

Systematic Review

Simvastatin Embedded into Poly(Lactic-Co-Glycolic Acid)-Based Scaffolds in Promoting Preclinical Bone Regeneration: A Systematic Review

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Abstract: Simvastatin embedded into poly(lactic-co-glycolic acid) (PLGA)-based scaffolds can stimulate bone regeneration in preclinical models. However, the ideal pharmacological dose has not been evaluated. This systematic review reports on the simvastatin doses used in preclinical studies and evaluates the regeneration of critical-sized bone defects. References were selected in a two-phase process. Electronic databases (Embase, LILACS, LIVIVO, PubMed, SCOPUS, and Web of Science) and grey literature databases (Google Scholar, Open Grey, and ProQuest) were searched until September 2022. The risk of bias was considered to be low based on the SYRCL tool. We identified four studies in rat, two in parietal and two in calvaria bone, one in mouse parietal bone, and one in rabbit femur bone. Simvastatin, ranging from 8 to 100 µg, significantly increased bone formation in five studies, as compared to the scaffold alone based on µ-computed tomography, histomorphometric, and radiography analysis. The median increase in bone formation caused by simvastatin was 2.1-fold compared to the PLGA-based scaffold alone. There was, however, no significant correlation between the relative bone gain and the doses of simvastatin ($p = 0.37$). The data suggest that relatively lower doses of simvastatin can consistently promote preclinical bone regeneration. However, the interpretation of these data must consider the heterogeneity of the PLGA-scaffolds, the defect anatomy, the observation period, and the evaluation method.

Keywords: drug delivery systems; simvastatin; bone regeneration; polylactic acid-polyglycolic acid copolymer; systematic review



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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 of installing the dental implant in the correct position [2]. Therefore, bone regeneration procedures have been performed in several clinical situations of tooth loss. However, vertical bone reconstructions are clinically unpredictable and hard to achieve. Autologous bone grafts have limitations, including the morbidity of the donor site, limited bone availability in some harvesting areas, high rates of bone remodeling, and unpredictable degradation rate over time. Consequently, approaches, including drug delivery systems with different graft materials and growth factors/active substances, have been proposed. However, despite the efforts made, biomaterials and surgical techniques that enhance bone regeneration are still lacking in clinical applications. Consequently, vertical bone augmentation is still a challenge in craniomaxillofacial region [3–5].

Among the biodegradable polymers for drug delivery, the poly(lactic-co-glycolic acid) (PLGA) has shown great potential due to its 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 properties 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 dose to achieve the desired dosage and release interval [7,11].

Simvastatin (SIM), a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is used to reduce blood cholesterol levels [11,12], and has been extensively investigated due to its pleiotropic effects, such as angiogenic, immunomodulatory, and anti-inflammatory properties [12–14]. Additionally, SIM promotes osteogenic differentiation and increases bone formation [8,15–17]. Furthermore, it is relevant to emphasize that SIM in-site applications require a suitable carrier to allow for its controlled release, thus preventing burst release, drug degradation, and exacerbated inflammatory responses [14,18].

Despite several studies reporting the efficacy of SIM embedded into PLGA-based scaffolds for bone formation, the dose of SIM required to promote bone formation through local drug delivery systems is not fully elucidated. This systematic review aimed to critically discuss the available scientific evidence to answer the following focused question: “What is the appropriate SIM dose embedded into PLGA-based scaffolds required to promote bone formation in critical-sized bone defects?”

2. Methods

2.1. Protocol and Registration

This systematic review followed the checklist Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [19]. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) under the identification number CRD42021206667.

2.2. Inclusion Criteria

The PICOS acronym (population, intervention, comparison, outcome, and type of study) was used to create the focused question of this systematic review; Population (P): Animals who received SIM embedded into PLGA-based scaffolds in critical bone defects. Intervention (I): Local delivery of SIM embedded 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 (S) were considered eligible when they met the following inclusion criteria: Evaluate the bone formation through the local delivery of SIM embedded into PLGA-based scaffolds in critical in vivo bone defects, as compared to PLGA-based scaffolds without SIM. No publication time restrictions were applied.

