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Review

3D printing with polylactic acid (PLA) in bone regeneration using animal models: a systematic review

Rafael Álvarez-Chimal [1,*], Lucía Pérez-Sánchez [1], Janeth Serrano-Bello [1], Febe Carolina Vázquez-Vázquez [2] and Marco Antonio Álvarez-Pérez [1,*]

[1] Tissue Bioengineering Laboratory, Postgraduate Studies and Research Division, Faculty of Dentistry, National Autonomous University of Mexico, University City, Coyoacán 04510, Mexico City, Mexico

[2] Laboratory for Research in Dental Materials and Biomaterials, Postgraduate Studies and Research Division, Faculty of Dentistry, National Autonomous University of Mexico, University City, Coyoacán 04510, Mexico City, Mexico

* **Correspondence:** Email: rachimal65@comunidad.unam.mx; marcoalv@unam.mx.

Abstract: Background: Bone regeneration is a critical area of regenerative medicine that faces significant challenges, such as bone defects and fractures. 3D printing offers a promising solution through customized scaffolds that mimic the natural architecture of bone and support tissue healing. Polylactic acid (PLA) is a biodegradable and biocompatible polymer widely used in biomedical 3D printing. Preclinical animal models are essential to evaluate the performance of PLA-based scaffolds before their clinical use. Objective: This systematic review aimed to assess the current applications of 3D-printed PLA scaffolds for bone regeneration in animal models, focusing on PLA, animal models, biological performance, and in vivo outcomes. Methods: A comprehensive search was conducted across databases, covering studies published between January 2009 and January 2025, following the PRISMA guidelines. Studies were included if they reported 3D-printed PLA scaffold constructs for bone regeneration that were validated in animal models. Data on the animal species, defect types, biomaterials, and outcomes were extracted and analyzed. Results: This review included 38 studies that used animal models, such as rodents, rabbits, canines, and sheep, to assess the performance of the 3D-printed PLA scaffolds. Cells and compounds such as hydroxyapatite, drugs, nanoparticles, proteins, and polymers enable active scaffold fabrication that enhances regeneration from 1 to 12 weeks on the defect created in the chosen animal model. Conclusion: 3D printing based on PLA offers significant potential for advancing bone regeneration, with promising preclinical outcomes in animal models.

Further preclinical and clinical studies are required to confirm the safety, effectiveness, and scalability for human applications.

Keywords: 3D printing; polylactic acid; bone regeneration; animal model; biomaterials; regenerative medicine

1. Introduction

Bone regeneration is a critical aspect of orthopedic and reconstructive medicine that addresses challenges such as bone defects, fractures, and diseases that impair the body's natural healing processes [1]. Traditional approaches, including grafting and implants, often face limitations, such as donor site morbidity, immune rejection, and mechanical incompatibility [2]. Recent advances in biomedical engineering have led to innovative solutions, with 3D printing emerging as a transformative technology in tissue engineering and regenerative medicine [3].

The precision and versatility of 3D printing enable the fabrication of patient-specific scaffolds and implants that mimic the natural bone's complex architecture and properties [4]. By integrating biomaterials and bioactive components, these constructs promote osteointegration and support bone healing [5]. Despite their promise, translating 3D-printed solutions from laboratory settings to clinical applications requires extensive validation in preclinical models, making animal studies fundamental in this field [6].

Polylactic acid (PLA) is one of the most widely used biodegradable polymers in 3D printing for tissue regeneration, because of its biocompatibility, ease of processing, and mechanical stability [7,8]. However, their inherent brittleness, hydrophobic nature, and limited bioactivity have driven extensive research on composite formulations to enhance their regenerative potential [9]. The incorporation of hydroxyapatite (HA) into PLA scaffolds has been widely explored to improve osteoconductivity, mimic the mineral composition of natural bone, and promote cellular attachment and differentiation [10,11]. In addition, PLA can be functionalized with bioactive molecules, such as drugs [12,13], and proteins [14,15], such as bone morphogenetic proteins (BMPs) [16], nanoparticles [17], and growth factors, such as insulin-like growth factor-1 (IGF1) [12], into PLA scaffolds, which have demonstrated enhanced osteoinduction and vascularization in preclinical models because of the controlled release systems that enhance tissue healing.

Furthermore, blending PLA with other biodegradable polymers such as polycaprolactone (PCL) [19], poly (lactic-co-glycolic acid) (PLGA) [18,19], or polyethylene glycol (PEG) [12,17] can improve mechanical flexibility, degradation kinetics, and drug-loading capacity. These hybrid scaffolds not only offer better biomimetic properties but also allow tailored degradation rates that match the needs of specific tissues [20]. The combination of PLA with ceramics [21], natural polymers [16,22,23] (e.g., collagen, gelatin, and alginate), and therapeutic agents represent a promising strategy for optimizing scaffold performance, making PLA-based 3D-printed constructs highly versatile for bone regeneration.

Animal models provide a critical platform for evaluating the biological, mechanical, and functional performances of 3D-printed constructs [24]. They help bridge the gap between in vitro research and human clinical trials by offering insights into material biocompatibility, scaffold degradation, and regenerative response of the host tissue [24,25].

Critical-sized bone defects, defined as defects that cannot heal spontaneously within an animal's lifespan, are commonly used experimental models to rigorously evaluate the osteogenic and regenerative capabilities of scaffolds [26]. The consistent use of critical-size defects across studies allows for reliable comparisons of scaffold efficacy and facilitates meaningful interpretations of their potential clinical applicability [27] (Figure 1).

