applied sciences

MDPI

Review

Preclinical Evaluation of Bioactive Scaffolds for the Treatment of Mandibular Critical-Sized Bone Defects: A Systematic Review

Jana Desnica [1], Sanja Vujovic [1], Dragana Stanisic [1], Irena Ognjanovic [1], Bojan Jovicic [2], Momir Stevanovic [1,*] and Gvozden Rosic [3,*]

[1] Department of Dentistry, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia; jana.desnica@gmail.com (J.D.); sanja.994@live.com (S.V.); stanisic92@yahoo.com (D.S.); irena.ognjanovic@gmail.com (I.O.)
[2] Dental Clinic, Military Medical Academy, University of Defence, Crnotravska 17, 11000 Belgrade, Serbia; jovicicdrbojan@gmail.com
[3] Department of Physiology, Faculty of Medical Sciences, University of Kragujevac, Svetozara Markovića 69, 34000 Kragujevac, Serbia
* Correspondence: momirstevanovic7@gmail.com (M.S.); grosic@medf.kg.ac.rs (G.R.)

Abstract: This systematic review evaluated current in vivo research on regenerating critical-sized mandibular defects and discussed methodologies for mandibular bone tissue engineering. Out of the 3650 articles initially retrieved, 88 studies were included, and all studies that used a scaffold reported increased bone formation compared to negative controls. Combining scaffolds with growth factors and mesenchymal stem cells improved bone formation and healing. Bone morphogenic proteins were widely used and promoted significant bone formation compared to controls. However, discrepancies between studies exist due to the various methodologies and outcome measures used. The use of scaffolds with bioactive molecules and/or progenitor cells enhances success in mandibular bone engineering. Scaffold-based mandibular bone tissue engineering could be introduced into clinical practice due to its proven safety, convenience, and cost-effectiveness.

Keywords: bone regeneration; growth factors; mandible; mesenchymal stem cells; scaffold; tissue engineering

Citation: Desnica, J.; Vujovic, S.; Stanisic, D.; Ognjanovic, I.; Jovicic, B.; Stevanovic, M.; Rosic, G. Preclinical Evaluation of Bioactive Scaffolds for the Treatment of Mandibular Critical-Sized Bone Defects: A Systematic Review. *Appl. Sci.* **2023**, *13*, 4668. https://doi.org/10.3390/app13084668

Academic Editor: Vittorio Checchi

Received: 15 March 2023
Revised: 4 April 2023
Accepted: 5 April 2023
Published: 7 April 2023

1. Introduction

The reconstruction of mandibular bone defects following traumatic injuries, postoperative defects from tumor removal, or infection treatment is the most common reconstructive procedure in oral and maxillofacial surgery. Due to the significant impact of the craniofacial area on patients' well-being and quality of life, the proper reconstruction of defects represents a significant challenge for surgeons [1]. The reconstruction or augmentation of craniofacial bones is one of the most frequent surgical procedures in oral and maxillofacial surgery. Special consideration of mandible reconstruction exists in cases of critical-sized defects where a high quantity of bone is lost and intrinsic regeneration is not possible [1].

Standard bone augmentation procedures comprise the clinical use of autografts, allografts, and xenografts. Alloplastic materials, such as titanium load-bearing plates, were widely used as reconstruction plates to reestablish mandibular segmental defects. Still, numerous studies on titanium reconstruction plates have reported high failure rates of up to 52% due to the resorption and infection of the bone [1]. Microvascular bone grafts harvested from the fibula, scapula, radial bone, and iliac crest are currently used as standard grafts to reconstruct extensive mandibular bone defects. However, the use of autografts is complex and requires a second surgical intervention, causing donor-site morbidity and possible graft rejection [1]. To overcome these limitations, extensive research on bone tissue engineering (BTE) using bioactive and biocompatible bone substitutes has been performed in recent years. The techniques for mandibular BTE should be adjusted to

certain features that are unique to mandible physiology. Firstly, materials for regeneration may come into contact with saliva and intraoral pathogens, leading to inflammation and infection. Secondly, materials used for mandibular BTE must withstand significant forces of mastication (200–400 N). Thirdly, the BTE of the mandible should restore the contour of the bone in order to ensure proper patients' appearance. Fourthly, the restored bone should be available for dental implant placement. Techniques for mandibular bone tissue engineering may be categorized into scaffolds, bioactive substances, and cell therapy [2].

Scaffolds used as synthetic or autologous bone substitutes act as matrices mimicking the artificial extracellular matrix (ECM) to promote bone healing until they are partially or wholly replaced by newly formed bone [3]. Biomaterials are mainly categorized into natural and synthetic materials. Synthetic materials are classified into polymers, bioceramics, metals, and composite scaffolds. Numerous studies reported a range of advantages and disadvantages for each scaffold type [4,5]. Natural polymeric scaffolds have excellent biocompatibility and controlled biodegradation, but their weak mechanical properties are their major drawback as bone scaffold materials [1]. Synthetic polymeric scaffolds are biocompatible, biodegradable, easily fabricated into different shapes, and provide mechanical support and a controlled degradation time. Their use in bone engineering is limited because of their decreased mechanical strength due to their rapid degradation in vivo and immune reaction to acidic degradation products [1]. Bioceramic scaffolds with excellent biocompatibility and bioactivity are extensively used in bone tissue engineering. They have high compressive strength and osteogenic properties but a low tensile strength and toughness, fabrication hitches, brittleness, and slow resorption rate [2]. Metals have been used in manufacturing biomimetic scaffolds, fixation plates, screws, pins, wires, and stents. Biodegradable metals have a variety of properties that are essential for bone regeneration scaffolds, including biodegradability properties, mechanical strength, formability, osteogenic capacity, and antibacterial properties. The term "biomimetic" refers to the process of designing materials, structures, or systems that mimic natural biological processes or structures. In the context of scaffolds for bone regeneration, biomimetic scaffolds are designed to mimic the extracellular matrix (ECM) of natural bone tissue, both in terms of its chemical composition and its physical structure [2]. Even though metallic materials are not superior to other material combinations because of the increased number of failure cases demanding revision procedures, they are still utilised in some developing countries due to their reasonable cost and availability [6]. Composite scaffolds of bioceramics with polymers have been extensively used as materials for bone repair studies. The natural bone matrix is a combination of organic/inorganic composites; thus, the composite scaffolds for bone regeneration are designed by combining the advantages of both components. The most common composite scaffolds for bone substitution are bioceramic and polymer scaffolds [4,5].

Bioactive molecules such as bone morphogenetic proteins (BMPs) promote bone healing, usually in combination with a scaffold as a carrier. Several commercial products are based on recombinant human bone morphogenetic proteins (rhBMPs) and are used for alveolar bone augmentation, sinus lift procedures, and periodontal defects [7]. The direct administration of growth factors into the bone defect is considered an excellent strategy for bone tissue regeneration. The critical point when using bioactive molecules is selecting a suitable scaffold carrier system to tailor the localized and sustained release of these molecules [8]. Stem cells (SCs) therapy is an up-and-coming technique for BTE. In BTE, SCs have been used due to their ability to produce and differentiate into osteoblasts. SCs have several advantages, including the ability to differentiate directly into osteoblasts, modulate immune responses, promote angiogenesis, and exhibit plasticity. [9]. Various scaffolds are used to mimic native ECM for SCs seeding, providing a conducive framework for the attachment and growth of cells [10]. The common sources from which SCs can be obtained are bone marrow, dental pulp, embryo, and adipose tissue [10].

1.1. Role of Scaffolds in BTE

The capacity of bone to regenerate represents a unique feature, influenced by various factors, but spontaneous healing is not possible in cases of bone damage with a critical shape [11]. Scaffolds are porous 3D structures that simulate native ECM features; thus, their functions are similar to those of ECM in natural tissues. They are a fundamental component of BTE, alongside SCs and growth factors, as their role is to provide structural and mechanical support for tissue formation and regeneration [12]. By producing different biophysical and chemical signals, scaffolds create a stimulative microenvironment for multiple processes, including the adhesion, migration, proliferation, and differentiation of osteoblast progenitors [13]. In such a functional three-dimensional space, bone development can occur. They may also serve as a reservoir for various growth factors required for successful bone regeneration. The main goal of scaffold-based tissue engineering is to fabricate a biomimetic structure that can prompt the directed growth of cells toward an organ-like formation [14].

1.2. Properties of an Ideal Scaffold for Bone Regeneration

Bone is a natural composite consisting of inorganic components, mainly calcium apatite, and organic components, mainly collagen type I. Bone has a complex architecture to withstand diverse mechanical, biological, and chemical functions. The specialized structure involves the complex arrangement of macrostructures (proportion of medullar and cortical bone), microstructures (arrangement of osteons and trabeculae), and nanostructures (collagen fibers and apatite mineral crystals) [4,5,15,16]. Scaffolds are 3D structures that imitate the in vivo environment and stimulate the formation of new tissue [12]. They promote SCs' adhesion, proliferation, and differentiation by creating a suitable microenvironment [13]. Porous scaffolds with optimal 3D architecture serve as artificial bone ECM with osteoconductive properties to promote bone healing. A scaffold should possess good biological properties, including (Table 1):

- Biocompatibility;
- Mechanical stability;
- Architecture;
- Biodegradability;
- Bioactivity [4,5,15,16].

In order to promote natural bone healing, an equilibrium between the biological properties of the scaffold, osteoprogenitor cells, and signaling molecules must exist [17,18]. The ideal 3D scaffold for bone regeneration should be composed of a biocompatible, biodegradable material with similar mechanical properties to bone ECM to provide enough mechanical support to the host cells [4,5,15,16]. Scaffolds should mimic the bone ECM to facilitate the osteogenic host cells to deposit natural ECM and replace the scaffold material. Thus, the rate of scaffold resorption should be controlled, complementing host cells' ingrowth. This is particularly important for bone regeneration, where mechanical stability of the scaffold is expected, especially in the load-bearing areas [17,18]. The 3D architecture of the scaffold should be highly porous with a high index of porosity (number of interconnected pores/mm^2) to allow for osteoprogenitor cell ingrowth and nutrient migration [19]. The scaffold surface should also be optimized to promote cell attachment, mainly its wettability and roughness. A high number of interconnected pores allows for the diffusion of nutrients and oxygen into the avascular scaffold. The size of the pores is essential, as well as the proportion of nano-, micro-, and macropores in the scaffold. Pores greater than 300 nm promote osteogenesis due to the ingrowth of osteoprogenitor cells and angiogenesis [19]. Micro- and nanopores with a size <10 nm increase the scaffold's overall surface, enabling protein attachment and improving cell–scaffold binding. The porous 3D architecture of the scaffold allows for the incorporation of growth factors such as BMPs, vascular endothelial growth factor (VEGF), transforming growth factor β (TGF-β), and insulin-like growth factor 1 (IGF-1) [4,17].

Table 1. Ideal properties of the scaffold to promote bone regeneration.

Scaffold Property	Desirable Properties
Biocompatibility	• Non-toxic breakdown products; • Non-inflammatory scaffold components; • Without immune rejection; • Non-carcinogenic.
Biodegradability	• Controlled degradation complementing tissue ingrowth and allowing host cells to produce extracellular matrix; • Sufficient support to the newly formed tissue; • Degradable by host biological processes.
Mechanical stability	• Enough compressive, elastic and fatigue strength for osteogenic cell migration; • Scaffold material allows easy surgical manipulation; • Material adaptive to the individual bone defect.
Bioactivity	• Scaffold material should interact and bind to the host tissuel • Osteoconductive properties; • Stimulation of cell ingrowth, attachment and differentiation; • Stimulation of neoangiogenesis.
Architecture	• Macroporosity to allow cell migration and angiogenesis; • Microporosity to increase surface area for cell–scaffold connections; • High porosity index and interconnected pores allow for diffusion of nutrients and cell migration; • Adequate pore size for osteogenic cells.
Surface of the scaffold	• Hydrophilicity and surface roughness of the scaffold surface enhances protein adsorption and host cell binding.
Sterilibility	• Sterilibility without loss of bioactivity.

The most significant advantage of scaffolds is their ease of production, enabling researchers to modify numerous materials and combine them into composite grafts [17,18,20]. Thus, it is crucial that the scaffold material allows for easy surgical manipulation and is composed of a material that is adaptive to the individual bone defect. The mechanical properties of the scaffold should be comparable to the compressive strength of the cortical bone (100–250 MPa) to withstand physiological mechanical forces on the bone [20].

Previous systematic reviews of the literature concluded that preclinical in vivo studies demonstrated the clinical potential of scaffolds as an alternative to autogenous bone grafting [21,22]. Boysuni et al. [21] performed a systematic review investigating the results of mandibular BTE in animal studies. This review reported a constant increase in the frequency of publications regarding mandibular BTE, reflecting the growing interest in the field. Despite promising results in bone regeneration, clinical translation is still impossible due to a lack of understanding of the biological interplay between scaffolds, biomolecules, exogenous cells, and host immune reactions. In addition, a qualitative and quantitative comparison of outcomes between the animal and clinical studies was impossible due to the significant differences between the studies regarding the methodology, definition of critical-sized defects, follow-up period, and evaluation of outcomes. Still, there is much controversy regarding defining what constitutes a critical-sized defect. In general, a "critically-sized" defect is regarded as one that would not heal spontaneously despite surgical stabilization and requires further surgical intervention, such as autologous bone grafting [12]. Differences among studies using the same animal models for mandibular bone defects allow for an objective comparison of outcomes. The clinical evaluation of scaffolds for mandibular bone regeneration is limited to case reports or single-center case series with limited follow-up periods and questionable results. This review aimed to evaluate current research on the regeneration of mandibular defects and discuss the further development of mandibular BTE methodologies, focusing on a better understanding of the

clinical use of different scaffold types in the BTE process to overcome mandibular defects. Furthermore, the goal was to compare the result of scaffold-based BTE in conjunction with various SCs and growth factors in bone reconstruction to aid reconstructive surgeons in determining the most suitable scaffolds for mandibular bone reconstruction.