2.3. 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 28 days 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; and (11) duplicate data (e.g., dissertations/thesis in which correspondent published articles were available).

2.4. Information Sources

A detailed research strategy was developed for each following electronic database: Embase, Latin American and Caribbean Health Sciences (LILACS), Leibniz Information Centre for Life Sciences (LIVIVO), PubMed, SCOPUS, and Web of Science. As additional literature, a search strategy for Google Scholar web search (first 100 references), Open Grey, and ProQuest (Dissertations and Thesis) was elaborated. Other than that, reference lists of potentially relevant articles were hand-searched to identify any studies that could have been missed in the previous steps. All databases search was conducted in September 2022. Detailed search strategies are available in Figure A1. Reference lists of included studies were manually searched, as recommended by Greenhalgh and Peacock [20]. A software (EndNote X7, Thomson Reuters, Canada) was used to manage the references.

2.5. Study Selection

A two-phase selection process using online software was performed (Rayyan, Qatar Computing Research Institute, Qatar). In phase 1, 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, disagreements were solved in a consensus discussion. A third reviewer (R.B.C.) was involved in making the final decision if a consensus was not reached. If data were missing or unclear, an attempt to contact the corresponding authors was made to resolve or clarify the issue.

2.6. Data Collection Process

Data collection was performed by one author (E.B.M) and a second author (L.O.M) cross-checked the information. Disagreements were resolved by discussion. If needed, a third author (R.B.C.) was involved in making the final decision. The following data were recorded: Study characteristics (author, year, and country of publication), population characteristics (total animals/defects, control group, test group, animal species, bone defect area, and bone defect dimension), scaffold properties (SIM dose, drug delivery system, and PLA/PGA ratio), and outcome measures (analyses methods, experimental time, main findings, and *p*-value). In the case of uncertainty, the authors were contacted.

2.7. 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 [21]. 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 (Figure 1).

2.8. Summary Measures

A qualitative analysis of the results based on the quantification of the bone formation in critical defects in pre-clinical models due to the SIM embedded into PLGA-based scaffolds was performed. The articles that described the quantification of the bone formation using histological, μ -computed tomographic (μ CT), and/or radiographic analyses were considered.

