Which substances loaded onto collagen scaffolds influence oral tissue regeneration?—an overview of the last 15 years

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Abstract

Background Collagen scaffolds are widely used for guided bone or tissue regeneration. Aiming to enhance their regenerative properties, studies have loaded various substances onto these scaffolds. This review aims to provide an overview of existing literature which conducted in vitro, in vivo, and clinical testing of drug-loaded collagen scaffolds and analyze their outcome of promoting oral regeneration.

Materials and methods PubMed, Scopus, and Ovid Medline® were systematically searched for publications from 2005 to 2019. Journal articles assessing the effect of substances on oral hard or soft tissue regeneration, while using collagen carriers, were screened and qualitatively analyzed. Studies were grouped according to their used substance type—biological medical products, pharmaceuticals, and tissue-, cell-, and matrix-derived products.

Results A total of 77 publications, applying 36 different substances, were included. Collagen scaffolds were demonstrating favorable adsorption behavior and release kinetics which could even be modified. BMP-2 was investigated most frequently, showing positive effects on oral tissue regeneration. BMP-9 showed comparable results at lower concentrations. Also, FGF2 enhanced bone and periodontal healing. Antibiotics improved the scaffold's anti-microbial activity and reduced the penetrability for bacteria.

Conclusion Growth factors showed promising results for oral tissue regeneration, while other substances were investigated less frequently. Found effects of investigated substances as well as adsorption and release properties of collagen scaffolds should be considered for further investigation.

Clinical relevance: Collagen scaffolds are reliable carriers for any of the applied substances. BMP-2, BMP-9, and FGF2 showed enhanced bone and periodontal healing. Antibiotics improved anti-microbial properties of the scaffolds.

Keywords Collagen · Dentistry · Oral surgery · Maxillofacial surgery · Periodontics

Introduction

Collagen is an important biomaterial which is frequently applied in oral and maxillofacial tissue engineering and

regenerative medicine approaches that aim to restore, maintain, or enhance damaged or substantially reduced tissues [1, 2]. Different fields in dentistry face specific objectives referring to hard and soft tissue regeneration, and collagen has been successfully implemented into various treatment strategies [2, 3]. As the main component of extracellular matrix (ECM), collagen is essential for the structural maintenance of oral tissues and plays an important role in numerous cellular processes [4, 5]. In tissue engineering application, collagen exhibits excellent biocompatibility and low antigenicity [6]. As collagen is biodegradable and shows poor mechanical properties, non-enzymatic or enzymatic cross-linking procedures using glutaraldehyde or formaldehyde for example can be performed to modify collagen membranes. Consequently, these procedures prevent rapid degradation and enhance mechanical properties [7, 8]. Collagen membranes are used in guided tissue regeneration (GTR) and guided bone

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regeneration (GBR), which are methods used in oral surgery as augmentative techniques. Both GBR and GTR are based on the principle to cover periodontal epithelium and bone as well as to maintain the tissue defect space in order to exclude epithelium and gingival connective tissue due to their faster growing properties compared with hard tissue. While GTR focuses on regenerating soft tissue of the periodontal attachment, GBR, in addition, is aiming to support new bone tissue growth [9]. Favorable properties of a membrane include biocompatibility, impermeability for epithelial cells or bacteria, and adequate mechanical properties to allow proper handling during surgery and stiffness for space maintenance and less pressure to the bone graft during healing. Also, the time of resorption should allow the bone graft to regenerate, before degradation of the membrane causes the loss of the stated properties. In the beginning of GTR development, a bacterial filter consisting of cellulose acetate was placed over the treated area to serve as an occlusive membrane [10]. Aiming to improve clinical application, further studies investigated the use of non-resorbable expanded polytetrafluorethylen (e-PTFE) in membranes, which could also have been reinforced by titanium. Although mechanical stability can be enhanced using titanium, a second surgical entry is required to remove the membrane [11]. The next generation of barrier membranes were focused on resorbable materials, including collagen, to avoid a surgical removal [12]. Further advances aimed to improve the regeneration process and wound healing, and to serve as local drug carriers. To release specific substances at the wound site, collagen membranes can be loaded with various agents, such as antibiotics, growth factors, or adhesion molecules [13]. Progress in the field of tissue engineering has resulted in the development of innovative scaffold designs that act as a biological platform to enable reparation and restoration of injured tissues during the healing process. Collagen can easily be used to build 3D scaffolds and sponges, and can also serve as a carrier for the delivery of substances, such as growth factors or bone morphogenetic proteins, aiming to enhance healing and support regeneration [14].

In the past years, the number of publications investigating the role of drug-loaded collagen scaffolds in tissue engineering has increased, indicating a growing interest in this topic. The primary aim of this review is to give an overview of different cell-free regenerative approaches which utilize collagen as a biomaterial carrier by summarizing existing studies from the past 15 years. At the time of creating this publication, there was no existing literature reviewing this topic. The secondary aim was to provide information about the outcome of those studies according to their influence on oral tissue regeneration. This information could allow suggestions of future applications in dentistry and oral surgery or the need for further research.

Materials and methods

Study design

A study protocol for a qualitative literature review was developed by all authors based on recommended methods [15]. The focused PICO (problem, intervention, comparison, outcome) question was: Does loading of substances onto collagen scaffolds influence oral tissue regeneration parameters *in vitro* and/or *in vivo*, compared with unloaded control? The whole review process was designed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement [16].

Inclusion and exclusion criteria

Literature research was performed by applying specific search terms, aiming to find publications about drug-loaded collagen scaffolds with the purpose to promote oral soft or hard tissue regeneration. Journal articles published from January 1, 2005 to December 31, 2019 with original data from *in vitro* and/or *in vivo* and/or clinical testing were included. Reasons for exclusion were review articles, cell-based therapeutic approaches, if the collagen scaffold was not loaded, if other biomaterials than collagen were used as substance carriers, or if the study did not reflect the topic of this review. Aiming to widen the scope of this review and to be able to discuss articles which primarily focused on release properties without using negative controls, we did not exclude studies without unloaded collagen scaffolds as control group.

Search strategy

Three scientific databases—PubMed, Scopus, and Ovid Medline®—were used for the search. The following search term was used: ("collagen" AND ("loaded" OR "soaked") AND ("dentistry" OR "oral surgery" OR "maxillofacial surgery" OR "periodontics" OR "periodontology")). Filters or limits for publication date starting from January 1, 2005 to December 31, 2019, language (English), and type of article (original journal article) were applied. References of each publication were searched for additional records.

Study selection

Duplicates were automatically removed, and remaining papers were manually screened for their content. Publications were then selected according to the inclusion and exclusion criteria. Studies were subdivided—depending on utilized substances—into biological medical products, pharmaceuticals, and tissue-, cell-, and matrix-derived products.

Data Extraction

Two authors independently extracted data from the full texts of included articles, using specifically designed tables. Descriptive summaries were entered, and a qualitative synthesis was conducted. Data extracted were author(s), study design, substance, type of collagen scaffold, aim(s), methods, and outcomes or main findings.

Risk of bias and quality assessment

The risk of bias and the quality of the included studies was assessed independently by two authors according to the revised recommendation of the CONSORT statement. After scores were calculated, an overall estimation risk of bias (low, all criteria met; moderate, one or more criteria partly met; or high, one or more criteria were not met) was performed for each selected study. Any disagreement between the reviewers during study selection, data extraction, and quality assessment was resolved by discussion until consensus among all authors was reached. For in vitro and in vivo studies, no further risk of bias was assessed as no validated bias risk assessment tool was available.

Results

Search results and study characteristics

The initial search procedure using PubMed ($n = 248$), Scopus ($n = 56$), and Ovid Medline® ($n = 20$) led to 324 search results overall. After discarding of duplicates, the total findings were reduced to 259 unique journal articles. Following the inclusion and exclusion criteria, 182 studies had to be excluded leading to a total of 77, which were further investigated and later synthesized in this review (Fig. 1).

Out of these 182 excluded studies, 4 were review articles, 22 were using cell-based approaches, and 28 used collagen scaffolds but did not load them with their substance as they mostly used a collagen membrane only as a cover. Another 53 articles were evaluating different aspects like mechanical testing, implant survival after alveolar socket preservation and implant placement, systemic therapy, and others. From 72 studies which utilized other biomaterials than collagen as drug carriers, 18 were actually using collagen but in a mixed combination with deproteinized bovine bone mineral (DBBM), a widely used type of scaffold in dentistry and oral surgery (Fig. 1). The timeline of the past 15 years reveals a steadily increasing number of scientific publications in this field. Starting from zero to three publications from 2005 to 2010, to around 10 publications per year in the last 5 years (Fig. 2).

In all included publications, in vitro and in vivo testing was performed in 34% ($n = 32$) and 55% ($n = 52$), respectively.

Clinical testing of loaded collagen was carried out in only 11% ($n = 10$) of the publications, where BMP-2, doxycycline, alendronate, simvastatin, and platelet-rich fibrin have been tested in humans.

Drug types and types of collagen scaffolds

All of the 77 included studies were assigned to subgroups according to their defined category: biological medical products, pharmaceuticals, or tissue-, cell-, and matrix-derived products (Table 1).

The numerical values show how often the specific substance was used in one of the 77 studies

In total, 36 different drugs were utilized as therapeutic agents in 77 included publications. Biologicals were the most frequently used substance-category ($n = 60$), followed by pharmaceuticals ($n = 27$) and tissue-, cell-, and matrix-derived products ($n = 10$). BMP-2 ($n = 33$), followed by FGF2 ($n = 5$) and BMP-9 ($n = 4$) together with SDF-1 ($n = 4$) were to most often used substances. Looking at the types of collagen scaffolds which were utilized as carrier materials, sponges, or matrices were used in 50, membranes in 23, and gels in only 4 studies (Tables 2, 3, and 4).

Risk of bias and quality of the included studies

Sixty-seven out of 77 included studies were conducting in vitro and in vivo testing which are regarded as the lowest level of evidence. Ten out of 67 pre-clinical studies did not use unloaded collagen scaffolds as control. These studies utilized different substance dosages as positive control [40, 63, 68, 82], other substances [64, 85], blood clot formation after surgical interventions [33, 39], or no control group at all [23, 66]. Four out of ten clinical studies were case series with five to ten patients [46–48, 89], whereas one was a pilot study with 12 patients [90] and five were clinical trials with 20 to 64 patients [45, 49, 69, 71, 76]. Eight out of ten clinical studies did not use an unloaded collagen scaffold as control [45–49, 69, 89, 90]. A CONSORT-based quality analysis of the ten included clinical studies showed a high risk of bias due to missing sample size calculation, unreported or missing methods of randomization and allocation concealment, incompleteness of follow-up, and lack of masking.

Biological medical products

Biopharmaceuticals or biological medical products are manufactured pharmaceutical drugs which are semi-synthesized or extracted from biological sources. This drug category was most commonly applied in the included studies (Table 1). The following table lists all studies which used different biological medical products loaded onto different types of collagen scaffolds (Table 2).

Fig. 1 Flow diagram of the review process according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement [16]

Multifunctional growth and differentiation factors

- Bone morphogenetic protein-2 (BMP-2)

Collagen membranes loaded with BMP-2 at a concentration of 10 ng/mL increased human osteoblast proliferation up to 5 days post-seeding. Osteoblast differentiation markers, such as osterix, collagen I, and osteocalcin, were increased and mineralization of primary osteoblasts was pronounced (Miron R.J. et al. 2013 [17]). Another study used BMP-2 loaded collagen gel as control, comparing a designed chitosan–alginate–hydroxyapatite (CAH) scaffold as carrier for BMP-2. Release kinetics revealed a faster release from

collagen compared with CAH (84 to 64% within 7 days). Alkaline phosphatase (ALP) activity and Alizarin red staining were significantly increased in mesenchymal stem cells (MSC) cultured with loaded collagen compared with unloaded CAH. Other results are not included in this review when collagen was not used as the substance carrier and BMP-2 was loaded in combination with MSC in the vivo part of this study (He X. et al. 2014 [18]). When loading BMP-2 at a low (10 ng/mL) or high (100 ng/mL) concentration onto collagen membranes, the substance was fully absorbed and slowly released within 10 days. Seeding murine ST2 stromal bone marrow cells onto these scaffolds revealed that ALP mRNA levels were significantly induced at 3 days and bone

Fig. 2 Timeline of included publications per year including a linear trend line demonstrating an increasing number of publications in that field over the past 15 years

Table 1 Overview of drugs and their categories which have been used in the included articles