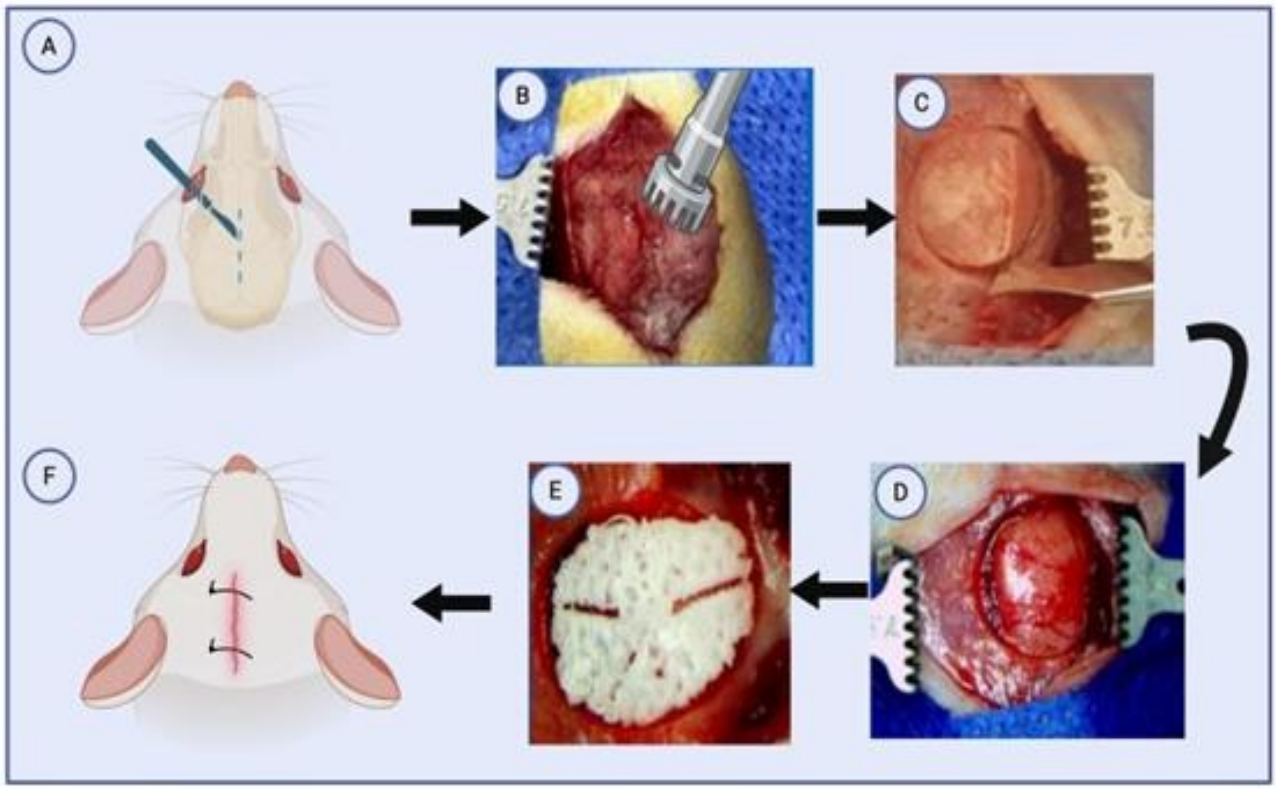

Figure 1. Critical-sized bone defect formation in a rat calvarial model. A) Selection of the area where the defect will be made and initiation of surgery. B) Exposed bone and the beginning of defect generation, created using a rotary instrument (trephine). C) Removal of the cut bone. D) Generated defect. E) Placement of the PLA-based scaffold in the defect zone. F). Closure of the defect zone. (Created and owned by the authors.)

Various species, including rodents [28], rabbits [29], and large animals, such as sheep [15], canines [30], and pigs [31], have been used to assess the biological performance of 3D-printed constructs. Rodents, particularly rats and mice, are commonly employed because of their cost-effectiveness, short lifespan, and well-characterized genetic background [32]. They are frequently used in calvarial defect models, where 3D-printed scaffolds are implanted to evaluate bone regeneration over weeks or months [32,33]. Despite their advantages, rodent models have limitations, particularly in replicating the mechanical and physiological properties of human bones [33]. Rabbits serve as intermediate models, providing larger defect sites for testing and more accurate mechanical load-bearing conditions [34]. In rabbits, critical-sized femoral and tibial defects are widely used to assess the osteointegration and biodegradation of 3D-printed biomaterials, such as polylactic acid (PLA) and composite scaffolds infused with bioactive components [35].

Larger animals, including canines, sheep, and pigs, offer a more translational approach to human bone healing because of their close anatomical and biomechanical similarities [25]. Sheep and pigs are preferred as long-bone defect models, allowing researchers to evaluate vascularization, bone remodeling, and scaffold integration under weight-bearing conditions [36]. Canines have also been used to test the maxillofacial and dental applications of 3D-printed scaffolds, particularly for periodontal and mandibular regeneration [37]. Although large animal models provide invaluable insights, their high cost, long healing time, and ethical considerations pose challenges for their widespread use [25,38].

Overall, the selection of an appropriate animal model depends on the research objectives, scaffold characteristics, and specific tissue engineering applications being studied, highlighting the importance of comparative studies to optimize the translational potential.

The evaluation of the 3D-printed PLA scaffold performance in animal models may depend on the regenerated tissue type, animal species used, and specific study objectives [39]. Tracking the behavior of the printed scaffolds in the regeneration process in animal models over time allows the evaluation of the performance of the scaffolds. Short-term studies (4–8 weeks) have typically focused on early-stage biocompatibility, inflammatory responses, and the initial cell attachment and proliferation on scaffolds. Medium-term studies (8–16 weeks) have assessed scaffold degradation, osteointegration, and progression of new tissue formation, providing insights into the performance of the material under physiological conditions. Long-term studies (16–52 weeks or more) are crucial for evaluating complete scaffold resorption, bone remodeling, and functional restoration, particularly in large animal models, where the healing process closely resembles human bone regeneration [24,40,41]. The replacement rate of PLA or its composites depends on factors such as the components added and test conditions, which can take weeks to years to be absorbed by the animal body [42]. The choice of evaluation period is critical for understanding how the scaffold supports tissue regeneration over time, ensuring that it maintains its structural integrity during the healing phase before being gradually replaced by natural tissue [43].

This systematic review aimed to comprehensively analyze the current applications of 3D printing using PLA for bone regeneration validated in animal models. This study sought to highlight advancements, identify gaps, focus on regeneration evaluation periods not considered in previous studies, and propose future directions to enhance the translational potential of 3D-printed constructs based on PLA in regenerative medicine, supported by their performance in animal models.

2. Materials and methods

The systematic review was registered in the Open Science Framework (OSF) Registries.

2.1. Study design

- Type of review: Systematic
- Focus: 3D-printed PLA scaffolds applied in bone regeneration in animal models.

2.2. Research strategy

- Databases: PubMed, Scopus, and Web of Science

- Keywords used: 3D printing, polylactic acid, PLA, bone regeneration, animal model.
- Filters applied: Original studies published from January 2009 to January 2025.

2.3. Inclusion criteria

- Studies have considered animal models for evaluating bone regeneration.
- Use of 3D printing for scaffold generation.
- Use of PLA as a base polymer for 3D printing.

2.4. Exclusion criteria

- Studies that do not involve animal models.
- In vitro studies without validation in animal models.
- Studies without the use of 3D printing.
- Studies that do not use PLA in the printing of the scaffolds.
- Duplicate studies.
- Literature and systematic reviews.

2.5. Selection process

- Elimination of duplicates.
- Selection by title and abstract.
- Complete evaluation of the text.

2.6. Quality assessment

- Use of the PRISMA guide to perform the systematic review.
- Use of the Systematic Review Centre of Laboratory Animal Experimentations (SYRCLE) Risk of Bias Tool to assess the risk of bias in the included animal studies.

3. Results

3.1 Selection of studies

The studies were selected according to the PRISMA guidelines (Figure 2).