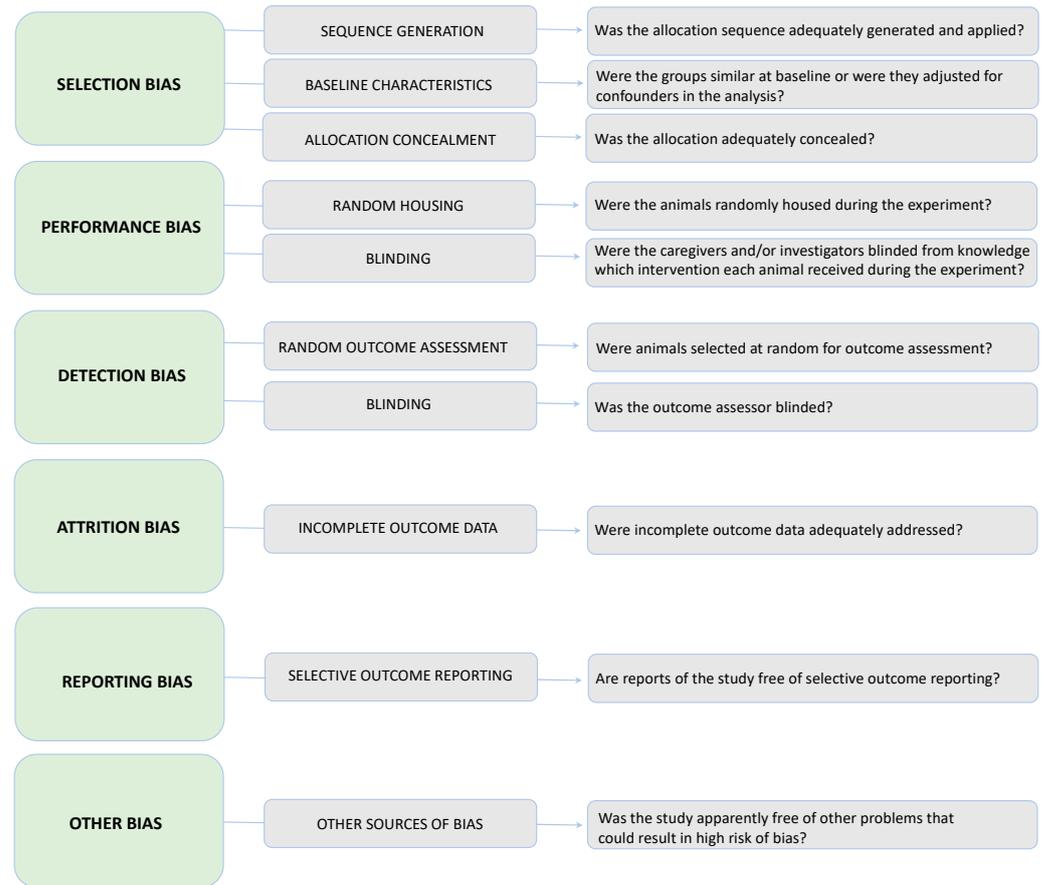


Figure 1. Graphical risk of bias summary assessed by systematic review center for laboratory animal experiments.

2.9. Synthesis of Results

A qualitative analysis of the results based on the SIM dose embedded into PLGA-based scaffolds, required to promote bone formation in critical defects in pre-clinical models (reported or calculated), was performed. The 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

In phase 1, 738 references were retrieved electronically from the following databases: EMBASE (169), LILACS (03), LIVIVO (75), PubMed (144), SCOPUS (96), Web of Science (67), ProQuest (43), Google Scholar (140), and Open Grey (01). Additional references were not identified manually. After removing duplicates, 266 references remained. Subsequently, title and abstract evaluation were performed, and 18 articles were included in phase 2 for full-text reading. Finally, after full-text analyses, six studies matched the inclusion criteria and were included for further analyses, while 12 articles were excluded (Table A1). Figure 2 shows a flowchart describing the complete process of identification, inclusion, and exclusion of studies.

