group, or treated only with PLGA microspheres without SIM and covered with membrane (MM) group. The ratio of lactide to glycolic used to produce the PLGA scaffold was 50:50. The defects were evaluated after 30 or 60 days by conventional and scanning electron microscopies, immunohistochemistry for osteopontin (OPN), bone sialoprotein (BSP), and osteoadherin (OSAD), and immunocytochemistry for OPN. PLGA microspheres loaded with SIM demonstrated osteoinductive capacity. PLGA microspheres loading SIM and covered with PLGA membrane (MSI) showed the highest newly formed bone (p <0.05).

In the Jiang et al. (2013) [17] study, critical-size defects were created in the calvaria bone of 34 female Wistar rats (6 weeks old), randomly divided into three groups: PLGA+HA; PLGA+HA+SIM (8  $\mu\text{g}/\text{scaffold}$ ); and a control group that did not receive any material. The ratio of lactide to glycolic used to produce the PLGA scaffold was 85:15. On 4 and 8-weeks post-implantation, H&E staining and  $\mu\text{CT}$  analyses were performed to quantify the bone formation. PLGA+HA+SIM group stimulated the highest new bone formation ( $p < 0.05$ ). 14.8% of SIM was released in the first 2 days, 20% of SIM was released over the first fifteen days and over 8 weeks, when the experiment was terminated, 23.2% of SIM loaded was released from the scaffold.

Liu et al. (2014) [24] evaluated 4-mm diameter critical-sized defect created at the left side of the calvarium of 32 ICR mice (4 weeks old), divided into four groups: PLGA scaffold; PLGA+SIM (35  $\mu\text{g}/\text{scaffold}$ ); PLGA+Stromal cell-derived factor-1a (SDF-1a); and PLGA+SIM+SDF-1a. The ratio of lactide to glycolic used to produce the PLGA scaffold was 75:25. According to the  $\mu\text{CT}$ , H&E staining, and immunohistochemistry analyses performed six weeks after the implantation, PLGA+SIM+ SDF-1a increased non-collagenous protein expression in the bone matrix the highest newly formed bone tissue ( $p < 0.05$ ).

Mendes et al. (2017) [8] evaluated 8-mm bone defect in the calvaria of 35 Wistar rats (3 months old) divided into five groups: control (blank defect); PLGA-based scaffold; PLGA+SIM (40  $\mu\text{g}/\text{scaffold}$ ); PLGA+Mesenchymal stem cells (MSC); and PLGA+SIM+MSC. The ratio of lactide to glycolic used to produce the PLGA was not reported. After eight weeks, the histological and histomorphometric analyses were performed. PLGA+SIM group promoted the highest bone formation ( $p < 0.05$ ). Control (empty defect), PLGA, PLGA+MSC, and PLGA+SIM+MSC groups promoted similar new bone formation ( $p > 0.05$ ). The SIM release occurred slowly, 4% in 24 hours. The cumulative release was approximately 30% in 30 days.

Zhang et al. (2015) [25] created critical defects (6 mm x 10 mm) on the lateral femoral condyle of 30 New Zealand rabbits weighing about 1000 g that were divided into three groups as follows: Sham-operation; PLGA+Calcium phosphate composite (CPC); PLGA+CPC+SIM (100  $\mu\text{g}/\text{scaffold}$ ). The ratio of lactide to glycolic used to produce the PLGA scaffold was 50:50. After 6- and 12-weeks post-implantation,  $\mu\text{CT}$  and histomorphology analyses were performed. SIM+PLGA+CPC+SIM group showed the highest bone tissue formation ( $p < 0.05$ ), and the defects were almost repaired as early as 12 weeks after implantation. SIM-loaded PLGA

microspheres exhibited a significant initial burst of SIM, >60% was released within the first 7 days. The SIM release was almost completed in 21 days.

### 3.5 Synthesis of results

It was impossible to carry out a meta-analysis due to the heterogeneity among the studies concerning the biomaterial composition, animal model, and SIM concentration.

Six studies demonstrated that SIM embedded into PLGA-based scaffolds promoted bone formation in critical-size defects *in vivo* [8,17,22–25]. One study evaluated PLGA scaffold loading SIM [22], one study evaluated SIM loaded into PLGA microspheres and covered with the PLGA membrane [23]. In contrast, four studies analyzed a combination of SIM, PLGA, and other substances, such as HA [17], SDF-1a [24], MSC [8], and CPC [25]. The concentrations of SIM analyzed on these studies ranged from 8 to 200 µg/scaffold with the follow-up periods from 4 to 12 weeks. All seven studies evaluated new bone formation by histological analyses. In addition to this method, µCT analyses were performed [17,24,25].

In only one study [21] the loaded SIM into the scaffold did not lead to bone neoformation. Other studies showed more significant bone formation in the PLGA-based scaffolds embedding SIM [8,17,22–25].

## 4. Discussion

Considering that bone regeneration is still a challenge in several approaches to oral and maxillofacial surgeries, the search for biomaterials and predictable techniques that enhance the bone regeneration process continues [26]. Additionally, since SIM has been described for promoting osteogenic differentiation and increasing bone formation, drug delivery systems SIM-based have been evaluated [23,27]. Thus, it is appropriate to determine the SIM concentration required to promote bone formation through local delivery systems. This SR aimed to critically evaluate available literature concerning the SIM dose loaded into PLGA-based scaffolds necessary to stimulate bone formation in critical *in vivo* bone defects. In summary, we observed that, from the seven included studies evaluating rat, mouse, and rabbit models, six studies demonstrated the enhanced capacity of SIM loaded into PLGA-based scaffolds to promoted bone formation in critical bone defects. The required SIM concentration ranged from 8 to 50 µg/scaffold in rat and mouse models, and 100 µg/scaffold in rabbit models. In one of the included studies, SIM loaded into the PLGA scaffold did not lead to bone neoformation. It is important to highlight that determining the SIM concentration loaded into

PLGA-based scaffolds required to promote bone formation in critical defects in different animal models may contribute to the experimental design of future translational studies in bone tissue engineering.

SIM stimulates BMP expression levels in bone cells and promotes bone formation [21,29–32]. Interestingly, the clinical use of SIM presents a reduced risk of side-effects compared to growth factors or gene therapies [33] and lower costs [21]. However, the success of SIM application in bone regeneration is related to a slow and controlled release process since high SIM concentrations *in vivo* are associated with an exacerbated inflammatory responses and impaired bone formation [24,32,34] due to the cytotoxicity and the reduction of cholesterol production in cell membranes [31,35]. On the other hand, low-dose of SIM may not reach the minimum concentration necessary to stimulate bone formation.