2. Materials and Methods

The systematic review of the BTE concepts for the reconstruction of critical-size mandibular defects was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.

2.1. Hypothesis

Hypothesis 0 (H0). *There is no significant difference between the various scaffold types, stem cells (SCs), and growth factors in bone reconstruction for critical-sized mandibular defects.*

Hypothesis 1 (H1). *There is a significant difference between the various scaffold types, SCs, and growth factors in bone reconstruction for critical-sized mandibular defects.*

2.2. Research Question

The research question for this review was: "What are the characteristics and the results of the existing studies on the use of biomimetic scaffolds in the reconstruction of critical-sized mandibular bone defects?".

2.3. Search Strategy

In November 2022, electronic searches were conducted in the following databases: PubMed (National Library of Medicine), Web of Science (Clarivate Analytics), and Scopus (Elsevier). These comprised both MeSH and free-text terms. The following search strategy was applied: ((“bone tissue engineering” [MeSH Terms] OR (“tissue” [All Fields] AND “engineering” [All Fields] AND “mandible” [All Fields]) OR “bone tissue engineering” [All Fields]) OR (“tissue scaf-folds” [MeSH Terms] OR (“tissue” [All Fields] AND “scaffolds” [All Fields]) OR “tissue scaffolds” [All Fields] OR (“tissue” [All Fields] AND “scaffold” [All Fields]) OR “tissue scaffold” [All Fields]) OR (“mandible reconstruction” [MeSH Terms AND “engineer-ing” [All Fields]] OR (“reconstructive” [All Fields] AND “surgical” [All Fields] AND “pro-cedures” [All Fields]) OR “mandible reconstruction” [All Fields] OR “reconstruction” [All Fields]) OR (“bone morphogenetic proteins” [MeSH Terms] OR (“bone” [All Fields] AND “morphogenetic” [All Fields] AND “proteins” [All Fields]) OR “bone morphogenetic pro-teins” [All Fields] OR (“bone” [All Fields] AND “morphogenetic” [All Fields] AND “pro-tein” [All Fields]) OR “bone morphogenetic protein” [All Fields]) OR (“bone marrow cells” [MeSH Terms] OR (“bone” [All Fields] AND “marrow” [All Fields] AND “cells” [All Fields]) OR “bone marrow cells” [All Fields]) OR (“intercellular signaling peptides and proteins” [MeSH Terms] OR (“intercellular” [All Fields] AND “signaling” [All Fields] AND “peptides” [All Fields] AND “proteins” [All Fields]) OR (“growth” [All Fields] AND “factors” [All Fields]) OR “growth factors” [All Fields])) AND ((“mandible” [MeSH Terms] OR “mandible” [All Fields]) OR ((“mandible” [MeSH Terms] OR “mandible” [All Fields] OR “mandibular” [All Fields]) AND (“critical sized defect” [All Fields]) AND defect [All Fields])). The retrieved references were exported to the EndNote software 20.5 (Windows) (Clarivate Analytics, Philadelphia, PA, USA) to identify the duplicates.

Searches were limited to studies in the English language. The reference lists of the articles identified from the initial search were screened for further relevant studies. No restrictions were imposed on the date or the type of publication.

2.4. Eligibility Criteria

Only full-text papers that reported original data from in vivo studies on the regeneration of mandibular critical size defects in animal models were included. Animal in vivo studies presenting macroscopical, histological, or histomorphometric data on the amount

of bone-defect bridging, bone ingrowth, results of biomechanical testing, histological or histomorphometric data of scaffold degradation, and radiographic evidence of restoration of mandibular continuity were included. The following inclusion criteria were applied: 1. research papers presenting in vivo animal data; 2. critical-sized defects; 3. reconstructive technique clearly described; 4. follow-up and healing period clearly stated; 4. the animal model used clearly described; 5. clearly presented data on bone regeneration and the methods for the evaluation of bone growth. Conference abstracts, review papers, letters to the editor, opinion pieces, and studies on animal or human tissues in vitro were excluded. Papers investigating periodontal regeneration, dental implants' osseointegration, distraction osteogenesis, autologous bone grafts or free flaps, and treatment of fracture healing were also excluded.

2.5. Data Extraction

Retrieved references were read for titles and abstracts by all authors. If the title/abstract met the inclusion criteria, the article was included for a full article reading. If the full text of the article was not available, the paper was not included. After the evaluation of full texts, the references that met the eligibility criteria were included. All authors performed the quality assessment and evaluated the published data. Any disagreements in judgment were resolved by a final discussion. If the published data were insufficient, the study was excluded. The data extracted for review included: study characteristics and setting (sample size, design, animal species, type and mechanism of bony defect), method of bone engineering (scaffolds, bioactive factors, and cell therapy), and outcome measures.

3. Results

A total of 3872 articles were initially identified using the search algorithm. After the screening of the titles and abstracts, 265 full texts were retrieved, and a total of 88 studies were included (Figure 1) [22–109]. An overview of the included trials is depicted in Supplementary Table S1.

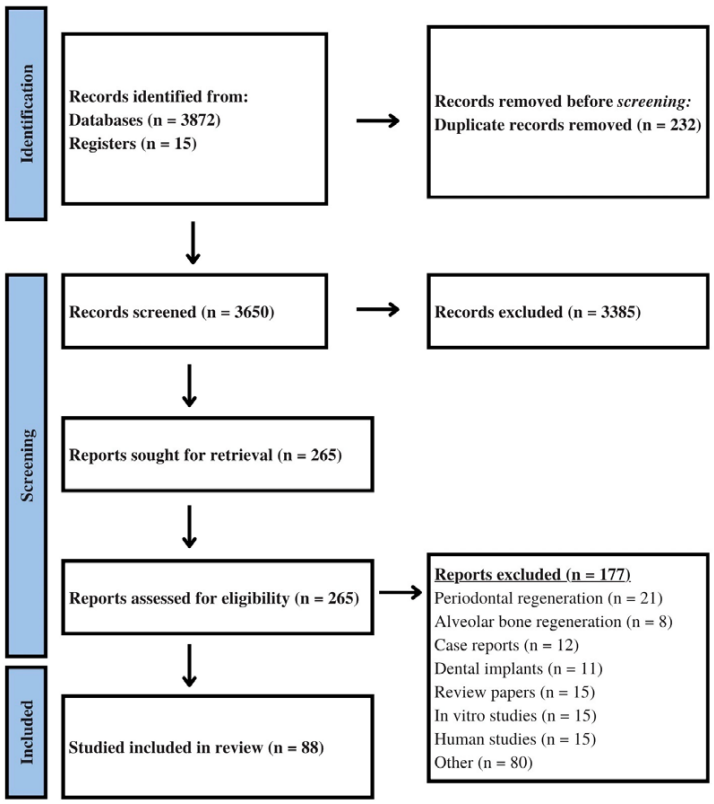

Figure 1. PRISMA flow diagram of studies included in the systematic review.

3.1. Study Design

The review included studies with critical-size mandibular defects. Regeneration modalities were categorized into tissue scaffolds, cell therapies, and bioactive substances. Most studies comprised a combination of these modalities (Figure 2). Most reported a combined use of scaffold with bioactive substances. Animal models included dogs, mini-pigs, non-human primates (nHPs), swine, rabbits, rats, sheep, and mice, which were used in 15, 7, 6, 2, 23, 26, 8, and 1 study, respectively (Table 2).

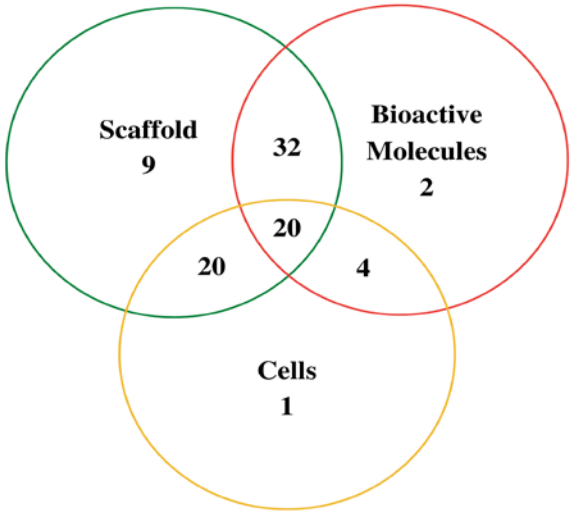

Figure 2. Diagram indicating the number of studies that investigated each component of the regenerative triad (bioactive molecules, scaffolds, and cells).

Table 2. Overview of the animal models.

Animals	Number of Studies (%)	Critical-Sized Defect (mm) Mean (SD)	Follow Up (Weeks) Mean (SD)	References
Rats	26 (29.5)	4.76 (0.3)	10.1 (5.8)	[24,30,39–41,49–54,56–58,60–62,65,77,80,83,87,91,95,99,104]
Rabbits	23 (26.1)	17.2 (5.3)	12.4 (4.1)	[22,23,25–29,32,33,44,59,69,71,75,76,79,85,98,100–102,105,108]
Dogs	15 (17)	25.3 (7.8)	27.1 (24.5)	[31,37,38,46,67,78,81,82,86,88,90,96,97,106,107]
Sheep	8 (9.1)	34.3 (4.6)	22.1 (16.3)	[47,64,72–74,84,89,94]
Mini-pigs	7 (8)	33.5 (6.9)	34.6 (12.9)	[34,35,48,63,92,93,109]
nHPs *	6 (6.8)	29.1 (12.2)	43.2 (34.5)	[45,55,66,68,70,103]
Swine	2 (2.3)	24.3 (5.6)	10.0 (4.4)	[36,43]
Mice	1 (1.1)	10.0	4.0	[42]

* nHPs—non-human primates.

The duration of follow-up (the time at which the mandibles were analyzed for bone regeneration—either using radiographic imaging of the animal or at the time of sacrifice) varied widely depending on the animal model used (Table 2). The size of the critical defect was not uniform, not even within a single animal model.

The diagnostic modalities used for characterizing the new bone formation included micro-CT examination, histology, radiography, immunohistochemistry, real-time PCR, fluorescence microscopy, biomechanical testing, and scanning electron microscopy (Table 3). The most common diagnostic modalities used were micro-CT or other X-ray modalities (computed tomography) and histological analysis.

Table 3. Overview of diagnostic modalities used to characterize new bone formation.

Characterization Approach	Number of Studies (%)	References
Micro-CT	35 (39.8)	[24,27,30,33,35,39–41,43–45,47,49–51,62,65,69,76–80,83–86,88,90,93,99–102,107]
Histology	79 (89.8)	[22,23,25–39,41–46,48–73,75–87,90–92,94–107]
Radiography	38 (43.2)	[22,23,25,26,28,32,34,35,37–39,43,46,48,56,60–63,65–68,70,71,75,79,89,91–94,97,98,102,103,105,106]
Immunohistochemistry	28 (31.8)	[30,35,36,41,46–50,52,57,60,61,64,67,72,73,76,83,86,88,92,93,96,97,101,102,106]
RT-PCR [1] (mRNA quantification)	6 (6.8)	[23,57,80,88,92,106]
Fluorescence microscopy	4 (4.5)	[53,54,72,109]
Biomechanical testing	11 (12.5)	[24,28,37,38,42,63,73,74,92,102,103]
SPECT [2]	1 (1.1)	[109]
SEM [3]	10 (11.3)	[37,38,59,64,82,83,86,90,107,108]
CBCT [4]	4 (4.5)	[36,59,71,96]

[1] RT-PCR—reverse transcriptase polymerase chain reaction; [2] SPECT—single-photon-emission computed tomography; [3] SEM—scanning electron microscopy; [4] CBCT—cone beam computed tomography.

3.2. Scaffolds

Scaffolds were used in 81 studies, either as the primary variable of investigation or as delivery vectors for bioactive molecules and cell seeding (Table 4). Most studies used synthetic 3D porous scaffolds of a synthetic polymer, such as beta-tricalcium phosphate, PLGA, PCL, or their combination, to create composite scaffolds.

Table 4. Overview of scaffold types used for mandibular bone regeneration.

Scaffold	Number of Studies (%)	References
Natural polymers		
Hyaluronic-acid-based	3 (3.4)	[52,53,77]
Collagen	23 (26.1)	[22,24,39,45,52,54,55,63–65,68,72–74,79–81,88,90,97,100–102]
Chitosan-based	6 (6.8)	[49,59,76,87,99,100]
Synthetic polymers		
PLGA [1]	15 (17)	[26,33,35,36,40,41,43,46,47,50,51,66,70,103,108]
PLA-based	3 (3.4)	[32,60,96]
PDLLA/$CaCO_3$ [2]	2 (2.3)	[56,57]
<PEI [3]	1 (1.1)	[36]
PCL [4]	7 (8)	[27,59,82,85,98,106,107]
PTFE membrane [5]	1 (1.1)	[94]
PU [6]	1 (1.1)	[93]
Bioceramics		
βTCP-based [7]	12 (13.3)	[30,35,43–45,69,78,85,86,90,93,107]
HAP [8]	16 (18.2)	[23,26–28,36,42,45,52,54,78,86,90,96,101,102,104]
Autologous (any tissue)	3 (3.4)	[34,67,84]
Xenogenic graft (any tissue)	3 (3.4)	[36,83,109]

When a conjugate scaffold was used, references are placed under both categories for completion. [1] PLGA—poly(lactide-co-glycolic acid); [2] PDLLA/$CaCO_3$—poly-dl-lactic acid/calcium carbonate; [3] PEI—polyethylenimine; [4] PCL—polycaprolactone; [5] PTFE—polytetrafluoroethylene; [6] PU—polyurethane; [7] TCP—tricalciumphosphate; [8] HAP—hydroxyapatite.