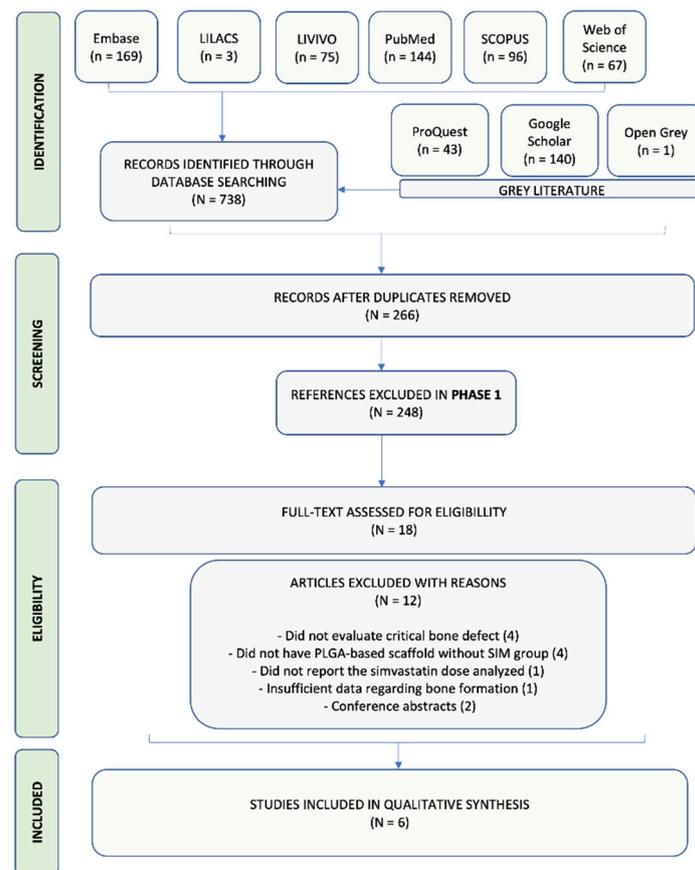


Figure 2. Flowchart of the literature search and selection criteria adapted from PRISMA. References were selected in a two-phase process. Electronic databases (Embase, LILACS, LIVIVO, PubMed, SCOPUS, and Web of Science) and grey literature databases (Google Scholar, Open Grey, and ProQuest) were searched up to September 2022.

3.2. Study Characteristics

The characteristics of the selected studies are shown in Table 2 and Figure 3. The included studies were published in the English language from 2013 up to 2017. The studies were conducted in China (3) and Brazil (3). Different experimental animal models were tested, including rats (4), rabbits (1), and mice (1). In total, 333 animals were analyzed. As expected, due to the selection criteria, all the studies evaluated critical-size defects to assess the osteogenic capacity of the implanted drug delivery systems. The bone defects were made in the parietal [22–24], calvaria [8,18], and femur [25] bones. PLGA-based scaffolds, PLGA-based scaffolds embedding SIM, and no treatment were used in the bone defects. The bone formation was evaluated by histological [8,23], μ CT [18,24,25], and radiographic [22] analyses.

Table 1. Summary of descriptive characteristics of included studies (n = 06).

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/Experimental Time	Main Findings (p Value)
Assaf K. et al. (2013) [23]; 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/28 and 56 days.	PLGA-SIM promoted more bone formation than PLGA or blank control groups on 28 and 56 days ($p < 0.05$).
Encarnação I.C. et al. (2016) [22]; Brazil	180/360	Naive (n = 6) Blank control (n = 6)	PLGA-HA-βTCP-SIM (n = 6) PLGA-HA-βTCP-SIM (n = 6) SIM (n = 6) Vehicle (ethyl alcohol + phosphate-buffered saline) (n = 6)	Wistar Rats/Parietal Bone	5 mm diameter	200 µg/scaffold	82:18	Radiographical analysis/1, 7, 15, 30, and 60 days.	SIM incorporated into PLGA-HA-βTCP scaffold did lead to bone formation ($p < 0.05$).
Jiang L. et al. (2013) [18]; 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/28 and 56 days	PLGA-HA-SIM group stimulated more bone formation than PLGA-HA or Blank control on 28 and 56 days ($p < 0.05$).

Table 2. Summary of descriptive characteristics of included studies (n = 06).

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 Meth- ods/Experimental Time	Main Findings (<i>p</i> Value)
Liu Y.S. et al. (2014) [24]; China	32/32	PLGA (n = 8)	PLGA-SIM (n = 8) PLGA-SDF1a (n = 8) PLGA-SDF1a- SIM (n = 8)	Mice/Calvaria bone	4 mm diameter	35 µg/scaffold	75:25	Micro- computed tomogra- phy/56 days	PLGA-SDF1α- SIM promoted more bone formation than PLGA, PLGA-SDF1, or PLGA-SIM on day 56 (<i>p</i> < 0.05).
Mendes J.D. et al. (2017) [8]; 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	40 µg/scaffold	Not reported	Histological analy- sis/56 days	PLGA-SIM promoted more bone formation than PLGA, PLGA-MSC, or PLGA-SIM- MSC (<i>p</i> < 0.05).
Zhang H.X. et al. (2015) [25]; China	30/30	Blank Control (n = 10)	PLGA-CPC (n = 10) PLGA-CPC-SIM (n = 10)	Rabbits/Femur	6 mm diameter × 10 mm length	100 µg/scaffold	50:50	Micro- computed tomography analysis/42 and 84 days	PLGA-CPC-SIM scaffolds promoted more bone formation than PLGA-CPC or blank control on days 42 and 84 (<i>p</i> < 0.05).