Biological medical products	Count
Multifunctional growth and differentiation factors	$\Sigma = 60$
Bone morphogenetic protein-2 (BMP-2)	33
Bone morphogenetic protein-6 (BMP-6)	1
Bone morphogenetic protein-7 (BMP-7)	1
Bone morphogenetic protein-9 (BMP-9)	4
Erythropoietin (EPO)	1
Fibroblast growth factor 2 (FGF2)	5
Human growth hormone (HGH)	1
Interferon gamma (IFN- γ)	1
Platelet-derived growth factor (PDGF)	3
Receptor activator of NF- κ B ligand (RANKL)	1
Stromal cell-derived factor 1/C-X-C motif chemokine 12 (SDF1)	4
Transforming growth factor beta 1 (TGF β 1)	3
Vascular endothelial growth factor (VEGF)	2
Pharmaceuticals	$\Sigma = 27$
Antibiotics	
β -Lactam antibiotic (amoxicillin)	2
Tetracycline (doxycycline)	3
Bisphosphonates	
Alendronate	3
Corticosteroid	
Dexamethasone	1
Hypoxia mimetic agents	
Deferoxamine (DFO)	2
Dimethylxalylglycine (DMOG)	2
L-Mimosine (L-MIM)	1
Statin	
Simvastatin	2
Others	
Anti-tumor necrosis factor- α (anti-TNF- α)	1
<i>Calendula officinalis</i> flower extract	1
Cyanoacrylate (CA)	1
Glycosaminoglycan/hyaluronic acid (GAG/HA)	3
Human albumin (nitric oxide donor)	1
Mussel adhesive protein (MAP)	1
Parathyroid hormone (PTH)	1
Progranulin (anti-tumor necrosis factor- α)	1
Vitamin D	1
Tissue-, cell-, and matrix-derived products	$\Sigma = 10$
Bone conditioned medium (BCM)	2
Cell conditioned medium (CCM)	1
Platelet-rich plasma (PRP)	2
Platelet-rich fibrin (PRF)	2
Platelet secretome (PSEC)	1
Enamel matrix derivative (EMD)	2

sialoprotein (BSP) at 14 days. Compared with BMP-9 loaded scaffolds, messenger RNA (mRNA) levels of ALP, collagen1, BSP, and osteocalcin were significantly lower in BMP-2 groups. Effects on cell attachment, proliferation, and osteoblast differentiation were not significant. Alizarin red staining was three times less compared with BMP-9 (Fujioka-Kobayashi M. et al. 2016 [19], Fujioka-Kobayashi M. et al. 2017 [20]). Elastin-like polypeptide-collagen hydrogels were loaded with BMP-2 and doxycycline together. Release of BMP-2 assayed by ELISA showed a linearity over 7 days. Adhesion, proliferation, and migration of human adipose-derived stem cells on hydrogel surfaces was higher for drug-loaded samples compared with unloaded gels (Pal P. et al. 2019 [21]). In vivo, collagen sponges were soaked with 100, 500, or 1000 μ g/mL BMP-2 and used in a calvarial defect model in rabbits. After 1 month, height and amount of newly generated tissue were significantly higher at any concentrations of BMP-2. The amount of mineralized bone was only significantly increased in the 1000 μ g/mL group compared with control (Hasegawa Y. et al. 2008 [22]). Another study in 2008 used gene transfer in bone marrow stromal cells (BMSC) to promote BMP-2 production. Human BMP-2 complementary DNA (cDNA) was subcloned into the plasmid pcDNA3.1 and BMSCs were transfected with it. Results showed that the level of expressed and secreted BMP-2 was significantly higher in transfected cells than in untransfected cells. Collected cultured medium from transfected BMSCs induced the expression of ALP and osteocalcin in non-transfected cells. When incorporating BMP-2-cDNA3.1 complexes into collagen sponges and implanting them into rabbit calvarial defects at both 4 and 8 weeks, experimental groups showed significantly more newly formed bone. No negative control was utilized (Seol Y.J. et al. 2008 [94]). In a mid-diaphyseal defect model of diabetic rats, collagen sponges loaded with BMP-2 were inserted. Qualitative microradiography and assessment showed that BMP-2 increased new bone formation after 6 weeks. Histomorphometric analysis revealed a significantly higher area of new bone formation. Histologically, platelet endothelial cell adhesion molecule-1 staining at 3 weeks was higher in BMP-2 treated rats. All defects in the control group without BMP-2 had nonunion, so mechanical testing could only be executed in the BMP-2 group (Azad V. et al. 2009 [24]). Another study used critical-sized calvarial defects as orthotopic model and created subcutaneous pouches in rats as ectopic model. The aim was to assess the effect of collagen sponges loaded with different doses of BMP-2 (25, 50, 100, and 200 μ g/mL). Bone formation was enhanced at all doses of BMP-2 compared with control. After 2 weeks, bone formation could be observed in the ectopic model which was less at 8 weeks post operatively but showed advanced bone remodeling and maturity (Lee J.H. et al. 2010 [25]). In 2011, a study combined BMP-2 together with glycosaminoglycans (GAG) of biglycan (BGN) on

Table 2 Overview of studies applying biological medical products onto collagen scaffolds sorted by study methods (in vitro, in vivo, clinical) and year of publication

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Bone morphogenetic protein-2 Miron R.J. et al. [17]	2013	X			BMP-2/TGFβ1	Membrane	Bone regeneration	Human osteoblasts. Scanning electron microscopy (SEM). Real-time (RT) PCR. Alizarin red staining	Effects for BMP-2: Osteoblast proliferation ↑ Osterix ↑ Collagen I ↑ Osteocalcin ↑ Mineralization of primary osteoblasts ↑ Burst-like initial release followed by sustained release over 28d Osteoblast differentiation ↑ Osteopontin ↑ Collagen I ↑ Osteocalcin ↑ Adhesion = Proliferation = Differentiation ↑ BSP ↑ Higher ALP and Alizarin red staining in BMP-9.
He X. et al. [18]	2014	X			BMP-2	Gel	Release kinetics using a new scaffold compared to collagen carrier. (In vitro part using stem cells not included) Bone regeneration	Viability and differentiation of bone marrow mesenchymal stem cells (BMSCs) by SEM, MTS -, alkaline phosphatase (ALP) assay, alizarin-red staining and RT PCR Adhesion, proliferation and differentiation of stromal cell line (ST2) pre-osteoblasts. RT PCR. Alizarin red staining	BMP-2 and -9 fully adsorbed and slowly released over 10d. Adhesion = Proliferation = Collagen I ↑ Osteocalcin ↑ BSP ↑ Higher ALP and Alizarin red staining in BMP-9.
Fujioka-Kobayashi M. et al. [19]	2016	X			BMP-2/BMP-9	Membrane	Bone regeneration	Adsorption and release kinetics by ELISA. Adhesion, proliferation and differentiation of stromal cell line (ST2) pre-osteoblasts. RT PCR. ALP activity. Alizarin red staining	BMP-2 and -9 fully adsorbed and slowly released over 10d. Adhesion = Proliferation = Collagen I ↑ Osteocalcin ↑ ALP ↑ Bone sialoprotein ↑ mRNA levels all higher in BMP-9. Higher Alizarin red staining in BMP-9.
Fujioka-Kobayashi M. et al. [20]	2017	X			BMP-2/BMP-9	Sponge/Matrix	Extraction socket healing	Adhesion, proliferation, and migration of human adipose-derived stem cell (hASC). DNA and protein quantification. SEM, spectroscopy, Alizarin red staining, mechanical testing	BMP-2 exhibits a nearly linear release profile. Osteocalcin ↑ ALP ↑ Cell differentiation ↑ All hydrogels supported hASC attachment, proliferation, and differentiation. New bone formation ↑ Mineralized bone ↑
Pal P. et al. [21]	2019	X			BMP-2/doxycycline	Gel	Bone regeneration	Calvarial defect model in 18 Japanese white rabbits. Histomorphometry (μCT), histology.	Transfected cells were able to incorporate the BMP-2 gene into their nuclei. Secreted BMP-2 ↑
Hasegawa Y. et al. [22]	2008		X		BMP-2	Sponge/matrix	Bone regeneration	Calvarial defect model in 22 New Zealand white rabbits. RT PCR. Histomorphometry (μCT), histology	
Scol Y.J. et al. [23]	2008	X	X		BMP-2 gene transfer	Sponge/matrix	Bone regeneration		

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Azad V. et al. [24]	2009	X	X		BMP-2	Sponge/matrix	Bone regeneration in diabetes mellitus	Femoral defect model in 62 diabetes mellitus Wistar rats. Histomorphometry (μCT), histology, immunohistochemistry, mechanical testing	Osteocalcin ↑ ALP ↑ New bone formation ↑ New bone formation ↑ Angiogenesis ↑ Torsional rigidity ↑
Lee J.H. et al. [25]	2010	X	X		BMP-2	Sponge/matrix	Bone regeneration	Subcutaneous ectopic model in 32 and calvarial critical-sized defect model in 44 male Sprague-Dawley rats.	Bone formation ↑
Miguez P.A. et al. [26]	2011	X	X		BMP-2/glycosaminoglycan of biglycan (BGN)	Sponge/matrix	Bone regeneration	Histomorphometry (μCT), histology BMP signaling and function in C2C12 myogenic cells ALP activity Mandible defect model in 16 Sprague-Dawley rats. Histomorphometry (μCT)	BMP-2 + BGN: BMP signaling ↑ BMP function ↑ Bone formation ↑ (only with de-glycanated BGN)
Jung I.H. et al. [27]	2011	X	X		BMP-2	Sponge/matrix	Bone regeneration	Calvarial defect model in 60 Sprague-Dawley rats. Histomorphometry (μCT), histology	Augmented area ↑ New bone area ↑ New bone ratio ↑ Collagen scaffold completely resorbed after 8 weeks.
Choi Y. et al. [28]	2012	X	X		BMP-2	Sponge/matrix	Bone regeneration after sinus floor augmentation	Sinus model in 6 male New Zealand white rabbits. Histomorphometry (μCT), histology	Bone formation ↑ Bony window closure ↑
Lai C.H. et al. [29]	2013	X	X		BMP-2	Membrane	Vertical guided bone regeneration	Calvarial vertical guided bone regeneration model in 8 female New Zealand rabbits. Histomorphometry (μCT), histology	Bone formation ↑ From the surface of the native bone and from the superficial structures.
Stancoven B.W. et al. [30]	2013	X	X		BMP-2 + systemic parathyroid hormone (PTH)	Sponge/matrix	Bone regeneration	Calvarial defect model in 160 Sprague-Dawley rats. Histomorphometry (μCT), radiographic analysis	Bone formation ↑ No significant effect on bone formation by PTH
Chang Y.Y. et al. [31]	2015	X	X		BMP-2	Membrane	Bone regeneration	Guided bone regeneration at the buccal aspect of edentulous maxillary alveolar ridges in 5 male mongrel dogs. Histomorphometry (μCT), histology	Bone formation ↑ Bone-to-residual bone substitute contact ratio lower in collagen vs hydroxyapatite incorporated with collagen as carrier material
Hwang H.D. et al. [32]	2015	X	X		BMP-2/SDF-1	Sponge/matrix	Sequential use of BMP-2 and SDF-1 for bone regeneration	Osteoblastic differentiation and cell migration in C2C12, C3H10T1/2 and BMSC. Subcutaneous ectopic model in 32 and calvarial critical-sized defect model in 36 female C57BL/6 mice. Histomorphometry (μCT), histology	Strongest osteoblastic differentiation and enhanced cell migration with BMP-2 after SDF-1. SDF-1 followed by BMP-2 treatment showed highest degree of new bone regeneration.
Jung I.H. et al. [33]	2015	X	X		BMP-2	Sponge/matrix	Bone regeneration	Calvarial defect model in 10 male New Zealand white rabbits. SEM. Histomorphometry (μCT), histology	Early bone regeneration ↑

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Lee C.H. et al. [34]	2015	X	X		BMP-2/SDF-1	Sponge/matrix	Bone regeneration	In vitro assays with mesenchymal cells and endothelial cells Subcutaneous ectopic model and calvarial defect model in 32 C57BL/6 mice. Histomorphometry (μCT), histology	Effects for BMP-2: Osteogenic induction ↑ Cell migration ↑ Angiogenesis ↑ Bone formation ↑ No additive effect of SDF-1. Mineralized tissue formation ↑
Lee J.S. et al. [35]	2015		X		BMP-2	Sponge/matrix	Bone regeneration and mineralized/adipose tissue formation	Subcutaneous ectopic model in 25 immunodeficient mice and calvarial defect model in 50 male Sprague-Dawley rats. Histomorphometry (μCT), histology Cleft defect model in 30 Wistar rats. Histomorphometry (μCT), histology Calvarial defect model in 56 male Sprague-Dawley rats. Pulsed electromagnetic fields (PEMF) after surgery. Histomorphometry (μCT), histology and immunohisto-chemistry	Bone formation ↑ Bone regeneration ↑ PEMF accelerated bone regeneration in lower BMP-2 dose groups.
Mostafa N.Z. et al. [36]	2015	X	X		BMP-2	Sponge/matrix	Cleft palate defects	Calvarial defect model in 20 Sprague-Dawley rats. Histomorphometry (μCT), histology	New bone area ↑ Bone density ↑ New bone formation ↑
Yang H.J. et al. [37]	2015		X		BMP-2 + pulsed electromagnetic fields	Sponge/matrix	Bone regeneration	Calvarial defect model in 16 male New Zealand white rabbits. Histomorphometry (μCT), histology	
Lee J.S. et al. [38]	2016		X		BMP-2	Sponge/matrix	Bone regeneration	Femur defect model in 18 Wistar rats. ELISA for BMP-2. Histomorphometry (μCT) and histology	Rapid release after 1 day followed by gradual release over 14 days. New bone formation ↑ (concentration dependent)
You H., et al. [39]	2016		X		BMP-2	Sponge/matrix	Bone regeneration	Differentiation and the mineralization of rat BMSCs. Calvarial defect model in 80 Sprague-Dawley rats. Histomorphometry (μCT), histology. RT PCR. Alizarin red staining. Immunohistochemistry	Burst release in first 8h followed by gradual release. BMP-2 + ALN: RANKL ↑ OPG ↓ Cathepsin K ↑ TRAP pos. cells ↓ ALP ↑ Osterix ↑ VEGF ↑ Bone mass ↑ Bone density ↑
Zhang Y. et al. [40]	2016	X	X		BMP-2	Membrane	Bone regeneration	Calvarial defect model in 39 C57BL/6 mice. Histomorphometry (μCT), histology	Bone regeneration ↑ Cell presence within the healing defect ↑
Cho T.H. et al. [41]	2017	X	X		BMP-2/alendronate (ALN)	Sponge/matrix	Bone regeneration	Peri-implant defect in 5 mongrel dogs. Histomorphometry (μCT) and radiographic analysis	No significant change in bone regeneration
Durham E.L. et al. [42]	2018		X		BMP-2	Sponge/matrix	Large craniofacial defects		
Sun Y.K. et al. [43]	2018		X		BMP-2	Membrane	Peri-implant dehiscence defects		

