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Systematic Review

Mechanisms of Degradation of Collagen or Gelatin Materials (Hemostatic Sponges) in Oral Surgery: A Systematic Review

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Abstract: Objective: The goal of this systematic review was to identify the mechanisms associated with the enzymatic degradation of collagen and gelatin biomaterials and the possible associated flaws. Methods: Four databases (PubMed, B-On, Cochrane Library, and ResearchGate) were used for the bibliographic search of articles. The research question was formulated using the PCC method, (P): collagen or gelatin sponges, hydrogels, and scaffolds; concept (C): enzymatic degradation of collagen or gelatin sponges, hydrogels, and scaffolds; and context (C): effect of enzymatic action on degradation time of collagen or gelatin sponges, hydrogels, and scaffolds. The search was contextualized according to PRISMA recommendations. The identification and exclusion of evidence followed the PRISMA criteria, with specific inclusion and exclusion factors being stipulated for the selection of articles. The risk of bias assessment was performed using the QUIN Scale. Results: The initial search was composed of 13,830 articles after removing duplicates; 56 articles followed for the full-text reading; 45 were excluded; then, 11 articles were obtained, constituting the results of this systematic review. All studies evaluated the materials using gravimetric analysis, and collagenases were the proteases used for the degradation solution. The materials tested were as follows: human-like collagen (HLC) hydrogel with microbial transglutaminase (MTGase), gelatin sponges subjected to different types of crosslinking, and collagen scaffolds with different types of crosslinking. The period of analysis varied between 0.25 h and 35 days. It was possible to highlight the lack of uniformity in the protocols used, which varied largely, thus influencing the degradation times. The risk of bias was low in nine studies and medium in two studies. Conclusions: This systematic review identified a gap in the literature, highlighting the absence of in vitro studies using human saliva and a collagenase concentration close to the physiological levels to simulate oral dynamics. However, based on existing literature, the mechanisms associated with collagen enzymatic degradation in collagen and gelatin biomaterials were comprehensively understood, answering the first research question postulated. In response to the second research question, the main shortcomings identified in the laboratory evaluation of mechanisms associated with collagen enzymatic degradation in collagen and gelatin biomaterials included the lack of standardization in degradation test protocols; this limited inter-study comparisons, which increased heterogeneity. Additionally, variations in collagenase concentrations and types influenced collagen degradation rates, and inappropriate evaluation intervals hindered the identification of total degradation time.

Keywords: biodegradation; collagen; gelatin; enzymatic degradation; collagenase; in vitro; biodegradation assays

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1. Introduction

Collagen is a protein of around 29 types, consisting of more than 1000 amino acids, and among many functions, it is essential for resistance to mechanical stress. The most important are as follows: type I is found in the skin, bone, teeth, tendon, ligament, and vascular ligature; type II in cartilage; type III in muscle and blood vessels; type IV in the basal lamina, the epithelium-secreted layer of the basement membrane; and type V in hair, cell surfaces, and placenta [1]. Type I is the most abundant form and main component of the extracellular matrix (ECM) [2], comprising 30% of bodily proteins. It has a high biocompatibility, permitting it to be used in treatments [3]. Its structure involves a triple-helical domain, self-twisted into a rope-like form. To guarantee stability, hydrogen bonds the structure; it can only be cleavage and disrupted by collagenases [4].

Gelatin is a natural polymer obtained from a partial hydrolysis of a non-soluble native collagen. It practically has the same characteristics as collagen and can be metabolized by human tissues without any immune response (rapid degradation); it can be used as a barrier against hydrophilicity but has poor mechanical strength [5,6]. Together, collagen and gelatin are common substances used in oral surgery due to their extensive effect on hemostasis, biocompatibility, and biodegradability. Moreover, these substances serve as natural matrices for osteoblast migration, aiding in soft tissue healing and hard tissue reconstruction [7,8].

Collagen and gelatin are widely used natural biomaterials in treatments in medicine and dentistry. Therefore, some differences exist between them (Table 1) [9].

Table 1. Differences found between collagen and gelatin.