3.3. Bioactive Substances

Bioactive molecules were used in 58 studies (66%), either alone or in combination with a scaffold or cell therapy (Table 5). The most common growth factor was BMP-2, which was used alone in 31 studies and in combination with other growth factors in 8 studies. The growth factor dosage depended on the graft size and the animal model. Several studies reported using various concentrations of growth factors for bone regeneration.

Table 5. Overview of bioactive molecules.

Bioactive Molecules	Number of Studies (%)	References
BMP-2	31 (35.2)	[22,23,27,30,40,45,46,49,51,52,55–57,60,61,66,68,70,76–79,86,87,93,95,97,100,103,107,108]
BMP-7	5 (5.6)	[28,71,72,74,75]
BMP–4	1 (1.1)	[29]
BMP-2 + BMP-4	1 (1.1)	[53]
BMP-2 + phenamil	1 (1.1)	[41]
BMP-2 + VEGF	3 (3.4)	[43,88,92]
BMP-6 + VEGF	1 (1.1)	[50]
BMP-2 + FGF	3 (3.4)	[25,58,105]
FGF	1 (1.1)	[80]
TGF1 + IGF-1	1 (1.1)	[91]
Insulin	1 (1.1)	[33]
L-PRF	1 (1.1)	[47]
rHOP-1	4 (4.5)	[63,65,73,109]
Dipyridamole	1 (1.1)	[69]
CGF	2 (2.3)	[101,102]
SDF 1α	1 (1.1)	[24]

BMP—bone morphogenetic protein; VEGF—vascular endothelial growth factor; FGF—fibroblast growth factor; TGF—transforming growth factor; IGF-1—insulin-like growth factor 1; L-PRF—leucocyte and platelet-rich fibrin; rHOP-1—recombinant human osteogenic protein 1; CGF—concentrated growth factor; SDF 1α—stromal-derived factor 1α.

3.4. Cell Therapy

Cell therapies were used for mandibular regeneration in 45 studies (51%). Bone marrow stem cells (BMSCs) were mostly used to promote bone regeneration. Regarding SCs, adipose-derived stem cells (ADSCs), stem cells derived from human exfoliated deciduous teeth (SHED), and dental-pulp-derived stem cells (DPSCs) were used in 7, 1, and 1 study, respectively (Table 6). Other cell therapies included lipid-free dedifferentiated fat cells (DFAT) and alveolar osteoblasts (AOB).

Table 6. Overview of cell therapies.

Cell Therapy	Number of Studies (%)	References
BMSCs	22 (25)	[23,25,29–31,33,34,37,38,42,43,48,61,68,70,71,77,80,84,95,98,105]
DPSCs	1 (1.1)	[26]
ADSCs	7 (8)	[35,40,49,62,99,104,106]
MSCs	11 (12.5)	[28,41,50,67,75,79,82,85,94,100,101]
SHED	1 (1.1)	[81]
DFAT	1 (1.1)	[39]
Osteoblasts/osteocytes	2 (2.3)	[65,83]

BMSCs—bone-marrow-derived stem cells; DPSCs—dental pulp derived stem cells; ADSCs—adipose-tissue-derived stem cells; MSCs—mesenchymal stem cells; SHED—stem cells from human exfoliated deciduous teeth; DFAT—dedifferentiated adipocyte-derived progeny cells.

4. Discussion

Bone healing is a complex process, where there is a temporospatial interaction between the ECM, growth factors, and osteogenic cells, resulting in bone regeneration. During natural bone healing, the body generates a natural scaffold on which MSCs differentiate into osteoblasts under the influence of growth factors to regenerate bone tissue [3]. Nonetheless, critical-sized defects that cannot heal spontaneously within a patient's lifetime impair this process. In these cases, it is necessary to provide biomimetic scaffolds to bridge the defect and provide the repair site with sufficient osteogenic progenitor cells or growth factors in a suitable carrier to ensure osteoblastic differentiation [2]. The present review evaluated current therapeutic approaches regarding the BTE of critically sized mandibular defects. BTE is a promising alternative in mandibular regeneration based on in vivo studies; however, translation to clinical use is to be performed. The results of in vivo trials should be taken with caution because, for successful mandibular bone regeneration, specific clinical problems unique to the mandible should be considered. These issues include the contact between materials and saliva and intraoral pathogens, which may lead to inflammation and infection, significant forces of mastication in load-bearing locations, restoration of the normal contour of the bone for the esthetic appearance of the patient, and dental implant placement.

4.1. Scaffolds

Based on the results of the present review, the use of scaffolds in mandibular bone regeneration, alone or in combination with bioactive molecules and/or cell therapies, resulted in improved osteogenesis of critical-sized mandibular defects in various animal models. The most promising results in mandibular bone regeneration were observed with composite scaffolds. Scaffolds for BTE can be classified into four classes: polymeric, ceramic, composite, and metallic scaffolds.

Natural polymeric scaffolds have excellent biocompatibility and controlled biodegradation [20]. Natural biopolymers are used for maxillofacial bone regeneration because they mimic the structure, chemical composition, and biochemical properties of the natural ECM bone organic matrix and have osteoinductive and osteoconductive properties. This was demonstrated by their ability to induce bone regeneration in mandibular bone defects. The major drawbacks of natural polymers are their poor mechanical properties, which do not approach those of natural bone tissue, and the high degradation rate of natural polymers, since they are naturally metabolizable [110]. The most commonly used natural polymeric scaffolds implemented for mandibular bone regeneration were hyaluronic-acid-based and collagen. Due to their excellent biological properties, natural polymers are extensively studied in composite scaffolds for bone repair. Several investigations have indicated that incorporating inorganic components, such as hydroxyapatite (HAP), or tricalcium phosphate (TCP), into hybrid hyaluronic-acid-based (HA) scaffolds results in enhanced osteoinductivity, osteoconductivity, and improved mechanical properties [111,112]. Natural polymers are versatile, encapsulating bioactive osteogenic factors (growth factors, drugs, hormones, peptides, nucleic acids, and cells) via a cross-linking reaction [112–114]. Recent studies reported excellent the bone regeneration of rabbit mandibular bone defects using nano-hydroxyapatite/collagen (nHAC) scaffolds with concentrated growth factors (CGF). The results of these studies showed degradation of the scaffold within 24 weeks, a high rate of new bone formation, and higher compressive strength and elastic modulus on biomechanical tests of the nHAC/CGF group compared to those of the nHAC group [99,100].

Synthetic polymers are aliphatic polyesters, and their copolymers are commonly utilized polymers in bone tissue engineering due to their mechanical properties. They are biocompatible, biodegradable, easily fabricated into different shapes, and provide mechanical support and a controlled degradation time. Their use in bone engineering is limited due to their decrease in mechanical strength due to rapid degradation in vivo and immune reaction to acidic degradation products. The chemical modification of synthetic

polymers allows for the incorporation of bioactive molecules to produce biocompatible and functional materials to enhance osteogenesis. One of the major advantages of synthetic polymers is that they can be mass-produced and fabricated for individual bone sites. The traditional preparation methods of synthetic-polymer-based bone scaffolds include particle leaching, phase separation, gas forming, and fiber bonding. The 3D printing technology produces porous scaffolds by managing the arrangement of the scaffold, the thickness of each layer, and the porosity [115]. The 3D-printed scaffolds achieved better cell adhesion, proliferation, and differentiation while possessing suitable mechanical properties and a sufficiently long degradation time [116]. Recent studies have developed grafts for the mandible, skull, femoral head, tibia, fibula, and others [117].

Poly(lactic-co-glycolic acid) (PLGA), a linear copolymer of lactic acid and glycolic acid monomers, was the most widely used biodegradable polymer. PLGA was investigated as a single scaffold biomaterial for several reasons: it posesses excellent biocompatibility, non-toxic metabolites that can be safely eliminated from the metabolic cycle, and excellent processability; the degradation time of PLGA can be controlled to be consistent with bone regeneration; PLGA has suitable mechanical strength to adequately support the defect area at the early stage; grafts can be loaded with a variety of bioactive factors, such as SCs, growth factors, and drugs, to promote the regeneration of bone defects [118–120]. Inflammation is one of the main reasons for bone graft resorption. Synthetic porous polymers can be loaded with anti-inflammatory medicines (such as ibuprofen) and growth factors to reduce inflammatory reactions and enhance bone regeneration [121]. In the present review, we found that the application of synthetic polymer scaffolds, alone or in combination with bioactive molecules and/or mesenchymal SCs, resulted in improved bone regeneration.

The bioceramic scaffolds have high compressive strength, excellent biocompatibility and stability, bioactivity, and osteogenic properties. The mechanical strength of ceramics is superior to polymers, but is still inferior to natural bones, especially in terms of tensile and torsional strength [9]. Disadvantages include low tensile strength and toughness, fabrication hitches, brittleness, and slow resorption rate. Hydroxyapatite (HAP) ($Ca_{10}(PO_4)_6(OH)_2$) is the main mineral component of bone tissue and, as a scaffold, it is a bioactive ceramic material of high biocompatibility because it forms direct chemical connections with bone tissue. It has excellent bioactive, and bioresorbable properties and can be synthesized in various forms, such as ceramic plates, blocks, granules, and powder for various bone tissue applications [122,123]. In the bone tissue, HAP is deposited around and within the collagen fibers in the form of thin slabs and sticks (length 20–40 nm, width 15 nm, and thickness of 1.5–3 nm) in regular spaces [122,123]. Synthetic HAP is similar to a natural mineral apatite in bones and is widely used for bone repairs. Its structural composition is the same as natural bone, with a nominal stoichiometric Ca/P atomic ratio of 1:67 [122,123]. Still, mechanical properties are very low compared to natural bone [41,42]. Due to brittleness and fragility, HAP is commonly mainly used in the form of composite scaffolds with polymer materials [124,125]. Numerous studies have shown, that after applying a HAP scaffold, the rapid activation and binding of osteoblasts to the scaffold surface occurred due to the rapid deposition of the biological carbonate HAP, which is the primary substrate for binding of the osteoprogenitor cells [126]. The osteoprogenitor cells are known to better bond to rough surfaces than smooth surfaces. For this purpose, in addition to good biological characteristics, the topography of the HAP scaffold has an impact on cell adhesion, proliferation, and differentiation [127]. Cells on rough surface show phenotypes that are similar to osteoblasts and release pro-osteoblastic mediators such as prostaglandins and LTGF-β. In vitro research showed that osteoprogenitor cells do not differ in terms of the surface of synthetic and biological HAP [127,128].

Combining the advantages of two or more different scaffold materials into a composite scaffold has been a matter of research in many previous trials [129,130]. Composite scaffolds of bioceramics with synthetic polymers have been used in the majority of studies, either as a single regenerative therapy or as a carrier of bioactive molecules or SCs. These

scaffolds mimic the natural bone matrix, a combination of organic/inorganic composites. Composite scaffolds combine the excellent biological activity of HAP and mechanical properties and 3D architecture of synthetic polymers. The HAP/PLGA scaffold was extensively studied for bone regeneration. Jokanovic et al. [110] examined the PLGA/HAP scaffold for critical-sized calvaria defects and found numerous biological advantages compared to commercially available xenograft, which were reflected mainly by the lower number of giant cells surrounding implanted material and a higher degree of mineralization in newly formed bone. Micic et al. [114] evaluated the nHAP/PLGA coating scaffold for large segmental defects in the rabbit's ulna. The authors reported almost complete bone regeneration and excellent histological parameters: new bone formation with both endochondral and endosomal types of ossification, high concentrations of BMPs, osteocalcin, and osteopontin within the newly formed bone. Zhang et al. [108] evaluated the HAP/PLGA scaffold for the repair of large segmental defects of rabbit radius and exhibited rapid and strong mineralization and osteoconductivity. Previous studies have shown that both the biocompatibility and mandibular bone regeneration performance of composite polymer/bioceramic scaffolds are enhanced compared to polymeric or bioceramic scaffolds [32]. Regarding mandible reconstruction, Stevanovic et al. [36] investigated the HAP/PLGA and HAP/PEI scaffolds for mandibular bone regeneration in a swine model and demonstrated improved biological behavior compared to conventional xenograft in the treatment of swine's mandibular defects in terms of bone density and bone tissue histological characteristics. An interesting finding in this study was the significant activation of osteocalcin, the most abundant noncollagenous protein in bone tissue, produced by osteoblasts in the HAP/PEI cohort. Osteocalcin is an important molecule for the regulation of bone mineral deposition, and its expression can serve as a marker of mineralized matrix formation [36]. Therefore, the ability of composite scaffolds to induce osteoblasts to produce more osteocalcin may be an interesting feature of these biomaterials.

Biodegradable metals (BMs) have a variety of properties that are essential for bone regeneration scaffolds, including biodegradability properties, mechanical strength, formability, osteogenic capacity, and antibacterial properties [131]. The most representative BMs are Mg-based, Zn-based, and Fe-based. These materials have been used in manufacturing biomimetic scaffolds, fixation plates, screws, pins, wires, and stents [131]. Mg-based biomaterials are widely used because of their elastic modulus, which is similar to human bones, biosafety, and biodegradability. Fe-based biomaterials have a relatively low degradation rate and insoluble degradation products, which greatly limit their application [132]. Zn-based biomaterials possess a moderate degradation rate and excellent mechanical properties for orthopedic and cardiovascular applications [51]. Critical-sized mandibular defects imply the use of load-bearing tissue scaffolds due to significant masticatory forces. Bioresorbable metallic scaffolds may potentially be used to overcome the mechanical properties of conventional tissue scaffolds. Magnesium and its alloys have outstanding mechanical and biological properties for bone regeneration, including mechanical properties and Young's modulus close to that of cortical bone [133–136]. Magnesium stimulates bone growth due to the formation of bone-apatite-like HAP crystals [136]. In vitro studies showed excellent mechanical properties of porous magnesium scaffolds with a modified Young's modulus to adjust to one of the cancellous bones (0.01–2.0 Gpa) [134,135]. The use of Mg-based bone scaffolds may be a promising strategy in future research on mandibular bone regeneration.