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Tenkumo T. et al. [44]	2018		X		BMP-2 gene transfer/BMP-2/naked plasmid	Membrane	Enhancing the release kinetics by gene transfection using non-viral vectors	Subcutaneous ectopic model in 96 Wistar rats. ELISA for BMP-2, ALP, OCN. Histomorphometry (μ CT), histology	Larger and longer release when using BMP-2-encoding plasmid DNA-functionalized calcium phosphate nanoparticles.
Boyne P.J. et al. [45]	2005		X		BMP-2	Sponge/matrix	Phase II study	BMP-2 in maxillary sinus augmentation. 0.75 mg/mL (n=18), 1.50 mg/mL (n=17), or without (n=13). Radiographic analysis, histology	ALP $\uparrow$ Normal bone formation. Similar bone dimension increases in each group but sign. lower bone densities in BMP-2 groups.
Carter T.G. et al. [46]	2008		X		BMP-2	Sponge/matrix	Treatment of mandibular bone defects as an alternative to autogenous bone grafting	Case series with 5 patients who had mandibular defects reconstructed using loaded scaffolds. Radiographic analysis and clinical examination	Bone regeneration and restoration in 3 of 5 patients
Levin B.P. et al. [47]	2012		X		BMP-2	Sponge/matrix	Extraction socket healing before implant placement.	Case series with 6 patients receiving a loaded scaffold after extraction and before delayed implant placement. Clinical examination	All seven implants achieved primary stabilization. No early implant failures or adverse events.
Kumar M.S. et al. [48]	2016		X		BMP-2	Sponge/matrix	Treatment of bony defect secondary to periodontal surgery	Case series with 10 patients receiving apicectomy and scaffold implantation at surgical defect. Radiographic analysis and clinical examination	Regeneration of bony defect on all 10 patients without any case of graft failure
Jo D.W. et al. [49]	2019		X		BMP-2	Sponge/matrix	Clinical trial - alveolar ridge preservation	Loaded scaffold placement at implant site of 64 patients. Histomorphometry (CBCT)	No significant differences in alveolar bone width and histomorphometric analysis.
Bone morphogenetic protein-6 Chiu H.C. et al. [50]	2013		X		BMP-6	Sponge/matrix	Periodontal wound healing	Supraalveolar periodontal defects in 11 beagle dogs. Histomorphometry (μ CT), histology	No severe adverse events New bone formation $\uparrow$ New cementum formation $\uparrow$ Functionally oriented periodontal ligament. No ankylosis
Bone morphogenetic protein-7 Jin Q. and Giannobile W. [51]	2014		X		BMP-7/PDGF/SDF-1/VEGF	Membrane	Bone development and repair of a large bone defect	Subcutaneous ectopic bone formation in 32 and calvarial critical-sized defect model in 36 mice. Histomorphometry (μ CT), histology	Effects for BMP-7: Bone mineral content $\uparrow$
Bone morphogenetic protein-9 Fujioaka-Kobayashi M. et al. [19]	2016	X			BMP-9/BMP-2	Membrane	Bone regeneration	Adhesion, proliferation of ST2 stromal bone marrow cells. RT PCR for Runx2, ALP, bone sialoprotein (BSP). Alizarin red staining	Adhesion = Proliferation = Differentiation $\uparrow$ BSP $\uparrow$ Higher ALP and Alizarin red staining in BMP-9

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Fujioka-Kobayashi M. et al. [20]	2017	X			BMP-9/BMP-2	Sponge/matrix	Extraction socket healing	Adsorption and release kinetics by ELISA. Adhesion, proliferation and differentiation of stromal cell line (ST2) preosteoblasts. RT-PCR. ALP activity. Alizarin red staining	BMP-2 and -9 fully adsorbed and slowly released over 10d. Adhesion = Proliferation = Collagen I ↑ Osteocalcin ↑ ALP ↑ Bone sialoprotein ↑ mRNA levels all higher in BMP-9. Higher Alizarin red staining in BMP-9.
Fujioka-Kobayashi M. et al. [52]	2017	X	X		BMP-9	Membrane	Bone regeneration	Calvarial defect model in 10 New Zealand white rabbits. Histomorphometry (μCT)	New bone height ↑ New bone area ↑ Volume of native host bone ↑ Complete horizontal bone defect closure
Saulacic N. et al. [53]	2017		X		BMP-9	Membrane	Guided bone regeneration	Calvarial defect model in 9 New Zealand white rabbits. Histomorphometry (μCT), histology	New bone formation ↑ Complete horizontal bone defect closure
Erythropoietin Schwarz F. et al. [54]	2014		X		EPO/FGF/HGH/PDGF-BB	Sponge/matrix	Impact on organization and biodegradation of porcine-derived collagen matrix.	Subcutaneous pouches on the back of 112 Wistar rats. Histology and immunohisto-chemistry	All factors caused pronounced organization of CM by C3 fibers and a biodegradation of the matrix body.
Fibroblast growth factor 2/β D'Mello S. et al. [55]	2015	X			FGF2 gene transfer	Sponge/matrix	Testing of a gene activated matrix encoding basic human fibroblast growth factor 2	Cellular recruitment, attachment, and proliferation of bone marrow stromal cells (BMSCs). Scanning electron microscopy and confocal imaging	Recruitment and attachment of BMSCs to scaffolds Proliferation ↑
Lee S.H. et al. [56]	2017	X	X		FGF2	Membrane	Guided bone regeneration	Calvarial defect model in 18 New Zealand white rabbits. Histomorphometry (μCT), histology	Bone regeneration ↑
Schwarz F. et al. [54]	2014		X		FGF/HGH/PDGF-BB/EPO	Sponge/matrix	Impact on organization and biodegradation of porcine-derived collagen matrix.	Subcutaneous pouches on the back of 112 Wistar rats. Histology and immunohisto-chemistry	All factors caused pronounced organization of CM by C3 fibers and a biodegradation of the matrix body.
Momose T. et al. [57]	2016		X		FGF2	Gel + Sponge/matrix	Periodontal wound healing	Class II furcation defects in 6 beagle dogs. Histomorphometry (μCT), histology	Cell and tissue ingrowth ↑ Periodontal attachment repaired No ankylosis or root resorption
Poudel S.B. et al. [58]	2019		X		FGF2	Sponge/matrix	Biological activity and bone regeneration	Proliferation, mineralization, and osteogenic marker expression in MC3T3-E1 cells, Calvarial defect model in 54 male C57BL/6 mice	Proliferation = Runx2 ↑ Osterx ↑ Bone sialoprotein ↑ Collagen IAI ↑

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Human growth hormone Schwarz F. et al. [54]	2014		X		HGH/PDGF-BB/EPO/FGF	Sponge/matrix	Impact on organization and biodegradation of porcine-derived collagen matrix.	Subcutaneous pouches on the back of 112 Wistar rats. Histology and immunohisto-chemistry	Osteocalcin ↑ Osteopontin ↑ Bone healing ↑ All factors caused pronounced organization of CM by C3 fibers and a biodegradation of the matrix body.
Interferon gamma Jansen R.G. et al. [59]	2011	X	X		IFN-γ	Sponge/matrix	Improve wound contraction and scar formation after cleft palate repair	Submucosa-periosteal scaffold placement in the palate of 25 Wistar rats. Histology and immunohisto-chemistry	Inflammatory and foreign body response Influx of host cells ↑ Myofibroblasts ↓↓
Platelet-derived growth factor Elangovan S. et al. [60]	2014	X	X		PDGF-B gene transfer	Sponge/matrix	Develop and test a non-viral gene delivery system for bone regeneration	In vitro cytotoxicity and transfection efficacy tested in human bone marrow stromal cells (BMSCs). Calvarial defect model in 13 Fisher 344 rats. SEM, histomorphometry (μCT), histology	Proliferation ↑ New bone volume/total volume (BV/TV) ↑
Jin Q. and Giannobile W. [51]	2014		X		PDGF/SDF-1/VEGF/BMP-7	Membrane	Bone regeneration	Calvarial critical-sized defect model in 36 mice. Histomorphometry (μCT), histology	PDGF enhanced calvarial critical-sized bone defect healing similar to SDF-1, and VEGF.
Schwarz F. et al. [54]	2014		X		PDGF-BB/EPO/FGF/HGH	Sponge/matrix	Impact on organization and biodegradation of porcine-derived collagen matrix.	Subcutaneous ectopic model in 112 Wistar rats. Histology and immunohisto-chemistry	All factors caused pronounced organization of CM by C3 fibers and a biodegradation of the matrix body.
Receptor activator of NF-κB ligand Chang A.R. et al. [61]	2017		X		RANKL	Sponge/matrix	Establishing local osteoporotic model without ovariectomy using RANKL.	Mandible defect model in 5 beagles dogs. Histomorphometry (μCT)	Bone resorption at over 40μg of RANKL
Stromal cell-derived factor 1/C-X-C motif chemokine 12 Hwang H.D. et al. [32]	2015	X	X		SDF-1/BMP-2	Sponge/matrix	Sequential use of SDF-1 and BMP-2 for bone regeneration	Osteoblastic differentiation and cell migration in C2C12, C3H10T1/2 and bone-marrow stromal cells (BMSC). Subcutaneous ectopic model in 32 and calvarial defect model in 36 female C57BL/6 mice. Histomorphometry (μCT), histology.	Strongest osteoblastic differentiation and enhanced cell migration with BMP-2 after SDF-1. SDF-1 followed by BMP-2 showed highest new bone regeneration
Lee C.H. et al. [34]	2015	X	X		SDF-1/BMP-2	Sponge/matrix	Bone regeneration	In vitro assays with mesenchymal and endothelial cells Subcutaneous pockets and critical-size calvarial defect model in 40 mice. Histology, and radiographic analysis	SDF-1 has no own or additive effect with BMP-2 in osteogenic induction, cell migration or angiogenesis

Table 2 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Liu H. et al. [62]	2015	X	X		SDF-1	Membrane	Recruitment of host stem cells and periodontal bone defect repair	Buccal bone defects in bilateral mandibles in 40 Wistar rats. Histology, immunohistochemistry, and radiographic analysis	SDF-1 alone does not enhance bone formation CD11b+inflammatory cell response ↓ Vascular formation ↑ Early bone osteoclastogenesis ↑ Scaffold degradation ↑ Bone regeneration ↑ SDF-1 had no effect in the ectopic bone model. SDF-1 enhanced calvarial critical-sized bone defect healing similar to VEGF, and PDGF
Jin Q. and Giannobile W. [51]	2014		X		SDF-1/VEGF/BMP-7/PDGF	Membrane	Bone regeneration	Subcutaneous ectopic model in 32 and calvarial defect model in 36 mice. Histomorphometry (μCT), histology	
Transforming growth factor beta Prenaraj S. et al. [63]	2006	X			TGFβ3	Gel	Release kinetics and bioactivity	ELISA measurements of Tgf-β3 collected in media. Mink lung epithelial cell proliferation and osteoblast (rat calvaria) alkaline phosphatase (AP) activity and AlamarBlue assay	Initial large release followed by sustained release over 14 days. Epithelial cell proliferation ↓ AP activity ↓ Collagen gel inhibited osteoblast proliferation. Effects for TGFβ1: Osteoblast proliferation ↑
Miron R.J. et al. [17]	2013	X			TGFβ1/BMP-2	Membrane	Bone regeneration.	Human osteoblasts. Scanning electron microscopy. Real-time PCR. Alizarin red staining.	
Caballe-Serrano J. et al. [64]	2017	X			TGFβ1/bone conditioned medium (BCM)	Membrane	Adsorption of TGF-β expression provoking molecules from BCM.	Gingival fibroblasts on BCM carrier. Expression of adrenomedullin, pentraxin 3, interleukin 11, and proteoglycan 4. Cell viability and morphology. TGF-β1 as control	No changes in viability or morphology of fibroblasts. Adrenomedullin ↓ Pentaxin 3 ↓ Interleukin 11 ↑ Proteoglycan 4 ↑
Vascular endothelial growth factor Jin Q. and Giannobile W. [51]	2014		X		VEGF/BMP-7/PDGF/SDF-1	Membrane	Bone regeneration	Calvarial defect model in 36 mice. Histomorphometry (μCT), histology	VEGF enhanced calvarial critical-sized bone defect healing similar to SDF-1, and PDGF.
Kim J.H. et al. [65]	2016	X	X		VEGF	Sponge/matrix	VEGF release, biocompatibility and angiogenesis	Rat bone marrow derived mesenchymal stem cell (rMSC) culture. Chorioallantoic membrane (CAM) model. Subcutaneous ectopic model in 12 Sprague-Dawley rats	Gradual release over 28 days Cell proliferation ↑ Blood vessel complexes ↑ Good biocompatibility

Table 3 Overview of studies applying pharmaceuticals onto collagen scaffold sorted by study methods (in vitro, in vivo, clinical) and year of publication

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Antibiotics									
Cheng C.F. et al. [66]	2009	X			Amoxicillin/tetracycline	Membrane	Antibacterial effects for guided tissue regeneration	Testing the membrane penetration of <i>Streptococcus mutans</i> and <i>Aggregatibacter actinomycetemcomitans</i>	Bacterial penetration ↓ Inhibitory effect of tetracycline on <i>S. mutans</i> was greater than of amoxicillin
Cheng C.F. et al. [67]	2015	X			Amoxicillin/tetracycline	Membrane	Bacterial adhesion on guided tissue regeneration membranes	Adhesion of <i>Streptococcus mutans</i> and <i>Aggregatibacter actinomycetemcomitans</i> analyzed using SEM	<i>S. mutans</i> and <i>A. actinomycetemcomitans</i> attached best on collagen membranes. Incorporation of tetracycline or amoxicillin greatly reduced the adhesion.
Anderson T.R. et al. [68]	2015	X			Doxycycline	Gel	Post-surgical infections and wound healing	Disk diffusion method with <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> , <i>Streptococcus sanguinis</i> , and methicillin-resistant <i>Staphylococcus aureus</i> . Spectroscopy	Gradual release over 5 days. Effective against all four bacterial strains
Pal P. et al. [21]	2019	X			Doxycycline/BMP-2	Gel	Bone regeneration	Adhesion, proliferation, and migration of human adipose-derived stem cell (hASC). DNA and protein quantification, SEM, spectroscopy, Alizarin red staining, mechanical testing	Doxycycline shows an initial burst followed by a gradual release over 7 days. All hydrogels supported hASC attachment, proliferation, and differentiation.
Gamal A.Y. et al. [69]	2012		X		Doxycycline	Membrane	Influence of root surface etching on release kinetics	Open flap debridement in 30 patients with contralateral interproximal intrabony defects. High-performance liquid chromatography of gingival crevicular fluid	Released doxycycline shows anti-microbial activity against <i>Pseudomonas aeruginosa</i> , <i>Streptococcus sanguinis</i> , and <i>Escherichia coli</i> Fast release in first 3 days followed by sustained release over 21 days Higher Doxycycline availability after 24% EDTA root surface etching
Bisphosphonates									
Cho T.H. et al. [41]	2017	X	X		BMP-2/alendronate (ALN)	Sponge/matrix	Bone regeneration	Differentiation and the mineralization of rat BMSCs. Calvarial defect model in 80 Sprague-Dawley rats. Histomorphometry (μCT), histology. RT PCR. Alizarin red staining, Immunohistochemistry	Burst release in first 8h followed by gradual release. BMP-2 + ALN: RANKL ↑ OPG ↓ Cathepsin K ↑ TRAP pos. cells ↓ ALP ↑ Osterix ↑