Commonly, the materials used as bone grafts demonstrate osteoconduction capacity only [27,36]. The addition of bioactive substances into the scaffolding materials is intended to lead to osteoinduction, thus stimulating the differentiation of bone-forming cells and stimulating the angiogenesis, which is essential for integrating the implanted system [27,36,37]. In this sense, efforts to find an appropriate delivery system to increase the SIM circulating concentrations are continuous since the bone formation *in vivo* enhanced by SIM depends on an adequate local concentration [38]. In our findings, three of the included articles evaluated SIM release [8,17,25], showing an initial release varying from 4% (1 day) [8], 14.8% (2 days) [17], and >60% (7 days) [25], and reaching SIM release of approximately 30% in 30 days [8], 23% in 56 days [17], and 100% in 21 days [25]. It is possible to control the bioactive substance release rate from PLGA according to the molecular weight and the ratio of lactide to glycolide [11]. Among the included studies, different ratios of lactide to glycolic were used to produce the PLGA scaffolds. The proportion most employed was 50:50 [22,23,25], followed by 82:18 [21], 85:15 [17], and 75:25 [24]. Only one article did not report the lactide to glycolic ratio applied [8].

This SR raises a question on which animal model most accurately represents human alveolar bone conditions. A recent study [28] compared structural and functional characteristics of alveolar bone among humans, mini pigs, rats, and mice. The same anatomic location was analyzed in all species and the findings demonstrated that bone volume differed among the species, while bone mineral density was equal. All species showed a similar density of alveolar osteocytes, with a highly conserved pattern of collagen organization. Bone remodeling was also

morphologically similar among species, with comparable mineral apposition rates. No evidence for the superiority of pig models over rodent models in representing human bone biology was found. However, the size of the animal model may impact the SIM concentration applied to the scaffold, as observed from the results obtained in this SR.

Concerning the RoB judgment, low RoB was attributed to most items evaluated in included studies. It is important to mention that low RoB judgments denote that none or minor methodological flaws occurred in the assessed studies. Consequently, none or small deviations from the true effect estimation befallen, providing confidence in interpreting the results [39]. However, in an attempt to improve future studies, it is worth mentioning that all the included studies failed to report if the allocation sequence was adequately generated and applied, as well as if the caregivers/investigators and outcome assessors were blinded to knowledge the received intervention of each animal during the experiment [8,17,21–25]. Also, the question related to the animals selected at random for outcome assessment was unclear for all the studies [8,17,21–25]. Therefore, future experiments concerning bone formation *in vivo* should be more careful regarding these methodological details.

Regarding the limitations of this SR, only PLGA-based scaffolds were evaluated since we aimed to evaluate the SIM dose loaded into PLGA-based scaffolds required to promote bone formation in critical *in vivo* bone defects. Therefore, further studies assessing the dose of SIM needed loaded into scaffolds based on different chemical compositions are indicated. The extrapolation of this synthesis into human models is controversial.

## **5. Conclusion**

Based on *in vivo* studies, the concentration of SIM embedded into PLGA-based scaffolds required to promote bone formation ranged from 8 to 50  $\mu\text{g}/\text{scaffold}$  in rat and mouse models and 100  $\mu\text{g}/\text{scaffold}$  in rabbit models *in vivo* bone defects.

## **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.

Eduarda Blasi Magini and Luiza de Oliveira Matos are supported with scholarship by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (Coordination for the Improvement of Higher Education Personnel), Brazil.

#### **ETHICAL APPROVAL**

This article does not contain any studies with human participants or animals performed by any of the authors.

#### **INFORMED CONSENT**

Formal consent is not required for this type of study.

#### **ACKNOWLEDGMENTS**

The authors thank Ms. Karyn Munik Lehmkuhl, for the instructions regarding the search strategy construction.

#### **AUTHORS CONTRIBUTION**

Eduarda Blasi Magini, Luiza de Oliveira Matos, Raissa Borges Curtarelli, Mariane Beatriz Sordi, Gabriel Leonardo Magrin, Carlos Flores Mir, and Ariadne Cristiane Cabral Cruz, contributed to the conception and the design of the study; Eduarda Blasi Magini, Luiza de Oliveira Matos, Raissa Borges Curtarelli, and Ariadne Cristiane Cabral Cruz collected the data; Eduarda Blasi Magini, Luiza de Oliveira Matos, Raissa Borges Curtarelli, and Ariadne Cristiane Cabral Cruz analyzed the data; and Eduarda Blasi Magini, Luiza de Oliveira Matos, Raissa Borges Curtarelli, Mariane Beatriz Sordi, Gabriel Leonardo Magrin, Carlos Flores Mir, and Ariadne Cristiane Cabral Cruz drafted and critically revised the manuscript.



## Reference

- [1] M.G. Araújo, J. Lindhe, Dimensional ridge alterations following tooth extraction. An experimental study in the dog, *J. Clin. Periodontol.* 32 (2005) 212–218. <https://doi.org/10.1111/j.1600-051X.2005.00642.x>.
- [2] K.Y. Kim T., Kim S., Sándor G., Comparison of platelet-rich plasma (PRP), platelet -rich fibrin (PRF), and concentrated growth factor (CGF) in rabbit- skull defect healing, *Arch. Oral Biol.* 59 (2014) 550–558.
- [3] S. Jankovic, Z. Aleksic, P. Klokkevold, V. Lekovic, B. Dimitrijevic, E.B. Kenney, P. Camargo, Use of platelet-rich fibrin membrane following treatment of gingival recession: a randomized clinical trial., *Int. J. Periodontics Restorative Dent.* 32 (2012) e41-50. <http://www.ncbi.nlm.nih.gov/pubmed/22292152>.
- [4] A. Puisys, T. Linkevicius, The influence of mucosal tissue thickening on crestal bone stability around bone-level implants. A prospective controlled clinical trial, *Clin. Oral Implants Res.* 26 (2015) 123–129. <https://doi.org/10.1111/clr.12301>.
- [5] C. Landsberg, O. Bender, M. Weinreb, R. Wigler, T. Chackartchi, E. Weinberg, S. Matalon, Postextraction ridge width alterations following socket seal surgery—A retrospective study, *Appl. Sci.* 11 (2021) 1–9. <https://doi.org/10.3390/app11010324>.
- [6] M.J.R. Virilan, D. Miricescu, A. Totan, M. Greabu, C. Tanase, C.M. Sabliov, C. Caruntu, B. Calenic, Current Uses of Poly ( lactic-co-glycolic acid ) in the Dental Field : A Comprehensive Review, *J. Chem.* 2015 (2015) 1–12. <https://doi.org/http://dx.doi.org/10.1155/2015/525832>.
- [7] Hirenkumar K. Makadia, S.J. Siegel, Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier, *Polym.* 3 (2011) 1377–1397. <https://doi.org/10.3390/polym3031377.Poly>.
- [8] D. Mendes, J.A. Domingues, M.A. Hausen, S.M.M. Cattani, A. Aragones, A.L.R. Oliveira, R.F. Inácio, M.L.P. Barbo, E.A.R. Duek, Study of mesenchymal stem cells cultured on a poly(Lactic-co-glycolic acid) scaffold containing simvastatin for bone healing, *J. Appl. Biomater. Funct. Mater.* 15 (2017) e133–e141. <https://doi.org/10.5301/jabfm.5000338>.
- [9] F. Danhier, E. Ansorena, J. M. Silva, R. Coco, A. Le Breton, V. Prétat, PLGA-based nanoparticles : An overview of biomedical applications, *J. Control. Release.* 161 (2012) 505–522. <https://doi.org/10.1016/j.jconrel.2012.01.043>.