4.2. Bioactive Molecules

Bioactive molecules and the cell therapies incorporated in the scaffolds significantly improved bone regeneration in comparison to the scaffold alone in the majority of studies. Scaffolds with BMPs significantly promoted bone regeneration in a dose-dependent manner [22,24,31,46,51,56,57,83,84]. BMPs activate MSCs differentiation during bone formation through the activation of the Smad-dependent signaling pathway or the MAPK pathway [10]. The protracted slow release of BMP-2 using microspheres resulted in a better

bone quality compared to the early deleterious effect of the supraphysiological dose of BMP [2]. The timing of the application of BMP-2 is important, especially for critical-sized defects, due to the initial burst release of rhBMP-2 and the insufficient number of SCs from the boundary of the bone defect in such cases [137]. Kim et al. [79] found the application of BMP-2 to the mandibular defect one week after surgery, compared with admixing with a hyaluronic acid scaffold before the operation, resulted in significant increases in the mineral density, total volume, and trabecular volume of bone. Çakır-Özkan et al. [92] found that the combined use of VEGF + BMP-2 with the PLLA/PEG scaffold for rabbit mandibular defect resulted in increased osteoblastic activity and neovascularization when compared with the use of BMP-2 or VEGF alone. Similarly, the combined use of FGF + BMP-2 with PLGA/PCL/nHA scaffold for rabbit mandibular defect resulted in significantly promoted proliferation and osteogenic differentiation of BMSCs and osteogenesis than when BMP-2 and FGF were used alone [25]. In a study on sub-human primates of different ages, the host recipient tissue had the same capacity to respond to BMP-2 induction regardless of the subject's age [55]. In addition, previous studies have shown good bone regeneration of scaffolds with BMP-7 or BMP-6 factors [50,63,109]. Das et al. [83] found that the combined use of VEGF + BMP-6 with the PLGA scaffold for rat mandibular defects resulted in significantly enhanced bone repair through the enhancement of angiogenesis and the differentiation of endogenously recruited MSCs into the bone repair site.

Despite the encouraging results of BMPs for bone regeneration in the maxillofacial area for the bridging and ingrowth of bone, the results of biomechanical testing were not uniform. The mechanical tests performed in previous papers showed that the regeneration in the mandibular critical-size defects reconstructed with BMP-7 and BMP-2 showed a wide range of mechanical properties due to the varied proportions of woven and lamellar bone formation that were histologically shown, as well as possible variations in the concentration of BMPs within the grafts [73–75].

A study with porous nHAP/collagen/PLGA scaffolds with incorporated insulin-loaded microspheres showed a higher bone restoration capacity than the defects that were filled with nHAC scaffolds [33]. This finding may be important in the bone regeneration of patients diagnosed with diabetes.

Several studies investigated the use of concentrated growth factor (CGF), a third-generation platelet concentrate extracted from blood, featuring a wide range of sources, low cost, absorbability, lack of immunogenicity, and bone inducibility [101,102]. Zhu et al. [102] investigated the HAP/collagen scaffold in combination with CGF to repair mandibular rabbit defects. The results showed a higher rate of new bone formation, better bone quality, higher osteocalcin and BMP-2 expression, and higher compressive strength and elastic modulus of the nHAC/CGF cohort.

Due to the positive influence of BMPs on mandibular bone regeneration in in vivo studies, several case reports and case series reported the human application of BMPs [138–146]. Moghadam et al. [138] successfully used a BMP bioimplant for the primary reconstruction of a 6 cm mandibular critical-size defect after the segmental resection of ameloblastoma. Warnke et al. [139] used a titanium mesh cage filled with bone mineral blocks, recombinant human BMP-7, and the patient's bone marrow implanted in the *latissimus dorsi* muscle for the growth of the grafted bone. The graft was successfully transplanted as a free bone–muscle flap to reconstruct the mandibular defect. A similar study was performed by Heliotis et al. [140], who used a vascularized pedicled bone flap created with a HAP/BMP-7 composite implant; however, an unfavorable outcome was achieved due to graft failure occurring five months after the transplantation due to MRSA infection. Herford and Boyne [141] reported successful results in 14 patients treated with BMP-2 in a collagen carrier for various mandibular defects. Chao et al. [142] reported successful mandibular reconstruction with BMP-2, applied with collagen sponge and granules of 85% TCP and 15% HAP, following a hemimandibulectomy due to aggressive juvenile ossifying fibroma in a 9-year-old boy.

4.3. Cell Therapy

Seeding scaffolds with biologically active cells in order to promote bone regeneration directly at the implant site is a commonly employed bone tissue engineering strategy. All the studies with scaffolds serving as carriers for MSCs reported an increased bone formation compared to scaffold-only cohorts [23,26,34,39,71,81,82,85,98,99,106]. Scaffolds can function as a substitute for native ECM for the conduction, attachment, and growth of encapsulated MSCs cells and prevent anoikis, a form of apoptosis [10]. Furthermore, scaffolds are optimized to protect against host immune attack and induce major cellular processes that are necessary for tissue regeneration [10]. The most commonly used cell therapy used the bone-marrow-derived MSCs (BMSCs), which are capable of differentiating multiple mesodermal lineages, including bone and cartilage [23]. Guo et al. [23] investigated the bioactivity of an n-HA/PA composite implant seeded with ectogenic BMSCs and found that ectogenic BMSCs had a significant impact on bone regeneration in bone-marrow-poor locations such as mandible angle. The authors suggested that the presence of endogenic BMSCs at the implantation site was found to be a critical factor in determining the outcome of the bone regeneration process, since ectogenic BMSCs were not found to enhance bone regeneration in the rich-marrow sites such as the mandibular body, implicating different tissue engineering strategies for bone defects in marrow-rich and marrow-poor sites.

Dental pulp stem cells (DPSCs) offer certain advantages over other SCs, such as accessibility, availability and multipotency, maintain an undifferentiated state upon long-term cultivation, and are little influenced by the number of passages [146]. Gutiérrez-Quintero et al. [26] reported HA/PLGA/DPSCs scaffolds without offering improved bone regeneration due to significant differences in the mRNA levels of osteogenic markers capable of determining osteoblastic stage differentiation (Runx2, OPN, COL1, and ALP). Adipose-derived SCs (ADSCs) have certain advantages compared to BMSCs, such as easy harvesting, a huge number of cells can be obtained from fat tissue, cells that are easy to cultivate and a higher proliferation capacity [147]. Probst et al. [35] found that ADSCs seeding on ceramic/polymer scaffolds improve bone regeneration in large mandibular defects and results in significantly higher bone volume and an increased amount of osteocalcin deposition.

The combination of MSCs with BMP-2 and composite ceramic/polymer scaffolds resulted in significantly more bone regeneration than when using MSCs/scaffold and BMP-2/scaffold complexes [25,28,43,49]. Local gene therapy using BMPs transfected on MSCs and porous n-HA/PA scaffolds in the repair of mandibular defects in rabbits, as well as their response during various periods, showed good biocompatibility, more significant bone formation and earlier mineralization in the implant area. BMP7-transfected MSCs resulted in significantly higher-end elastic modulus, ultimate stress, and ultimate strain four weeks after implantation of the mandibular explant [28]. In addition, no differences were observed between the MSC-only and BMP7-transfected MSC groups at 16 weeks, impying that BMP-7 enhances bone formation in the early phases of mandibular bone regeneration [28]. However, in a study investigating the efficacy of PLGA scaffolds, alone and in combination with BMP-2 and ASCs, when healing a critical-sized segmental mandibular defect in a rat model, bone regeneration was most robust in the BMP-2-treated scaffolds [40]. In this study, bone regeneration scores were graded according to a previously described semi-quantitative scale based on micro-CT images without histological examination.

In a study that compared liposome-mediated gene transfer with MSCs and adenoviral-mediated MSC transduction with BMP-2, the authors found that adenovirus-transfected MSCs resulted in nearly complete bone healing within four weeks of the scaffold being implanted [61]. Still, the authors suggested that liposomes offer several advantages compared to other vectors, such as ease of preparation, no limitation on the DNA size, and fewer immunological and safety problems; thus, they may represent the best vector systems for trials of bone regeneration by BMP-2 gene therapy [61]. Regarding the clinical translation of the MSCs therapy in human mandibular reconstruction, Sandor et al. reported the successful reconstruction of a 10 cm anterior mandibular ameloblastoma resection defect with b-TCP granules, BMP-2, and autologous ASCs [148]. Thus, we may conclude that

the preclinical evaluation of MSCs is insufficient to demonstrate the clinical efficacy in mandibular bone regeneration. Presently, the main challenges that need to be resolved before the clinical application of cell therapy for mandibular defects include the choice of optimal MSC source, route of administration, and understanding of interactions between the scaffolds, host tissue, and cells.

4.4. Animal Models

Different animal models have been used to investigate mandibular BTE, including rats, rabbits, mini-pigs, domestic swine, dogs, sheep, and primates. Rats are cost-effective, easy to handle, and may be genetically engineered to construct different pathological states. However, rats have a higher metabolic rate and osteogenic potential compared with humans. Furthermore, the bone mineral density of rat cortical bone differs greatly from that of humans, and the operating space of rats is limited by their small size [149]. Rabbits are the most commonly used laboratory animals, as they are easy to acquire, house, and handle, with a short developmental cycle, reaching skeletal maturity at 6 months of age [150]. Rabbits exhibit faster cortical bone remodeling and bone turnover compared with rodents or primates. Due to their faster bone remodeling and large amounts of adipose tissue in the medullary spaces of the mandible, it is difficult to extrapolate in vivo results to human trials [151]. Dog models are widely used in maxillofacial research due to similarities with humans regarding the similar bone mineral density of the mandible and periodontal tissue and comparable intracortical remodeling, similar levels of collagen, and insulin-like growth factor-1 in cortical and cancellous bone. However, the rate of trabecular bone turnover in dogs is highly variable and higher compared to humans [152,153]. Pig bone models are used due to similarities in terms of bone mineral density and bone mineral concentration and the similar bone remodeling processes. Furthermore, mature swine have a similar bone structure to humans, with a well-developed Haversian system and a similar bone regeneration rate [154]. Swine are large animals, which are difficult to handle and maintain under experimental conditions [155]. The sheep model for bone regeneration was used due to its similar bone turnover and bone modeling rates to those of humans [156]. Sheep bones are sufficiently large to compare multiple surgical procedures simultaneously. However, the bones of sheep have a different microstructure and undergo seasonal periods of bone loss [157]. Non-human primates and humans share significant genetic homology, have comparable osteonal remodeling in skeletally mature individuals, and develop similar bone diseases and age-associated bone loss. Primates are generally used in bone biomechanics and loading studies when other large animal models do not adequately represent human bone. The use of non-human primates is expensive and is associated with some cultural and ethical questions [135]. The primary rationale for nHPs is the opportunity to match the genetic background and, therefore, the biological responsiveness of the model, as closely as possible to that of humans. Nonetheless, it has been assumed that the non-human primate model will provide the most predictive model for immunological and biological response due to its genetic background, particularly in the setting of delivery of BMPs, although the variation in response between primates limits these investigations [5,158]. The follow-up duration and the critical defect size varied widely and depended strongly on the animal used. Furthermore, the critical defect size was not uniform, even within a single animal model. Establishing uniform critical-sized defects is particularly difficult with small animals. Thus, it is difficult to fuse the results of the studies into unique conclusions to extrapolate to clinical conditions. Future studies on mandibular bone regeneration should maintain standardized animal models, possibly with larger animals (sheep, swine, and dogs), with standardized defects and follow-up periods.

4.5. Study Outcomes

Another important issue was the heterogeneity in the approaches used to evaluate in vivo bone regeneration, which, in many cases, enabled a direct comparison of outcomes between different treatment groups. Standard diagnostic approaches to the evaluation of

bone regeneration in vivo comprise radiological, histological, and mechanical testing [2]. The majority of studies used a histological analysis of the explanted mandibular defect to measure the quantity and quality of bone regeneration. Micro-CT and plain radiography were commonly used to quantitatively assess new bone formation. Micro-CT offers certain advantages for new bone formation, and several objective variables could be extracted: bone mineral density, trabecular thickness, and the percentage of newly formed bone [2]. Cone-beam computed tomography (CBCT) may be an interesting alternative because it is available, economical, and less expensive than CT or micro-CT [36]. CBCT can calculate the amount of new bone from grey-scale changes; however, the results are unpredictable, and more objective means of bone characterization are still not possible [36]. Immunohistochemistry was used along with histology and radiologic examinations to evaluate the maturity and formation rate of new bone [2]. Noting that the mandible, during the function of mastication, has to withstand significant muscle forces, the biomechanical characteristics of newly formed bone are extremely substantial. Still, only 12.5% of studies performed a biomechanical testing of mandibular bone defects.

This research has several limitations. The significant variations among the included studies, which made any reasonable comparison complex, were as follows: differences in animal models, various scaffold types, various means of producing scaffolds, differences in cell source, and inconsistent evaluation methods. In addition, several studies did not have control groups. Due to variations in animal models, there were significant differences in the concentrations of growth factors used, and only a few studies determined the dose–response curve. This review included studies with various animal models with substantial differences regarding the follow-up duration and the critical defect size, even within a single animal model. Due to a lack of consistency in the defect size, it is difficult to compare the outcomes between the studies. Similarly, there is no standard animal model for mandibular bone regeneration. There was considerable heterogeneity in the evaluation of in vivo bone regeneration, which enables the direct comparison of outcomes between treatment groups. Future studies should provide standardized measurements of bone regeneration, including histological analysis, imaging results (micro-CT), and biomechanical testing of the newly formed bone for a reliable comparison of the results.