Table 3 (continued)

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Möller B. et al. [70]	2014		X		Alendronate	Membrane	Resorption of onlay bone grafts	Ridge augmentation model in 8 adult female domestic pigs using mandibular bone blocks. Split-mouth design. Histology, fluorescent labeling	VEGF ↑ Bone mass ↑ Bone density ↑ In five cases, necrosis of the overlying periosteal tissues. Loss in graft height ↓ Resorption lacunae ↓
De Sarkar A. et al. [71]	2015		X		Alendronate	Sponge/matrix	Extraction socket healing	Tooth extraction in 20 patients. Radiographic analysis	Less bone loss: 23% with collagen 44% with loaded collagen
Corticosteroids									
Piao Z.G. et al. [72]	2014	X	X		Dexamethasone (DEX)	Membrane	Bone regeneration	Spectrophotometry for DEX. Calvarial defect model in 48 Sprague-Dawley rats. UV Histomorphometry (μCT), histology	Initial burst release followed by a sustained release over 28 days. Bone volume ↑ Bone formation ↑ New bone ↑
Hypoxia mimetic agents									
Edelmayer M. et al. [73]	2017	X			DFO/DMOG	Membrane	Angiogenesis	Viability and proliferation in RAW 264.7 and MC3T3-E1 cells. MTT and BrdU assays. ELISA for VEGF	Burst like release within first hours. VEGF ↑ (DFO or DMOG) Cell proliferation and metabolic activity ↓ (DFO) Osteoclastogenesis ↓ VEGF ↑↑ (DFO or L-MIM) No changes in viability and proliferation-
Al-Habbal D. et al. [74]	2018	X			DFO/DMOG/L-MIM	Membrane	Angiogenesis	Viability and proliferation in human gingival fibroblasts. MTT and BrdU assays. ELISA for VEGF	
Statins									
Calixto J.C. et al. [75]	2011		X		Simvastatin	Sponge/matrix	Bone regeneration	Calvarial defect model in 64 Wistar rats. Histomorphometry (μCT), histology	No differences after 30 days. Lower radiographic densities after 60 days.
Suthanthiran T. et al. [76]	2019		X		Simvastatin	Membrane	Periodontal regeneration	Intrabony defects in 60 patients with split-mouth design. ELISA for RANKL and OPG in gingival crevicular fluid	RANKL ↓ OPG ↑
Others									
Millan D. et al. [77]	2016	X	X		<i>Calendula Officinalis</i>	Sponge/matrix	Skin wound regeneration	Full-thickness skin wound model in 39 male New Zealand rabbits. Bicinehonic acid (BCA) method for collagenase digestion	Wound healing ↓ Collagen cross-linking ↑

Table 3 (continued)

Authors	Year	<i>In vitro</i>	<i>In vivo</i>	Clinical	Drug(s)/substance(s)	Type of collagen scaffold	Aim(s)	Methods	Outcomes/main findings
Chen Q. et al. [78]	2019		X		Anti-tumor necrosis factor- α (anti-TNF- α)/Progranulin (PGRN)	Membrane	Periodontal bone defect repair	Inflammatory periodontal bone defect model in 6 Wistar rats. Histomorphometry (μ CT), histology, immunohistochemistry, Immunofluorescence staining	New bone $\uparrow$ ALP $\uparrow$ Runx2 $\uparrow$ Tumor necrosis factor receptor 2 $\uparrow$ Expression of TNF- α $\downarrow$ TRAP pos. cells $\downarrow$
Song W.K. et al. [79]	2018		X		Cyanoacrylate (CA)/mussel adhesive protein	Membrane	Biocompatibility and barrier function	Calvarial defect model in 8 New Zealand white rabbits. Histomorphometry (μ CT), histology	CA adhesive remained, showing inflammatory reaction. No significant difference in new bone.
Miguez P.A. et al. [46]	2011	X	X		BMP-2/Glycosaminoglycan of biglycan (BGN)	Sponge/matrix	Bone regeneration	BMP signaling and function in C2C12 myogenic cells ALP activity Mandible defect model in 16 Sprague–Dawley rats. Histomorphometry (μ CT)	BMP-2 + BGN: BMP signaling $\uparrow$ BMP function $\uparrow$ Bone formation $\uparrow$ (only with de-glycanated BGN)
de Brito Bezerra B. et al. [80]	2012		X		Hyaluronic acid	Sponge/matrix	Bone regeneration	Calvarial defect model in 32 Wistar rats. Histomorphometry (μ CT), histology	Connective tissue $\uparrow$ Bone formation $\uparrow$ New bone $\uparrow$
Eliezer M. et al. [81]	2019	X	X		Hyaluronic acid	Sponge/matrix	Release kinetics and scaffold degradation	Subcutaneous ectopic model in 32 diabetic Wistar rats. ELISA for adsorption and HA release. Histomorphometry (μ CT), histology	80% adsorption. Gradual release over 10 days. Residual collagen $\uparrow$ Significant effect on keeping the membrane thickness.
Fügl A. et al. [82]	2014		X		S-NO-HSA/human albumin	Sponge/matrix	Bone regeneration	Calvaria augmentation model in 40 male New Zealand white rabbits. Histomorphometry (μ CT), histology	Bone formation $\uparrow$
Song W.K. et al. [79]	2018		X		Mussel adhesive protein (MAP)/cyano-acrylate	Membrane	Biocompatibility and barrier function	Calvarial defect model in 8 New Zealand white rabbits. Histomorphometry (μ CT), histology	MAP was completely absorbed. No significant difference in new bone.
Auersvald C.M. et al. [83]	2017		X		Parathyroid hormone (PTH)	Sponge/matrix	Bone regeneration	Calvarial defect model in 42 Wistar rats. Histomorphometry (μ CT), histology	Bone formation $\uparrow$ New bone $\uparrow$
Chen Q. et al. [78]	2019		X		Progranulin (PGRN)/anti-tumor necrosis factor- α (anti-TNF- α)	Membrane	Periodontal bone defect repair	Inflammatory periodontal bone defect model in 6 Wistar rats. Histomorphometry (μ CT), histology, immunohistochemistry, Immunofluorescence staining	New bone $\uparrow$ ALP $\uparrow$ Runx2 $\uparrow$ Tumor necrosis factor receptor 2 $\uparrow$ Expression of TNF- α $\downarrow$ TRAP positive cells $\downarrow$
Fügl A. et al. [84]	2015		X		Vitamin D	Sponge/matrix	Bone regeneration	Maxilla and mandible defects in 60 Sprague–Dawley rats. Histomorphometry (μ CT). Serum levels of vitamin D and PTH	No significant effects

Table 4 Overview of studies applying tissue-, cell-, or matrix-derived products onto collagen scaffolds sorted by study methods (in vitro, in vivo, clinical) and year of publication

Authors	Year	In vitro	In vivo	Clinical	Drug(s)/ substance(s)	Type of collagen scaffold	Aim(s)/outcome(s)	Methods	Outcomes/main findings
Bone conditioned medium									
Caballe-Serrano J. et al. [64]	2017	X			Bone conditioned medium/TGF- β 1	Membrane	Adsorption of TGF- β expression provoking molecules from BCM	Gingival fibroblasts: Cell viability and morphology Expression of adrenomedullin, pentraxin 3, interleukin 11, and proteoglycan 4 TGF- β 1 as control	Viability or morphology of fibroblasts = Adrenomedullin $\downarrow$ Pentaxin 3 $\downarrow$ Interleukin 11 $\uparrow$ Proteoglycan 4 $\uparrow$
Kuchler U. et al. [85]	2018		X		Bone conditioned medium	Membrane	Bone regeneration	Calvarial defect model in 20 Sprague-Dawley rats. Histomorphometry (μ CT), histology	Osteoconductive competence. Bony islands $\uparrow$ Bone connected to the host bone $\downarrow$
Cell conditioned medium									
Katagiri W. et al. [86]	2013	X			Cell conditioned media from mesenchymal stem cells (MSCs)	Sponge/matrix	Bone regeneration and endogenous stem cell mobilization	Cell mobilization and expression of osteogenic-related genes in rat bone-marrow-derived stem cells (rMSCs). Calvarial defect model in 24 Wistar rats. Histomorphometry (μ CT), histology	Migration $\uparrow$ Proliferation $\uparrow$ Alkaline phosphatase $\uparrow$ Osteocalcin $\uparrow$ Runx2 $\uparrow$ Insulinlike growth factor-1, VEGF, TGF- β 1, and hepatocyte growth factor present. Early bone regeneration in rat calvaria
Platelet-rich plasma									
Pryor M.E. et al. [87]	2005		X		Platelet-rich plasma	Sponge/matrix	Bone regeneration	Calvarial defect model in 30 Sprague-Dawley rats. Radiographic image analysis. Histologic and histometric analysis	Limited potential to promote local bone formation.
Pryor M.E. et al. [88]	2005		X		Platelet-rich plasma	Sponge/matrix	Bone regeneration	Calvarial defect model in 30 Sprague-Dawley rats. Radiographic image analysis	No significant effect on osteogenesis
Platelet-rich fibrin									
Ghanaati S. et al. [89]	2019			X	Platelet-rich fibrin	Sponge/matrix	Testing of a novel clinical protocol	Case series of 7 patients with alveolar ridge atrophy. Open healing concept of soft tissue gap after augmentation. Histological evaluation	Well-vascularized newly formed bone. No fibrosis. Clinically good functional and aesthetic results.
Gulsen U. et al. [90]	2019			X	Platelet-rich fibrin	Sponge/matrix	Bone regeneration after sinus floor augmentation	Pilot study with 12 patients. Sinus augmentation with i-PRF soaked collagen. Radiographic evaluation	New bone formation $\uparrow$
Platelet secretome									
Mozgan E.M. et al. [91]	2017	X			Platelet secretome	Membrane	Mitogenic capacity of collagen loaded with secretome of washed and unwashed platelet preparations	Supernatants 1–48h. Mitogenic effect on periodontal fibroblasts. Cell attachment based on fluorescence microscopy with DiI-labeled cells. Release of total protein, PDGF-BB, TGF- β 1	Cell attachment was not modulated. Highest level of highest levels of total protein, PDGF-BB and TGF- β 1 after 1h with declining release kinetics
Enamel matrix derivative									
Miron R.J. et al. [92]	2017	X			Enamel matrix derivative	Sponge/matrix	Bone regeneration	ELISA for amelogenin adsorption and release. ST2 pre-osteoblasts for cellular attachment and cell proliferation. Osteoblast differentiation by real-time PCR and alizarin red staining	Nearly 100% adsorption. Gradual release over 10 days. Cell attachment $\uparrow$ mRNA levels of osteoblast differentiation markers $\uparrow$ Induced alizarin red staining when combined with collagen
Shirakata Y. et al. [93]	2017		X		Enamel matrix derivative	Sponge/matrix	Periodontal wound healing/regeneration in furcation defects	ELISA for amelogenin adsorption and release. Chronic class III furcation defects in three monkeys. Histology	Connective tissue attachment $\uparrow$ Bone formation $\uparrow$ New attachment formation, cementum and bone area $\uparrow$

collagen sponges. In C2C12 myogenic cells, biglycan and BMP-2 loaded collagen scaffolds induced BMP signaling and function at most. When critical-sized mandible defects of 16 Sprague–Dawley rats received loaded scaffolds, bone formation was significantly increased when BMP-2 was combined only with de-glycanated BGN, indicating that the GAG component of BGN functions as a suppressor for the BGN-assisted BMP function (Miguez P.A. et al. 2011 [26]). In another study of Jung et al., BMP-2 soaked collagen blocks were implanted in calvarial defects in 60 rats. Groups with loaded scaffolds were compared with blood-filled defects. Bone formation was higher at 8 weeks after invention compared with 2 weeks. Collagen was completely resorbed after 8 weeks. BMP-2 led to a significantly greater augmented area, new bone area, and new bone ratio (Jung J.H. et al. 2011 [27]). In a standardized maxillary sinus model in rabbits, BMP-2 loaded collagen sponges were implanted into left and right maxillary sinuses. After 8 weeks, radiographic analysis showed new bone formation independently of BMP-2. The maximum augmented height did not differ, but maxillary window closure was more advanced in the BMP-2 group (Choi Y. et al. 2012 [28]). For the assessment of vertical GBR purposes, four titanium cylinders were implanted into the external cortical bone of rabbit calvaria. Cylinders were filled with mineralized bone matrix and collagen membranes loaded with BMP-2 or BMP-2 with collagen-binding domain (CBD) were used as coverage. After 6 weeks, new bone trabeculae were observed in the superficial layer when covered with membranes with BMP-2-CBD. Moreover, the average area of newly formed bone was significantly higher in this group compared with BMP-2 or control (Lai C.H. et al. 2013 [29]). Investigating a possible synergistic effect of local BMP-2 and systemic PTH therapy, collagen sponges were loaded with BMP-2 and applied in rat calvarial defects. One group received systemic PTH in addition. BMP-2 loaded collagen scaffolds significantly increased histologic bone formation, whereas PTH did not show a significant additional effect (Stancoven B.W. et al. 2013 [30]). When a GBR procedure was conducted at the buccal side of edentulous maxillary alveolar ridges in dogs, onlay grafts containing bovine hydroxyapatite incorporated with collagen matrix were used and covered by a collagen membrane. In one group, the graft was loaded with BMP-2; in the other group, BMP-2 was loaded onto the collagen membranes. Newly formed bone could be observed in both groups without significant differences. Only the bone-to-residual bone substitute contact ratio was significantly lower in the group where only collagen membranes carried BMP-2 (Chang Y.Y. et al. 2015 [31]). The sequential treatment using SDF-1 before BMP-2 loaded onto collagen scaffold showed an enhanced osteoblastic differentiation, cell migration, and the highest degree of new bone formation in calvarial defects in mice (Hwang H.D. et al. 2015 [32]). When different carrier systems were compared in vivo, collagen