- [10] Z.Z. Zhang, H.Z. Zhang, Z.Y. Zhang, 3D printed poly( $\epsilon$ -caprolactone) scaffolds function with simvastatin-loaded poly(lactic-co-glycolic acid) microspheres to repair load-bearing segmental bone defects, *Exp. Ther. Med.* 17 (2019) 79–90. <https://doi.org/10.3892/etm.2018.6947>.
- [11] I.C. Tai, Y.C. Fu, C.K. Wang, J.K. Chang, M.L. Ho, Local delivery of controlled-release simvastatin/PLGA /HA p microspheres enhances bone repair, *Int. J. Nanomedicine.* 8 (2013) 3895–3905. <https://doi.org/10.2147/IJN.S48694>.
- [12] G.J.S. Littuma, M.B. Sordi, R. Borges Curtarelli, Á. Aragonês, A.C.C. da Cruz, R.S. Magini, Titanium coated with poly(lactic-co-glycolic) acid incorporating simvastatin: Biofunctionalization of dental prosthetic abutments, *J. Periodontal Res.* 55 (2020) 116–124. <https://doi.org/10.1111/jre.12695>.
- [13] I. Özeç, E. Kiliç, C. Gümüş, F. Göze, Effect of local simvastatin application on mandibular defects, *J. Craniofac. Surg.* 18 (2007) 546–550. <https://doi.org/10.1097/scs.0b013e318052ff05>.
- [14] K. Papadimitriou, G. Karkavelas, I. Vouros, E. Kessopoulou, A. Konstantinidis, Effects of local application of simvastatin on bone regeneration in femoral bone defects in rabbit, *J. Cranio-Maxillofacial Surg.* 43 (2015) 232–237. <https://doi.org/10.1016/j.jcms.2014.11.011>.
- [15] Y. Lee, M.J. Schmid, D.B. Marx, M.W. Beatty, D.M. Cullen, M.E. Collins, R.A. Reinhardt, The effect of local simvastatin delivery strategies on mandibular bone formation in vivo, *Biomaterials.* 29 (2008) 1940–1949. <https://doi.org/10.1016/j.biomaterials.2007.12.045>.
- [16] A. Oryan, A. Kamali, A. Moshiri, Potential mechanisms and applications of statins on osteogenesis: Current modalities, conflicts and future directions, *J. Control. Release.* 215 (2015) 12–24. <https://doi.org/10.1016/j.jconrel.2015.07.022>.
- [17] L. Jiang, H. Sun, A. Yuan, K. Zhang, D. Li, C. Li, C. Shi, X. Li, K. Gao, C. Zheng, B. Yang, H. San, Enhancement of osteoinduction by continual simvastatin release from poly(lactic-co-glycolic acid)-hydroxyapatite-simvastatin nano-fibrous scaffold, *J. Biomed. Nanotechnol.* 9 (2013) 1921–1928. <https://doi.org/10.1166/jbn.2013.1692>.
- [18] D. Moher, A. Liberati, J. Tetzlaff, D.G. Altman, P. Grp, Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement (Reprinted from *Annals of Internal Medicine*), *Phys. Ther.* 89 (2009) 873–880.

- <https://doi.org/10.1371/journal.pmed.1000097>.
- [19] T. Greenhalgh, R. Peacock, Effectiveness and efficiency of search methods in systematic reviews of complex evidence: Audit of primary sources, *Br. Med. J.* 331 (2005) 1064–1065. <https://doi.org/10.1136/bmj.38636.593461.68>.
- [20] C.R. Hooijmans, M.M. Rovers, R.B.M. De Vries, M. Leenaars, M. Ritskes-Hoitinga, M.W. Langendam, SYRCLE's risk of bias tool for animal studies, *BMC Med. Res. Methodol.* 14 (2014) 1–9. <https://doi.org/10.1186/1471-2288-14-43>.
- [21] 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+ $\beta$ TCP scaffold, *Implant Dent.* 25 (2016) 140–148. <https://doi.org/10.1097/ID.0000000000000359>.
- [22] K. Assaf, E.A. De Rezende Duek, N.M. Oliveira, Efficacy of a combination of simvastatin and poly(DL-lactic-co-glycolic acid) in stimulating the regeneration of bone defects, *Mater. Res.* 16 (2013) 215–220. <https://doi.org/10.1590/S1516-14392012005000159>.
- [23] L.B. Ferreira, V. Bradaschia-Correa, M.M. Moreira, N.D.M. Marques, V.E. Arana-Chavez, Evaluation of bone repair of critical size defects treated with simvastatin-loaded poly(lactic-co-glycolic acid) microspheres in rat calvaria, *J. Biomater. Appl.* 29 (2015) 965–976. <https://doi.org/10.1177/0885328214550897>.
- [24] Y.S. Liu, M.E. Ou, H. Liu, M. Gu, L.W. Lv, C. Fan, T. Chen, X.H. Zhao, C.Y. Jin, X. Zhang, Y. Ding, Y.S. Zhou, The effect of simvastatin on chemotactic capability of SDF-1 $\alpha$  and the promotion of bone regeneration, *Biomaterials.* 35 (2014) 4489–4498. <https://doi.org/10.1016/j.biomaterials.2014.02.025>.
- [25] H.X. Zhang, G.Y. Xiao, X. Wang, Z.G. Dong, Z.Y. Ma, L. Li, Y.H. Li, X. Pan, L. Nie, Biocompatibility and osteogenesis of calcium phosphate composite scaffolds containing simvastatin-loaded PLGA microspheres for bone tissue engineering, *J. Biomed. Mater. Res. - Part A.* 103 (2015) 3250–3258. <https://doi.org/10.1002/jbm.a.35463>.
- [26] W. Wang, K.W.K. Yeung, Bone grafts and biomaterials substitutes for bone defect repair: A review, *Bioact. Mater.* 2 (2017) 224–247. <https://doi.org/10.1016/j.bioactmat.2017.05.007>.
- [27] M.B. Sordi, A.C.C. da Cruz, Á. Aragones, M.M. Rodríguez Cordeiro, R. de Souza Magini, PLGA+HA/ $\beta$ TCP scaffold incorporating simvastatin: A promising biomaterial for bone tissue engineering, *J. Oral Implantol.* 47 (2021) 93–101.