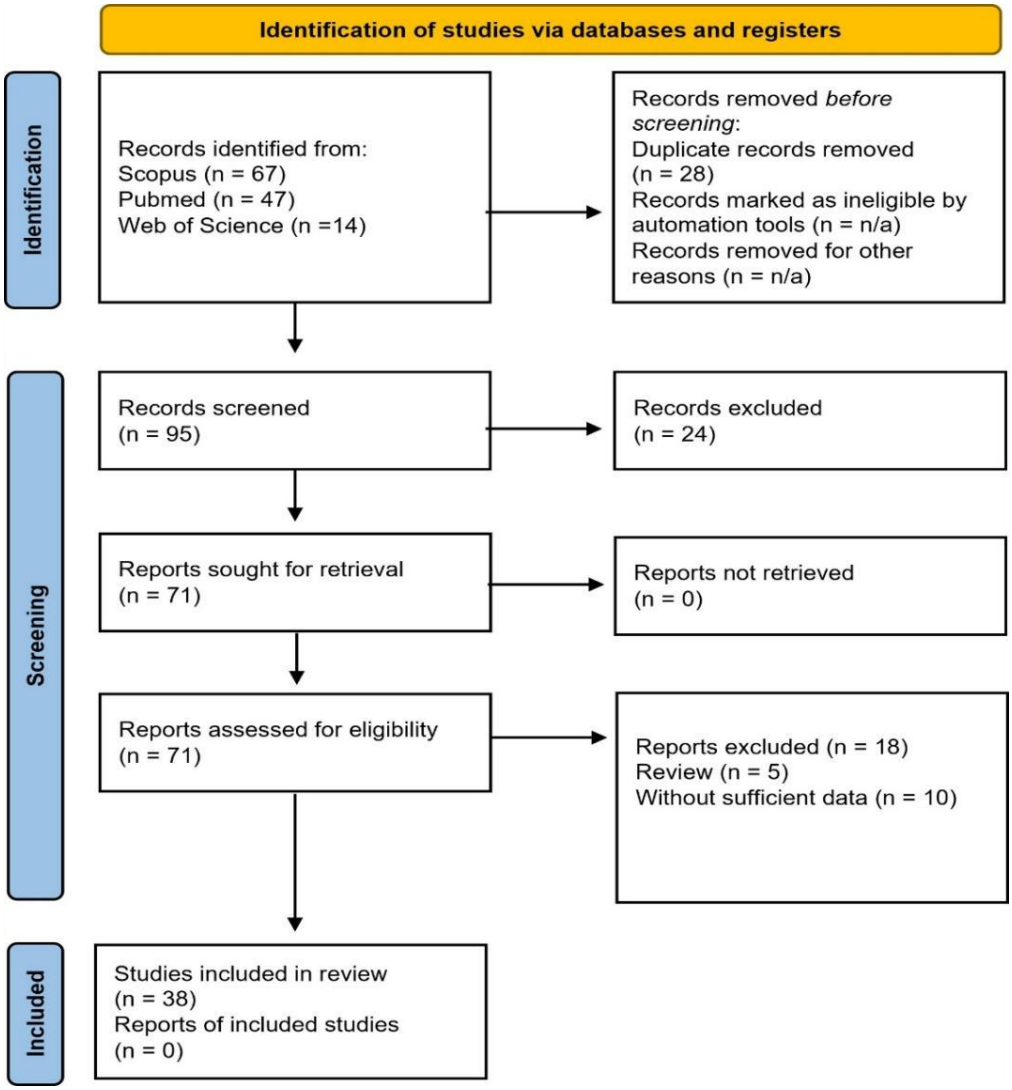

Figure 2. Flowchart for selecting studies to be considered in the systematic review.

3.2 Quality assessment

SYRCLE is a methodological approach based on the development, application, and dissemination of systematic reviews of animal studies to advance responsible animal-based research by synthesis of evidence [44]. The quality assessment of this systematic review indicated that most of the 38 included studies achieved moderate-to-high methodological quality according to the SYRCLE's Risk of Bias tool (Table 1). Domains such as incomplete outcome data (81.6% low risk), baseline characteristics (71.0% low risk), and blinding of outcome assessors (57.9% low risk) were consistently well addressed. Likewise, random outcome assessment (55.3%) and random housing (52.6%) were judged as low risk in more than half of the studies, suggesting efforts to reduce bias. However, some methodological gaps persisted: sequence generation and allocation concealment were insufficiently reported in a proportion of studies, while blinding of caregivers/investigators was inconsistently described. Overall, these findings suggest that, although limitations remain in reporting and methodological transparency, the body of evidence supporting PLA-based scaffolds in animal models is based on studies of acceptable-to-high quality, strengthening confidence in the observed regenerative outcomes.

Table 1. Results of the quality assessment using the SYRCLE Risk of Bias tool.

SYRCLE domain	Low risk (n, %)	High risk (n, %)	Unclear risk (n, %)
Sequence generation (randomization)	19 (50.0%)	4 (10.5%)	15 (39.5%)
Baseline characteristics	27 (71.0%)	3 (7.9%)	8 (21.1%)
Allocation concealment	15 (39.5%)	5 (13.2%)	18 (47.3%)
Random housing	20 (52.6%)	6 (15.8%)	12 (31.6%)
Blinding of caregivers/investigators	16 (42.1%)	8 (21.1%)	14 (36.8%)
Random outcome assessment	21 (55.3%)	4 (10.5%)	13 (34.2%)
Blinding of outcome assessor	22 (57.9%)	5 (13.2%)	11 (28.9%)
Incomplete outcome data	31 (81.6%)	2 (5.3%)	5 (13.1%)
Selective outcome reporting	25 (65.8%)	3 (7.9%)	10 (26.3%)
Other bias	23 (60.5%)	6 (15.8%)	9 (23.7%)

3.3 Characteristics of the included studies

3.3.1 The use of PLA in 3D printing

Only one study in this systematic review evaluated PLA alone, highlighting its potential as a polymer that can lead to osteoinductive performance (Figure 3). However, 37 studies evaluated PLA combined with other compounds, such as hydroxyapatite, drugs, nanoparticles, growth factors, proteins, cells, or other biodegradable polymers (Table 2) to enhance its properties, biocompatibility, and osteoinductive potential. This finding suggests that, while PLA is a promising base material for 3D-printed scaffolds, its combination with bioactive components is essential for optimizing its effectiveness in tissue regeneration.

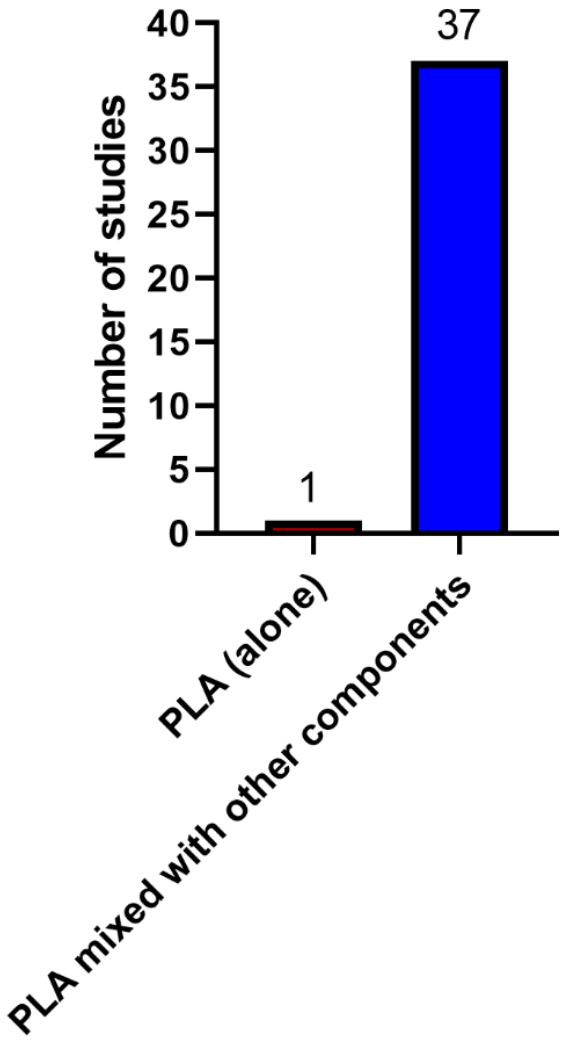

Figure 3. Studies that involve PLA alone and PLA mixed with other components.

Most studies have combined PLA with various bioactive compounds, primarily drugs, nanoparticles, organic compounds, and plant extracts to enhance its biological functionality and therapeutic potential. These modifications were followed by the incorporation of hydroxyapatite, which improved osteoconductivity [45], making the scaffolds more suitable for bone regeneration. Additionally, several studies have explored the combination of PLA with other polymers, such as polyethylene glycol (PEG), poly ε-caprolactone (PCL), polyglycolic acid (PGA), chitosan, hyaluronic acid, and collagen, to enhance the degradation rates [46], hydrophilicity [47], and strength [48]. However, very few studies have included the integration of cells or bioactive compounds, such as proteins, growth factors, or extracellular matrix components, despite their potential to significantly enhance osteoinduction and tissue regeneration [15] (Table 2). This suggests that synthetic and natural additives are preferred for extensively optimizing PLA-based scaffolds.