Legend: βTCP: β-tricalcium phosphate; CPC: calcium phosphate composite; HA: hydroxyapatite; MSC: mesenchymal stem cells; PLGA: poly(lactic-co-glycolic) acid; SDF1a: stromal cell-derived factor 1a; SIM: Simvastatin; Blank control: empty defect; Naive: Incision and detachment of the periosteum.

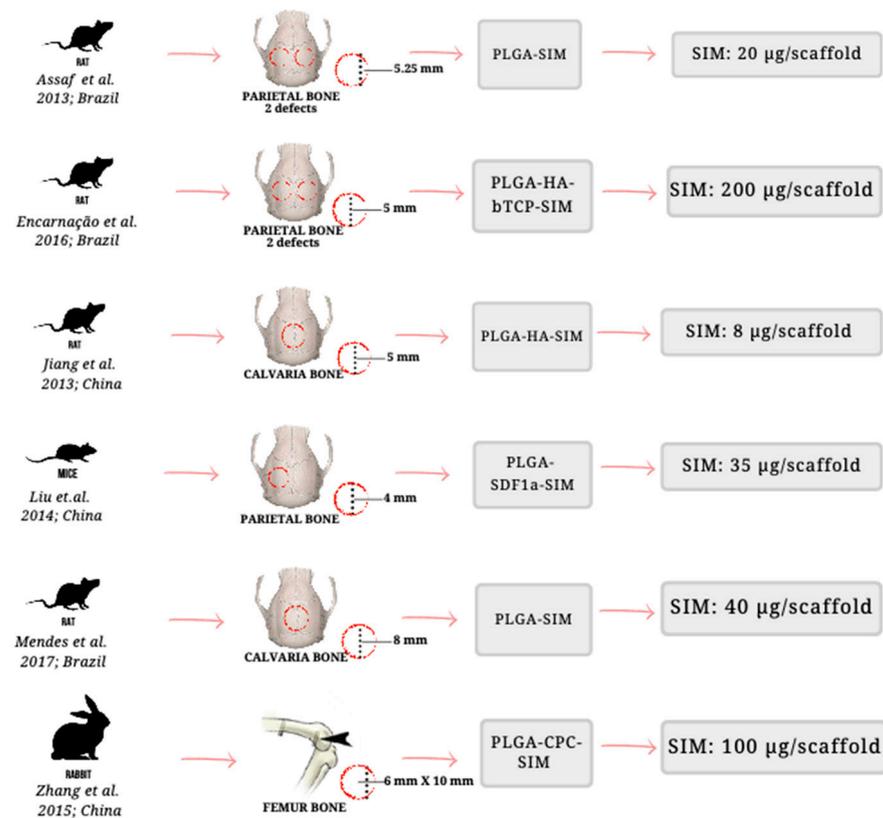


Figure 3. Scheme of characteristics of included studies (n = 6). Four studies analyzed bone regeneration in a rat model, two in the parietal and two in calvaria bones, one used mouse parietal bone, and one rabbit femur bone. Simvastatin dose ranges from 8 to 200 µg per scaffold. Assaf et al. (2013) [23], Encarnação et al. (2016) [22], Jiang et al. (2013) [18], Liu et al. (2014) [24], Mendes et al. 2017 [8], Zhang et al. 2015 [25].

Description of items:

- (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?).

3.3. Risk of Bias (RoB) in Individual Studies

The RoB was assessed using the SYRCLE tool [21]. In summary, the RoB was considered low for most items evaluated in the studies (Table 3). 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 of the received intervention of each animal during the experiment [8,18,22–25]. Additionally, the question related to the animals selected at random for outcome assessment was unclear for all the studies [8,18,22–25].

Table 3. Risk of bias summary assessed by the Systematic Review Center for Laboratory Animal Experiments.