- <https://doi.org/10.1563/AAID-JOI-D-19-00148>.
- [28] I. Pilawski, U.S. Tulu, P. Ticha, P. Schüpbach, H. Traxler, Q. Xu, J. Pan, B.R. Coyac, X. Yuan, Y. Tian, Y. Liu, J. Chen, Y. Erdogan, M. Arioka, M. Armario, M. Wu, J.B. Brunski, J.A. Helms, Interspecies Comparison of Alveolar Bone Biology, Part I: Morphology and Physiology of Pristine Bone, *JDR Clin. Transl. Res.* 6 (2021) 352–360. <https://doi.org/10.1177/2380084420936979>.
- [29] I.C. Encarnação, M.B. Sordi, Á. Aragones, C.M.O. Müller, A.C. Moreira, C.P. Fernandes, J. V. Ramos, M.M.R. Cordeiro, M.C. Fredel, R.S. Magini, Release of simvastatin from scaffolds of poly(lactic-co-glycolic) acid and biphasic ceramic designed for bone tissue regeneration, *J. Biomed. Mater. Res. - Part B Appl. Biomater.* 107 (2019) 2152–2164. <https://doi.org/10.1002/jbm.b.34311>.
- [30] S.P. Lyu, D. Untereker, Degradability of polymers for implantable biomedical devices, *Int. J. Mol. Sci.* 10 (2009) 4033–4065. <https://doi.org/10.3390/ijms10094033>.
- [31] R.A. Jain, The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices, *Biomaterials.* 21 (2000) 2475–2490. [https://doi.org/10.1016/S0142-9612\(00\)00115-0](https://doi.org/10.1016/S0142-9612(00)00115-0).
- [32] W.L. Grayson, M. Fröhlich, K. Yeager, S. Bhumiratana, M.E. Chan, C. Cannizzaro, L.Q. Wan, X.S. Liu, X.E. Guo, G. Vunjak-Novakovic, Engineering anatomically shaped human bone grafts, *Proc. Natl. Acad. Sci. U. S. A.* 107 (2010) 3299–3304. <https://doi.org/10.1073/pnas.0905439106>.
- [33] S.H. Barbanti, A.R. Santos, C.A.C. Zavaglia, E.A.R. Duek, Porous and dense poly(L-lactic acid) and poly(D,L-lactic acid-co-glycolic acid) scaffolds: In vitro degradation in culture medium and osteoblasts culture, *J. Mater. Sci. Mater. Med.* 15 (2004) 1315–1321. <https://doi.org/10.1007/s10856-004-5740-6>.
- [34] C.T. Lee, Y. Der Lee, Preparation of porous biodegradable poly(lactide-co-glycolide)/hyaluronic acid blend scaffolds: Characterization, in vitro cells culture and degradation behaviors, *J. Mater. Sci. Mater. Med.* 17 (2006) 1411–1420. <https://doi.org/10.1007/s10856-006-0617-5>.
- [35] M. Lebourg, J. Suay Antón, J.L. Gomez Ribelles, Characterization of calcium phosphate layers grown on polycaprolactone for tissue engineering purposes, *Compos. Sci. Technol.* 70 (2010) 1796–1804. <https://doi.org/10.1016/j.compscitech.2010.07.017>.
- [36] M. Özcan, D. Hotza, M.C. Fredel, A. Cruz, C.A.M. Volpato, Materials and

- manufacturing techniques for polymeric and ceramic scaffolds used in implant dentistry, *J. Compos. Sci.* 5 (2021) 1–22. <https://doi.org/10.3390/jcs5030078>.
- [37] M.B. Sordi, A. Cruz, M.C. Fredel, R. Magini, P.T. Sharpe, Three-dimensional bioactive hydrogel-based scaffolds for bone regeneration in implant dentistry, *Mater. Sci. Eng. C.* 124 (2021). <https://doi.org/10.1016/j.msec.2021.112055>.
- [38] Z. Wu, C. Liu, G. Zang, H. Sun, The effect of simvastatin on remodelling of the alveolar bone following tooth extraction, *Int. J. Oral Maxillofac. Surg.* 37 (2008) 170–176.
- [39] 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 J. Evidence-Based Med.* 9 (2009) 8–11.

**LIST OF TABLES, FIGURES AND LEGENDS**

Table 1 Summary of descriptive characteristics of included studies (n=7)

Table 2 Risk of bias summary, assessed by Systematic Review Centre for Laboratory Animal Experiments (SYRCLE) tool

Figure 1 Flowchart of the literature search and selection criteria. Adapted from PRISMA

Figure 2 Scheme of characteristics of included studies (n=7)

Appendix 1 Data search strategy

Appendix 2 Articles excluded and the reasons for exclusion (n=11)

# CAPÍTULO III

## CONSIDERAÇÕES FINAIS

A partir desta revisão sistemática, foi possível confirmar que a incorporação de sinvastatina nos arcabouços impulsionou a regeneração óssea na maioria dos artigos incluídos. Foi concluído que a dose necessária de sinvastatina incorporada em arcabouços à base de PLGA para promover a formação óssea em defeitos ósseos críticos *in vivo*, variou de 8-50  $\mu\text{g}$  em modelos de ratos e camundongos, e 100  $\mu\text{g}$  em modelo de coelhos.



# CAPÍTULO IV

## REFERÊNCIAS

- ANITUA, E. et al. Morphogen and proinflammatory cytokine release kinetics from PRGF-Endoret fibrin scaffolds: Evaluation of the effect of leukocyte inclusion. **Journal of Biomedical Materials Research - Part A**, v. 103, n. 3, p. 1011–1020, 2015.
- ARAÚJO, M. G. et al. Alveolar socket healing: What can we learn? **Periodontology 2000**, v. 68, n. 1, p. 122–134, 2015.
- ARAÚJO, M. G.; LINDHE, J. Dimensional ridge alterations following tooth extraction. An experimental study in the dog. **Journal of Clinical Periodontology**, v. 32, n. 2, p. 212–218, 2005.
- DANHIER, F. et al. PLGA-based nanoparticles : An overview of biomedical applications. **Journal of Controlled Release**, v. 161, n. 2, p. 505–522, 2012.
- ELGALI, I. et al. Guided bone regeneration: materials and biological mechanisms revisited. **European Journal of Oral Sciences**, v. 125, n. 5, p. 315–337, 2017.
- FRÖHLICH, M. et al. Tissue Engineered Bone Grafts: Biological Requirements, Tissue Culture and Clinical Relevance. **Curr Stem Cell Res Ther**, v. 3, n. 4, p. 254–264, 2008.
- HIRENKUMAR K. MAKADIA; SIEGEL, S. J. Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier. **Polymers (Basel)**, v. 3, n. 3, p. 1377–1397, 2011.
- HO, M. L. et al. Simvastatin increases osteoblasts and osteogenic proteins in ovariectomized rats. **European Journal of Clinical Investigation**, v. 39, n. 4, p. 296–303, 2009.
- LANDSBERG, C. et al. Postextraction ridge width alterations following socket seal surgery—A retrospective study. **Applied Sciences (Switzerland)**, v. 11, n. 1, p. 1–9, 2021.
- LEE, Y. et al. The effect of local simvastatin delivery strategies on mandibular bone formation in vivo. **Biomaterials**, v. 29, p. 1940–1949, 2008.
- LITTUMA, G. J. S. et al. Titanium coated with poly(lactic-co-glycolic) acid incorporating simvastatin: Biofunctionalization of dental prosthetic abutments. **Journal of Periodontal Research**, v. 55, n. 1, p. 116–124, 2020.
- LIU, Y. S. et al. The effect of simvastatin on chemotactic capability of SDF-1 $\alpha$  and the promotion of bone regeneration. **Biomaterials**, v. 35, n. 15, p. 4489–4498, 2014.
- MCALLISTER, B. S.; HAGHIGHAT, K. Bone Augmentation Techniques. **Journal of Periodontology**, v. 78, n. 3, p. 377–396, 2007.
- MENDES, D. et al. Study of mesenchymal stem cells cultured on a poly(Lactic-co-glycolic acid) scaffold containing simvastatin for bone healing. **Journal of Applied Biomaterials and**