Property	Collagen	Gelatin
Origin	Animals/human tissues	Collagen from bones/skin
Number of Amino acids	≅1050	<20
Types	fibril-forming and non-fibrillar-forming	A and B
Solubility	NaCI solution/dilute acid	H_2O
Mechanical strength	Poor	Poor
Gelling properties	No	Yes
Degradation	In vitro: serine protease, pepsin-cleaving enzyme, gelatinase, and collagenase In vivo: endopeptidase	Collagenase/MMP-2 and MMP-9
Usage	Burns, hemostasis, tissue defects, wound dressings, augmentation of soft tissue, artificial dermis skin replacement, bone regeneration	Adhesive of soft tissues, artificial skin, wound dressings

While its biodegradability is advantageous, its degradation rate often needs to be regulated through crosslinking techniques [10]. Proper biodegradation is crucial for the success of regenerative or therapeutic processes resulting from the implantation of biomaterials into damaged tissues. Selecting materials with appropriate biodegradation characteristics is essential, allowing synchronization of material degradation with new tissue formation [11–13].

The selection of inappropriate materials can lead to clinical complications. Early degradation is associated with the loss of mechanical strength and therapeutic properties, affecting function and viability. Conversely, late degradation can trigger immune reactions and fibrous encapsulation, impacting regeneration and healing and potentially leading to chronic inflammation [11–13]. Thus, the characterization of the material's biodegradability is crucial. Evaluating the degradation dynamics of implantable materials is essential for predicting and assessing expected clinical effects [11–13].

Matrix metalloproteinases (MMPs) are a group of over 25 endopeptidases with a significant role in extracellular dynamics. They can be grouped into matrilysins, stromelysins, membrane-type MMPs, collagenases, gelatinases, and others [14]. Collagenases, namely MMP-1, MMP-8, and MMP-13, are primarily responsible for in-vivo collagen degradation. MMP collagenolytic activity is critical in fibrillar collagen catabolism, which is the main constituent of the periodontium and alveolar bone. They are also responsible for degrading collagen and gelatin biomaterials and are preferred enzymes for degradation studies. It is worth noting that collagen biomaterials stimulate higher collagenolytic activity than native collagen, and each collagenase has a different collagenolytic rate [10,15].

In vitro study results are limited by the methodologies used, particularly in simulating physiological conditions. The most accurate way to simulate the oral environment involves using human saliva since artificial saliva does not exactly replicate the components and contaminants of saliva, hindering the translation of results into clinical conclusions. Saliva's interaction with materials is inevitable and alters their characteristics [16].

Within this background, this systematic review aimed to explore mechanisms associated with collagen and gelatin biomaterial degradation in laboratory studies and identify potential associated failures. Specifically, it sought to respond to the following research questions: (1) what are the mechanisms associated with enzymatic degradation of collagen in collagen and gelatin biomaterials in laboratory studies? (2) What are the main failures in the laboratory evaluation of mechanisms associated with enzymatic degradation of collagen in collagen and gelatin biomaterials? The clinical relevance of this study is grounded in the routine clinical use of collagen and gelatin as hemostatic agents in sinus surgery, vascular surgery, cardiovascular surgery, ophthalmic surgery, and in dental procedures as hemostatic and barrier agents such as in extractions, tissue biopsies, and regenerative techniques for soft tissue and bone.

2. Materials and Methods

2.1. Protocol and Focus Question

This systematic review followed the preferred reporting items for systematic reviews and meta-analyses (PRISMA) standards. The research question was developed using the PCC method, representing population (P): collagen or gelatin sponges, hydrogels, and scaffolds; concept (C): enzymatic degradation of collagen or gelatin sponges, hydrogels, and scaffolds; and context (C): effect of enzymatic action on degradation time of collagen or gelatin sponges, hydrogels, and scaffolds. The search was contextualized according to PRISMA recommendations.

2.2. Database and Keywords

For the development of this systematic review, the articles were searched and screened in the following databases: PubMed, B-On, Science Direct, and Cochrane Library, between September 2022 and August 2023. The search was performed by combining the following search terms: collagen, gelatin, sponge, scaffold, hydrogel, biodegradability, degradation, and collagenase, using Boolean operators AND and OR, arranged in the following search key: (((collagen) AND ((sponge) OR (scaffold) OR (hydrogel))) OR ((gelatin) AND ((sponge) OR (scaffold) OR (hydrogel)))) AND ((biodegradability) OR (degradation) OR (collagenase)).