5. Conclusions

Mandibular BTE can be considered a highly promising treatment for the reconstruction of critical-sized bone defects. It could become an alternative to microvascular bone grafts, which are considered a gold-standard treatment. In vivo trials are critical for translating from experimental to randomized clinical trials. The review aimed to systematically review in vivo studies and analyze this concept's effectiveness in treating mandibular critical-sized defects.

Currently, there are significant discrepancies between the studies due to various study methodologies, review periods, outcome measures, and different control groups, with significant differences occurring even within a single animal model. The standardization of the animal models, operative techniques, and definition of critical-sized defects for each model is needed, as well as the duration of follow-up and the evaluation of study outcomes. The results of this review support the use of biocompatible scaffolds, especially composite polymer/ceramic scaffolds, for bone regeneration, as they obtain significant results compared to blank controls. In addition, the success of mandibular bone engineering is significantly enhanced by the use of scaffolds with bioactive molecules and/or progenitor cells. However, the clinical application of biomolecules and progenitor cells is limited by its high costs, side effects, and unpredictable responses in humans. Therefore, further research is required to understand the biological fundamentals of the interplay between scaffolds, regulatory molecules, and progenitor cells to translate these experimental findings into clinical practice.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13084668/s1, Table S1: An overview of included trials.

Author Contributions: Conceptualization, J.D. and G.R.; writing—original draft preparation, J.D., S.V., D.S., I.O., B.J. and M.S.; writing—review and editing, J.D., S.V. and G.R.; supervision, M.S. and G.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: This work was supported by Internal Project N^0 15/21 of Faculty of Medical Sciences, University of Kragujevac, Serbia (01-773/1, 21 January 2022).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Vujovic, S.; Desnica, J.; Stanisic, D.; Ognjanovic, I.; Stevanovic, M.; Rosic, G. Applications of Biodegradable Magnesium-Based Materials in Reconstructive Oral and Maxillofacial Surgery: A Review. *Molecules* **2022**, *27*, 5529. [CrossRef]
2. Basyuni, S.; Ferro, A.; Santhanam, V.; Birch, M.; McCaskie, A. Systematic scoping review of mandibular bone tissue engineering. *Br. J. Oral Maxillofac. Surg.* **2020**, *58*, 632–642. [CrossRef]
3. Liu, L.; Shi, G.; Cui, Y.; Li, H.; Li, Z.; Zeng, Q.; Guo, Y. Individual construction of freeform-fabricated polycaprolactone scaffolds for osteogenesis. *Biomed. Tech.* **2017**, *62*, 467–479. [CrossRef]
4. Amini, A.R.; Laurencin, C.T.; Nukavarapu, S.P. Bone tissue engineering: Recent advances and challenges. *Crit. Rev. Biomed. Eng.* **2012**, *40*, 363–408. [CrossRef]
5. Oryan, A.; Alidadi, S.; Moshiri, A. Current concerns regarding healing of bone defects. *Hard Tissue* **2013**, *2*, 13. [CrossRef]
6. Jamari, J.; Ammarullah, M.I.; Saad, A.P.M.; Syahrom, A.; Uddin, M.; van der Heide, E.; Basri, H. The Effect of Bottom Profile Dimples on the Femoral Head on Wear in Metal-on-Metal Total Hip Arthroplasty. *J. Funct. Biomater.* **2021**, *12*, 38. [CrossRef]
7. Kretlow, J.D.; Young, S.; Klouda, L.; Wong, M.; Mikos, A.G. Injectable biomaterials for regenerating complex craniofacial tissues. *Adv. Mater.* **2009**, *21*, 3368–3393. [CrossRef]
8. Azevedo, H.S.; Pashkuleva, I. Biomimetic supramolecular designs for the controlled release of growth factors in bone regeneration. *Adv. Drug Deliv. Rev.* **2015**, *94*, 63–76. [CrossRef]
9. Oryan, A.; Kamali, A.; Moshiri, A.; Baghaban Eslaminejad, M. Role of Mesenchymal Stem Cells in Bone Regenerative Medicine: What Is the Evidence? *Cells Tissues Organs* **2017**, *204*, 59–83. [CrossRef]
10. Thrivikraman, G.; Athirasala, A.; Twohig, C.; Boda, S.K.; Bertassoni, L.E. Biomaterials for Craniofacial Bone Regeneration. *Dent. Clin. N. Am.* **2017**, *61*, 835–856. [CrossRef]
11. Putra, R.U.; Basri, H.; Prakoso, A.T.; Chandra, H.; Ammarullah, M.I.; Akbar, I.; Syahrom, A.; Kamarul, T. Level of Activity Changes Increases the Fatigue Life of the Porous Magnesium Scaffold, as Observed in Dynamic Immersion Tests, over Time. *Sustainability* **2023**, *15*, 823. [CrossRef]
12. Stevanovic, M.; Vujovic, S.; Stanisic, D.; Desnica, J.; Ognjanovic, I. The Use of Newly Synthesized Composite Scaffolds for Bone Regeneration—A Review of Literature. *Exp. Appl. Biomed. Res. EABR* **2022**. [CrossRef]
13. Venkataiah, V.S.; Yahata, Y.; Kitagawa, A.; Inagaki, M.; Kakiuchi, Y.; Nakano, M.; Suzuki, S.; Handa, K.; Saito, M. Clinical Applications of Cell-Scaffold Constructs for Bone Regeneration Therapy. *Cells* **2021**, *10*, 2687. [CrossRef]
14. Valdoz, J.C.; Johnson, B.C.; Jacobs, D.J.; Franks, N.A.; Dodson, E.L.; Sanders, C.; Cribbs, C.G.; Van Ry, P.M. The ECM: To Scaffold, or Not to Scaffold, That Is the Question. *Int. J. Mol. Sci.* **2021**, *22*, 12690. [CrossRef]
15. Velasco, M.A.; Narváez-Tovar, C.A.; Garzón-Alvarado, D.A. Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. *BioMed Res. Int.* **2015**, *2015*, 729076. [CrossRef]
16. Liu, J.; Kerns, D.G. Mechanisms of guided bone regeneration: A review. *Open Dent. J.* **2014**, *8*, 56–65. [CrossRef]
17. Nandi, S.K.; Roy, S.; Mukherjee, P.; Kundu, B.; De, D.K.; Basu, D. Orthopaedic applications of bone graft & graft substitutes: A review. *Indian J. Med. Res.* **2010**, *132*, 15–30.
18. Alam, S.; Ueki, K.; Marukawa, K.; Ohara, T.; Hase, T.; Takazakura, D.; Nakagawa, K. Expression of bone morphogenetic protein 2 and fibroblast growth factor 2 during bone regeneration using different implant materials as an onlay bone graft in rabbit mandibles. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontology* **2007**, *103*, 16–26. [CrossRef]
19. Sheikh, Z.; Hamdan, N.; Ikeda, Y.; Grynpas, M.; Ganss, B.; Glogauer, M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. *Biomater. Res.* **2017**, *21*, 9. [CrossRef]
20. Elgali, I.; Omar, O.; Dahlin, C.; Thomsen, P. Guided bone regeneration: Materials and biological mechanisms revisited. *Eur. J. Oral Sci.* **2017**, *125*, 315–337. [CrossRef]

21. Boyce, T.; Edwards, J.; Scarborough, N. ALLOGRAFT BONE: The influence of processing on safety and performance. *Orthop. Clin. N. Am.* **1999**, *30*, 571–581. [CrossRef]
22. Zhang, L.; Mu, W.; Chen, S.; Yang, D.; Xu, F.; Wu, Y. The enhancement of osteogenic capacity in a synthetic BMP-2 derived peptide coated mineralized collagen composite in the treatment of the mandibular defects. *Biomed. Mater. Eng.* **2016**, *27*, 495–505. [CrossRef]
23. Guo, J.; Meng, Z.; Chen, G.; Xie, D.; Chen, Y.; Wang, H.; Tang, W.; Liu, L.; Jing, W.; Long, J.; et al. Restoration of critical-size defects in the rabbit mandible using porous nanohydroxyapatite-polyamide scaffolds. *Tissue Eng. Part A* **2012**, *18*, 1239–1252. [CrossRef] [PubMed]
24. Liu, Y.; Liu, S.; Fu, Y.; Chang, D.T.; Zhou, Y.H. Mineralised Collagen Scaffolds Loaded with Stromal Cell-derived Factor-1 Improve Mandibular Bone Regeneration. *Chin. J. Dent. Res.* **2014**, *17*, 23–29. [PubMed]
25. Su, J.; Xu, H.; Sun, J.; Gong, X.; Zhao, H. Dual delivery of BMP-2 and bFGF from a new nano-composite scaffold, loaded with vascular stents for large-size mandibular defect regeneration. *Int. J. Mol. Sci.* **2013**, *14*, 12714–12728. [CrossRef] [PubMed]
26. Gutiérrez-Quintero, J.G.; Durán Riveros, J.Y.; Martínez Valbuena, C.A.; Pedraza Alonso, S.; Munévar, J.C.; Viafara-García, S.M. Critical-sized mandibular defect reconstruction using human dental pulp stem cells in a xenograft model-clinical, radiological, and histological evaluation. *Oral Maxillofac. Surg.* **2020**, *24*, 485–493. [CrossRef]
27. Liu, X.; Zhao, K.; Gong, T.; Song, J.; Bao, C.; Luo, E.; Weng, J.; Zhou, S. Delivery of growth factors using a smart porous nanocomposite scaffold to repair a mandibular bone defect. *Biomacromolecules* **2014**, *15*, 1019–1030. [CrossRef]
28. Li, J.; Li, Y.; Ma, S.; Gao, Y.; Zuo, Y.; Hu, J. Enhancement of bone formation by BMP-7 transduced MSCs on biomimetic nano-hydroxyapatite/polyamide composite scaffolds in repair of mandibular defects. *J. Biomed. Mater. Res. A* **2010**, *95*, 973–981. [CrossRef]
29. Jiang, X.; Gittens, S.A.; Chang, Q.; Zhang, X.; Chen, C.; Zhang, Z. The use of tissue-engineered bone with human bone morphogenetic protein-4-modified bone-marrow stromal cells in repairing mandibular defects in rabbits. *Int. J. Oral Maxillofac. Surg.* **2006**, *35*, 1133–1139. [CrossRef]
30. Zhao, J.; Hu, J.; Wang, S.; Sun, X.; Xia, L.; Zhang, X.; Zhang, Z.; Jiang, X. Combination of beta-TCP and BMP-2 gene-modified bMSCs to heal critical size mandibular defects in rats. *Oral Dis.* **2010**, *16*, 46–54. [CrossRef]
31. Wu, W.; Chen, X.; Mao, T.; Chen, F.; Feng, X. Bone marrow-derived osteoblasts seeded into porous beta-tricalcium phosphate to repair segmental defect in canine's mandibula. *Ulus Travma Acil Cerrahi Derg* **2006**, *12*, 268–276. [PubMed]
32. Liu, X.; Liu, H.Y.; Lian, X.; Shi, X.L.; Wang, W.; Cui, F.Z.; Zhang, Y. Osteogenesis of mineralized collagen bone graft modified by PLA and calcium sulfate hemihydrate: In vivo study. *J. Biomater. Appl.* **2013**, *28*, 12–19. [CrossRef] [PubMed]
33. Wang, X.; Wu, X.; Xing, H.; Zhang, G.; Shi, Q.; E, L.; Liu, N.; Yang, T.; Wang, D.; Qi, F.; et al. Porous Nanohydroxyapatite/Collagen Scaffolds Loading Insulin PLGA Particles for Restoration of Critical Size Bone Defect. *ACS Appl. Bio. Mater.* **2017**, *9*, 11380–11391. [CrossRef] [PubMed]
34. Cui, Y.; Lu, C.; Chen, B.; Han, J.; Zhao, Y.; Xiao, Z.; Han, S.; Pan, J.; Dai, J. Restoration of mandibular bone defects with demineralized bone matrix combined with three-dimensional cultured bone marrow-derived mesenchymal stem cells in minipig models. *J. Mater. Sci. Mater. Med.* **2018**, *29*, 147. [CrossRef]
35. Probst, F.A.; Fliefel, R.; Burian, E.; Probst, M.; Eddicks, M.; Cornelsen, M.; Riedl, C.; Seitz, H.; Aszódi, A.; Schieker, M.; et al. Bone regeneration of minipig mandibular defect by adipose derived mesenchymal stem cells seeded tri-calcium phosphate-poly(D,L-lactide-co-glycolide) scaffolds. *Sci. Rep.* **2020**, *10*, 2062. [CrossRef]
36. Stevanovic, M.; Selakovic, D.; Vasovic, M.; Ljujic, B.; Zivanovic, S.; Papic, M.; Zivanovic, M.; Milivojevic, N.; Mijovic, M.; Tabakovic, S.Z.; et al. Comparison of Hydroxyapatite/Poly(lactide-co-glycolide) and Hydroxyapatite/Polyethyleneimine Composite Scaffolds in Bone Regeneration of pigs Mandibular Critical Size Defects: In Vivo Study. *Molecules* **2022**, *27*, 1694. [CrossRef]
37. He, Y.; Zhang, Z.Y.; Zhu, H.G.; Qiu, W.; Jiang, X.; Guo, W. Experimental study on reconstruction of segmental mandible defects using tissue engineered bone combined bone marrow stromal cells with three-dimensional tricalcium phosphate. *J. Craniofac Surg.* **2007**, *18*, 800–805. [CrossRef]
38. Yuan, J.; Cui, L.; Zhang, W.J.; Liu, W.; Cao, Y. Repair of canine mandibular bone defects with bone marrow stromal cells and porous beta-tricalcium phosphate. *Biomaterials* **2007**, *28*, 1005–1013. [CrossRef]
39. Tateno, A.; Asano, M.; Akita, D.; Toriumi, T.; Tsurumachi-Iwasaki, N.; Kazama, T.; Arai, Y.; Matsumoto, T.; Kano, K.; Honda, M. Transplantation of dedifferentiated fat cells combined with a biodegradable type I collagen-recombinant peptide scaffold for critical-size bone defects in rats. *J. Oral Sci.* **2019**, *61*, 534–538. [CrossRef]
40. Lee, M.K.; DeConde, A.S.; Lee, M.; Walthers, C.M.; Sepahdari, A.R.; Elashoff, D.; Grogan, T.; Bezouglaia, O.; Tetradis, S.; St John, M.; et al. Biomimetic scaffolds facilitate healing of critical-sized segmental mandibular defects. *Am. J. Otolaryngol.* **2015**, *36*, 1–6. [CrossRef]
41. Fan, J.; Guo, M.; Im, C.S.; Pi-Anfruns, J.; Cui, Z.K.; Kim, S.; Wu, B.M.; Aghaloo, T.L.; Lee, M. Enhanced Mandibular Bone Repair by Combined Treatment of Bone Morphogenetic Protein 2 and Small-Molecule Phenamil. *Tissue Eng. Part A.* **2017**, *23*, 195–207. [CrossRef] [PubMed]
42. Mankani, M.H.; Kuznetsov, S.A.; Wolfe, R.M.; Marshall, G.W.; Robey, P.G. In vivo bone formation by human bone marrow stromal cells: Reconstruction of the mouse calvarium and mandible. *Stem. Cells* **2006**, *24*, 2140–2149. [CrossRef] [PubMed]