**Functional Materials**, v. 15, n. 2, p. e133–e141, 2017.

MEZZOMO, L. A. et al. Alveolar ridge preservation after dental extraction and before implant placement: A literature review Preservação do rebordo alveolar após a extração dentária e antes da colocação de implante: revisão da literatura. **Rev Odonto Cienc**, v. 26, n. 1, p. 77–83, 2011.

MONTERO, J.; MANZANO, G.; ALBALADEJO, A. The role of topical simvastatin on bone regeneration : A systematic review. **Biomaterials and Bioengineering in Dentistry**, v. 6, n. 3, p. 286–290, 2014.

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

OLIVEIRA, S. M. et al. Engineering Endochondral Bone : In Vivo Studies. **Tissue Engineering Part A**, v. 15, n. 3, p. 635–643, 2009.

ORYAN, A.; KAMALI, A.; MOSHIRI, A. Potential mechanisms and applications of statins on osteogenesis: Current modalities, conflicts and future directions. **Journal of Controlled Release**, v. 215, p. 12–24, 2015.

PARK, J. B. The use of simvastatin in bone regeneration. **Medicina Oral, Patologia Oral y Cirugia Bucal**, v. 14, n. 9, p. 485–488, 2009.

PRECHEUR, H. V. Bone Graft Materials. **Dent Clin N Am**, v. 51, p. 729–746, 2007.

RUHAIMI, K. A. AL. Bone Graft Substitutes : A Comparative Qualitative Histologic Review of Current Osteoconductive Grafting Materials. **INT J ORAL MAXILLOFAC IMPLANTS**, v. 16, p. 105–114, 2001.

SADIASA, A.; KIM, M. S.; LEE, B. T. Poly(lactide-co-glycolide acid)/biphasic calcium phosphate composite coating on a porous scaffold to deliver simvastatin for bone tissue engineering. **J Drug Target**, v. 21, n. 8, p. 719–729, 2013a.

SADIASA, A.; KIM, M. S.; LEE, B. T. Poly(lactide-co-glycolide acid)/biphasic calcium phosphate composite coating on a porous scaffold to deliver simvastatin for bone tissue engineering. **Journal of Drug Targeting**, v. 21, n. 8, p. 719–729, 2013b.

SANZ, M. et al. The effect of placing a bone replacement graft in the gap at immediately placed implants: a randomized clinical trial. **Clinical Oral Implants Research**, v. 28, n. 8, p. 902–910, 2017.

SCHROPP, L. et al. Bone healing and soft tissue contour changes following single-tooth extraction: A clinical and radiographic 12-month prospective study. **The International Journal of Periodontics & Restorative Dentistry**, v. 23, n. 4, p. 313–323, 2003.

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

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, 2013b.

TAN, W. L. et al. A systematic review of post-extractional alveolar hard and soft tissue dimensional changes in humans. **Clinical Oral Implants Research**, v. 23, n. SUPPL. 5, p. 1–21, 2012.

VIRLAN, M. J. R. et al. Current Uses of Poly ( lactic-co-glycolic acid ) in the Dental Field : A Comprehensive Review. **Journal of Chemistry**, v. 2015, n. 2, p. 1–12, 2015.

WANG, W.; YEUNG, K. W. K. Bone grafts and biomaterials substitutes for bone defect repair: A review. **Bioactive Materials**, v. 2, n. 4, p. 224–247, 2017.

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. **Journal of Endocrinology**, v. 196, n. 3, p. 601–613, 2008.

# CAPÍTULO V

## LISTA DE FIGURAS

Figure 1 - Flowchart of the literature search and selection criteria. Adapted from PRISMA.