Table 2 summarizes the different components incorporated into PLA for printing scaffolds, grouped by their structural relationship, bioactivity, or composition.

In 37 studies that combined PLA with other components, more than one component was combined to reinforce the properties of the scaffold (Figure 4).

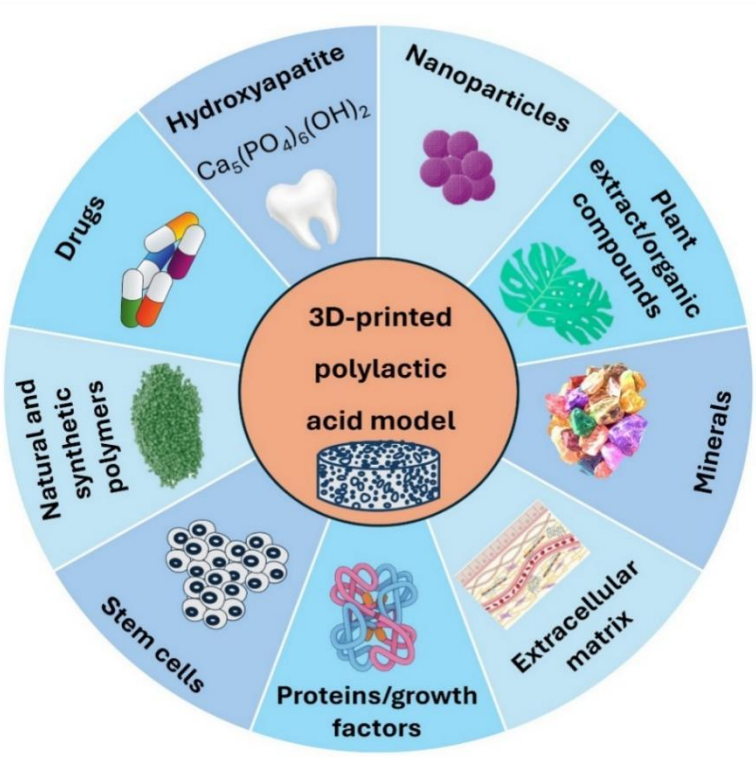

Figure 4. Compounds added to the 3D-printed PLA models to reinforce their properties. (Created and owned by the authors.)

Table 2. Compounds added to the 3D-printed PLA scaffold.

Compounds	Number of studies
Drugs, nanoparticles, minerals, extracts, and organic compounds	20
Hydroxyapatite (nanoparticles and bulk)	17
Polymers (PEG, PCL, PGA, chitosan, collagen, gelatine, and hyaluronic acid)	16
Cells	8
Proteins, growth factors, and extracellular matrix components	8

3.3.2 Animal models

This systematic review revealed that the rat calvarial model was the most frequently used model, with 19 studies employing this approach to evaluate PLA-based scaffolds for bone regeneration. This preference is likely due to the well-established protocol, cost-effectiveness, and ability to assess osteointegration in a controlled environment [32,33]. Rabbit models have also been widely utilized, particularly in femoral (4 studies), cranial (4 studies), tibial (2 studies), and radial (3 studies) defect models, highlighting their relevance in studying weight-bearing bone regeneration [34]. The use of rat

femur (2 studies) and rat radial (1 study) models was comparatively less common, suggesting a more limited application of these models in PLA-based scaffold research. Additionally, a few studies have explored alternative models, including osteochondral tissue in mice (1 study), beagle dogs (1 study), and sheep (1 study), which are likely to assess different zones of administration, long-term performance, scaffold degradation, and stability under more physiologically relevant conditions [25] (Figure 5) (Table 3).

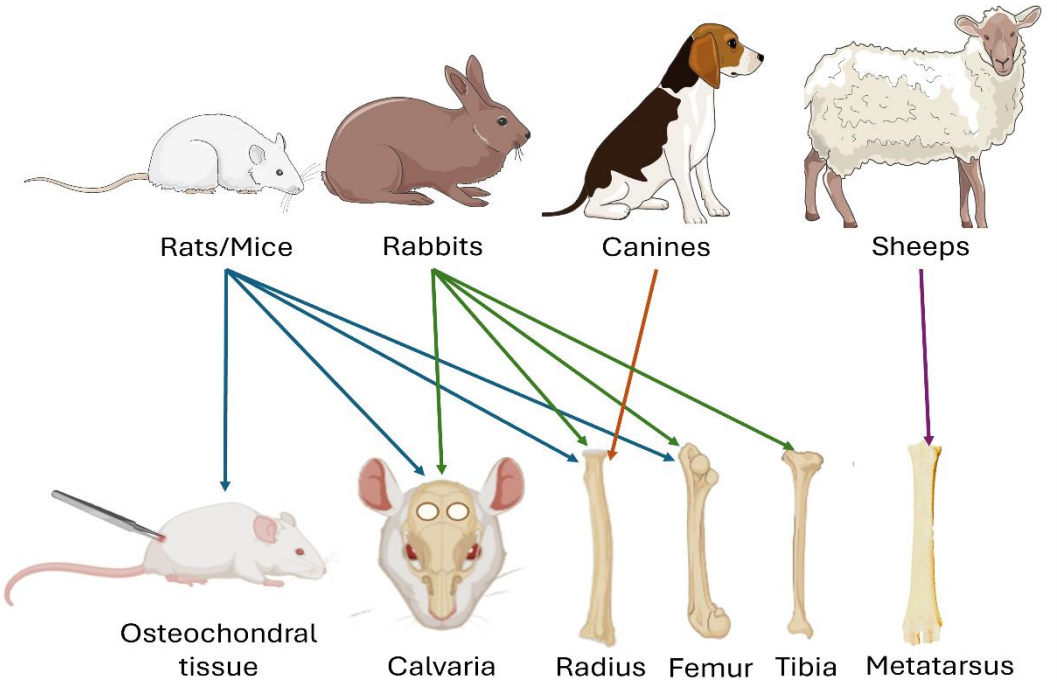

Figure 5. The most common animal models used to evaluate the regeneration process of 3D-printed PLA models in vivo. (Created and owned by the authors.)

Variations in animal models highlight the importance of selecting an appropriate model based on specific research objectives, ranging from early-stage biocompatibility assessments in rodents to large animal models that better simulate human bone regeneration.

Table 3. Animal models considered in the studies.

Animal model	Number of studies
Rat calvaria	19
Rabbit femur	4
Rabbit calvaria	4
Rabbit radius	3
Rabbit tibia	2
Rat femur	2
Beagle radius	1
Mouse osteochondral tissue (subcutaneous implantation)	1
Rat radius	1
Sheep metatarsus	1

3.3.3 Biological performance

All studies included in this systematic review consistently confirmed positive outcomes regarding the performance of PLA-based scaffolds in bone regeneration. Specifically, the scaffolds could effectively stimulate and accelerate the repair process of the defects, resulting in complete bone repair and formation of new bone tissue, accompanied by enhanced vascularization (Figure 5) [49]. Additionally, the scaffolds exhibited favorable biological properties, such as regulating inflammatory and immune responses and alleviating tissue hypoxia [16,50], thereby creating an optimal environment for tissue regeneration.

Histological assessments further indicated that bone regeneration typically occurred progressively from the edges of the defect toward the center, demonstrating a consistent healing pattern [14]. Importantly, none of the studies reported any adverse effects associated with scaffold implantation, thereby supporting the safety and biocompatibility of PLA-based materials for potential clinical applications.