Study	SYRCLE'S Quality Assessment of the Reviewed Papers Item									
	Item									
	1	2	3	4	5	6	7	8	9	10
Assaf K. et al. (2013) [23]	No	Yes	Yes	Yes	No	Unclear	No	Yes	Yes	Yes
Encarnação I.C. et al. (2016) [22]	No	Unclear	Unclear	Unclear	No	Unclear	No	Yes	Yes	No
Jiang L. et al. (2013) [18]	No	Yes	Yes	Unclear	No	Unclear	No	Yes	Yes	Yes
Liu Y.S. et al. (2014) [24]	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes
Mendes J.D. et al. (2017) [8]	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes
Zhang H.X. et al. (2015) [25]	No	Yes	Unclear	Unclear	No	Unclear	No	Yes	Yes	Yes

3.4. Results of Individual Studies

Assaf et al. (2013) [23] evaluated 32 male Wistar rats (250–300 g) divided in two groups (n = 16 each). In each rat, two critical-size defects of 5.3 mm in diameter were created in the dorsal part of the parietal bone. The defect on the right side was the experimental group, while the left side was the control (no treatment). In the first group, the right-side defect was filled with a PLGA scaffold, and the second group received a PLGA-based scaffold embedding SIM (20 µg/scaffold). According to the histological analysis, the PLGA-SIM group promoted the highest length of the bone formation, filling the defects on days 28 and 56 (0% and 96%, respectively, $p < 0.05$). The control group showed 38% and 52% of bone formation on days 28 and 56, respectively, while the PLGA group filled 71% of the defects on days 28 and 56.

Encarnação et al. (2016) [22] 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 in phosphate-buffered saline); PLGA-HA-βTCP; PLGA-HA-βTCP-SIM (200 µg/scaffold); and 200 µg SIM only. Radiographs were carried out and bone densitometry was determined on days 1, 7, 15, 30, and 60. On day 60, according to the gray scale values, PLGA-HA-βTCP-SIM, PLGA-HA-βTCP, naive, sham, vehicle, and SIM groups showed approximately 85, 75, 105, 72, 80, and 72 of bone densitometry, respectively ($p > 0.05$). Therefore, SIM alone or embedded into PLGA-HA-βTCP scaffolds failed to support bone formation.

Jiang et al. (2013) [18] created critical-size defects in the calvaria bone of 34 female Wistar rats (6 weeks old), randomly divided into three groups: PLGA-HA; PLGA-HA-SIM (8 µg/scaffold); and a control group that did not receive any material. On days 28 and 56 post-implantation, µCT analyses were performed to quantify bone formation. The PLGA-HA-SIM group exhibited the highest bone formation (approximately 4% and 10% on days 28 and 56, respectively, $p < 0.05$). The control group showed an average of approximately 0.5% and 1.8% of bone formation on days 28 and 56, respectively, while PLGA-HA group demonstrated 0.5% and 3.9% on days 28 and 56, respectively.

Liu et al. (2014) [24] evaluated a 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 µg/scaffold); PLGA-stromal cell-derived factor 1α (SDF1α); and PLGA-SIM-SDF1α. According to the µCT performed 42 days after the implantation, the PLGA-SIM-SDF1α group promoted the highest volume of bone formation (1.1 mm³, $p < 0.05$). Conversely, the PLGA group did not lead to bone formation. PLGA-SIM and PLGA-SDF1α showed 0.18 mm³ and 0.41 of bone formation, respectively.

Mendes et al. (2017) [8] evaluated an 8-mm bone defect in the calvaria of 35 Wistar rats (three months old) divided into five groups: control (blank default); PLGA-based scaffold; PLGA-SIM (40 µg/scaffold); PLGA-mesenchymal stem cells (MSC); and PLGA-SIM-MSC. After 56 days, according to the histomorphometric analyses, the PLGA-SIM

group promoted the highest area of bone formation ($1.5 \times 10^4 \text{ mm}^2$, $p < 0.05$). Control, PLGA, PLGA-MSC, and PLGA-SIM-MSC groups showed $4 \times 10^3 \text{ mm}^2$, $7 \times 10^3 \text{ mm}^2$, $5 \times 10^3 \text{ mm}^2$, and $2 \times 10^3 \text{ mm}^2$ of bone formation, respectively ($p > 0.05$).