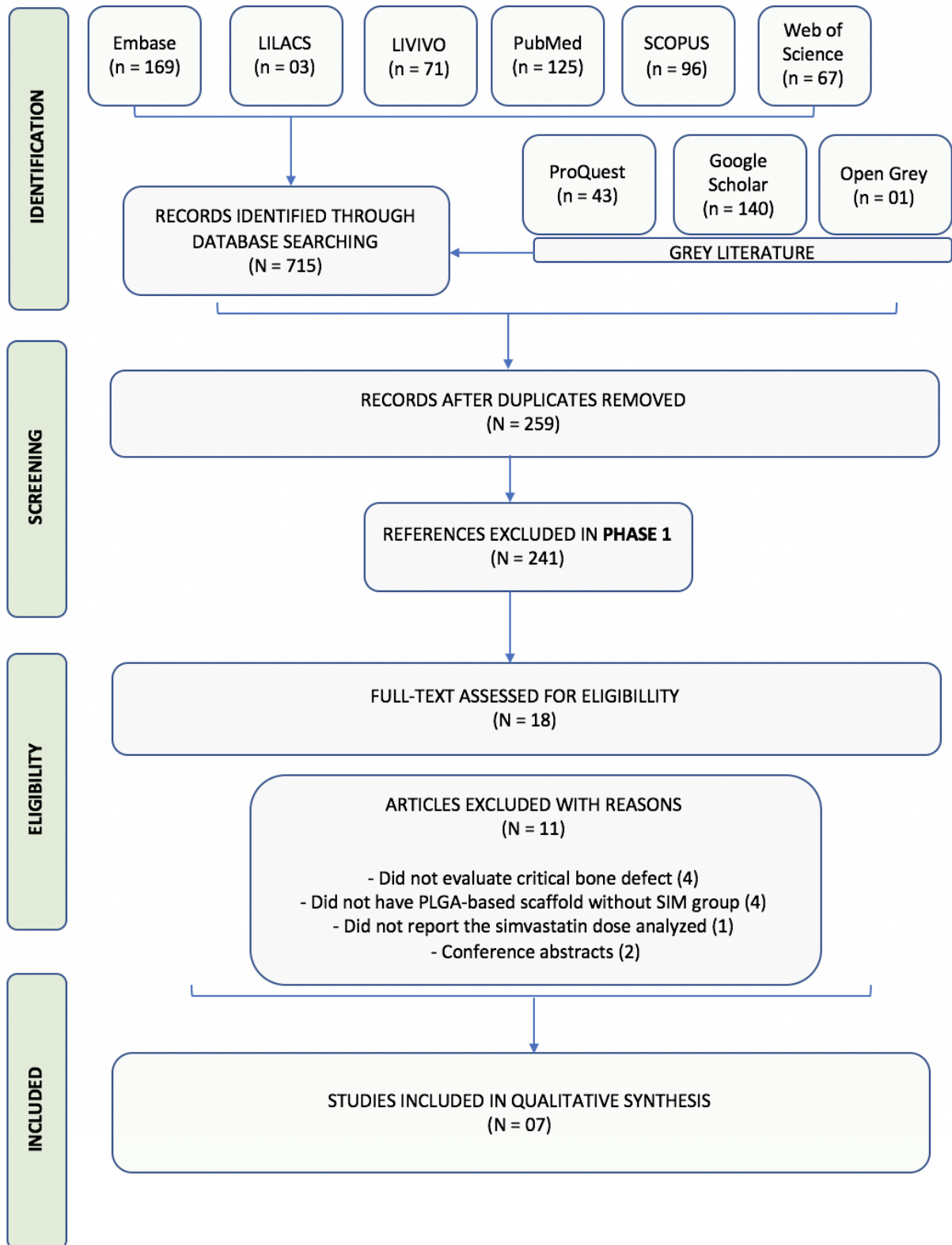
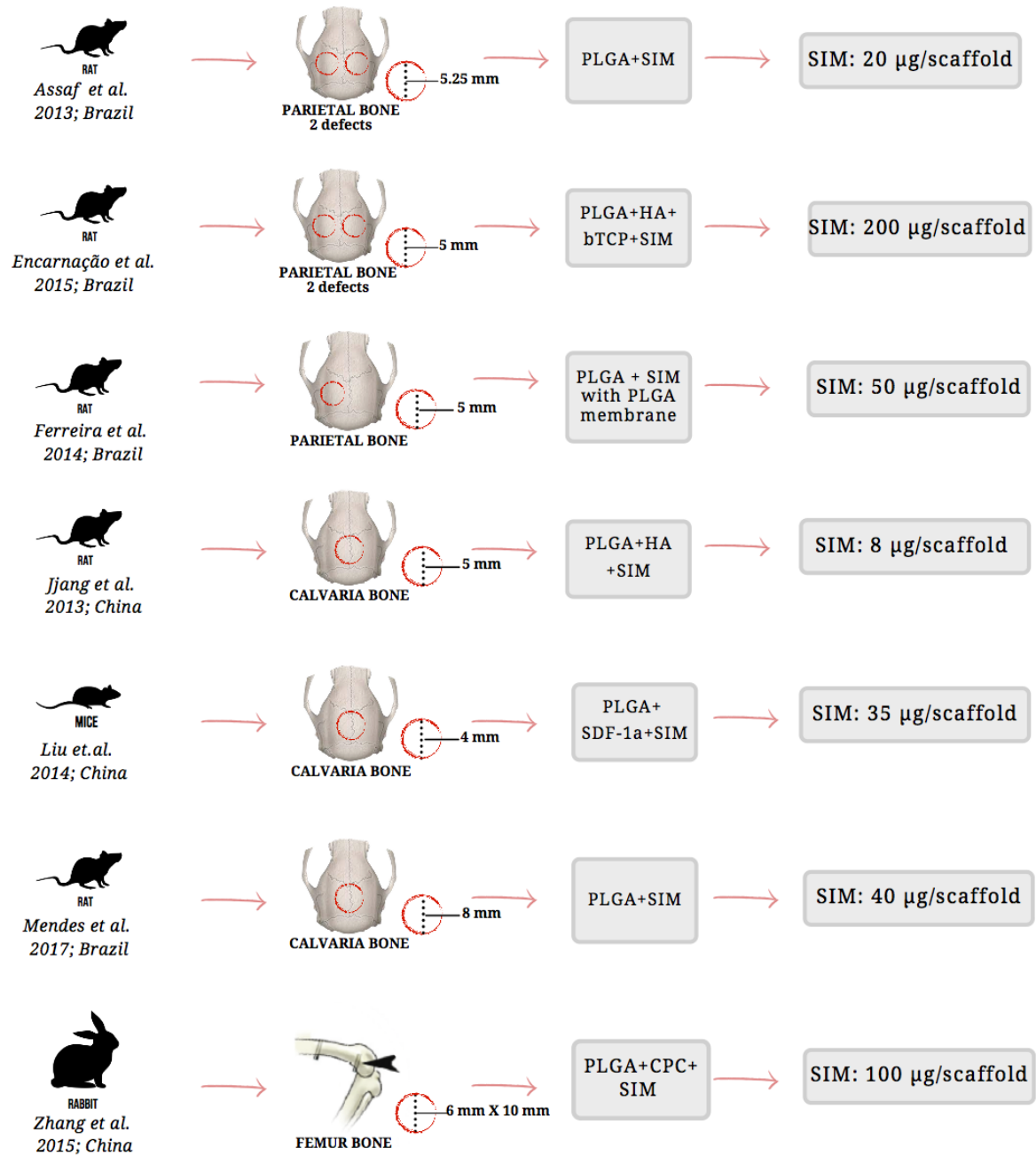


Figure 2 - Scheme of characteristics of included studies (n=7)