Some studies have evaluated specific treatment times to evaluate the efficacy of PLA-based scaffolds. Of the studies analyzed, 30 explicitly included treatment timeframes, allowing for a detailed assessment of scaffold performance over defined periods. These studies have facilitated a better understanding of the degradation rates, bone regeneration progression, and long-term biocompatibility. However, 8 studies did not specify the treatment duration, limiting the ability to compare outcomes across different experimental designs and reducing the reliability of the conclusions regarding scaffold effectiveness over time (Figure 6).

The lack of standardized reporting on treatment duration highlights a critical gap in current research, emphasizing the need for future studies to consistently document experimental timelines to enhance reproducibility and facilitate comparisons between different studies.

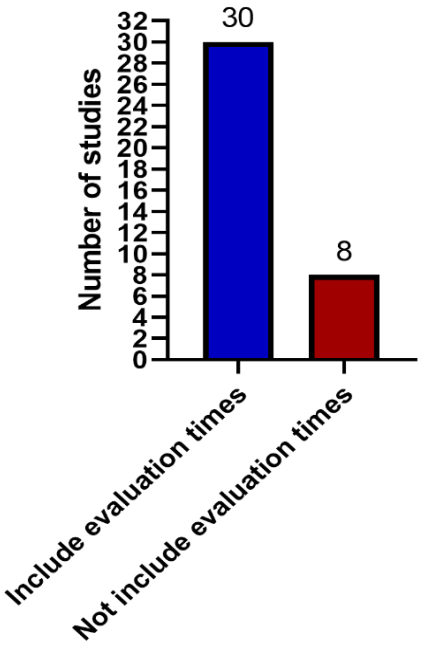

Figure 6. Studies that included or did not include evaluation times of the scaffold in animal models.

Among the studies that included treatment duration, considerable variation was observed in the evaluation time points across the different animal models. The rat calvarial model was the most extensively studied model, with evaluations conducted from 1 to 12 weeks, suggesting a focus on both short- and medium-term bone regeneration. The rat femur model had only one study reporting a treatment duration of 6 weeks, limiting comparative insights into its long-term performance. In the rabbit models, calvarial defect studies included evaluations at 4 and 12 weeks, whereas the radial defect model was assessed at 8 weeks. The rabbit femur model, which is more relevant for weight-bearing bone regeneration, was evaluated at 4, 8, and 12 weeks, providing a broader temporal assessment of the scaffold performance. These variations highlight the diversity in study designs and the standardized evaluation periods focused on comparing the results and optimizing the translation of PLA-based scaffolds for clinical applications (Table 4).

Of the ten animal models included in this systematic review, only five reported specific evaluation time points, limiting the ability to compare the temporal effectiveness of PLA-based scaffolds across all studies. The models that included treatment duration were the rat calvarial, rat femur, rabbit calvarial, rabbit radial, and rabbit femur. These studies have provided crucial insights into scaffold performance over different periods, ranging from early assessments at 1 week to long-term evaluations at 12 weeks. However, the absence of time-specific data in rabbit tibial, beagle, sheep, mouse osteochondral tissue, and rat radial models reduces the ability to comprehensively analyze long-term degradation rates and tissue regeneration in large animals, such as canines and sheep, and different bones, such as the tibia.

Table 4. Number of studies that reported evaluation times for the different animal models considered in this systematic review.

Evaluation times (weeks)	Rat calvaria	Rat femur	Rabbit calvaria	Rabbit radius	Rabbit femur
1	2	-	-	-	-
4	3	-	1	-	3
6	-	1	-	-	-
8	10	-	-	1	3
12	4	-	3	-	3

4. Discussion

This systematic review demonstrates that PLA-based scaffolds are widely used in bone tissue engineering, often in combination with other bioactive compounds, to enhance their mechanical and biological properties. Most studies have incorporated PLA with hydroxyapatite, polymers such as PEG and chitosan, or bioactive molecules such as drugs and nanoparticles, demonstrating that pure PLA alone may not support optimal bone regeneration [51,52].

Incorporating drugs such as anti-inflammatory agents, antibiotics, or osteogenic stimulators into PLA scaffolds enhances bone healing by providing local and sustained release at the defect site [53]. This modification not only accelerates the resolution of inflammation but also reduces the risk of infection and stimulates osteoblastic activity [54]. Drug-loaded PLA scaffolds are particularly beneficial for critical-size defects, where systemic administration alone is insufficient to promote complete healing.

Nanoparticles, such as hydroxyapatite, and minerals, such as magnesium silicate, hardystonite, and wollastonite, are used as additives because of their chemical similarity to the mineral phase of bone. Hydroxyapatite improves osteoconductivity and enhances the mechanical stiffness of the scaffold, which is crucial for load-bearing and larger defects [55]. Additionally, other nanoparticles can contribute to antimicrobial effects and improve bioactivity [56,57]. Their role is particularly relevant in critical-sized defects, where mechanical reinforcement and robust osteoconduction are required.

Blending PLA with polymers such as PEG, PCL, PGA, chitosan, collagen, gelatin, or hyaluronic acid modifies the mechanical and degradation properties of the scaffold [58]. Hydrophilic polymers (PEG, hyaluronic acid) improve wettability and cell attachment [59], whereas natural biopolymers (chitosan, collagen, and gelatin) enhance biocompatibility and mimic extracellular matrix components, thereby promoting osteogenesis and angiogenesis. These modifications are particularly advantageous for medium-to-large defects because they balance scaffold resorption with new bone formation and reduce the risk of fibrous tissue infiltration [60].

The incorporation of bioactive plant extracts or small organic molecules contributes to antioxidant, anti-inflammatory, and osteogenic effects [61]. These compounds can modulate the local microenvironment, reduce oxidative stress, and promote vascularization, which are essential for stable bone regeneration [62]. While evidence is still emerging, these additives may be particularly useful in challenging healing environments such as infected or hypoxic large defects [63].

Using cells, proteins, growth factors, and extracellular matrix components is the most biologically potent strategy, involving seeding PLA scaffolds with osteoprogenitor cells, stem cells, or incorporating proteins, growth factors (BMP-2, VEGF), and extracellular matrix components [64]. These biologically active modifications provide osteogenic and angiogenic signals, which directly enhance bone formation and vascular ingrowth [65]. Although technically complex, these approaches have shown the greatest potential to achieve full regeneration of critical-size defects, particularly in calvarial or long-bone models, by mimicking the natural bone healing cascade [66].

The predominant use of rat calvarial models suggests a focus on small, controlled defect studies, which provide valuable insights into scaffold integration and early bone formation but may not fully replicate the complexities of weight-bearing bone healing [67]. The inclusion of larger animal models, such as rabbits, canines, and sheep, although less frequent, is essential for assessing the stability and long-term performance of the scaffold, which is crucial for clinical translation.

One of the strengths of the included studies was the diversity of the animal models used, ranging from small rodents to large mammals, which allowed for a comprehensive understanding of scaffold performance under different physiological conditions [68]. Furthermore, combining PLA with bioactive materials enhances osteogenic potential, which is a significant step toward clinical application. However, there were notable limitations to the analyzed studies. A few studies did not specify the treatment duration, making it difficult to compare outcomes over time. The lack of standardized reporting on treatment duration highlights a critical gap in current research, emphasizing the need for future studies to consistently document experimental timelines to enhance reproducibility and facilitate comparisons between different studies. Very few studies have incorporated cell-based or protein-based strategies that could further enhance bone regeneration [69–71]. Although preclinical models provide valuable insights, differences in bone metabolism and healing between animals and humans must be carefully considered before their clinical application [72,73].