43. Tee, B.C.; Desai, K.G.; Kennedy, K.S.; Sonnichsen, B.; Kim, D.G.; Fields, H.W.; Mallery, S.R.; Schwendeman, S.P.; Sun, Z. Reconstructing jaw defects with MSCs and PLGA-encapsulated growth factors. *Am. J. Transl. Res.* **2016**, *8*, 2693–2704.
44. Lopez, C.D.; Diaz-Siso, J.R.; Witek, L.; Bekisz, J.M.; Cronstein, B.N.; Torroni, A.; Flores, R.L.; Rodriguez, E.D.; Coelho, P.G. Three dimensionally printed bioactive ceramic scaffold osseoconduction across critical-sized mandibular defects. *J. Surg. Res.* **2018**, *223*, 115–122. [CrossRef]
45. Herford, A.S.; Lu, M.; Buxton, A.N.; Kim, J.; Henkin, J.; Boyne, P.J.; Caruso, J.M.; Rungcharassaeng, K.; Hong, J. Recombinant human bone morphogenetic protein 2 combined with an osteoconductive bulking agent for mandibular continuity defects in nonhuman primates. *J. Oral Maxillofac. Surg.* **2012**, *70*, 703–716. [CrossRef]
46. Toriumi, D.M.; O'Grady, K.; Horlbeck, D.M.; Desai, D.; Turek, T.J.; Wozney, J. Mandibular reconstruction using bone morphogenetic protein 2: Long-term follow-up in a canine model. *Laryngoscope* **1999**, *109*, 1481–1489. [CrossRef]
47. Witek, L.; Tian, H.; Tovar, N.; Torroni, A.; Neiva, R.; Gil, L.F.; Coelho, P.G. The effect of platelet-rich fibrin exudate addition to porous poly(lactic-co-glycolic acid) scaffold in bone healing: An in vivo study. *J. Biomed. Mater. Res. B Appl. Biomater.* **2020**, *108*, 1304–1310. [CrossRef]
48. Konopnicki, S.; Sharaf, B.; Resnick, C.; Patenaude, A.; Pogal-Sussman, T.; Hwang, K.G.; Abukawa, H.; Troulis, M.J. Tissue-engineered bone with 3-dimensionally printed β-tricalcium phosphate and polycaprolactone scaffolds and early implantation: An in vivo pilot study in a porcine mandible model. *J. Oral Maxillofac. Surg.* **2015**, *73*, 1016.e1–1016.e11. [CrossRef]
49. Fan, J.; Park, H.; Lee, M.K.; Bezouglaia, O.; Fartash, A.; Kim, J.; Aghaloo, T.; Lee, M. Adipose-derived stem cells and BMP-2 delivery in chitosan-based 3D constructs to enhance bone regeneration in a rat mandibular defect model. *Tissue Eng. Part A* **2014**, *20*, 2169–2179. [CrossRef]
50. Das, A.; Fishero, B.A.; Christophel, J.J.; Li, C.J.; Kohli, N.; Lin, Y.; Dighe, A.S.; Cui, Q. Poly(lactic-co-glycolide) polymer constructs cross-linked with human BMP-6 and VEGF protein significantly enhance rat mandible defect repair. *Cell Tissue Res.* **2016**, *364*, 125–135. [CrossRef]
51. DeConde, A.S.; Sidell, D.; Lee, M.; Bezouglaia, O.; Low, K.; Elashoff, D.; Grogan, T.; Tetradis, S.; Aghaloo, T.; St John, M. Bone morphogenetic protein-2-impregnated biomimetic scaffolds successfully induce bone healing in a marginal mandibular defect. *Laryngoscope* **2013**, *123*, 1149–1155. [CrossRef]
52. Arosarena, O.; Collins, W. Comparison of BMP-2 and -4 for rat mandibular bone regeneration at various doses. *Orthod. Craniofac. Res.* **2005**, *8*, 267–276. [CrossRef] [PubMed]
53. Arosarena, O.A.; Collins, W.L. Bone regeneration in the rat mandible with bone morphogenetic protein-2: A comparison of two carriers. *Otolaryngol. Head Neck Surg.* **2005**, *132*, 592–597. [CrossRef]
54. Arosarena, O.A.; Falk, A.; Malmgren, L.; Bookman, L.; Allen, M.J.; Schoonmaker, J.; Tatum, S.; Kellman, R. Defect repair in the rat mandible with bone morphogenic proteins and marrow cells. *Arch. Facial Plast. Surg.* **2003**, *5*, 103–108. [CrossRef]
55. Boyne, P.J.; Salina, S.; Nakamura, A.; Audia, F.; Shabahang, S. Bone regeneration using rhBMP-2 induction in hemimandibulectomy type defects of elderly sub-human primates. *Cell Tissue Bank.* **2006**, *7*, 1–10. [CrossRef]
56. Lohse, N.; Moser, N.; Backhaus, S.; Annen, T.; Epple, M.; Schliephake, H. Continuous delivery of rhBMP2 and rhVEGF165 at a certain ratio enhances bone formation in mandibular defects over the delivery of rhBMP2 alone–An experimental study in rats. *J. Control. Release* **2015**, *220*, 201–209. [CrossRef]
57. Moser, N.; Lohse, N.; Golstein, J.; Kauffmann, P.; Sven, B.; Epple, M.; Schliephake, H. Do we need retarded delivery of bone growth factors in facial bone repair? An experimental study in rats. *Eur. Cells Mater.* **2017**, *34*, 162–179. [CrossRef]
58. Springer, I.N.; Niehoff, P.; Açil, Y.; Marget, M.; Lange, A.; Warnke, P.H.; Pielenz, H.; Roldán, J.C.; Wiltfang, J. BMP-2 and bFGF in an irradiated bone model. *J. Craniomaxillofac. Surg.* **2008**, *36*, 210–217. [CrossRef]
59. Yahia, S.; Khalil, I.A.; El-Sherbiny, I.M. Sandwich-Like Nanofibrous Scaffolds for Bone Tissue Regeneration. *ACS Appl. Mater. Interfaces* **2019**, *11*, 28610–28620. [CrossRef]
60. Schliephake, H.; Weich, H.A.; Dullin, C.; Gruber, R.; Frahse, S. Mandibular bone repair by implantation of rhBMP-2 in a slow release carrier of polylactic acid–an experimental study in rats. *Biomaterials* **2008**, *29*, 103–110. [CrossRef]
61. Park, J.; Ries, J.; Gelse, K.; Kloss, F.; von der Mark, K.; Wiltfang, J.; Neukam, F.W.; Schneider, H. Bone regeneration in critical size defects by cell-mediated BMP-2 gene transfer: A comparison of adenoviral vectors and liposomes. *Gene Ther.* **2003**, *10*, 1089–1098. [CrossRef] [PubMed]
62. Streckbein, P.; Jäckel, S.; Malik, C.Y.; Obert, M.; Kähling, C.; Wilbrand, J.F.; Zahner, D.; Heidinger, K.; Kampschulte, M.; Pons-Kühnemann, J.; et al. Reconstruction of critical-size mandibular defects in immunoincompetent rats with human adipose-derived stromal cells. *J. Craniomaxillofac. Surg.* **2013**, *41*, 496–503. [CrossRef] [PubMed]
63. Wang, H.; Springer, I.N.; Schildberg, H.; Acil, Y.; Ludwig, K.; Rueger, D.R.; Terheyden, H. Carboxymethylcellulose-stabilized collagenous rhOP-1 device-a novel carrier biomaterial for the repair of mandibular continuity defects. *J. Biomed. Mater. Res. Part A* **2004**, *68*, 219–226. [CrossRef] [PubMed]
64. Schliephake, H.; Knebel, J.W.; Aufderheide, M.; Tauscher, M. Use of cultivated osteoprogenitor cells to increase bone formation in segmental mandibular defects: An experimental pilot study in sheep. *Int. J. Oral Maxillofac. Surg.* **2001**, *30*, 531–537. [CrossRef]
65. Ozaki, M.; Takayama, T.; Yamamoto, T.; Ozawa, Y.; Nagao, M.; Tanabe, N.; Nakajima, A.; Suzuki, N.; Maeno, M.; Yamano, S.; et al. A collagen membrane containing osteogenic protein-1 facilitates bone regeneration in a rat mandibular bone defect. *Arch. Oral Biol.* **2017**, *84*, 19–28. [CrossRef]