## APÊNDICES

### Appendix 1 - Data search strategy

(("Drug carriers"[MeSH Terms] OR "drug carriers" OR "drug carrier" OR "drug" OR "carrier" OR "drugs" OR "carriers" OR "Drug delivery systems"[MeSH Terms] OR "Drug delivery systems" OR "Drug delivery system" OR "Drug Targeting" OR "Delivery system, Drug" OR "Delivery systems, Drug" OR "Bone Substitutes"[MeSH Terms] OR "Bone Substitutes" OR "Bone Substitute" OR "Substitute, Bone" OR "Substitutes, Bone" OR "Bone Replacement Material" OR "Bone Replacement Materials" OR "Tissue Scaffolds"[MeSH Terms] OR "Tissue Scaffolds" OR "Scaffold, Tissue" OR "Scaffolds, Tissue" OR "Tissue Scaffold" OR "Tissue Scaffolding" OR "Scaffolding, Tissue" OR "Tissue Scaffoldings") AND ("Simvastatin"[MeSH Terms] OR "Simvastatin" OR "lovastatin/analogs and derivatives" OR "simvastatin/analogs and derivatives" OR "zocor" OR "mk 733" OR "mk733" OR "MK-733" OR "synvinolin" OR "Lovastatin"[MeSH Terms] OR "Lovastatin" OR "Mevinolin" OR "Monacolin K" OR "MK-803" OR "MK 803" OR "MK803" OR "Mevacor") AND ("bone regeneration"[MeSH Terms] OR "bone regeneration" OR "Bone Regenerations" OR "Regeneration, Bone" OR "Osteoconduction" OR "Bone Remodeling"[MeSH Terms] OR "Bone Remodeling" OR "Remodeling, Bone" OR "Bone Turnover" OR "Bone Turnovers" OR "Turnover, Bone" OR "Turnovers, Bone" OR "osteoinductive factor" OR "Osteogenesis"[MeSH Terms] OR "Osteogenesis" OR "Bone Formation" OR "Ossification" OR "Ossifications" OR "Biom mineralization"[MeSH Terms] OR "Biom mineralization" OR "Biom mineralizations" OR "Biocrystallization") AND ("Polylactic Acid-Polyglycolic Acid Copolymer"[MeSH Terms] OR "Polylactic Acid-Polyglycolic Acid Copolymer" OR "PLGA" OR "Polylactic Acid Polyglycolic Acid Copolymer" OR "Poly (Lactic-co-glycolic Acid)" OR "Poly(Lactic-co-glycolic Acid)" OR "Polylactic-co-glycolic Acid Copolymer" OR "Copolymers, Polylactic-co-glycolic Acid" OR "Polylactic co glycolic Acid Copolymer" OR "PLGA Compound" OR "PLGA Compounds" OR "Poly(Glycolide-co-lactide)" OR "Poly (D,L-lactic-co-glycolic Acid)" OR "Poly(D,L-lactide-co-glycolide)" OR "Poly(DL-lactide-co-glycolic Acid)" OR "Poly-L-lactic-polyglycolic Acid" OR "Poly L lactic polyglycolic Acid" OR "Poly(L-lactide)-co-glycolide" OR "Polymers"[MeSH Terms] OR "Polymers" OR "Polymer" OR "Polyglycolic Acid"[MeSH Terms] OR "Polyglycolic Acid" OR "PGA" OR "Polyglycolide" OR "Dexon" OR "Dexon-S" OR "Dexon S" OR "DexonS" OR "Biofix" OR "poly(lactide)" OR "Polylactic Acid" OR "PLA" OR "PLLA" OR "poly(DL-lactide)" OR "polylactide" OR "poly(D,L-lactide)" OR "poly(lactic acid)" OR "polylactic acid" OR "poly(L-lactide-co-D,L-lactide)" OR "poly(L-lactide)" OR "poly-L-lactide" OR "poly(rac-lactide)" OR "poly-96L 4D-lactide" OR "PLA96" OR "PolyMax" OR "Resomer R208" OR "Resorb-X" OR "SR PLA96" OR "SR-PLA96" OR "poly-L-D-lactide" OR "ticron" OR "poly(L-lactide-co-D-lactide)" OR "Atrisorb" OR "poly-d,l-lactic acid" OR "poly-L-lactic acid" OR "PLLA polymer" OR "Resomer R104" OR "polylactate" OR "70-30 poly-L-lactide-Co-D,L-lactide" OR "hydrosorb")



**Appendix 2 - Articles excluded and the reasons for exclusion (n = 11)**

<b>Reference</b>	<b>Author</b>	<b>Reasons for Exclusion*</b>
[1]	CHANG et al., 2013	1
[2]	CHANG et al., 2020	1
[3]	FU et al., 2015	3
[4]	LEE et al., 2018	2
[5]	L.C et al., 2010	4
[6]	MASAELI et al., 2016	2
[7]	NAITO et al., 2014	2
[8]	SENON GUVEN et al., 2015	4
[9]	TERUKINA et al., 2016	2
[10]	VENKATESAN et al., 2019	1
[11]	ZHANG; ZHANG; ZHANG, 2019	1

\*Legend:

1. Studies that did not evaluate critical bone defect (4)
2. Studies that did not have PLGA-based scaffold without SIM group (4)
3. Studies that did not report the simvastatin concentration analyzed (1)
4. Conference abstracts (2)

## Appendix 2 – References

- [1] P.C. Chang, A.S. Dovban, L.P. Lim, L.Y. Chong, M.Y. Kuo, C.H. Wang, Dual delivery of PDGF and simvastatin to accelerate periodontal regeneration *in vivo*, *Biomaterials*. 34 (2013) 9990–9997. <https://doi.org/10.1016/j.biomaterials.2013.09.030>.
- [2] P. Chang, W. Tai, H. Luo, C. Lai, H. Lin, Z. Lin, Y. Chang, B. Lee, Core-Shell poly- ( D , L -Lactide- co -Glycolide ) -chitosan Nanospheres with simvastatin-doxycycline for periodontal and osseous repair, *Int. J. Biol. Macromol.* 158 (2020) 627–635.
- [3] Y.C. Fu, Y.H. Wang, C.H. Chen, C.K. Wang, G.J. Wang, M.L. Ho, Combination of calcium sulfate and simvastatin-controlled release microspheres enhances bone repair in critical-sized rat calvarial bone defects, *Int. J. Nanomedicine*. 10 (2015) 7231–7240. <https://doi.org/10.2147/IJN.S88134>.
- [4] T.C. Lee, Y.H. Wang, S.H. Huang, C.H. Chen, M.L. Ho, Y.C. Fu, C.K. Wang, Evaluations of clinical-grade bone substitute-combined simvastatin carriers to enhance bone growth: *In vitro* and *in vivo* analyses, *J. Bioact. Compat. Polym.* 33 (2018) 160–177. <https://doi.org/10.1177/0883911517720813>.
- [5] T. L.C, W. C.J, F. Y.C, C. J.K, E. Rajalakshmanan, H. M.L., Effect of controlled local delivery of simvastatin/PLGA carrier on bone healing, in: 56th Annu. Meet. Orthop. Res. Soc., 2010: p. 1230.
- [6] R. Masaeli, T.S. Jafarzadeh Kashi, R. Dinarvand, V. Rakhshan, H. Shahoon, B. Hooshmand, F. Mashhadi Abbas, M. Raz, A. Rajabnejad, H. Eslami, K. Khoshroo, M. Tahriri, L. Tayebi, Efficacy of the biomaterials 3 wt%-nanostrotrium-hydroxyapatite-enhanced calcium phosphate cement (nanoSr-CPC) and nanoSr-CPC-incorporated simvastatin-loaded poly(lactic-co-glycolic-acid) microspheres in osteogenesis

- improvement: An explorative multi-phases, *Mater. Sci. Eng. C.* 69 (2016) 171–183. <https://doi.org/10.1016/j.msec.2016.06.033>.
- [7] Y. Naito, T. Terukina, S. Galli, Y. Kozai, S. Vandeweghe, T. Tagami, T. Ozeki, T. Ichikawa, P.G. Coelho, R. Jimbo, The effect of simvastatin-loaded polymeric microspheres in a critical size bone defect in the rabbit calvaria, *Int. J. Pharm.* 461 (2014) 157–162. <https://doi.org/10.1016/j.ijpharm.2013.11.046>.
- [8] G. Senol Guven, D. Morina, E. Cevher, G. Ak, Local administration of simvastatin on bone regeneration: an in vivo experimental study in Guinea pigs, in: *Int. J. Oral Maxillofac. Surg.*, 2015: p. e140. <https://doi.org/10.1016/j.ijom.2015.08.790>.
- [9] T. Terukina, Y. Naito, T. Tagami, Y. Morikawa, Y. Henmi, W. Prananingrum, T. Ichikawa, T. Ozeki, The effect of the release behavior of simvastatin from different PLGA particles on bone regeneration in vitro and in vivo: Comparison of simvastatin-loaded PLGA microspheres and nanospheres, *J. Drug Deliv. Sci. Technol.* 33 (2016) 136–142. <https://doi.org/10.1016/j.jddst.2016.03.005>.
- [10] N. Venkatesan, A.D.T. Liyanage, J. Castro-Núñez, T. Asafo-Adjei, L.L. Cunningham, T.D. Dziubla, D.A. Puleo, Biodegradable polymerized simvastatin stimulates bone formation, *Acta Biomater.* 93 (2019) 192–199. <https://doi.org/10.1016/j.actbio.2019.04.059>.
- [11] Z.Z. Zhang, H.Z. Zhang, Z.Y. Zhang, 3D printed poly( $\epsilon$ -caprolactone) scaffolds function with simvastatin-loaded poly(lactic-co-glycolic acid) microspheres to repair load-bearing segmental bone defects, *Exp. Ther. Med.* 17 (2019) 79–90. <https://doi.org/10.3892/etm.2018.6947>.