The findings of this review emphasize the need for further research before PLA-based scaffolds

can be widely used in the clinical setting. Future studies should prioritize large animal models that better mimic human bone physiology to facilitate translation into human applications, particularly under weight-bearing conditions. Additionally, more research is needed to evaluate the long-term degradation of PLA scaffolds, their impact on surrounding tissues, and the controlled release of bioactive molecules to enhance bone healing. Integrating standardized protocols, including defined evaluation times and consistent defect models, will improve the comparability of studies and support regulatory approval for clinical trials [74]. Although PLA-based 3D-printed scaffolds show significant promise for bone regeneration, further optimization is necessary to ensure their safety, efficacy, and scalability in human applications. Nonetheless, their use involves important logistical and economic challenges, including animal availability, handling requirements, and associated costs [25,75].

5. Conclusions

This systematic review highlights the widespread use of PLA-based 3D-printed scaffolds for bone regeneration in animal models, emphasizing the importance of combining PLA with bioactive compounds, such as hydroxyapatite, cells, proteins, polymers (PEG, chitosan, and collagen), nanoparticles, and therapeutic agents to enhance their biological properties. Most studies have focused on rat calvarial models, whereas few have explored weight-bearing bone regeneration in larger animals, limiting direct translational applications to human clinical settings. Additionally, while many studies included specific treatment durations, a few did not report this information, reducing the ability to compare outcomes over time.

Future research should optimize scaffold composition to improve osteoinductive and osteoconductive properties while ensuring controlled degradation rates. The use of larger animal models that better mimic human bone physiology is essential to evaluate long-term efficacy. Standardized methodologies, including consistent defect models, scaffold fabrication techniques, and evaluation time points, will enhance the reproducibility and comparability of results. Furthermore, incorporating cell-based therapies, growth factors, and controlled drug delivery systems can significantly enhance the performance of the scaffolds. Although PLA-based 3D-printed scaffolds show promising potential for bone tissue engineering, further preclinical and clinical studies are required to ensure their safety, effectiveness, and scalability for human applications.

Use of AI tools declaration

The authors declare they have not used Artificial Intelligence (AI) tools in the creation of this article.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Study concept and design: Rafael Álvarez-Chimal and Lucía Pérez-Sánchez; analysis and interpretation of data: Rafael Álvarez-Chimal and Lucía Pérez-Sánchez; drafting of the manuscript: Janeth Serrano-Bello and Marco Antonio Álvarez-Pérez; critical revision of the manuscript for important intellectual content: Janeth Serrano-Bello, Febe Carolina Vázquez-Vázquez, and Marco Antonio Álvarez-Pérez; statistical analysis: Rafael Álvarez-Chimal and Lucía Pérez-Sánchez.

References

1. Henkel J, Woodruff MA, Epari DR, et al. (2013) Bone regeneration based on tissue engineering conceptions—a 21st century perspective. *Bone Res* 1: 216–248. https://doi.org/10.4248/BR201303002
2. De Pace R, Molinari S, Mazzoni E, et al. (2025) Bone regeneration: a review of current treatment strategies. *J Clin Med* 14: 1838. https://doi.org/10.3390/jcm14061838
3. Tracy T, Wu L, Liu X, et al. (2023) 3D printing: innovative solutions for patients and pharmaceutical industry. *Int J Pharm* 631: 122480. https://doi.org/10.1016/j.ijpharm.2022.122480
4. Gao Y, Lalevée J, Simon-Masseron A (2023) An overview on 3D printing of structured porous materials and their applications. *Adv Mater Technol* 8: 2300377. https://doi.org/10.1002/admt.202300377
5. Szwed-Georgiou A, Płociński P, Kupikowska-Stobba B, et al. (2023) Bioactive materials for bone regeneration: biomolecules and delivery systems. *ACS Biomater Sci Eng* 9: 5222–5254. https://doi.org/10.1021/acsbiomaterials.3c00609
6. Tosca EM, Ronchi D, Facciolo D, et al. (2023) Replacement, reduction, and refinement of animal experiments in anticancer drug development: the contribution of 3D in vitro cancer models in the drug efficacy assessment. *Biomedicines* 11: 1058. https://doi.org/10.3390/biomedicines11041058
7. Balla E, Daniilidis V, Karlioti G, et al. (2021) Balla E, Daniilidis V, Karlioti G, et al. Poly (lactic Acid): a versatile biobased polymer for the future with multifunctional properties—from monomer synthesis, polymerization techniques and molecular weight increase to PLA applications. *Polymers* 13: 1822. https://doi.org/10.3390/polym13111822
8. Wu Y, Gao X, Wu J, et al. (2023) Biodegradable polylactic acid and its composites: characteristics, processing, and sustainable applications in sports. *Polymers* 15: 3096. https://doi.org/10.3390/polym15143096
9. Södergård A, Stolt M (2002) Properties of lactic acid based polymers and their correlation with composition. *Prog Polym Sci* 27: 1123–1163. https://doi.org/10.1016/S0079-6700(02)00012-6
10. Gunathilake TMSU, Ching YC, Chuah CH, et al. (2020) Electro-stimulated release of poorly water-soluble drug from poly (lactic acid)/carboxymethyl cellulose/ZnO nanocomposite film. *Pharm Res* 37: 178. https://doi.org/10.1007/s11095-020-02910-z

11. Kronemberger GS, Palhares TN, Rossi AM, et al. (2023) A synergic strategy: adipose-derived stem cell spheroids seeded on 3D-printed PLA/CHA scaffolds implanted in a bone critical-size defect model. *J Funct Biomater* 14: 555. https://doi.org/10.3390/jfb14120555
12. Salehi S, Ghomi H, Hassanzadeh-Tabrizi SA, et al. (2025) Antibacterial and osteogenic properties of chitosan-polyethylene glycol nanofibre-coated 3D printed scaffold with vancomycin and insulin-like growth factor-1 release for bone repair. *Int J Biol Macromol* 298: 139883. https://doi.org/10.1016/j.ijbiomac.2025.139883
13. Kazemi N, Hassanzadeh-Tabrizi SA, Koupaei N, et al. (2024) Highly porous sildenafil loaded polylactic acid/polyvinylpyrrolidone based 3D printed scaffold containing forsterite nanoparticles for craniofacial reconstruction. *Int J Biol Macromol* 282: 137255. https://doi.org/10.1016/j.ijbiomac.2024.137255
14. Zhang X, Lou Q, Wang L, et al. (2019) Immobilization of BMP-2-derived peptides on 3D-printed porous scaffolds for enhanced osteogenesis. *Biomedical Materials* 15: 015002. https://doi.org/10.1088/1748-605X/ab4c78
15. Garot C, Schoffit S, Monfoulet C, et al. (2023) 3D-printed osteoinductive polymeric scaffolds with optimized architecture to repair a sheep metatarsal critical-size bone defect. *Adv Healthc Mater* 12: 2301692. https://doi.org/10.1002/adhm.202301692
16. Hong YR, Kim T-H, Lee K, et al. (2023) Bioactive bone substitute in a rabbit ulna model: preclinical study. *Tissue Eng Regen Med* 20: 1205–1217. https://doi.org/10.1007/s13770-023-00591-4
17. Salehi S, Ghomi H, Hassanzadeh-Tabrizi SA, et al. (2024) 3D printed polylactic acid/polyethylene glycol/bredigite nanocomposite scaffold enhances bone tissue regeneration via promoting osteogenesis and angiogenesis. *Int J Biol Macromol* 281: 136160. https://doi.org/10.1016/j.ijbiomac.2024.136160
18. Oryan A, Hassanajili S, Sahvieh S (2021) Effectiveness of a biodegradable 3D polylactic acid/poly(ε-caprolactone)/hydroxyapatite scaffold loaded by differentiated osteogenic cells in a critical-sized radius bone defect in rat. *J Tissue Eng Regen Med* 15: 150–162. https://doi.org/10.1002/term.3158
19. Shuai C, Yang W, Feng P, et al. (2021) Accelerated degradation of HAP/PLLA bone scaffold by PGA blending facilitates bioactivity and osteoconductivity. *Bioact Mater* 6: 490–502. https://doi.org/10.1016/j.bioactmat.2020.09.001
20. Zhang H, Mao X, Du Z, et al. (2016) Three dimensional printed macroporous polylactic acid/hydroxyapatite composite scaffolds for promoting bone formation in a critical-size rat calvarial defect model. *Sci Technol Adv Mater* 17: 136–148. https://doi.org/10.1080/14686996.2016.1145532
21. Tcacencu I, Rodrigues N, Alharbi N, et al. (2018) Osseointegration of porous apatite-wollastonite and poly(lactic acid) composite structures created using 3D printing techniques. *Mater Sci Eng* 90: 1–7. https://doi.org/10.1016/j.msec.2018.04.022
22. Bahraminasab M, Doostmohammadi N, Talebi A, et al. (2022) 3D printed polylactic acid/gelatin-nano-hydroxyapatite/platelet-rich plasma scaffold for critical-sized skull defect regeneration. *Biomed Eng Online* 21: 86. https://doi.org/10.1186/s12938-022-01056-w
23. Wang Y, Zhou X, Jiang J, et al. (2025) Carboxymethyl chitosan-enhanced multi-level microstructured composite hydrogel scaffolds for bone defect repair. *Carbohydr Polym* 348: 122847. https://doi.org/10.1016/j.carbpol.2024.122847