66. Marukawa, E.; Asahina, I.; Oda, M.; Seto, I.; Alam, M.; Enomoto, S. Functional reconstruction of the non-human primate mandible using recombinant human bone morphogenetic protein-2. *Int. J. Oral Maxillofac. Surg.* **2002**, *31*, 287–295. [CrossRef]
67. Yamada, Y.; Ueda, M.; Naiki, T.; Takahashi, M.; Hata, K.; Nagasaka, T. Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: Tissue-engineered bone regeneration. *Tissue Eng.* **2004**, *10*, 955–964. [CrossRef]
68. Seto, I.; Marukawa, E.; Asahina, I. Mandibular reconstruction using a combination graft of rhBMP-2 with bone marrow cells expanded in vitro. *Plast. Reconstr. Surg.* **2006**, *117*, 902–908. [CrossRef]
69. Lopez, C.D.; Diaz-Siso, J.R.; Witek, L.; Bekisz, J.M.; Gil, L.F.; Cronstein, B.N.; Flores, R.L.; Torroni, A.; Rodriguez, E.D.; Coelho, P.G. Dipyridamole Augments Three-Dimensionally Printed Bioactive Ceramic Scaffolds to Regenerate Craniofacial Bone. *Plast. Reconstr. Surg.* **2019**, *143*, 1408–1419. [CrossRef]
70. Seto, I.; Asahina, I.; Oda, M.; Enomoto, S. Reconstruction of the primate mandible with a combination graft of recombinant human bone morphogenetic protein-2 and bone marrow. *J. Oral Maxillofac. Surg.* **2001**, *59*, 53–63. [CrossRef]
71. Alfotawei, R.; Naudi, K.B.; Lappin, D.; Barbenel, J.; Di Silvio, L.; Hunter, K.; McMahon, J.; Ayoub, A. The use of TriCalcium Phosphate (TCP) and stem cells for the regeneration of osteoperiosteal critical-size mandibular bony defects, an in vitro and preclinical study. *J. Craniomaxillofac. Surg.* **2014**, *42*, 863–869. [CrossRef]
72. Abu-Serriah, M.M.; Odell, E.; Lock, C.; Gillar, A.; Ayoub, A.F.; Fleming, R.H. Histological assessment of bioengineered new bone in repairing osteoperiosteal mandibular defects in sheep using recombinant human bone morphogenetic protein-7. *Br. J. Oral Maxillofac. Surg.* **2004**, *42*, 410–418. [CrossRef] [PubMed]
73. Abu-Serriah, M.; Kontaxis, A.; Ayoub, A.; Harrison, J.; Odell, E.; Barbenel, J. Mechanical evaluation of mandibular defects reconstructed using osteogenic protein-1 (rhOP-1) in a sheep model: A critical analysis. *Int. J. Oral Maxillofac. Surg.* **2005**, *34*, 287–293. [CrossRef] [PubMed]
74. Kontaxis, A.; Abu-Serriah, M.; Ayoub, A.F.; Barbenel, J.C. Mechanical testing of recombinant human bone morphogenetic protein-7 regenerated bone in sheep mandibles. *Proc. Inst. Mech. Eng. H* **2004**, *218*, 381–388. [CrossRef] [PubMed]
75. Busuttil Naudi, K.; Ayoub, A.; McMahon, J.; Di Silvio, L.; Lappin, D.; Hunter, K.D.; Barbenel, J. Mandibular reconstruction in the rabbit using beta-tricalcium phosphate (β-TCP) scaffolding and recombinant bone morphogenetic protein 7 (rhBMP-7)—Histological, radiographic and mechanical evaluations. *J. Craniomaxillofac. Surg.* **2012**, *40*, e461–e469. [CrossRef]
76. Deng, N.; Sun, J.; Li, Y.; Chen, L.; Chen, C.; Wu, Y.; Wang, Z.; Li, L. Experimental study of rhBMP-2 chitosan nano-sustained release carrier-loaded PLGA/nHA scaffolds to construct mandibular tissue-engineered bone. *Arch. Oral Biol.* **2019**, *102*, 16–25. [CrossRef]
77. Jin, I.G.; Kim, J.H.; Wu, H.G.; Kim, S.K.; Park, Y.; Hwang, S.J. Effect of bone marrow-derived stem cells and bone morphogenetic protein-2 on treatment of osteoradionecrosis in a rat model. *J. Craniomaxillofac. Surg.* **2015**, *43*, 1478–1486. [CrossRef]
78. Yun, P.Y.; Kim, Y.K.; Jeong, K.I.; Park, J.C.; Choi, Y.J. Influence of bone morphogenetic protein and proportion of hydroxyapatite on new bone formation in biphasic calcium phosphate graft: Two pilot studies in animal bony defect model. *J. Craniomaxillofac. Surg.* **2014**, *42*, 1909–1917. [CrossRef]
79. Kim, J.; Yang, H.J.; Cho, T.H.; Lee, S.E.; Park, Y.D.; Kim, H.M.; Kim, I.S.; Seo, Y.K.; Hwang, S.J.; Kim, S.J. Enhanced regeneration of rabbit mandibular defects through a combined treatment of electrical stimulation and rhBMP-2 application. *Med. Biol. Eng. Comput.* **2013**, *51*, 1339–1348. [CrossRef]
80. Poudel, S.B.; Bhattarai, G.; Kim, J.H.; Kook, S.H.; Seo, Y.K.; Jeon, Y.M.; Lee, J.C. Local delivery of recombinant human FGF7 enhances bone formation in rat mandible defects. *J. Bone Miner. Metab.* **2017**, *35*, 485–496. [CrossRef]
81. Behnia, A.; Haghighat, A.; Talebi, A.; Nourbakhsh, N.; Heidari, F. Transplantation of stem cells from human exfoliated deciduous teeth for bone regeneration in the dog mandibular defect. *World J. Stem. Cells* **2014**, *6*, 505–510. [CrossRef] [PubMed]
82. Khojasteh, A.; Behnia, H.; Hosseini, F.S.; Dehghan, M.M.; Abbasnia, P.; Abbas, F.M. The effect of PCL-TCP scaffold loaded with mesenchymal stem cells on vertical bone augmentation in dog mandible: A preliminary report. *J. Biomed. Mater. Res. B Appl. Biomater.* **2013**, *101*, 848–854. [CrossRef]
83. Gallego, L.; Junquera, L.; García, E.; García, V.; Alvarez-Viejo, M.; Costilla, S.; Fresno, M.F.; Meana, A. Repair of rat mandibular bone defects by alveolar osteoblasts in a novel plasma-derived albumin scaffold. *Tissue Eng. Part A* **2010**, *16*, 1179–1187. [CrossRef]
84. Gallego, L.; Pérez-Basterrechea, M.; García-Consuegra, L.; Álvarez-Viejo, M.; Megías, J.; Novoa, A.; Costilla, S.; Meana, Á.; Junquera, L. Repair of segmental mandibular bone defects in sheep using bone marrow stromal cells and autologous serum scaffold: A pilot study. *J. Clin. Periodontol.* **2015**, *42*, 1143–1151. [CrossRef]
85. Park, J.H.; Jung, S.Y.; Lee, C.K.; Ban, M.J.; Lee, S.J.; Kim, H.Y.; Oh, H.J.; Kim, B.K.; Park, H.S.; Jang, S.H.; et al. A 3D-printed polycaprolactone/β-tricalcium phosphate mandibular prosthesis: A pilot animal study. *Laryngoscope* **2020**, *130*, 358–366. [CrossRef]
86. Li, X.; Song, T.; Chen, X.; Wang, M.; Yang, X.; Xiao, Y.; Zhang, X. Osteoinductivity of Porous Biphasic Calcium Phosphate Ceramic Spheres with Nanocrystalline and Their Efficacy in Guiding Bone Regeneration. *ACS Appl. Mater. Interfaces* **2019**, *11*, 3722–3736. [CrossRef]
87. Issa, J.P.; do Nascimento, C.; Bentley, M.V.; Del Bel, E.A.; Iyomasa, M.M.; Sebald, W.; de Albuquerque, R.F., Jr. Bone repair in rat mandible by rhBMP-2 associated with two carriers. *Micron* **2008**, *39*, 373–379. [CrossRef] [PubMed]
88. Hussein, K.A.; Zakhary, I.E.; Elawady, A.R.; Emam, H.A.; Sharawy, M.; Baban, B.; Akeel, S.; Al-Shabrawey, M.; Elsalanty, M.E. Difference in soft tissue response between immediate and delayed delivery suggests a new mechanism for recombinant human bone morphogenetic protein 2 action in large segmental bone defects. *Tissue Eng. Part A* **2012**, *18*, 665–675. [CrossRef] [PubMed]

89. Ekholm, M.; Hietanen, J.; Tulamo, R.M.; Muhonen, J.; Lindqvist, C.; Kellomäki, M.; Suuronen, R. The copolymer of epsilon-caprolactone-lactide and tricalcium phosphate does not enhance bone growth in mandibular defect of sheep. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 139–145. [CrossRef]
90. Jégoux, F.; Goyenvalle, E.; Cognet, R.; Malard, O.; Moreau, F.; Daculsi, G.; Aguado, E. Mandibular segmental defect regenerated with macroporous biphasic calcium phosphate, collagen membrane, and bone marrow graft in dogs. *Arch. Otolaryngol. Head Neck Surg.* **2010**, *136*, 971–978. [CrossRef]
91. Srouji, S.; Rachmiel, A.; Blumenfeld, I.; Livne, E. Mandibular defect repair by TGF-beta and IGF-1 released from a biodegradable osteoconductive hydrogel. *J. Craniomaxillofac. Surg.* **2005**, *33*, 79–84. [CrossRef] [PubMed]
92. Çakır-Özkan, N.; Eğri, S.; Bekar, E.; Altunkaynak, B.Z.; Kabak, Y.B.; Kıvrak, E.G. The Use of Sequential VEGF- and BMP2-Releasing Biodegradable Scaffolds in Rabbit Mandibular Defects. *J. Oral Maxillofac. Surg.* **2017**, *75*, 221.e1–221.e14. [CrossRef] [PubMed]
93. Carlisle, P.; Guda, T.; Silliman, D.T.; Burdette, A.J.; Talley, A.D.; Alvarez, R.; Tucker, D.; Hale, R.G.; Guelcher, S.A.; BrownBaer, P.R. Localized low-dose rhBMP-2 is effective at promoting bone regeneration in mandibular segmental defects. *J. Biomed. Mater. Res. B Appl. Biomater.* **2019**, *107*, 1491–1503. [CrossRef] [PubMed]
94. Stigler, R.G.; Schimke, M.M.; Bigus, S.; Steinmüller-Nethl, D.; Tillmann, K.; Lepperdinger, G. Pervasion of beta-tricalcium phosphate with nanodiamond particles yields efficient and safe bone replacement material amenable for biofunctionalization and application in large-size osseous defect healing. *Nanomed. Nanotechnol. Biol. Med.* **2019**, *16*, 250–257. [CrossRef]
95. Wang, Y.; Cai, X.; Huang, J.; Zhou, Y.; Jiang, T.; Wang, Y. Bone regeneration in critically sized rat mandible defects through the endochondral pathway using hydroxyapatite-coated 3D-printed Ti6Al4V scaffolds. *RSC Adv.* **2018**, *8*, 31745–31754. [CrossRef]
96. Helal, M.H.; Hendawy, H.D.; Gaber, R.A.; Helal, N.R.; Aboushelib, M.N. Osteogenesis ability of CAD-CAM biodegradable polylactic acid scaffolds for reconstruction of jaw defects. *J. Prosthet. Dent.* **2019**, *121*, 118–123. [CrossRef]
97. Khojasteh, A.; Hosseinpour, S.; Dehghan, M.M.; Mashhadiabbas, F.; Rezai Rad, M.; Ansari, S.; Farzad Mohajeri, S.; Zadeh, H.H. Antibody-Mediated Osseous Regeneration for Bone Tissue Engineering in Canine Segmental Defects. *BioMed Res. Int.* **2018**, *2018*, 9508721. [CrossRef]
98. Nuntanaranont, T.; Promboot, T.; Sutapreyasri, S. Effect of expanded bone marrow-derived osteoprogenitor cells seeded into polycaprolactone/tricalcium phosphate scaffolds in new bone regeneration of rabbit mandibular defects. *J. Mater. Sci. Mater. Med.* **2018**, *29*, 24. [CrossRef]
99. Maglione, M.; Spano, S.; Ruaro, M.E.; Salvador, E.; Zanconati, F.; Tromba, G.; Turco, G. In vivo evaluation of chitosan-glycerol gel scaffolds seeded with stem cells for full-thickness mandibular bone regeneration. *J. Oral Sci.* **2017**, *59*, 225–232. [CrossRef]
100. Song, W.Y.; Liu, G.M.; Li, J.; Luo, Y.G. Bone morphogenetic protein-2 sustained delivery by hydrogels with microspheres repairs rabbit mandibular defects. *Tissue Eng. Regen. Med.* **2016**, *13*, 750–761. [CrossRef]
101. Mao, Y.; Hu, M.; Chen, L.; Chen, X.; Liu, M.; Zhang, M.; Nie, M.; Liu, X. CGF-HLC-I repaired the bone defect repair of the rabbits mandible through tight junction pathway. *Front. Bioeng. Biotechnol.* **2022**, *10*, 976499. [CrossRef] [PubMed]
102. Zhu, Y.; Cao, N.; Zhang, Y.; Cao, G.; Hao, C.; Liu, K.; Li, X.; Wang, W. The Ability and Mechanism of nHAC/CGF in Promoting Osteogenesis and Repairing Mandibular Defects. *Nanomaterials* **2022**, *12*, 212. [CrossRef] [PubMed]
103. Cao, S.S.; Li, S.Y.; Geng, Y.M.; Kapat, K.; Liu, S.B.; Perera, F.H.; Li, Q.; Terheyden, H.; Wu, G.; Che, Y.J.; et al. Prefabricated 3D-Printed Tissue-Engineered Bone for Mandibular Reconstruction: A Preclinical Translational Study in Primate. *ACS Biomater. Sci. Eng.* **2021**, *7*, 5727–5738. [CrossRef]
104. Barrientos-Lezcano, F.J.; Redondo-González, L.M.; Alberca-Zeballos, M.; Sánchez-García, A.M.; García-Sancho, J. Mandibular bone regeneration with autologous adipose-derived mesenchymal stem cells and coralline hydroxyapatite: Experimental study in rats. *Br. J. Oral Maxillofac. Surg.* **2021**, *59*, 1192–1199. [CrossRef] [PubMed]
105. Xu, H.Z.; Su, J.S. Restoration of critical defects in the rabbit mandible using osteoblasts and vascular endothelial cells co-cultured with vascular stent-loaded nano-composite scaffolds. *J. Mech. Behav. Biomed. Mater.* **2021**, *124*, 104831. [CrossRef] [PubMed]
106. Lee, J.S.; Park, T.H.; Ryu, J.Y.; Kim, D.K.; Oh, E.J.; Kim, H.M.; Shim, J.H.; Yun, W.S.; Huh, J.B.; Moon, S.H.; et al. Osteogenesis of 3D-Printed PCL/TCP/bdECM Scaffold Using Adipose-Derived Stem Cells Aggregates; An Experimental Study in the Canine Mandible. *Int. J. Mol. Sci.* **2021**, *22*, 5409. [CrossRef]
107. Park, S.A.; Lee, H.J.; Kim, S.Y.; Kim, K.S.; Jo, D.W.; Park, S.Y. Three-dimensionally printed polycaprolactone/beta-tricalcium phosphate scaffold was more effective as an rhBMP-2 carrier for new bone formation than polycaprolactone alone. *J. Biomed. Mater. Res. A* **2021**, *109*, 840–848. [CrossRef]
108. Zhang, P.; Hong, Z.; Yu, T.; Chen, X.; Jing, X. In vivo mineralization and osteogenesis of nanocomposite scaffold of poly(lactide-co-glycolide) and hydroxyapatite surface-grafted with poly(L-lactide). *Biomaterials* **2009**, *30*, 58–70. [CrossRef]
109. Terheyden, H.; Warnke, P.; Dunsche, A.; Jepsen, S.; Brenner, W.; Palmie, S.; Toth, C.; Rueger, D.R. Mandibular reconstruction with prefabricated vascularized bone grafts using recombinant human osteogenic protein-1: An experimental study in miniature pigs. Part II: Transplantation. *Int. J. Oral Maxillofac. Surg.* **2001**, *30*, 469–478. [CrossRef]
110. Lynn, A.K.; Yannas, I.V.; Bonfield, W. Antigenicity and immunogenicity of collagen. *J. Biomed. Mater. Res. B Appl. Biomater.* **2004**, *71*, 343–354. [CrossRef]
111. Kim, M.H.; Kim, B.S.; Park, H.; Lee, J.; Park, W.H. Injectable methylcellulose hydrogel containing calcium phosphate nanoparticles for bone regeneration. *Int. J. Biol. Macromol.* **2018**, *109*, 57–64. [CrossRef] [PubMed]