2.3. Eligibility Criteria

As restrictions, the search looked up for articles published in English, with no temporal restrictions. Inclusion criteria were as follows: (1) in-vitro experimental studies, (2) studies where degradation occurs in $\geq$6 h, (3) studies describing the enzymolysis methodology and measurement, (4) studies using collagenase enzyme, (5) English language. Exclusion criteria were: (1) secondary studies, (2) human and in-vivo studies, (3) languages other than English, (4) studies where degradation occurs in <6 h, (5) studies not using collagenase enzyme, (6) studies where materials were not predominantly composed of collagen or

gelatin, (7) studies using subjective measurement methods, and (8) studies not presenting their results.

2.4. Articles Selection and Data Retrieved

All articles were screened by two independent authors (M.C. and F.C.). Initially, titles and abstracts were read. Duplicate articles were removed, and the eligible studies or those with insufficient data available in the abstract were included in the full-text reading. The data retrieved were stored in a spreadsheet (Excel®, Microsoft Office, v. 16.83), such as (i) author/year of publication, (ii) type of study, (iii) material, (iv) degradation solution, (v) interval for evaluations, (vi) methods for measurement, and (vii) statistical analysis. For any divergences, a third researcher was consulted (J.P.)

2.5. Risk of Bias

The QUIN tool (Quality Assessment Tool For In Vitro Studies) for in vitro assays [17] was used for the methodological quality assessment of the articles selected and included in this systematic review. It permits the classification of the reliability and validity of in vitro assays. All the processes were developed by two independent authors (M.C. and F.C.); as the tie-breaker, if necessary, a third author was consulted (J.P.).

The QUIN tool answered 12 questions (criteria); each one had four possible responses: "adequately specified", "inadequately specified", "not specified", or "not applicable". Each question of the QUIN checklist could receive a score of 2 (adequately specified), 1 (inadequately specified), 0 (not specified), or not applicable (no points assigned). The final score is obtained through the formula: QUIN score = (total score/2 × criteria numbers) × 100. The percentual result permitted to classify the in vitro study as (1) low risk of bias (>70%), (2) medium risk (50–70%), and high risk (<50%) [17].

3. Results

The results of the search strategy are presented in Figure 1. The initial search was composed of 13,830 articles after removing duplicates. A total of 56 articles were retrieved; after the full-text reading, 45 articles were excluded with the justification (Figure 1). Then, eleven articles were obtained, constituting the results of this systematic review.

Due to the heterogeneity observed among the studies, the results were described per article in order to better organize and present them. The systematization of the results is presented in Table 2. The methodological quality of the 11 included studies [7,8,18–26] was evaluated using the QUIN scale for in vitro studies, as detailed in Table 3.

3.1. Study 1 (Zhao et al., 2016) [18]

The enzymatic degradation of human-like collagen (HLC) hydrogels was examined. The researchers used microbial transglutaminase (MTG) with various MTG/HLC ratios, along with type I collagenase (100 U/mL) and type II collagenase (100 U/mL) enzymes, in a buffered solution at 37 °C. The experiment involved three types of HLC hydrogel samples with MTG: MTGH3 (5 mL HLC/75 mg MTG), MTGH4 (5 mL HLC/100 mg MTG), and MTGH5 (5 mL/125 mg MTG). In an incubator, these samples were immersed in tubes containing 2 mL of fresh enzymatic buffered solution at 37 °C. After specific immersion periods, they were removed, bathed in ultrapure water, and subjected to lyophilization. The dry material weights were obtained/measured. Additionally, the hydrogel structures post-degradation were examined using scanning electron microscopy (SEM). The study revealed that the hydrogel degradation rate had a reduction with an increase in the MTG/HLC ratio due to higher crosslinking density. Furthermore, collagenase II degraded the hydrogels more rapidly than type I collagenase, with hydrogels treated with type II collagenase completely degrading after 10, 11, and 12 h, respectively, in contrast to 12, 14, and 15 h required for degradation with type I collagenase.