24. Mukherjee P, Roy S, Ghosh D, et al. (2022) Role of animal models in biomedical research: a review. *Lab Anim Res* 38: 18. https://doi.org/10.1186/s42826-022-00128-1
25. Ribitsch I, Baptista PM, Lange-Consiglio A, et al. (2020) Large animal models in regenerative medicine and tissue engineering: to do or not to do. *Front Bioeng Biotechnol* 8: 972. https://doi.org/10.3389/fbioe.2020.00972
26. Schemitsch EH (2017) Size matters: defining critical in bone defect size! *J Orthop Trauma* 31: S20–S22. https://doi.org/10.1097/BOT.0000000000000978
27. Nauth A, Schemitsch E, Norris B, et al. (2018) Critical-size bone defects: is there a consensus for diagnosis and treatment? *J Orthop Trauma* 32: S7–S11. https://doi.org/10.1097/BOT.0000000000001115
28. Pérez-Sánchez L, Ortiz de la O MA, González-Alva P, et al. (2021) In vivo study on bone response to 3D-printed constructs designed from microtomographic images. *J Mater Eng Perform* 30: 5005–5012. https://doi.org/10.1007/s11665-021-05585-8
29. Fernández IA, Haugen HJ, Nogueira LP, et al. (2024) Bone regeneration in rabbit cranial defects: 3D printed polylactic acid scaffolds gradually enriched with marine bioderived calcium phosphate. *Materialia (Oxf)* 38: 102240. https://doi.org/10.1016/j.mtla.2024.102240
30. Yun J, Heo S, Lee M, et al. (2019) Evaluation of a poly(lactic-acid) scaffold filled with poly(lactide-co-glycolide)/hydroxyapatite nanofibres for reconstruction of a segmental bone defect in a canine model. *Vet Med (Praha)* 64: 531–538. https://doi.org/10.17221/80/2019-VETMED
31. Hou N, Du X, Wu S (2022) Advances in pig models of human diseases. *Animal Model Exp Med* 5: 141–152. https://doi.org/10.1002/ame2.12223
32. Vandamme T (2014) Use of rodents as models of human diseases. *J Pharm Bioallied Sci* 6: 2. https://doi.org/10.4103/0975-7406.124301
33. Gomes PS, Fernandes MH (2011) Rodent models in bone-related research: the relevance of calvarial defects in the assessment of bone regeneration strategies. *Lab Anim* 45: 14–24. https://doi.org/10.1258/la.2010.010085
34. Schlund M, Depeyre A, Kotagudda Ranganath S, et al. (2022) Rabbit calvarial and mandibular critical-sized bone defects as an experimental model for the evaluation of craniofacial bone tissue regeneration. *J Stomatol Oral Maxillofac Surg* 123: 601–609. https://doi.org/10.1016/j.jormas.2021.12.001
35. Thomas B, Bhat K, Mapara M (2012) Rabbit as an animal model for experimental research. *Dent Res J (Isfahan)* 9: 111. https://doi.org/10.4103/1735-3327.92960
36. Dias IR, Viegas CA, Carvalho PP (2018) Large animal models for osteochondral regeneration, In: Oliveira J, Pina S, Reis R, et al., *Osteochondral Tissue Engineering: Challenges, Current Strategies, and Technological Advances*, Cham: Springer, 441–501. https://doi.org/10.1007/978-3-319-76735-2_20
37. Kim C, Um Y, Chai J, et al. (2011) A canine model for histometric evaluation of periodontal regeneration. *Periodontol 2000* 56: 209–226. https://doi.org/10.1111/j.1600-0757.2010.00372.x
38. de Silva L, Longoni A, Staubli F, et al. (2023) Bone regeneration in a large animal model featuring a modular off-the-shelf soft callus mimetic. *Adv Healthc Mater* 12: 2301717. https://doi.org/10.1002/adhm.202301717
39. Cha M, Jin Y-Z, Park JW, et al. (2021) Three-dimensional printed polylactic acid scaffold integrated with BMP-2 laden hydrogel for precise bone regeneration. *Biomater Res* 25: 35. https://doi.org/10.1186/s40824-021-00233-7