Zhang et al. (2015) [25] created critical defects ($6 \text{ mm} \times 10 \text{ mm}$) 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); SIM-PLGA-CP ($100 \mu\text{g}/\text{scaffold}$). The bone formation was determined using μCT analysis on days 42 and 84. The SIM-PLGA-CPC group promoted the highest bone formation ($25.78 \pm 6.89\%$ and $68.0 \pm 11.62\%$ on days 42 and 84, respectively, $p < 0.05$). The sham-operation group showed $3.40 \pm 2.25\%$ and $6.10 \pm 4.48\%$ of bone defects repaired on days 42 and 84, respectively. The PLGA-CPC group demonstrated a bone coverage of $12.89 \pm 5.75\%$ and $29.24 \pm 9.25\%$ on days 42 and 84, respectively.

3.5. Synthesis of Results

The data were normalized and a correlation analysis was performed (Figures 4 and 5; $r = -0.48$, $p = 0.336$). These data suggest that there is no obvious impact of the SIM dose on the overall stimulation of bone regeneration. The impact of the species (mouse/rat versus rabbit) and defect location (femur versus calvaria/parietal) was also not significant ($r = -0.46$, $p = 0.429$). Thus, it is hard to predict the ideal dose of SIM using PLGA-based scaffolds. It does not require raising the SIM doses above $10 \mu\text{g}$, at least in rodent models. Nevertheless, this analysis must be interpreted with caution due to the heterogeneity of studies concerning the biomaterial composition, preclinical model, and SIM dose.

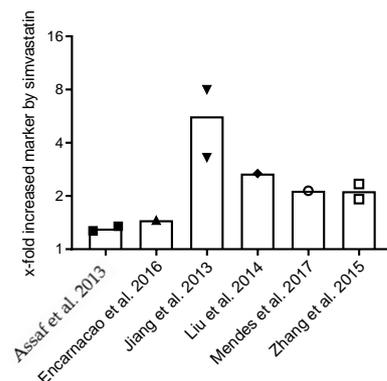


Figure 4. Summary of all normalized data showing the x-fold increase caused by the presence of simvastatin compared to the scaffold alone, independent of the evaluation method. The number of geometric shapes represents different observation times. Studies are in alphabetic order. Assaf et al. (2013) [23], Encarnação et al. (2016) [22], Jiang et al. (2013) [18], Liu et al. (2014) [24], Mendes et al. 2017 [8], Zhang et al. 2015 [25].

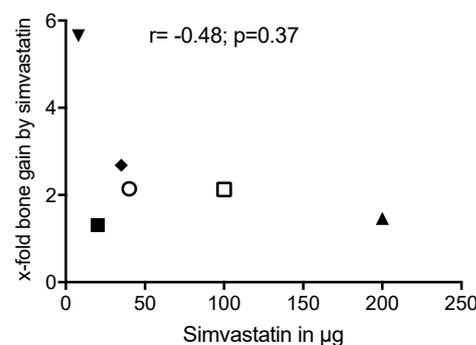


Figure 5. The correlation analysis between the x-fold bone gain by simvastatin and the simvastatin dose. The analysis showed a $r = -0.48$ and a $p = 0.336$, indicating that there is no obvious impact of the SIM dose on the overall stimulation of bone regeneration. Each geometric shape represents one study.

4. Discussion

Considering that bone regeneration is still a challenge in oral and maxillofacial surgeries, the search for biomaterials and predictable techniques that enhance bone regeneration continues [26]. Since SIM has been proposed to support bone formation, drug delivery systems were evaluated [27,28]. This systematic review evaluates the SIM dose embedded into PLGA-based scaffolds necessary to promote bone regeneration in preclinical models. We observed that, from the six included studies, five studies confirmed SIM embedded into PLGA-based scaffolds promotes bone formation. The required dose ranged from 8 to 50 μg SIM/scaffold in rodents, and 100 μg SIM/scaffold in rabbit. In one study, SIM failed to support bone regeneration. This review may contribute to the experimental design of future studies on SIM in bone regeneration.