3.2. Study 2 (Yang, 2018) [19]

The degradation of gelatin sponge prepared by different crosslinking agents was analyzed in this study. The agents were glutaraldehyde (GTA), genipin (GP), 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC), and microbial transglutaminase (MTG). Gelatin sponges' biological stability was ascertained by the exposition of them to enzymes to verify the degradation rates. In order to prepare the wet sponges, lyophilized sponges were soaked in PBS for 60 min. Then, pre-weighed wet sponges were exposed to solutions prepared in PBS: (1) 0.1% collagenase type I (>125 CDU/mg, Invitrogen, CA, USA), (2) 0.1% collagenase type II (>125 CDU/mg, Invitrogen), and (3) 0.1% collagenase type IV (>125 CDU/mg, Invitrogen) for 6 h. Enzymatic degradation tests were performed at 37 °C in a horizontal shaker. The remaining sponges were collected at 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h. Filter papers were used to gently remove the excess water from the sponge surface; then, the sponge was re-weighed.

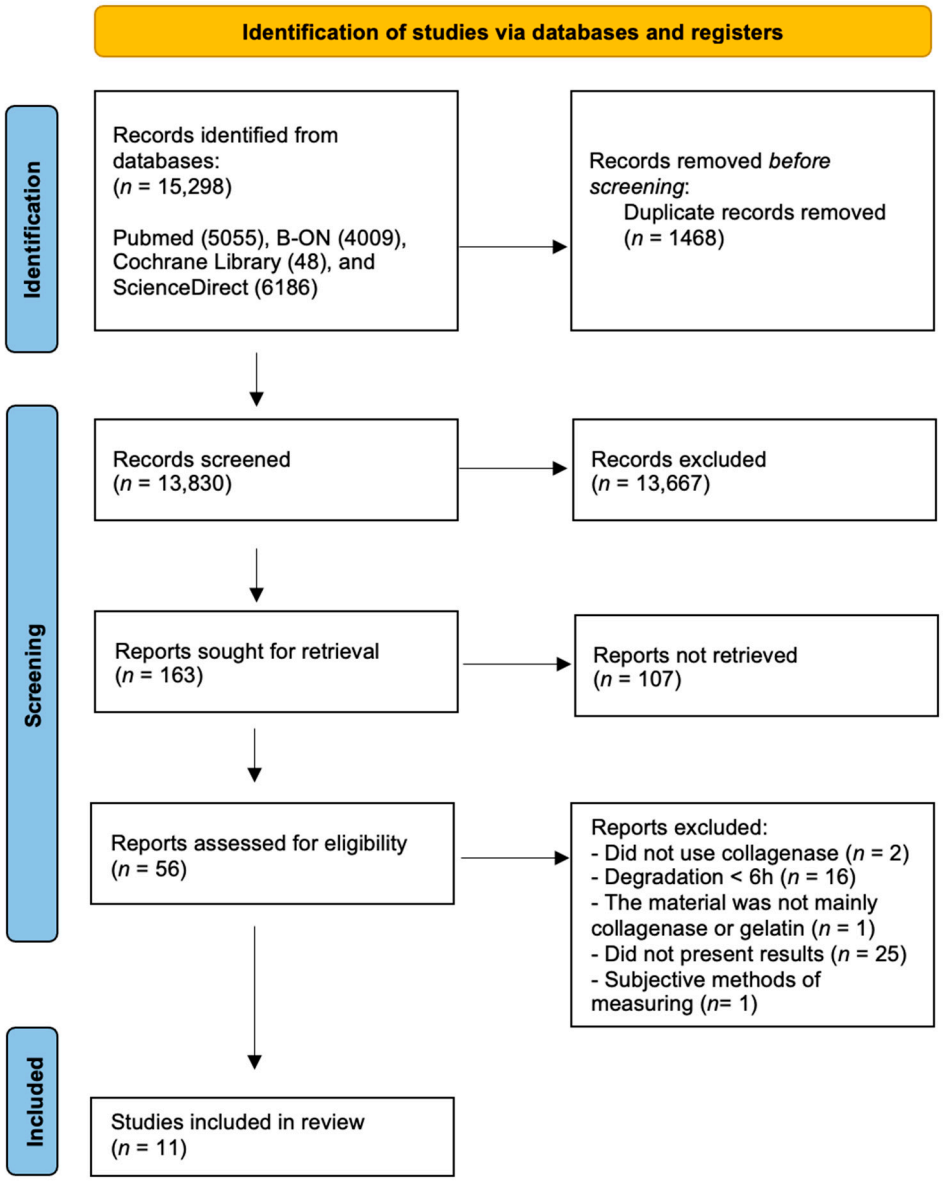

Figure 1. PRISMA flowchart for screening, selection, and inclusion of articles.