40. Van Norman GA (2019) Limitations of animal studies for predicting toxicity in clinical trials. *JACC Basic Transl Sci* 4: 845–854. https://doi.org/10.1016/j.jacbts.2019.10.008
41. van der Laan JW (2000) Current status and use of short/medium-term models for assessment of carcinogenicity of human pharmaceuticals: regulatory perspectives. *Toxicol Lett* 112–113: 567–572. https://doi.org/10.1016/S0378-4274(99)00228-3
42. Feng P, Jia J, Liu M, et al. (2021) Degradation mechanisms and acceleration strategies of poly (lactic acid) scaffold for bone regeneration. *Mater Des* 210: 110066. https://doi.org/10.1016/j.matdes.2021.110066
43. Tie S, Chen Y, Tan M (2024) An evaluation of animal models for using bioactive compounds in the treatment of inflammatory bowel disease. *Food Front* 5: 474–493. https://doi.org/10.1002/fft2.360
44. Hooijmans CR, Rovers MM, de Vries RB, et al. (2014) SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol* 14: 43. https://doi.org/10.1186/1471-2288-14-43
45. Anbu RT, Suresh V, Gounder R, et al. (2019) Comparison of the efficacy of three different bone regeneration materials: an animal study. *Eur J Dent* 13: 022–028. https://doi.org/10.1055/s-0039-1688735
46. Yao X, Yang X, Lu Y, et al. (2024) Review of the synthesis and degradation mechanisms of some biodegradable polymers in natural environments. *Polymers* 17: 66. https://doi.org/10.3390/polym17010066
47. Wang W, Wang L, Zhang B, et al. (2024) 3D printing of personalized magnesium composite bone tissue engineering scaffold for bone and angiogenesis regeneration. *Chem Eng J* 484: 149444. https://doi.org/10.1016/j.cej.2024.149444
48. Yetiş F, Liu X, Sampson WW, et al. (2023) Biodegradation of composites of polylactic acid and microfibrillated lignocellulose. *J Polym Environ* 31: 698–708. https://doi.org/10.1007/s10924-022-02583-2
49. Barbeck M, Serra T, Booms P, et al. (2017) Analysis of the in vitro degradation and the in vivo tissue response to bi-layered 3D-printed scaffolds combining PLA and biphasic PLA/bioglass components – guidance of the inflammatory response as basis for osteochondral regeneration. *Bioact Mater* 2: 208–223. https://doi.org/10.1016/j.bioactmat.2017.06.001
50. Zhang Y, Fang M, Zhu J, et al. (2024) Exosome-loaded hyaluronic acid hydrogel composite with oxygen-producing 3D printed polylactic acid scaffolds for bone tissue repair and regeneration. *Int J Biol Macromol* 274: 132970. https://doi.org/10.1016/j.ijbiomac.2024.132970
51. Alavi MS, Memarpour S, Pazhohan-Nezhad H, et al. (2023) Applications of poly(lactic acid) in bone tissue engineering: a review article. *Artif Organs* 47: 1423–1430. https://doi.org/10.1111/aor.14612
52. Hu X, Lin Z, He J, et al. (2022) Recent progress in 3D printing degradable polylactic acid-based bone repair scaffold for the application of cancellous bone defect. *MedComm Biomate Appl* 1: e14. https://doi.org/10.1002/mba2.14
53. Salehi Namini M, Mohandesnezhad S, Mohandesnezhad S, et al. (2025) Enhancing bone regeneration using kaempferol as an osteoprotective compound: signaling mechanisms, delivery strategies, and potential applications. *J Biol Eng* 19: 74. https://doi.org/10.1186/s13036-025-00545-5
54. Farjaminejad S, Farjaminejad R, Hasani M, et al. (2025) Innovative drug delivery systems in bone regeneration: benefits and applications in tissue engineering. *J Bionic Eng* 2025: 1–22. https://doi.org/10.1007/s42235-025-00739-z

55. Radha G, Manjubaashini N, Balakumar S (2023) Nano-hydroxyapatite/natural polymer composite scaffolds for bone tissue engineering: a brief review of recent trend. *In Vitro Models* 2: 125–151. https://doi.org/10.1007/s44164-023-00049-w
56. Shineh G, Mobaraki M, Afzali E, et al. (2024) Antimicrobial metal and metal oxide nanoparticles in bone tissue repair. *Biomed Mater Devices* 2: 918–941. https://doi.org/10.1007/s44174-024-00159-3
57. Álvarez-Chimal R, Arenas-Alatorre JÁ, Álvarez-Pérez MA (2024) Nanoparticle-polymer composite scaffolds for bone tissue engineering. A review. *Eur Polym J* 213: 113093. https://doi.org/10.1016/j.eurpolymj.2024.113093
58. Liu Y-Y, Echeverry-Rendón M (2025) 3D-printed biodegradable polymer scaffolds for tissue engineering: an overview, current stage and future perspectives. *Next Mater* 8: 100647. https://doi.org/10.1016/j.nxmate.2025.100647
59. Fan D, Staufer U, Accardo A (2019) Engineered 3D polymer and hydrogel microenvironments for cell culture applications. *Bioengineering* 6: 113. https://doi.org/10.3390/bioengineering6040113
60. Zarrintaj P, Seidi F, Youssefi Azarfam M, et al. (2023) Biopolymer-based composites for tissue engineering applications: a basis for future opportunities. *Compos B Eng* 258: 110701. https://doi.org/10.1016/j.compositesb.2023.110701
61. Zhou G, Xu R, Groth T, et al. (2024) The combination of bioactive herbal compounds with biomaterials for regenerative medicine. *Tissue Eng Part B Rev* 30: 607–630. https://doi.org/10.1089/ten.teb.2024.0002
62. Sodré LI, Gall MEC, Elias M de B, et al. (2025) Osteogenic effects of bioactive compounds found in fruits on mesenchymal stem cells: a review. *Nutr Rev* 83: 675–691. https://doi.org/10.1093/nutrit/nuae209
63. Guo Q, Yang S, Ni G, et al. (2023) The preparation and effects of organic–inorganic antioxidative biomaterials for bone repair. *Biomedicines* 12: 70. https://doi.org/10.3390/biomedicines12010070
64. Tollemar V, Collier ZJ, Mohammed MK, et al. (2016) Stem cells, growth factors and scaffolds in craniofacial regenerative medicine. *Genes Dis* 3: 56–71. https://doi.org/10.1016/j.gendis.2015.09.004
65. Chatakun P, Núñez-Toldrà R, Díaz López EJ, et al. (2014) The effect of five proteins on stem cells used for osteoblast differentiation and proliferation: a current review of the literature. *Cell Mol Life Sci* 71: 113–142. https://doi.org/10.1007/s00018-013-1326-0
66. Storti G, Scioli MG, Kim B-S, et al. (2019) Adipose-derived stem cells in bone tissue engineering: useful tools with new applications. *Stem Cells Int* 2019: 1–18. https://doi.org/10.1155/2019/3673857
67. Simon F, Oberhuber A, Schelzig H (2015) Advantages and disadvantages of different animal models for studying ischemia/reperfusion injury of the spinal cord. *Eur J Vasc Endovasc* 49: 744. https://doi.org/10.1016/j.ejvs.2015.03.041
68. Moran CJ, Ramesh A, Brama PAJ, et al. (2016) The benefits and limitations of animal models for translational research in cartilage repair. *J Exp Orthop* 3: 1. https://doi.org/10.1186/s40634-015-0037-x
69. Collins MN, Zamboni F, Serafin A, et al. (2022) Emerging scaffold- and cellular-based strategies for brain tissue regeneration and imaging. *In vitro models* 1: 129–150. https://doi.org/10.1007/s44164-022-00013-0

70. Sundelacruz S, Kaplan DL (2009) Stem cell- and scaffold-based tissue engineering approaches to osteochondral regenerative medicine. *Semin Cell Dev Biol* 20: 646–655. https://doi.org/10.1016/j.semcdb.2009.03.017
71. Rashidi N, Tamaddon M, Liu C (2024) Protein-based scaffolds for musculoskeletal tissue repair and regeneration, *Handbook of the Extracellular Matrix*, Cham: Springer International Publishing, 237–270. https://doi.org/10.1007/978-3-031-56363-8_12
72. Atkins JT, George GC, Hess K, et al. (2020) Pre-clinical animal models are poor predictors of human toxicities in phase 1 oncology clinical trials. *Br J Cancer* 123: 1496–1501. https://doi.org/10.1038/s41416-020-01033-x
73. Ruan Y, Robinson NB, Khan FM, et al. (2020) The translation of surgical animal models to human clinical research: a cross-sectional study. *Int J Surg* 77: 25–29. https://doi.org/10.1016/j.ijsu.2020.03.023
74. Li Y, Chen S-K, Li L, et al. (2015) Bone defect animal models for testing efficacy of bone substitute biomaterials. *J Orthop Translat* 3: 95–104. https://doi.org/10.1016/j.jot.2015.05.002
75. Webber MJ, Khan OF, Sydlik SA, et al. (2015) A perspective on the clinical translation of scaffolds for tissue engineering. *Ann Biomed Eng* 43: 641–656. https://doi.org/10.1007/s10439-014-1104-7