sponges, biphasic calcium phosphate blocks and collagenated biphasic calcium phosphate blocks were loaded with BMP-2. Critical-sized calvarial defects in ten rabbits were filled with loaded materials. Histological and histomorphometric analyses were performed at 2 or 8 weeks after surgery. At early stage, new bone formation was significantly less when collagen sponges were used and dimensional stability seemed to be greater in the other groups at both points in time (Jung I.H. et al. 2015 [33]). Lee et al. [34] investigated the effect of the combination of SDF-1 and BMP-2 loading onto collagen scaffolds. BMP-2 induced ectopic and orthotopic bone regeneration, whereas SDF-1 did not have a beneficial effect (Lee C.H. et al. 2015 [34]). Previous studies have revealed that BMP-2 is burst-like released from carriers in the early stage, leading to high peaks of BMP-2 onsite. These high peaks might result in unwanted effects relating to adipose tissue formation, consequently leading to less bone density. Aiming toward a more controlled continuous release, a heparin-conjugated fibrin gel was tested as a carrier of BMP-2. Loaded collagen sponges were used as control. In a critical-sized orthotopic calvarial defect model in rats, solidified gel and collagen sponges which contained BMP at various concentrations were implanted. There was no difference in bone formation and at lower levels of BMP-2 adipose tissue formation was less using loaded gel in comparison with collagen sponges. Findings of the ectopic model using subcutaneous transplantation in 25 immunodeficient mice are not being mentioned in our review as no collagen scaffold was used as carrier material (Lee J.S. et al. 2015 [35]). In 2015, Mostafa et al. (39)[36] compared BMP-2 loaded absorbable collagen sponges with nanofiber-based scaffolds with a collagen backbone to sustain release of BMP-2. In a cleft palate defect model in 30 Wistar rats, BMP-2 loaded carriers were placed into the defect and covered with a placental resorbable membrane. In vitro release testing revealed that scaffolds containing a higher content of 2% of nanofibers released BMP-2 more slowly. After 8 weeks, both BMP-2 treated groups showed higher relative bone filling, whereas the bridging of the bone defect happened to be earlier when using nanofibers (Mostafa N.Z. et al. 2015 [36]). Another study in 2015 investigated a possible additional effect of pulsed electromagnetic fields (PEMF) with BMP-2 treatment. Collagen sponges were soaked in different concentrations of BMP-2 and implanted in the area of rat calvarial defects. PEMF was applied for 8 h per day over 5 days in one group of animals. Four weeks after surgery, all groups statistically significantly increased bone formation parameters assessed by histology, histomorphometry, and immunohistochemistry irrespective of PEMF. When applying the highest dose of BMP-2 (10 µg), no additive effects were observed. Additional PEMF therapy accelerated bone regeneration in groups with lower BMP-2 doses, decreased soft tissue infiltrations into the defect site, and defect spaces were successfully bridged compared with groups without PEMF therapy

(Yang H.J. et al. 2015 [37]). In a study comparing onlay-type grafted human freeze-dried cortico-cancellous bone blocks (FDBB) and deproteinized bovine bone with collagen (DBBC) loaded with BMP-2 (2.5 µg), buffer and BMP-2 loaded collagen sponges acted as control. Scaffolds were placed in rat calvarial defects and 2 or 8 weeks post-surgery, histologic and histomorphometric analyses were performed. At both time points, all BMP-2 groups showed significantly higher new bone area and bone density compared with control. BMP-2 loaded FDBB and DBBC showed significantly higher total augmented area than loaded collagen, but highest bone density could be observed in BMP-2 loaded collagen sponges (Lee J.S. et al. 2016 [38]). Another study in 2016 assessed the effect of limited-dose BMP-2 (1.5 µg) loaded biphasic calcium phosphate (BCP) scaffolds on healing of rabbit calvarial bone defects and compared them with BMP-2 loaded collagen sponges. The control group received no filling of the defect at all. At 2 or 8 weeks post-operative, both groups showed extensive new bone formation, which was significantly greater after 8 weeks in all groups. In the loaded collagen group, bony collapse was found of the upper defect. New bone formation and the size of the augmented area were significantly greater when using loaded BCP compared with collagen (You H. et al. 2016 [39]). In a study which was aiming to evaluate a novel BCP scaffold, collagen membranes were used as control. Scaffolds were loaded with 20, 50, or 100 µg of BMP-2 and applied in cylindrical bone defects which were created bilaterally in the femurs of rats. No unloaded collagen membrane as a negative control was used. BMP-2 was gradually released within 14 days from collagen compared with a rapid release from BCP within 1 day. An increasing concentration of BMP-2 led to higher bone fill irrespective of the carrier system. Loaded collagen membranes at a BMP-2 concentration of 100 µg were promoting up to 30% bone volume over total volume (BV/TV), whereas loaded BCP reached up to 100%. TRAP staining and osteoclast numbers were less in defects receiving BMP-2 loaded collagen scaffolds compared with loaded BCP (Zhang Y. et al. 2016 [40]). BMP-2 combined with alendronate (ALN) led to enhanced osteoblast differentiation and mineralization of mesenchymal stromal cells. Resorption markers showed a higher expression while TRAP positive cells were reduced. In vivo, bone mass and density was increased demonstrating a possible positive effect of ALN together with BMP-2 (Cho T.H. et al. 2017 [41]). In 2018, Durham et al. [42] investigated the effects of collagen sponges loaded with BMP-2 in critical-sized defects in mice. BMP-2 led to greater healing compared with control emanating from the surgical margin. While BMP-2 treatment led to an increase in cell presence within the healing defect there was, however, no regional orientation of craniofacial stem cells or vascularization (Durham E.L. et al. 2018 [42]). To determine the effect on BMP-2 soaked collagen membranes on peri-implant dehiscence effects, implants were

placed in five dogs per group where the buccal bone was removed afterwards, resulting in a defect. This defect was filled with bone substitute material and covered with BMP-2 or saline collagen membranes. BMP-2 loaded scaffolds did not improve bone regeneration of this peri-implant bone defects compared with non-soaked membranes (Sun Y.K. et al. 2018 [43]). As the release of growth factors mainly depends on scaffold degradation, another study aimed to improve this condition by using scaffolds in combination with gene transfection using non-viral vectors. Nanohydroxyapatite–collagen scaffolds were loaded with BMP-2-encoding plasmid DNA-functionalized calcium phosphate nanoparticles, naked plasmid DNA, or BMP-2 solution, and implanted into subcutaneous tissue of the back of rats. Collagen membranes loaded with either of these substances in different concentrations were used as control. The amount and its releasing period of BMP-2, as well as alkaline phosphatase activity were significantly greater in scaffolds loaded with BMP-2-encoding calcium phosphate nanoparticles. Released BMP-2 could be observed on the macrophages or multinuclear giant cells surrounding the scaffold fragments. Tartrate-resistant acid phosphatase (TRAP)- or osteocalcin-positive sites were occurring in all groups and a mineralization area was observed in nanoparticle loaded collagen membranes (Tenkumo T. et al. 2018 [44]). In the first clinical phase II study, which evaluated the safety and efficacy of BMP-2, maxillary sinus floor augmentations were performed in patients who received rhBMP-2 via an absorbable collagen sponge, at concentrations of 0.75 or 1.50 mg/mL. Unloaded bone grafts but not collagen sponges were used as control. CT analysis 4 months after treatment and 6 months post-functional loading revealed similar bone dimension increases but significantly lower bone densities in BMP-2 groups. Bone biopsies at implant placement confirmed normal bone formation (Boyne P.J. et al. 2005 [45]). In another clinical study, five patients with mandibular bone defects have been reconstructed with BMP-2 soaked collagen sponges alone or with bone marrow cells and allogeneous cancellous bone chips. No unloaded sponges were used as control. Three cases were showing bone regeneration of the defect site. In two patients the grafts failed but were successfully reconstructed using autogenous bone from the iliac crest (Carter T.G. et al. 2008 [46]). In a case series, seven molar-site implants were placed in six consecutive patients. All sites were augmented with BMP-2 at 1.50 mg/mL loaded onto a collagen sponge after extraction to enhance bone regeneration prior to implantation. No negative controls were utilized. All sites achieved primary stability at implantation and were functionally loaded. No implants were lost or developed complications during the healing period of 3 to 8 months (Levin B.P. et al. 2012 [47]). Another case series of ten patients implanted collagen sponges loaded with 1.4 mL BMP-2 into the surgical defect after root resection. Panoramic radiographic and clinical assessments after 3 months revealed sufficient bone

regeneration. No unloaded scaffolds were used as comparative control (Kumar M.S. et al. 2016 [48]). In a 3-month clinical trial, two BMP-2 delivery systems for alveolar ridge preservation were tested. A total of 32 patients received a collagen sponge soaked with 1.05 mg rhBMP-2 and another 32 patients received β -tricalcium phosphate and hydroxyapatite scaffold immersed in BMP-2. No unloaded sponges were utilized as control. The loaded scaffolds were placed into the extraction sockets. Dimensional bone changes at 25, 50, and 75% of extraction socket level and histomorphometric results of bone biopsies before implant placement showed no significant differences (Jo D.W. et al. 2019 [49]).

- Bone morphogenetic protein-6 (BMP-6)

In critical-sized supra-alveolar periodontal defects in 11 Beagle dogs, collagen sponges were soak-loaded with BMP-6 at 0.25, 1, and 2 mg/mL and implanted into the defects. After 8 weeks, the lowest concentration of BMP-6 showed the best results compared with control. New bone regeneration and cementum height were significantly enhanced (Chiu H.C. et al. 2013 [50]).

- Bone morphogenetic protein-7 (BMP-7)

An *in vivo* study was combining SDF-1 with different growth factors (BMP-7, VEGF, PDGF) to evaluate the regenerative effect in critical-sized calvarial bone defects in mice. BMP-7 (1 μ g) and SDF-1 (1 μ g) alone or combined were loaded onto collagen membranes. Maximal bone formation was found in the BMP and the BMP + SDF-1 group at 2 and 4 weeks. After 4 weeks, the defects were almost completely healed. SDF-1 did not show a significant additional effect when combining with BMP-7 (Jin Q. and Giannobile W. 2014 [51]).

- Bone morphogenetic protein-9 (BMP-9)

Collagen membranes loaded with BMP-9 fully absorbed the substance and slowly released it for up to 10 days. When seeding murine ST2 stromal bone marrow cells onto collagen membranes which were carrying a low (10 ng/mL) or high (100 ng/mL) dose of BMP-9, ALP mRNA levels were significantly induced at 3 days and BSP at 14 days. ALP levels were significantly higher (5-fold increase at 3 days and 2-fold increase at 14 days) in groups where the scaffold was loaded with BMP-9 compared with BMP-2. In addition, mRNA levels of collagen1, BSP, and osteocalcin were significantly higher in BMP-9 compared with BMP-2 groups. Effects on cell attachment, proliferation, and on osteoblast differentiation were not significant. Alizarin red staining further showed that BMP-9 induced up to 3-fold more staining in comparison with BMP-2 (Fujioka-Kobayashi M. et al. 2016 [19], Fujioka-

Kobayashi M. et al. 2017 [20]). In a calvarial defect model in rabbits, deproteinized bovine bone mineral (DBBM) was used in combination with a collagen barrier membrane to investigate bone formation using BMP-9. BMP-9 was loaded onto DBBM or the collagen membrane and results were compared with unloaded materials and empty bone defects. Loaded samples promoted significantly higher mineralized tissue volume, new bone height, and new bone area which were evaluated by histomorphometry 8 weeks post-surgery. Multinucleated giant cells could be observed in contact with DBBM surfaces, whereas collagen membranes were not. BMP-9 loaded collagen membranes showed better horizontal wound closure with a faster regeneration of new host bone, suggesting that GBR procedures might benefit more from loaded collagen barrier membranes compared with DBBM (Fujioka-Kobayashi M. et al. 2017 [52], Saulacic N. et al. 2017 [53]).

- Erythropoietin (EPO)

Due to the known proangiogenic effects of EPO, Schwarz et al. [54] soak-loaded this substance onto collagen sponges. *In vitro* evaluation of the activity of human gingival fibroblast showed no significant change. When placed in subcutaneous pouches on the back of rats, organization of collagen type III fibers were pronounced and biodegradation of the collagen sponge speeded up after 14 days (Schwarz F. et al. 2014 [54]).

- Fibroblast growth factor 2/ β (FGF2/FGF β)

D'Mello S. et al. [55] used collagen scaffolds as carrier to deliver polyethylenimine-pDNA nano-sized complexes encoding the FGF2 protein in BMSCs. Recruitment and attachment of BMSCs to scaffolds could be observed using scanning electron microscopy. Complex-loaded scaffolds significantly increased cell proliferation and expression of FGF2 compared with controls (D'Mello S. et al. 2015 [55]). FGF2 loaded onto collagen membranes covering biphasic calcium phosphate bone grafts in calvarial defects in rabbits led to an increase of new bone formation and the amount of residual graft material decreased regardless of different FGF2 concentrations (Lee S.H. et al. 2017 [56]). When FGF2 was loaded onto collagen sponges, *in vitro* testing of the activity of human gingival fibroblast showed an increase which was not significant. Implanted into subcutaneous pouches on the back of rats, organization of collagen type III fibers was pronounced and biodegradation of the scaffold speeded up after 7 days (Schwarz F. et al. 2014 [54]). In another study, collagen hydrogel fabricated from bovine type I collagen was mixed with FGF2 and injected into sponge-form collagen. Those scaffolds were implanted into class II furcation defects in dogs. At 4 weeks, healing of alveolar bone including cementum-like tissue and periodontal attachment was significantly increased

(Momose T. et al. 2016 [57]). Collagen sponges impregnated with 5 µg of plant-derived rhFGF2 (p-FGF2) protein or *E. coli*-derived rhFGF2 (e-FGF2) were used to fill a calvarial defect in mice. Both groups, containing differently derived FGF2, enhanced bone healing to higher levels than without FGF2. Proliferation, mineralization, and osteogenic marker expression in MC3T3-E1 cells were also similar, suggesting the usefulness of a plant-based expression system for the production of biologically active rhFGF2 (Poudel S.B. et al. 2019 [58]).