112. Townsend, J.M.; Andrews, B.T.; Feng, Y.; Wang, J.; Nudo, R.J.; Van Kampen, E.; Gehrke, S.H.; Berkland, C.J.; Detamore, M.S. Superior calvarial bone regeneration using pentenoate-functionalized hyaluronic acid hydrogels with devitalized tendon particles. *Acta Biomater.* **2018**, *71*, 148–155. [CrossRef] [PubMed]
113. Zhai, P.; Peng, X. Li, B. Liu, Y.; Sun, H.; Li, X. The application of hyaluronic acid in bone regeneration. *Int. J. Biol. Macromol.* **2020**, *151*, 1224–1239. [CrossRef] [PubMed]
114. Micic, M.; Antonijevic, D.; Milutinovic-Smiljanic, S.; Trisic, D.; Colovic, B.; Kosanovic, D.; Prokic, B.; Vasic, J.; Zivkovic, S.; Milasin, J.; et al. Developing a novel resorptive hydroxyapatite-based bone substitute for over-critical size defect reconstruction: Physicochemical and biological characterization and proof of concept in segmental rabbit's ulna reconstruction. Biomedizinische Technik. *Biomed. Eng.* **2020**, *65*, 491–505. [CrossRef] [PubMed]
115. Zhao, D.; Zhu, T.; Li, J.; Cui, L.; Zhang, Z.; Zhuang, X.; Ding, J. Poly(lactic-co-glycolic acid)-based composite bone-substitute materials. *Bioact. Mater.* **2020**, *6*, 346–360. [CrossRef] [PubMed]
116. Liu, C.G.; Zeng, Y.T.; Kankala, R.K.; Zhang, S.S.; Chen, A.Z.; Wang, S.B. Characterization and Preliminary Biological Evaluation of 3D-Printed Porous Scaffolds for Engineering Bone Tissues. *Materials* **2018**, *11*, 1832. [CrossRef]
117. Dong, X.P.; Zhang, Y.W.; Pei, Y.J.; Wang, Z.; Zhang, X.X.; Yu, X.L.; Ai, Z.Z.; Mei, Y.X.; Li, J.N. Three-dimensional printing for the accurate orthopedics: Clinical cases analysis. *Bio-Des. Manuf.* **2022**, *3*, 122–132. [CrossRef]
118. Liang, X.; Qi, Y.; Pan, Z.; He, Y.; Liu, X.; Cui, S.; Ding, J. Design and preparation of quasi-spherical salt particles as water-soluble porogens to fabricate hydrophobic porous scaffolds for tissue engineering and tissue regeneration. *Mater. Chem. Front.* **2018**, *8*, 1539–1553. [CrossRef]
119. Lee, H.; Yoo, J.J.; Kang, H.W.; Cho, D.W. Investigation of thermal degradation with extrusion-based dispensing modules for 3D bioprinting technology. *Biofabrication* **2016**, *8*, 015011. [CrossRef]
120. Yu, Y.; Sun, B.; Yi, C.; Mo, X. Stem cell homing-based tissue engineering using bioactive materials. *Front. Mater. Sci.* **2017**, *11*, 93–105. [CrossRef]
121. Batool, F.; Strub, M.; Petit, C.; Bugueno, I.M.; Bornert, F.; Clauss, F.; Huck, O.; Kuchler-Bopp, S.; Benkirane-Jessel, N. Periodontal Tissues, Maxillary Jaw Bone, and Tooth Regeneration Approaches: From Animal Models Analyses to Clinical Applications. *Nanomaterials* **2018**, *8*, 337. [CrossRef] [PubMed]
122. Liu, J.; Ye, X.; Wang, H.; Zhu, M.; Wang, B.; Yan, H. The influence of pH and temperature on the morphology of hydroxyapatite synthesized by hydrothermal method. *Ceram. Int.* **2003**, *29*, 629–633. [CrossRef]
123. Rodríguez-Lugo, V.; Hernández, J.S.; Arellano-Jimenez, M.J.; Hernández-Tejeda, P.H.; Recillas-Gispert, S. Characterization of hydroxyapatite by electron microscopy. *Microsc. Microanal.* **2005**, *11*, 516–523. [CrossRef] [PubMed]
124. Jokanovic, V.; Jokanovic, B.; Markovic, D.; Zivojinovic, V.; Pasalic, S.; Izvonar, D.; Plavsic, M. Kinetics and sintering mechanisms of hydro-thermally obtained hydroxyapatite. *Mater. Chem. Phys.* **2008**, *111*, 180–185. [CrossRef]
125. Ungureanu, D.N.; Angelescu, N.; Bacinschi, Z.; Stoian, E.V.; Rizescu, C.Z. Thermal stability of chemically precipitated hydroxyapatite nanopowders. *Int. J. Biol. Biomed. Eng.* **2011**, *5*, 57–64.
126. Bodhak, S.; Bose, S.; Bandyopadhyay, A. Electrically polarized HAp-coated Ti: In vitro bone cell-material interactions. *Acta Biomater.* **2010**, *6*, 641–651. [CrossRef]
127. Zhang, B.G.; Myers, D.E.; Wallace, G.G.; Brandt, M.; Choong, P.F. Bioactive coatings for orthopaedic implants-recent trends in development of implant coatings. *Int. J. Mol. Sci.* **2014**, *15*, 11878–11921. [CrossRef]
128. Fitzpatrick, V.; Martín-Moldes, Z.; Deck, A.; Torres-Sanchez, R.; Valat, A.; Cairns, D.; Li, C.; Kaplan, D.L. Functionalized 3D-printed silk-hydroxyapatite scaffolds for enhanced bone regeneration with innervation and vascularization. *Biomaterial.* **2021**, *276*, 120995. [CrossRef]
129. Jokanović, V.; Čolović, B.; Marković, D.; Petrović, M.; Jokanović, M.; Milosavljević, P.; Sopta, J. In Vivo investigation of ALBO-OS scaffold based on hydroxyapatite and PLGA. *J. Nanomater.* **2016**, *2016*, 3948768. [CrossRef]
130. Furtos, G.; Rivero, G.; Rapuntean, S.; Abraham, G.A. Amoxicillin-loaded electrospun nanocomposite membranes for dental applications. *J. Biomed. Mater. Res. B Appl. Biomater.* **2017**, *105*, 966–976. [CrossRef]
131. Xia, D.; Yang, F.; Zheng, Y.; Liu, Y.; Zhou, Y. Research status of biodegradable metals designed for oral and maxillofacial applications: A review. *Bioact. Mater.* **2021**, *6*, 4186–4208. [CrossRef] [PubMed]
132. Hang, R.; Wang, C.; Yu, Z.; Li, Z.; Xiao, Y. Biodegradable metallic wires in dental and orthopedic applications: A review. *Materials* **2018**, *8*, 212. [CrossRef]
133. Rider, P.; Kačarević, Ž.P.; Elad, A.; Rothamel, D.; Sauer, G.; Bornert, F.; Windisch, P.; Hangyási, D.; Molnar, B.; Hesse, B.; et al. Biodegradation of a Magnesium Alloy Fixation Screw Used in a Guided Bone Regeneration Model in Beagle Dogs. *Materials* **2022**, *15*, 4111. [CrossRef] [PubMed]
134. Muschler, G.F.; Raut, V.P.; Patterson, T.E.; Wenke, J.C.; Hollinger, J.O. The design and use of animal models for translational research in bone tissue engineering and regenerative medicine. *Tissue Eng. Part B Rev.* **2010**, *16*, 123–145. [CrossRef]
135. Boden, S.D.; Grob, D.; Damien, C. Ne-Osteo bone growth factor for posterolateral lumbar spine fusion: Results from a nonhuman primate study and a prospective human clinical pilot study. *Spine* **2004**, *29*, 504–514. [CrossRef]
136. Chanchareonsook, N.; Junker, R.; Jongpaiboonkit, L.; Jansen, J.A. Tissue-engineered mandibular bone reconstruction for continuity defects: A systematic approach to the literature. *Tissue Eng. Part B Rev.* **2014**, *20*, 147–162. [CrossRef] [PubMed]

137. Kim, I.S.; Lee, E.N.; Cho, T.H.; Song, Y.M.; Hwang, S.J.; Oh, J.H.; Park, E.K.; Koo, T.Y.; Seo, Y.K. Promising efficacy of Escherichia coli recombinant human bone morphogenetic protein-2 in collagen sponge for ectopic and orthotopic bone formation and comparison with mammalian cell recombinant human bone morphogenetic protein-2. *Tissue Eng. Part A* **2011**, *17*, 337–348. [CrossRef]
138. Moghadam, H.G.; Urist, M.R.; Sandor, G.K.; Clokie, C.M. Successful mandibular reconstruction using a BMP bioimplant. *J. Craniofacial Surg.* **2001**, *12*, 119–128. [CrossRef]
139. Warnke, P.H.; Springer, I.N.; Wiltfang, J.; Acil, Y.; Eufinger, H.; Wehmöller, M.; Russo, P.A.; Bolte, H.; Sherry, E.; Behrens, E.; et al. Growth and transplantation of a custom vascularised bone graft in a man. *Lancet* **2004**, *364*, 766–770. [CrossRef]
140. Heliotis, M.; Lavery, K.M.; Ripamonti, U.; Tsiridis, E.; di Silvio, L. Transformation of a prefabricated hydroxyapatite/osteogenic protein-1 implant into a vascularised pedicled bone flap in the human chest. *Int. J. Oral Maxillofac. Surg.* **2006**, *35*, 265–269. [CrossRef]
141. Herford, A.S.; Boyne, P.J. Reconstruction of mandibular continuity defects with bone morphogenetic protein-2 (rhBMP-2). *J. Oral Maxillofac. Surg.* **2008**, *66*, 616–624. [CrossRef] [PubMed]
142. Chao, M.; Donovan, T.; Sotelo, C.; Carstens, M.H. In situ osteogenesis of hemimandible with rhBMP-2 in a 9-year-old boy: Osteoinduction via stem cell concentration. *J. Craniofac. Surg.* **2006**, *17*, 405–412. [CrossRef] [PubMed]
143. Clokie, C.M.; Sándor, G.K. Reconstruction of 10 major mandibular defects using bioimplants containing BMP-7. *J. Can. Dent. Assoc.* **2008**, *74*, 67–72.
144. Glied, A.N.; Kraut, R.A. Off-label use of rhBMP-2 for reconstruction of critical-sized mandibular defects. *N. Y. State Dent. J.* **2010**, *76*, 32–35.
145. Herford, A.S.; Cicciù, M. Recombinant human bone morphogenetic protein type 2 jaw reconstruction in patients affected by giant cell tumor. *J. Craniofac. Surg.* **2010**, *21*, 1970–1975. [CrossRef]
146. Scadden, D.T. The stem-cell niche as an entity of action. *Nature* **2006**, *441*, 1075–1079. [CrossRef]
147. Monaco, E.; Bionaz, M.; Hollister, S.J.; Wheeler, M.B. Strategies for regeneration of the bone using porcine adult adipose-derived mesenchymal stem cells. *Theriogenology* **2011**, *75*, 1381–1399. [CrossRef] [PubMed]
148. Sándor, G.K.; Tuovinen, V.J.; Wolff, J.; Patrikoski, M.; Jokinen, J.; Nieminen, E.; Mannerström, B.; Lappalainen, O.P.; Seppänen, R.; Miettinen, S. Adipose stem cell tissue-engineered construct used to treat large anterior mandibular defect: A case report and review of the clinical application of good manufacturing practice-level adipose stem cells for bone regeneration. *J. Oral Maxillofac. Surg.* **2013**, *71*, 938–950. [CrossRef]
149. Aerssens, J.; Boonen, S.; Lowet, G.; Dequeker, J. Interspecies differences in bone composition, density, and quality: Potential implications for in vivo bone research. *Endocrinology* **1998**, *139*, 663–670. [CrossRef]
150. Gilsanz, V.; Roe, T.F.; Gibbens, D.T.; Schulz, E.E.; Carlson, M.E.; Gonzalez, O.; Boechat, M.I. Effect of sex steroids on peak bone density of growing rabbits. *Am. J. Physiol.* **1988**, *255*, E416–E421. [CrossRef]
151. Castañeda, S.; Largo, R.; Calvo, E.; Rodríguez-Salvanés, F.; Marcos, M.E.; Díaz-Curiel, M.; Herrero-Beaumont, G. Bone mineral measurements of subchondral and trabecular bone in healthy and osteoporotic rabbits. *Skelet. Radiol.* **2006**, *35*, 34–41. [CrossRef] [PubMed]
152. Wancket, L.M. Animal Models for Evaluation of Bone Implants and Devices: Comparative Bone Structure and Common Model Uses. *Vet. Pathol.* **2015**, *52*, 842–850. [CrossRef] [PubMed]
153. Reitan, K.; Kvam, E. Comparative behavior of human and animal tissue during experimental tooth movement. *Angle Orthod.* **1971**, *41*, 1–14. [CrossRef]
154. Harper, R.A.; Pfeiffer, F.M.; Choma, T.J. The minipig as a potential model for pedicle screw fixation: Morphometry and mechanics. *J. Orthop. Surg. Res.* **2019**, *14*, 246. [CrossRef] [PubMed]
155. Schorn, L.; Sproll, C.; Ommerborn, M.; Naujoks, C.; Kübler, N.R.; Depprich, R. Vertical bone regeneration using rhBMP-2 and VEGF. *Head Face Med.* **2017**, *13*, 11. [CrossRef] [PubMed]
156. Jinno, Y.; Jimbo, R.; Lindström, M.; Sawase, T.; Lilin, T.; Becktor, J.P. Vertical Bone Augmentation Using Ring Technique with Three Different Materials in the Sheep Mandible Bone. *Int. J. Oral Maxillofac. Implants* **2018**, *33*, 1057–1063. [CrossRef] [PubMed]
157. Carrel, J.P.; Wiskott, A.; Moussa, M.; Rieder, P.; Scherrer, S.; Durual, S. A 3D printed TCP/HA structure as a new osteoconductive scaffold for vertical bone augmentation. *Clin. Oral Implants. Res.* **2016**, *27*, 55–62. [CrossRef]
158. Triplett, R.G.; Nevins, M.; Marx, R.E.; Spagnoli, D.B.; Oates, T.W.; Moy, P.K.; Boyne, P.J. Pivotal, randomized, parallel evaluation of recombinant human bone morphogenetic protein-2/absorbable collagen sponge and autogenous bone graft for maxillary sinus floor augmentation. *J. Oral Maxillofac. Surg.* **2009**, *67*, 1947–1960. [CrossRef]