## LISTA DE TABELAS

Table1- Summary of descriptive characteristics of included studies

STUDY	POPULATION					SCAFFOLD		OUTCOME MEASURES	
	Author (Year); Country	Total Animals / Defects (n/n)	Control Group (n)	Test Group (n)	Animal Model / Bone	Bone Defect Dimension	SIM dose per scaffold	PLA / PGA ratio (m:m)	Analysis Methods
Assaf et al. (2013); Brazil	32 / 64	Blank control (n=32)	PLGA (n=16) PLGA+SIM (n=16)	Wistar Rats / Parietal bone	5.25 mm diameter	20 µg/scaffold	50:50	Histological analysis to assess the biocompatibility, bone tissue regeneration, and the remained defect size on 4 and 8 weeks.	PLGA+SIM promoted more bone formation than PLGA or blank control groups on 4 and 8 weeks (p<0.05).
Encarnação et al. (2015); Brazil	180 / 360	Naive (n=6) Blank control (n=6)	PLGA+HA+βTCP+SIM (n=6) PLGA+HA+βTCP (n=6) SIM (n=6) Vehicle (ethyl alcohol+PBS) (n=6)	Wistar Rats / Parietal Bone	5 mm diameter	200 µg/scaffold	82:18	Densitometry Scanning electron microscopy	SIM incorporated into PLGA+HA+βTCP scaffold did not increase bone formation (p<0.05).
Ferreira et al. (2014); Brazil	66 / 66	Blank control (n=10)	Blank control with PLGA membrane (n=20) PLGA+SIM with PLGA membrane (n= 20) PLGA (n=6) PLGA with PLGA membrane (n=10)	Wistar Rats / Parietal bone	5 mm diameter	50 µg/scaffold	50:50	Scanning electron microscopy Light microscopy transmission Transmission electron microscopy Immunohistochemistry	PLGA+SIM with PLGA membrane group showed more newformed bone matrix inside the defect than blank control, blank control with PLGA membrane, PLGA or PLGA with PLGA membrane groups (Statistical analysis was not performed).
Jjang et al. (2013); China	24 / 24	Blank control (n=8)	PLGA+HA (n=8) PLGA+HA+SIM (n=8)	Wistar Rats / Calvaria bone	5 mm diameter	8 µg/scaffold	85:15	Micro-computed tomography Histological analysis	PLGA+HA+SIM group stimulated more bone formation than PLGA+HA or Blank control on 8 weeks (p<0.05).
Liu et al. (2014); China	32 / 32	PLGA (n=8)	PLGA+SIM (n=8) PLGA+SDF-1a (n=8)	Mice / Calvaria bone	4 mm diameter	35 µg/scaffold	75:25	Micro-computed tomography HE staining and	PLGA+SDF-1a+SIM promoted more bone formation than PLGA, PLGA+SDF-1a, or PLGA+SIM (p<0.05).

			PLGA+SDF-1a+SIM (n=8)					immunohistochemistry	
Mendes et al. (2017); Brazil	35 / 35	Blank control (n=7)	PLGA (n=7) PLGA+SIM (n=7) PLGA+MSC (n=7) PLGA+SIM+MSC (n=7)	Wistar Rats / Calvaria bone	8 mm diameter	40µg/scaffold	Not reported	Histomorphometric analysis Histological analysis	PLGA+SIM promoted more bone formation than PLGA, PLGA+MSC, or PLGA+SIM+MSC (p<0.05).
Zhang et al. (2015); China	30 / 30	Blank Control (n=10)	PLGA+CPC (n=10) PLGA+CPC+SIM (n=10)	Rabbits / Femur	6mm diameter x 10 mm length	100 µg/scaffold	50:50	Micro-radiograph Histological Micro-computed tomography 3D- computed tomography	PLGA+CPC + SIM scaffolds demonstrated more formed bone than PLGA+CPC or Blank control (p<0.05).

Legend:  $\beta$ TCP:  $\beta$ -tricalcium phosphate; CPC: calcium phosphate composite; HA: hydroxyapatite; HE: hematoxylin and eosin; MSC: mesenchymal stem cells; PLGA: poly(lactic-co-glycolic) acid; SDF1: stromal cell-derived factor 1; SIM: Simvastatin; Blank control: empty defect; Naïve: Incision and detachment of the periosteum. PBS: phosphate-buffered saline.

Table 2 – Risk of bias summary assessed by Systematic Review Center for Laboratory Animal Experiments (SYRCLE) tool

SYRCLE'S Quality Assessment of the Reviewed Papers Item										
STUDY	Item									
	1	2	3	4	5	6	7	8	9	10
Assaf et al. (2013)	No	Yes	Yes	Yes	No	Unclear	No	Yes	Yes	Yes
Encarnação et al. (2015)	No	Unclear	Unclear	Unclear	No	Unclear	No	Yes	Yes	No
Ferreira et al. (2014)	No	Yes	Unclear	Yes	No	Unclear	No	Yes	Yes	Yes
Jiang et al. (2013)	No	Yes	Yes	Unclear	No	Unclear	No	Yes	Yes	Yes
Liu et al. (2014)	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes
Mendes et al. (2017)	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes
Zhang et al. (2015)	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes

Legend:

- (1) Sequence generation (Was the allocation sequence adequately generated and applied?).
- (2) Baseline characteristics (Were all the animals similar at baseline [age, sex, weight]).
- (3) Allocation concealment (Was the allocation adequately concealed?).
- (4) Random housing (Were the animals randomly housed during the experiment?).
- (5) Blinding (Were the caregivers and /or investigators blinded from knowledge which intervention each animal received during the experiment?).
- (6) Random outcome assessment (Were animals selected at random for outcome assessment?).
- (7) Blinding (Was the outcome assessor blinded?).
- (8) Incomplete outcome data (Were incomplete outcome data adequately addressed?).
- (9) Selective outcome reporting (Are reports of the study free of selective outcome reporting?).
- (10) Other sources of bias (Was the study apparently free of other problems that could result in high risk of bias?).