- Human growth hormone (HGH)

Collagen sponges soak-loaded with HGH were surgically placed in subcutaneous pouches on the back of rats. Biological activity of human gingival fibroblasts in vitro and organization of collagen type III fibers was insignificantly pronounced. Degradation of the scaffold was accelerated (Schwarz F. et al. 2014 [54]).

- Interferon gamma (IFN-γ)

To evaluate the tissue response to IFN-γ by investigating a possible decrease in the differentiation of myofibroblasts, collagen scaffolds with or without IFN-γ were implanted submucoperiosteally in the palate of rats. Histological and immunohistochemical analyses were performed at 1, 2, 4, 8, and 16 weeks after implantation. IFN-γ caused a major numerical reduction in myofibroblasts and a faster influx of host cells (Jansen R.G. et al. 2011 [59]).

- Platelet-derived growth factor (PDGF)

Non-viral vectors in gene therapy can be a safe and efficient way to deliver growth factors. In a study by Elangovan et al. [60], the aim was to develop such a delivery system for bone regeneration using collagen scaffolds and polyethylenimine (PEI)–plasmid DNA complexes encoding PDGF-B. Low cytotoxicity could be observed and transfection efficacy testing in human BMSCs revealed significant proliferation in vitro. Complex loaded scaffolds showed significantly higher new bone volume to total volume ratio in calvarial defects in rats (Elangovan S. et al. 2014 [60]). An in vivo study was combining SDF-1 with PDGF and other growth factors like BMP-7, VEGF evaluating the effect in critical-sized calvarial bone defects in mice. PDGF (0.5 µg) and SDF-1 (1 µg) alone or combined were loaded onto collagen membranes. At 2 and 4 weeks, there were no differences in bone mineral content among the groups containing PDGF and SDF-1 alone or in combination, and VEGF. Groups containing BMP-7 significantly showed the maximal bone formation (Jin Q. and Giannobile W. 2014 [51]). Soak-loaded collagen sponges with PDGF-BB showed a significant increase

of human gingival fibroblast activity. When implanted in subcutaneous pouches on the back of rats, an ingrowth of collagen type III fibers was observed and biodegradation of the scaffold was significantly faster (Schwarz F. et al. 2014 [54]).

- Receptor activator of NF-κB ligand (RANKL)

To establish and test a local osteoporotic model without performing an ovariectomy, the mandibles of five beagle dogs were prepared by drilling a hole and implanting a collagen sponge with 20, 40, or 60 µg RANKL. The maximum bone density loss of about 40% could be observed at 40 µg RANKL. Dental implants combined with injectable β-tricalcium phosphate microsphere bone grafts loaded with BMP-2 were placed. The group with the lowest dosage of 5 µg BMP-2 showed the highest increase in bone areas and bone to implant contact. These changes in bone regeneration were statistically not significant (Chang A.R. et al. 2017 [61]).

- Stromal cell-derived factor 1 (SDF-1)/C-X-C motif chemokine 12

SDF-1 sequentially followed by BMP-2 soaked onto collagen sponges showed significantly enhanced osteoblastic differentiation and cell migration in vitro. In calvarial defects in mice this sequential treatment showed the highest degree of new bone regeneration. SDF-1 alone did not cause any changes (Hwang H.D. et al. 2015 [32]). A study by Lee et al. investigated the effects of simultaneous SDF-1 and BMP-2 treatment on bone formation. Collagen sponges with or without BMP-2 were loaded with different doses of SDF-1 (0.1, 0.5, or 1 µg). SDF-1 had no additive effect on osteoblastic differentiation and cell migration of mesenchymal cells, or capillary tube formation of endothelial cells compared with BMP-2 or SDF-1 alone. Loaded scaffolds were implanted into subcutaneous pockets and critical-sized calvarial defects in mice. SDF-1-only-treated implants did not lead to significant in vivo bone formation and SDF-1 treatment did not improve BMP-2-induced ectopic and orthotopic bone regeneration (Lee C.H. et al. 2015 [34]). To investigate the effects of SDF-1 on bone regeneration in periodontal diseases, collagen membranes loaded with SDF-1 were implanted into established bone defects on the buccal side of bilateral mandibles in rats. The results showed that SDF-1 recruited host-derived mesenchymal stem cells and hematopoietic stem cells to the wound area. CD11b+ inflammatory cell response was significantly reduced. Furthermore, SDF-1 led to an increase in vascular formation, promoted early osteoclastogenesis, accelerated scaffold degradation, and improved the quality and quantity of regenerated bone (Liu H. et al. 2015 [62]). In an in vivo study in mice, SDF-1 was loaded in different dosages onto collagen scaffolds containing BMP-7, VEGF, or PDGF, and implanted into critical-sized calvarial bone defects for 2

and 4 weeks. Part of this study, where SDF-1 was loaded onto demineralized bone matrix instead of collagen for further *in vivo* testing, is not being considered in our review. Biopsies were examined using micro-CT (μ CT) and histology. SDF-1 promoted calvarial critical-sized bone defect healing like VEGF and PDGF, without having significant additional effects when combining it with BMP-7, VEGF, and PDGF (Jin Q. and Giannobile W. 2014 [51]).

- Transforming growth factor beta (TGF- β)

Collagen gels loaded with 100 or 500 ng of TGF- β 3 demonstrated an initial large release, followed by a sustained release over 14 days which was determined by ELISA measurements. Proliferation of mink lung epithelial cell was not influenced by TGF- β 3 conditioned media, but alkaline phosphatase activity was decreased. No unloaded control was used (Premaraj et al. 2006 [63]). Collagen membranes soaked in TGF- β 1 at a concentration of 10 ng/mL allowed human osteoblasts to adhere and increased osteoblast proliferation at 3 and 5 days post-seeding (Miron R.J. et al. 2013 [17]). In another *in vitro* study, the authors were investigating if collagen membranes adsorb growth factors from bone conditioned medium. Recombinant human TGF- β 1 loaded onto collagen membranes served as control, which significantly provoked gene expression of TGF- β target genes. No negative controls were utilized (Caballe-Serrano J. et al. in 2017 [64]).

- Vascular endothelial growth factor (VEGF)

An *in vivo* study was combining SDF-1 with VEGF and growth factors BMP-7 and PDGF to evaluate the effect in critical-sized calvarial bone defects in mice. VEGF and SDF-1 either alone or combined were loaded onto collagen membranes. At 2 and 4 weeks after surgery, there were no differences in bone mineral content among the groups containing VEGF and SDF-1 alone or in combination, and PDGF. Groups containing BMP-7 showed the maximal bone formation (Jin Q. and Giannobile W. 2014 [51]). Kim J.H. et al. developed a composite scaffold consisting of VEGF loaded onto mesoporous silica nanoparticles, which were then incorporated within a type I collagen sponge. This composite material demonstrated good biocompatibility in a subcutaneous tissue model in rats. VEGF was sustainably released over the test period of 28 days and cell proliferation was increased. Testing with the chick chorioallantoic membrane (CAM) model showed significantly increased numbers of blood vessel complexes compared with scaffolds without VEGF (Kim J.H. et al. 2016 [65]).

Pharmaceuticals

Pharmaceuticals are fully synthesized drugs which are aiming to influence physiological pathways. In this review, included

studies used a wide variety of pharmaceuticals. Those substances were loaded onto collagen scaffolds aiming to influence oral hard and soft tissue regeneration. Some studies also evaluated if certain substances improved properties of the scaffold which indirectly could influence tissue regeneration (Table 3).

Antibiotics

The use of antibiotics, such as amoxicillin or tetracycline, loaded onto collagen membranes, demonstrated a lower penetration as well as a decreased adhesion of *Streptococcus mutans* and *Aggregatibacter actinomycetemcomitans* (Cheng C.F. et al. 2009 [66], Cheng C.F. et al. 2015 [67]). Doxycycline-containing collagen hydrogels showed a gradual time-dependent drug release over 5 days, and indicated efficacy against four bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus sanguinis*, and methicillin-resistant *Staphylococcus aureus*) (Anderson T.R. et al. 2015 [68]). Elastin-like polypeptide–collagen hydrogels loaded with doxycycline together with BMP-2 showed an initial burst release within the first 10 h, followed by a gradual release of doxycycline over 7 days, measured by spectrophotometry. Adhesion, proliferation, and migration of human adipose-derived stem cells on hydrogel surfaces was greater and all drug-loaded hydrogels showed bioactivity against *P. aeruginosa*, *S. sanguinis*, and *E. coli* (Pal P. et al. 2019 [21]). In a clinical setting, 30 patients with at least one pair of contralateral interproximal intrabony defects were treated with open flap debridement and placement of doxycycline containing gel in one group. In the other group, exposed root surfaces were etched using 24% EDTA (EDTA) before gel application. No negative control was carried out. Samples of gingival crevicular fluid of up to 21 days after the intervention were analyzed using high-performance liquid chromatography. In both groups, doxycycline was faster released in the first 3 days, followed by a sustained release over 21 days. After root surface etching, local doxycycline availability of doxycycline was higher (Gamal A.Y. et al. 2012 [69]).

Bisphosphonates

Alendronate (ALN) combined with BMP-2, loaded onto collagen sponges, enhanced osteoblast differentiation and mineralization of mesenchymal stromal cells *in vitro*. In a calvarial defect model in 80 rats, bone-forming and bone-resorbing markers showed a higher expression, while TRAP positive cells were reduced. Bone mass and density was also higher, suggesting a positive additive effect of ALN together with BMP-2 (Cho T.H. et al. 2017 [41]). *In vivo*, alendronate loaded on collagen membranes reduced the surface resorption of onlay bone grafts in a ridge augmentation model in eight pigs. However, in five cases, necrosis of the overlying periosteal

tissues was observed macroscopically (Möller B. et al. 2014 [70]). In a clinical study with 20 patients, collagen sponges were soaked with 20 mg/mL alendronate and placed into extraction sockets of incisors and premolars. Collagen sponges alone led to a 22.8% and loaded scaffolds to a 44.4% decrease in alveolar ridge resorption (De Sarkar A. et al. 2015 [71]).

Corticosteroids

Collagen membranes loaded with dexamethasone demonstrated a burst-like initial release, followed by a sustained release over 28 days. In a calvarial defect model in 48 male Sprague–Dawley rats, bone volume and quality of new bone formation was increased (Piao Z.G. et al. 2015 [72]).

Hypoxia mimetic agents (HMA)

HMAs such as deferoxamine (DFO), dimethyloxalylglycine (DMOG), or L-mimosine (L-MIM) loaded onto collagen barrier membranes showed a burst-like release in the first hours. In osteoblast- and osteoclast precursor cells (RAW 264.7 and MC3.T3-E1) DFO and DMOG inhibited cell proliferation and metabolic activity. Osteoclastogenesis was significantly reduced by DFO, suggesting a possible positive effect on oral tissue regeneration (Edelmayer M. et al. 2017 [73]). In human gingival fibroblasts, they did not significantly influence cell viability or proliferation (Al-Habbal D. et al. 2018 [74]). In both studies, DFO and DMOG led to an increase in the expression of vascular endothelial growth factor.

Statins

Simvastatin that was locally administered via collagen sponges at two different concentrations (0.5 mg/50 mL or 2.2 mg/50 mL) in a calvarial bone defect model in 64 Wistar rats showed no differences in radiographic densitometry and histometric analyses after 30 days. After 60 days, the group with lower concentrations even showed reduced bone formation, concluding that there was a detrimental effect on bone regeneration (Calixto J.C. et al. 2011 [75]). In a clinical split-mouth setting, collagen membranes loaded with 1.5 mg simvastatin were placed into intrabony defects of 60 patients, while the contralateral site was filled with an unloaded scaffold. Loaded scaffolds significantly increased OPG and decreased RANKL levels in the gingival crevicular fluid, demonstrating a potential role of simvastatin in periodontal regeneration (Suthanthiran T. et al. 2019 [76]).

Others

- Anti-tumor necrosis factor- α (anti-TNF- α)

Collagen membranes loaded with anti-TNF- α were implanted within inflammatory periodontal bone defects of six Wistar rats. The present study showed that anti-TNF- α promotes regeneration of those defects via anti-inflammation, osteoclastogenic inhibition, and osteogenic promotion. However, progranulin had a significantly higher regenerative effect than anti-TNF- α (Chen Q. et al. 2019 [78]).

- *Calendula officinalis* flower extract

Collagen scaffolds were loaded with calendula officinalis flower extract and implanted in full-thickness skin wounds in 39 rabbits. Results revealed an inferior wound healing and clinical outcome when compared with the non-loaded control. Collagen cross-linking was increased, leading to a decreased degradation behavior of the scaffolds (Millan D. et al. 2016 [77]).

- Cyanoacrylate

Evaluating new methods for membrane fixation, a study applied cyanoacrylate on collagen membranes in a rabbit calvarial GBR model. After 8 weeks, little amounts of cyanoacrylate adhesive remained on the collagen membrane, causing an inflammatory reaction. There was no difference in bone regeneration (Song W.K. et al. 2018 [79]).