Table 2. Summary of results.

Study	Material	Degradation Solution	Interval of Evaluation	Method for Measurement	Results	Statistical Analysis
S1. Zhao et al., 2016 [18]	Human-like collagen (HLC) hydrogel with microbial transglutaminase (MTGase)	Collagenase I (100U) and II (100U), separately, in PBS at 37 °C	Collagenase I—18 h; Collagenase II—14 h.	Gravimetric analysis	Time until complete degradation: MTGH3: collagenase I—12 h; collagenase II—10 h MTGH4: collagenase I—14 h; collagenase II—11 h MTGH5: collagenase I—15 h; collagenase II—12 h	Statistical significance was set at $p < 0.05$.
S2. Yang, et al., 2018 [19]	Gelatin sponges subjected to different types of crosslinking	0.1% collagenase type I (>125 CDU/mg, Invitrogen, Carlsbad, CA, USA), 0.1% collagenase type II (>125 CDU/mg, Invitrogen), and 0.1% collagenase type IV (>125 CDU/mg, Invitrogen) in PBS	0.5 h; 1 h; 1.5 h; 2 h; 3 h; 4 h; 5 h; and 6 h.	Gravimetric analysis	Time until complete degradation: MTG sponge—2 h; EDC sponge—6 h; GP sponge—6 h. The GA sponge preserved about 40% of its initial mass after 6 h.	Statistical significance was set at $p < 0.05$.
S3. Tihan et al., 2015 [20]	Type I collagen sponges (CGs) cross-linked with glutaraldehyde (GA) and loaded with chloramphenicol (CP)	Collagenase (10 mg/mL) in phosphate-buffered saline solution (PBS) at 37 °C (pH 7.4)	1 h; 2 h; 4 h; 8 h; 24 h; and 48 h.	Gravimetric analysis	Time until complete degradation: • CG-CP = 1 h; • CG-CP GA 0.25% = 4 h; • CG-CP GA 0.50% = 8 h; • CG-CP GA 0.75% = 48 h; • CG-CP GA 1% = 48 h.	No data were available.
S4. Kang, et al., 1999 [21]	Gelatin hydrogels cross-linked by glutaraldehyde	20 U/mL of collagenase solution in DPBS	0.25 h; 0.5 h; 0.75 h; 1 h; 1.5 h; 2 h; 3 h; 4 h; 8 h; 12 h; 18 h; 24 h; and 32 h.	Gravimetric analysis	Time until complete degradation: Frozen samples: 18 h; Samples with N2: 38 h.	No data were available.

Table 2. *Cont.*

Study	Material	Degradation Solution	Interval of Evaluation	Method for Measurement	Results	Statistical Analysis
S5. Rusu, et al., 2023 [22]	Enzymatically cross-linked gelatin-based hydrogels	Collagenase (0.1 mg/mL) in PBS pH 7.4 at 37 °C	Not disclosed	Gravimetric analysis	Time until complete degradation: HGel2 = 4 h; HGel3 = 8 h; HGel4 = 8 h.	For statistical analysis, the one-way ANOVA test and Tukey test were utilized.
S6. Salvatore et al., 2021 [8]	Collagen scaffolds subjected to various types of crosslinking	Degradation with collagenase (0.1 mg/mL) in phosphate buffer saline solution (PBS) at 37 °C	24 h	Gravimetric analysis	Time until complete degradation: Scaffold without crosslinking—5 min • DHT—1 h • DHT + GP—2 h Mass loss after 7 h: • DHT-GA—5% Mass loss after 8 h: • DHT + DMS—90% • GHT + EDC—50% Mass loss after 24 h: • DHT + FA—3% Half-life times: • DHT—12.6 min • DHT + GP—26.6 min • DHT + DMS—57.7 min • DHT + EDC—173.2 min	$p < 0.05$ was used as selection criteria. GP treatment did not significantly increase the resistance of the scaffolds ($p = 0.6$).
S7. Ribeiro et al., 2020 [23]	GelMA hydrogel loaded with ciprofloxacin	2 mL DPBS containing collagenase type A (1 U/mL) and incubated at 37 °C	Not disclosed (7 days)	Gravimetric analysis	Time for complete degradation: 2.5% GelMA-PDS-CIP-SF = 24 h; 2.5% GelMA-PDS-CIP/β-CD-IC-SF = 24 h; 10% GelMA-PDS-CIP-SF = 168 h; 10% GelMA-PDS-CIP/β-CD-IC-SF = 168 h.	The differences were considered as significant if $p < 0.05$.