Adequate drug release from scaffolds is critical for bone formation, and efforts to find an appropriate SIM dosing and delivery system are made [29]. High SIM doses are associated with an exacerbated inflammatory responses and impaired bone formation [24,30,31], also due to cytotoxicity and blocked cholesterol synthesis [32,33]. Conversely, low SIM doses may not reach the pharmacologically relevant concentration. In this context, three articles evaluated SIM release varying from 4% (1 day) [8], 15% (2 days) [18], to >60% (7 days) [25]. Moreover, SIM release of approximately 30% in 30 days [8], 23% in 56 days [18], and 100% in 21 days [25] was reported. Drug release from PLGA can be controlled by varying the molecular weight and the ratio of lactide to glycolide [11]. Different ratios of lactide to glycolic were used to produce the PLGA scaffolds. The proportion most often used was 50:50 [23,25,28], followed by 82:18 [22], 85:15 [18], and 75:25 [24]. One article did not report the lactide to glycolic ratio [8]. Thus, the lactide-to-glycolic ratios may affect the SIM release kinetic and, consequently, the bone regeneration capacity *in vivo*.

Concerning the RoB judgment, a low RoB was attributed to most items evaluated. 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 [34]. No study reports whether or not the allocation sequence was adequately generated and applied, as well as whether or not the caregivers/investigators and outcome assessors were blinded to the intervention [8,18,22,24,25]. The animals selected at random for outcome assessment also remained unclear [8,18,22,24,25]. Future studies should put more emphasis on reporting methodological details.

Regarding the limitations of this review, only PLGA-based scaffolds were evaluated. Thus, further studies assessing the dose and release of SIM embedded into different scaffolds are required. The included studies used μCT , histological, and radiographical analysis to quantify the bone regeneration. Due to high-resolution 3D information, μCT is the most reliable to evaluate bone regeneration [35,36]. Moreover, μCT provides information concerning the volume, texture, and external and internal structures of the implanted scaffold. Histological analysis, however, is ideal to study the cellular aspects of bone regeneration, hence any potential adverse effects of the scaffold [37]. Further studies assessing the dose of SIM embedded into scaffolds with different chemical compositions than PLGA are suggested. Additionally, the evaluation of SIM doses used clinically is proposed.

5. Conclusions

The collected data suggest that simvastatin at 10 to 100 μg /scaffold can approximately double the amount of bone regeneration in rodent and rabbit models. Moreover, the species and the location of the bone defect did not affect the simvastatin dose for stimulating bone regeneration. However, these data must be interpreted under the premise of the heterogeneity of PLGA-scaffolds, the defect anatomy, the observation period, and the evaluation method.

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Data Availability Statement: Data availability on request.

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Appendix A

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("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
"Biomineralization"[MeSH Terms] OR "Biomineralization" OR "Biomineralizations" 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")

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Figure A1. Data search strategy for the electronic databases (Embase, LILACS, LIVIVO, PubMed, SCOPUS, and Web of Science) and grey literature databases (Google Scholar, Open Grey, and ProQuest).

Table A1. Articles excluded and the reasons for exclusion (n = 12).

Reference	Author	Reasons For Exclusion *
(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)	TAI et al., 2010	5
(6)	MASAEI et al., 2016	2
(7)	NAITO et al., 2014	2
(8)	SENON et al., 2015	5
(9)	TERUKINA et al., 2016	2
(10)	VENKATESAN et al., 2019	1
(11)	ZHANG et al., 2019	1
(12)	FERREIRA et al., 2015	4

* 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. Studies with insufficient data regarding bone formation (1). 5. Conference abstracts (2).

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