- Glycosaminoglycan/hyaluronic acid

In 2011, a study combined glycosaminoglycans (GAG) of biglycan (BGN) together with BMP-2 and collagen scaffolds as a carrier. In C2C12 myogenic cells, biglycan and BMP-2 combined loaded collagen scaffolds induced BMP signaling and function at most. In vivo, loaded scaffolds were implanted in critical-sized mandibles defects of 16 Sprague–Dawley rats. Bone formation was significantly increased when BMP-2 was combined only with de-glycanated BGN, indicating that the GAG component of BGN functions as a suppressor for the BGN-assisted BMP function (Míguez P.A. et al. 2011 [26]). Collagen sponges that were loaded with 1% hyaluronic acid gel demonstrated significantly better bone fill of critical-sized calvarial defects in 32 adult Wistar rats (de Brito Bezerra B. et al. 2012 [80]). Collagen membranes soaked with hyaluronic acid showed nearly 80% adsorption and a gradual release over the observation period of 10 days. When implanted in the scalp pouch of diabetic rats, hyaluronic acid increased the residual collagen content in the diabetic group and delayed membrane degradation significantly (Eliezer M. et al. 2019 [81]).

- S-NO-HSA/human albumin (nitric oxide donor)

S-nitroso human serum albumin (S-NO-HAS) is a donor of nitric oxide which is a mediator involved in bone

regeneration. Collagen sponges soaked with 100 mL S-NO-HAS (5%, 20%) demonstrated higher bone formation in a calvarial augmentation model in 40 rabbits compared with the positive control group using human albumin (5%, 20%). No negative controls were used (Fügl A. et al. 2014 [82]).

- Mussel adhesive protein

Aiming to improve membrane fixation in GBR procedures, collagen membranes were loaded with mussel adhesive protein and compared with cyanoacrylate. Results suggested excellent biocompatibility of mussel adhesive protein loaded collagen membranes in guided bone regeneration of calvarial defects in rabbits. No difference in bone regeneration was found (Song W.K. et al. 2018 [79]).

- Parathyroid hormone

Collagen sponges soaked with parathyroid hormone significantly exhibited higher bone formation and new bone after 15 to 60 days of healing of calvarial defects in 42 male rats (Auersvald et al 2017⁶³).

- Progranulin

Recombinant human progranulin loaded onto collagen membranes were implanted within inflammatory periodontal bone defects of six Wistar rats. After 2 weeks, anti-inflammatory effects as well as inhibition of osteoclastogenesis and promotion of osteogenesis were seen (Chen Q. et al. 2019 [78]).

- Vitamin D

Topical application of calcitriol loaded onto collagen sponges did not promote bone regeneration in single maxillary or mandibular defects in vitamin D deficient rats (Fügl et al 2015⁴⁷).

Tissue-, cell-, and matrix-derived products

Bone conditioned medium (BCM)

In vitro, Caballe-Serrano et al. [64] generated bone conditioned medium (BCM) by placing demineralized harvested bone chips in cell culture medium. Aqueous soaked collagen membranes were placed in BCM to see if these scaffolds adsorb grow factors activating TGF- β signaling. Collagen membranes with BCM decreased the expression of adrenomedullin and pentraxin 3 and increased the expression of interleukin 11 and proteoglycan 4 in gingival fibroblasts. BCM did not influence the viability or morphology of the fibroblasts (Caballe-Serrano J. et al. in 2017 [64]). In a

calvarial defect model in rats, collagen membranes were soaked in bone conditioned medium or culture medium as control and implanted in the defect site. No unloaded control was used. The control group showed significantly more bone than the BCM group. Scanning electron microscopy and histology showed that BCM might shift bone formation toward the formation of bony islands rather than new bone connected to the host bone (Kuchler U. et al. 2018 [85]).

Cell conditioned medium (CCM)

Cultured conditioned media from human bone marrow-derived mesenchymal stem cells (MSC-CM) was collected to investigate its influence on endogenous stem cell mobilization and bone regeneration. Insulin-like growth factor-1, vascular endothelial growth factor, transforming growth factor- β 1, and hepatocyte growth factor were present in the MSC-CM. When MSC-CM was cultured with rat bone marrow-derived stem cells, migration, proliferation, and expression of alkaline phosphatase, osteocalcin, and Runx2 was increased. Collagen sponges soaked with MSC-CM were grafted into rat calvarial bone defects and showed early bone regeneration (Katagiri W. et al. 2013 [86]).

Platelet-rich plasma (PRP)

PRP preparations from donor rats were soak-loaded on absorbable collagen sponges and surgically implanted into contralateral critical-sized calvaria osteotomies in 18 rats. Histologic and histometric analysis after 4 or 8 weeks showed that bone formation was highly variable in PRP sites as well as in controls. Radiographic analysis did not show significant differences to the control group. Conclusively, PRP preparation did not enhance bone regeneration in vivo (Pryor M.E. et al. 2005 [87, 88]).

Platelet-rich fibrin (PRF)

In a clinical case series of seven patients with alveolar ridge atrophy of different etiologies, a novel augmentation protocol using a combination of xenogeneic bone substitutes covered with PRF loaded onto collagen carriers was carried out. No autologous bone transplantation or periosteum splitting was performed. The gap between the flap margins was bridged using the collagen matrix loaded with liquid PRF. No unloaded collagen matrices were used as control. The whole augmented site was finally covered by either a PTFE-based membrane or sterile latex. After 4 to 8 months, open healing resulted in newly formed soft tissue without any signs of scar formation or fibrosis. Biopsies during implant insertion histologically showed well-vascularized newly formed bone that incorporated the BSM granules (Ghanaati S et al. 2019 [89]). A retrospective clinical pilot study with 12 patients utilized

injectable platelet-rich fibrin (iPRF) preparations loaded onto collagen sponges as a graft for sinus augmentation. No negative controls were carried out. Minimum and maximum residual subantral bone heights were 2.0 and 9.8 mm. After 6 months, panoramic radiographic measurements showed significant new bone formation at all sites. Implants of 10.0 or 11.5 mm length were placed successfully (Gülksen U. et al. 2019 [90]).

Platelet secretome (PSEC)

In a study by Mozgan E.M. et al. [91], secretome of washed platelets (washed PSEC) and secretome of unwashed platelet preparations (unwashed PSEC) were lyophilized onto CBM. Supernatants were collected by adding and replacing medium after 1 to 48 h. Mitogenic response of human periodontal fibroblasts was induced by the supernatants and showed the highest levels of total protein, TGF β 1, and PDGF-BB in the first hour. These effects decreased rapidly in subsequent time points resulting in continuously declining release kinetics (Mozgan E.M. et al. 2017 [91]).

Enamel matrix derivative (EMD)

EMD demonstrated efficient adsorption properties on absorbable collagen sponges. Nearly 100% of the amelogenin proteins could be found after loading. Release kinetics showed a gradual release for up to 10 days. EMD significantly induced a 1.5-fold increase in cell attachment and resulted in a 2–6-fold increase in mRNA levels of runt-related transcription factor 2 (Runx2), collagen1a2, alkaline phosphatase, and bone sialoprotein. Alizarin red staining was pronounced when combined with ACS (Miron R.J. et al. 2017 [92]). In vivo, two different EMD carriers (Emdogain®/Osteogain®) soaked on separate absorbable collagen sponges (ACS) were applied upon periodontal wound regeneration in chronic class III furcation defects in monkeys. A significantly higher amount of total adsorbed amelogenin was found for ACS-loaded-Osteogain® when compared with Emdogain®. Combined with open flap debridement (OFD), both substances resulted in higher amounts of connective tissue attachment and bone formation, compared with treatment with OFD + ACS and OFD alone. Osteogain® showed higher new attachment formation, cementum, and new bone area (Shirakata Y. et al. 2017 [93]).

Discussion

Enhancing regenerative properties of scaffolds for oral tissue engineering remains an important study objective. The timeline of the past 15 years of scientific literature in this field (Fig. 1) together with the high number of 77 included journal

articles and the large variety of 36 different substances which have been used, demonstrate the importance of this topic (Tables 2, 3, and 4). One of the reasons might be the common use of collagen biomaterials in oral and maxillofacial surgery as well as in periodontal treatment. GBR or GTR procedures often require collagen scaffolds as tissue substitute or to cover bone grafts. Biocompatibility, impenetrability for epithelial cells and bacteria, degradation behavior and physical or architectural properties which allow mechanical stability are important elements for scaffolds. Also, adequate adsorption of loaded drugs and their diffusion as well as controlled release, are factors which are indispensable [95]. Improving regenerative capabilities of these scaffolds by additionally adding regenerative substances might seem to be the next logical step. Today, the clinical use of drug loaded scaffolds in oral and maxillofacial surgery is still limited as only few substances are clinically approved and therefore available.

Literature search and excluded studies

Out of 259 unique search results of all three database findings, 182 publications were excluded. The major reason was that in 72 of these studies, other biomaterial carriers than collagen were used in their investigations. These studies were matching the inclusion criteria and were therefore part of the initial findings. This might be explained due to the often-used method of assessing the effect of other biomaterials on the gene regulation of collagen, the collagen synthesis or the secretion level. In 28 studies, loading of the collagen scaffold did not take place. In these cases, bone substitute materials were loaded and unloaded collagen membranes were only used to cover the grafted site. As previously stated, only studies utilizing collagen as carrier material were taken into consideration for being included in this review. Since cell-based therapies are not approved for the clinical use for oral applications yet, the authors chose not to include this alternative approach, leading to 22 studies which had to be excluded.

Types, adsorption, and release kinetics of collagen scaffolds

Looking at the types of collagen scaffolds which were utilized as carrier materials, sponges or matrices were used in 50, membranes in 23, and gels in only 4 studies. The selection of the scaffold type might often have depended on the planned clinical application. In a clinical setting, sponges or matrices are usually used to fill soft or hard tissue defects, while membranes are used as a cover and gels for defects which require a more flowable consistency of the carrier. In our review, we found that nine out of ten clinical studies used sponges or matrices, while the remaining one was using membranes. Scaffold selection in in vitro testing was evenly distributed using sponges or matrices in 15 and membranes in 13

publications. In vivo experiments more often used sponges or matrices ($n = 37$) than membranes ($n = 15$). Gels were only used for in vitro testing (Tables 2–4). Adsorption behavior was not assessed in gels. In membranes, mussel adhesive protein was completely adsorbed and cyanoacrylate adhesive remained, showing an inflammatory reaction [79]. Sponges fully absorbed BMP-2 and BMP-9 [20], whereas hyaluronic acid was nearly 80% adsorbed [81] and amelogenin proteins were adsorbed by almost 100% [92]. Out of all 77 included articles, 18 studies investigated release properties of different substances from collagen carriers [18, 20, 21, 35, 36, 40, 41, 44, 63, 65, 68, 69, 72–74, 81, 92]. Release kinetics of sponges or matrices showed an initial burst-like release phase of BMP-2 and BMP-9 within the first 8 to 24 h and subsequently a slower continuous phase from deeper layers over 10 days [20, 41]. Hyaluronic acid and amelogenin were gradually released over 10 days and VEGF over 28 days [65, 81, 92]. Collagen membranes released BMP-2 fast in the first 24 h, followed by a gradual release over 28 days [40, 44]. Doxycycline was faster released in the first 3 days, followed by 21 days of sustained release. Dexamethasone, DMOG, and DFO were burst-like released from membranes in the first hours and while dexamethasone was continuously released for 28 days, DMOG was not detectable after 24 h and DFO rapidly declined after a period of 48 h [72–74]. PSEC burst-like released in the first hour, then declining over the 48-h observation period [91]. Collagen gels were tested for their release kinetics of BMP-2, TGF β 3, and doxycycline. One study demonstrated a burst-like release of BMP-2, followed by a gradual release over 28 days [18]. Another study showed a gradual release from the beginning for the period of 7 days [21]. Also, doxycycline release kinetics were different in three studies. While it was gradually released in one study for 5 days [68], another study demonstrated a burst-like release in the first hours, followed by a gradual release over 7 days [21]. In a clinical setting, doxycycline was faster released from collagen membranes within the first 3 days followed by a sustained release over 21 days [69]. The release of TGF β 3 from gels revealed to be characterized by an initial burst-like phase with a delayed release for up to 14 days [63]. Release periods were always the maximum time of observation, while the release was never complete at that time point unless otherwise stated. In vivo, collagen sponges were completely resorbed after 8 weeks [27]. Studies trying to establish a slower and more controlled release were successful by using nanofibers or gene transfection applying non-viral vectors, whereas heparin-conjugated fibrin gel did not improve release kinetics [35, 36, 44].

Biological medical products

Growth and differentiation factors, especially bone morphogenetic proteins, were by far the most frequently applied substances. BMPs belong to the transforming-growth-factor- β

superfamily and comprise 13 BMP family members according to the current nomenclature. BMP-2, BMP-4, BMP-6, BMP-7, and BMP-9 play an important role in ossification [96], while BMP-2, BMP-6, and BMP-9 are most potent in inducing differentiation of stem cells into osteoblasts [97]. Recombinant BMP-2 and BMP-7 are officially approved and available for clinical use. Although there are specific bone-related indications for treatment, up to 85% of usage is off-label and also the costs are very high [98]. Current clinical applications in oral and maxillofacial surgery are tooth extraction socket healing [99, 100], mandibular bone reconstruction [46, 101, 102], alveolar reconstruction for cleft repair [103, 104], or maxillary sinus augmentation procedures [105, 106]. In this review, studies were investigating the effects of BMP-2, BMP-6, BMP-7 and BMP-9. All types of bone morphogenetic proteins increased osteoblast differentiation markers and improved hard and soft tissue healing in general and when compared with other substances in vivo [17, 18, 22, 24, 25, 27, 28, 33, 35, 36, 39, 40, 42–44]. Previous studies have shown that adverse effects can occur, especially if the local concentrations of BMPs are too high. Side effects are ranging from inflammatory complications to osteoclast activation, induction of adipogenesis and bone cyst formation and wound complications [107–110]. In a clinical study, BMP-2 soaked collagen sponges in some cases supported the regeneration of mandibular bone defects [46]. Additional systemic PTH therapy or SDF-1 in combination with BMP-2 did not have any significant effects on tissue regeneration [30, 51]. The application of pulsed electromagnetic fields to stimulate bone regeneration resulted in increased bone volume and density and improved the microarchitecture of the regenerated bone in groups with lower concentrations of BMP-2 (2.5 and 5 μ g), suggesting its ability to reduce BMP-2 doses to avoid adverse effects of high concentrations. Schwartz et al. previously reported that PEMF enhanced the osteoinductive effects of BMP-2 on mesenchymal stem cells, which might be an interesting aspect to investigate in further studies [111]. In supra-alveolar periodontal defects in dogs, BMP-6 at the lowest applied concentration loaded onto collagen sponges showed the best results in bone regeneration and cementum height [50]. Previous studies have shown that the effects of BMP-9 on ossification are the strongest, followed by BMP-2 and BMP-7 [96]. In a study evaluating the additional effect of SDF-1 on local BMP-7 treatment utilizing collagen membranes, BMP-7 increased maximal bone formation without beneficial effects of SDF-1 [51]. Comparing BMP-9 to BMP-2 loaded collagen scaffolds, BMP-9 showed similar release kinetics but induced significantly greater ALP, collagen I mRNA, BSP, and osteocalcin levels in murine ST2 stromal bone marrow cells. Cell attachment, proliferation, and osteoblast differentiation did not differ, but alizarin red staining was three times higher compared with BMP-2 [19, 20]. In vivo, BMP-9 loaded onto collagen membranes enhanced

bone regeneration parameters and showed faster horizontal wound closure [52, 53]. When erythropoietin, which is well known for its proangiogenic effect, or human growth hormone or fibroblast growth factor 2 were loaded onto collagen sponges and implanted in subcutaneous pouches on the back of rats, scaffold degradation was faster and organization of collagen fibers was pronounced [54]. Collagen membranes loaded with FGF2, covering biphasic calcium phosphate bone grafts in vivo, led to an increase in new bone formation and decreased graft residuals [56]. Aiming to develop a new way of delivery, plasmid DNA complexes encoding for FGF2 carried by collagen scaffolds increased proliferation and FGF2 expression in BMSCs [55]. In a periodontal model, FGF2 mixed with collagen hydrogel and injected into furcation defects of dogs, bone healing and periodontal attachment were significantly increased [57]. Submucoperiosteally in rat palates IFN- γ loaded collagen scaffolds reduced the number of myofibroblasts and supported the influx of host cells [59]. PDGF soak-loaded on collagen sponges increase human gingival fibroblast activity and scaffold degradation [54]. In a rat calvarial defect model, PDGF increased bone mineral content similar to SDF-1 or VEGF alone [51]. Evaluating an efficient way to deliver growth factors, plasmid DNA complexes loaded onto collagen scaffolds, encoding PDGF, were shown to be effective and significantly enhanced new bone volume to total volume ratio in vivo [60]. Aiming to establish a local osteoporotic model without ovariectomy, collagen sponges loaded with RANKL were successfully used to cause bone loss [61]. SDF-1 is essential for BMP-2 induced osteogenic differentiation. In vivo, SDF-1 alone had no effect in a study but increased bone mineral content in vivo comparable with VEGF or PDGF alone in another study [51]. The sequential treatment of SDF-1 after BMP-2 increased bone regeneration and enhanced differentiation and migration of osteoblasts in vitro [32]. In combination with BMP-7, SDF-1 showed no additional effect [51]. In a periodontal defect model in rats, SDF-1 increased migration of MSCs, reduced inflammation, accelerated scaffold degradation, and improved bone regeneration [62]. TGF- β 1 soaked collagen membranes increased human osteoblast adhesion and proliferation [17]. VEGF is a potent inducer of angiogenesis, which is a key factor to repair damaged tissue. Loaded onto collagen membranes, VEGF significantly increased bone mineral content after 2 and 4 weeks in vivo, comparable with SDF-1 and PDGF [51].

Pharmaceuticals

In this particular subgroup, 16 different substances have been used in 24 studies. Evaluating the effect of antibiotics loaded onto collagen membranes and hydrogels, findings of the included studies demonstrated a lower penetration and decreased adhesion of different types of bacteria [66–69]. Bisphosphonate (alendronate) loaded collagen scaffolds

enhanced osteoblast differentiation and mineralization of MSCs in vitro [41] and significantly reduced bone loss of the alveolar ridge after tooth extraction clinically [71]. While alendronate also reduced surface resorptions of onlay bone grafts in vivo, in five of eight pigs, necrosis of the overlying periosteal tissues could be observed, suggesting that high local concentrations might be disadvantageous [70]. Corticosteroids, glycosaminoglycan, or hyaluronic acid, human serum albumin, parathyroid hormone, and progranulin enhanced bone regeneration in vivo in rat or rabbit calvarial defects [26, 72, 80, 82]. Hypoxia mimetic agents showed diverse effects on osteoblast and osteoclast precursor cells as well as VEGF expression in vitro [73]. Statins and extracts of the calendula officinalis flower loaded on collagen sponges or gels, respectively, had adverse effects on wound healing in bone defect models in vivo [75, 77]. However, a clinical study using simvastatin loaded membranes for periodontal regeneration showed a decrease in RANKL and an increase in OPG in gingival crevicular fluid, which might demonstrate a potential role of simvastatin in periodontal regeneration [76]. As collagen membranes often need to be fixated as part of GTR or GBR procedures, suturing and screw or tag placement might be necessary, which might be complicating the procedure. Also, additional interventions are needed as sutures and especially screws or tags might need to be removed. Application of resorbable surgical adhesives appear to be more convenient as cyanoacrylate has been used for decades to replace sutures, and also closures of sinus membrane perforations during sinus lift procedures [112] or gluing autologous bone grafts onto recipient beds after demineralization seem to be successful [113]. Aiming to use surgical adhesives instead, cyanoacrylate or mussel adhesive proteins loaded onto collagen membranes demonstrated that small remains of cyanoacrylate were still existent after 8 weeks, causing inflammation, while mussel adhesive protein showed excellent biocompatibility [79]. Due to previous studies, showing that cyanoacrylate is not fully adsorbed and causing adverse reactions [114, 115], mussel adhesive protein might have its advantages and therefore calls for further investigations.

Tissue-, cell-, and matrix-derived products

In 10 out of 77 included studies, investigators applied products derived from tissue, cells, or matrices. Bone conditioned medium loaded on collagen membranes was influencing TGF- β target genes but did not improve the formation of new bone in vivo [64, 85]. Conditioned medium of human bone marrow-derived mesenchymal stem cells increased activity, migration, and proliferation of rat bone-marrow-derived stem cells [86]. Collagen scaffolds were also successfully used as carriers of platelet-rich plasma, proved to preserve essential growth factors [116], and the use of platelet secretome induced mitogenic response and the expression of

growth factors in human periodontal fibroblasts [91]. Enamel matrix derivate demonstrated a positive effect in cell attachment, osteoblast differentiation, and proliferation [92]. In vivo, bone formation and connective tissue formation were increased when evaluating the effect of EMD on periodontal regeneration [93]. Platelet concentrates or “platelet-rich plasma” (PRP) were already introduced in the 1990s, aiming to promote local healing at surgical sites [117]. PRP requires the addition of anticoagulants during blood collection while PRF is obtained by centrifugation without anticoagulants and is therefore completely autologous. This fibrin matrix contains platelets and leukocytes as well as a variety of growth factors [118]. In a clinical case series, a novel augmentation protocol for alveolar ridge atrophy, using xenogeneic bone substitutes covered with PRF loaded onto collagen carriers, was performed. Results showed newly formed soft tissue without any signs of scar formation or fibrosis and well-vascularized newly formed bone [89]. A retrospective clinical pilot study aimed to use PRF without any hard tissue substitute for sinus floor augmentation. Injectable platelet-rich fibrin (iPRF) was prepared and loaded onto collagen sponges as a graft which were then implanted. Panoramic radiographic measurements showed significant new bone formation [90]. Follow-up data or three-dimensional measurements were missing and would be recommended for future studies.

Limitations

In our review, included literature most often conducted in vivo (55%) and in vitro (34%) experiments, whereas only 11% conducted clinical testing with patients. The evidence level of in vitro and animal experiments is regarded to be low, even though these studies are forming the basis for studies in humans. New substances and methods need to be evaluated in an in vitro and in vivo setting, before employing design and results from these studies. Out of ten clinical studies, four were case series with five to ten patients [46–48, 89], one was a pilot study with 12 patients [90], and five were clinical trials with 20 to 64 patients [45, 49, 69, 71, 76]. Contrary to our research question, we did not exclude studies without unloaded controls. The reason was that we aimed to provide a wider scope and to discuss articles which primarily focused on release properties without using negative controls. Articles which did not use negative controls were distinctly pointed out in our review. Only two out of ten clinical trials—which applied statins or bisphosphonates—used unloaded collagen scaffolds as control [71, 76], whereas the other eight studies in humans did not [45–49, 69, 89, 90]. The absence of negative controls leads to results which might show the feasibility and dose-dependent changes of certain methods but do not allow any further conclusions. Overall, the evidence level of included publications was low and the risk of bias was high. One reason for the low number of clinical studies might be the fact

that specific clinical applications require other bone substitute materials which have not been included in this review. Another reason might be the low number of clinically approved substances and therefore a lot of studies still focus on pre-clinical testing before clinical tests may follow. This review shows that clinically available BMP-2 was more often investigated. BMP-2 has been investigated in 33 studies, while other growth factors or substances which demonstrated positive effects on tissue regeneration were evaluated in only one to five studies. Today, the only stem cell-based treatment that is routinely reviewed and approved is hematopoietic stem cell transplantation. Cell-based therapy approaches might be a paradigm shift that may provide alternative therapeutic solutions for several diseases. Currently, many preclinical and clinical studies are conducted to approve cell-based therapies for diverse applications. As this approach is still under debate and the use in oral medicine is still not approved, we did not include studies which were focusing on these substances. While there are other bone substitute materials which are used for oral tissue regenerative applications, we focused on collagen scaffolds as they are commonly used and have been established in many applications as the gold standard. Another commonly used biomaterial for oral tissue engineering and regeneration is deproteinized bovine bone mineral alone or in combination with collagen. Evaluation of all available biomaterials in our review would have gone beyond the scope. A further review might be needed to address this aspect on its own.

Our research question primarily focused on the effects of substance loaded collagen scaffolds on oral tissue regeneration compared with unloaded scaffolds. Nevertheless, we did not exclude studies which were not using unloaded collagen scaffolds as control or even used loaded collagen scaffolds as a control for other loaded biomaterials. This way, we were able to include five additional journal articles which investigated release properties of carriers which did not use an unloaded scaffold [63, 68, 73, 74, 91]. The use of substance loaded bone substitute materials for applications in guided bone or tissue regeneration is still in an early phase of clinical use and needs further trials. A wide range of different bone substitute materials are widely used in clinical applications, whereas the availability of drug-loaded scaffolds is still low. Our review of the past 15 years of scientific experiments applying loaded collagen scaffolds includes 77 publications. These studies investigated 36 different substances. These facts might indicate that widespread investigations are aiming to find candidates to improve the regenerative potential of collagen scaffolds. Testing of all substances were performed in a pre-clinical setting (in vitro, in vivo), resulting in a low evidence level. Only BMP-2, doxycycline, alendronate, simvastatin, and platelet-rich fibrin were tested in humans, while the study design was qualitatively poor and had a high risk of bias. Overall, BMP-2 was most frequently investigated and low

doses of BMP-2 showed positive effects on oral regeneration in all studies. BMP-9 might have more pronounced effects compared with BMP-2, even at lower concentrations. FGF2 was tested in five studies, demonstrating an increase in bone and periodontal healing. Collagen scaffolds loaded with antibiotics improved the scaffold's anti-microbial activity and even reduced the penetrability for bacteria. Other biological medical products, pharmaceuticals, and tissue-, cell-, and matrix-derived products showed positive or diverse effects on hard tissue and soft tissue regeneration. Because of the low number of studies for each of these substances, results might not be reliable and need to be further investigated. Collagen scaffolds of any type have proven, in any of the included experiments, that they are functional and clinically acceptable carrier materials for any kind of substance without having any negative effect by itself.

Conclusion

Collagen scaffolds of any type have proven to be capable carriers for all 36 substances which were utilized in the 77 included publications. Adsorption and release kinetics of all assessed substances showed clinically favorable results. These properties were also influenced to optimize the burst-like initial release and prolong the release. Biological medical products were by far investigated most often. Especially BMP-2 was investigated most frequently and showed positive effects on oral regeneration in all 33 studies where it was applied on different types of collagen scaffolds. BMP-9 showed more pronounced effects compared with BMP-2, even at lower concentrations. FGF2 demonstrated an increase in bone and periodontal healing. Pharmaceuticals were used in 24 studies to be loaded onto collagen carriers. Collagen scaffolds loaded with antibiotics improved the scaffold's anti-microbial activity and even reduced the penetrability for bacteria. Hyaluronic acid might prolong collagen membrane stability. Tissue-, cell-, and matrix-derived products were investigated in only 10 out of 77 included articles. Enamel-matrix derivate and PRF showed promising results pre-clinically and clinically, respectively. In general, other biological medical products than BMPs, pharmaceuticals, and tissue-, cell-, and matrix-derived products were investigated much less frequently. Outcomes of those studies demonstrated positive or diverse effects in terms of influencing hard tissue and soft tissue regeneration. Due to the low number of studies, those results do not allow conclusive assumptions. Ten out of 77 studies tested doxycycline, alendronate, simvastatin, and platelet-rich fibrin clinically in humans. Five of them were prospective clinical trials while only two clinical studies, which investigated the effects of loaded alendronate and simvastatin, used unloaded collagen scaffolds as control. Because of the low number of investigations for each substance, besides BMP-2, and the low level of

evidence of studies using these substances, results might not be reliably reflecting the substances' influence on oral regeneration and need further investigation. In addition, more prospective clinical trials using appropriate study designs and higher sample sizes are needed. Still, findings of investigated substances, as well as physical properties (adsorption and release) of collagen scaffolds should be considered for further investigation and optimization. This review provides an overview of the last 15 years of scientific studies using drug-loaded collagen scaffolds for oral tissue regeneration and should be considered for further research in this field.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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

Informed consent For this type of study, formal consent is not required.

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