Table 2. *Cont.*

Study	Material	Degradation Solution	Interval of Evaluation	Method for Measurement	Results	Statistical Analysis
S8. Kishan et al., 2015 [24]	In situ cross-linked gelatin scaffolds	Degradation with collagenase (0.02 U/mL) in phosphate-buffered saline (PBS) solution at 37 °C	35 days	Gravimetric analysis: Mass loss	Time to complete degradation: Non-cross-linked gelatin—12 h; gelatin 1—10 days; gelatin 5—24 days; gelatin 10—35 days.	All tests were conducted with a 95% confidence interval ($p < 0.05$).
S9. Ribeiro et al., 2020 [25]	Photocrosslinkable gelatin methacryloyl (GelMA) hydrogel loaded with chlorhexidine (CHX)	5 mL DPBS containing 1 U/mL collagenase type I at 37 °C	Not disclosed (21 days)	Gravimetric analysis	Time until complete degradation: Non-CHX = 14 days; CHX-groups = 21 days.	*p*-value of less than 0.05 was statistically significant.
S10. Long et al., 2017 [7]	Gelatin sponges	0.1% collagenase I (>125 CDU/mg) in PBS at 37 °C	6 h	Gravimetric analysis	Time until complete degradation: 6 h	A value of $p < 0.05$ was considered statistically significant.
S11. Borrego-González et al., 2021 [26]	Atelocollagen sponge with different types of crosslinking	0.5 mL of 0.5 CDU/mL collagenase type I/buffer solution (0.1 M Tris-HCl and 5 mM $CaCl_2$, pH 7.4) at 37 °C.	0.5 h; 1.5 h; 3 h; 6 h; 24 h; 72 h; and 120 h.	Gravimetric analysis	Time to complete degradation: DCol-S: 3 h; DCol-S0.0015G: 72 h; After 120 h: DCol-S0.015G: 8.4% degraded; DCol-S0.03G: 7.0% degraded; DCol-S0.3G: 6.5% degraded.	No data were available.

Table 3. QUIN chart.

	Criterium	Details	S1 [18]	S2 [19]	S3 [20]	S4 [21]	S5 [22]	S6 [8]	S7 [23]	S8 [24]	S9 [25]	S10 [7]	S11 [26]
1	Clearly stated aims/objectives	Study should clearly state aims and/or objectives, which should then be followed throughout.	2	2	2	2	2	2	2	2	2	2	2
2	Detailed explanation of sample size calculation	Details regarding the method by which the given sample size calculated should be clearly stated. Details regarding the software program, formula, and parameters used for the calculation of the sample size should also be specified.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	Detailed explanation of the sampling technique	Details regarding the predefined population from the sample that has been selected. Details of the sampling technique and inclusion and exclusion criteria should be clearly stated.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	Details of the comparison group	Details of the comparison group (positive control, negative control, or standard) should be clearly specified.	0	1	0	0	1	0	1	1	2	2	0
5	Detailed explanation of the methodology	Clarity of procedure, method of standardization, and details of any universal standards used (if applicable) should be clearly stated.	2	2	2	2	2	2	2	2	2	2	2
6	Operator details	The number of operators and details regarding training and calibration of operator/s (inter-operator and intra-operator reliability) should be clearly specified.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	Randomization	Details regarding sequence generation and allocation concealment should be clearly stated.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	Method of measurement of outcome	Clarity of procedure and rationale for choosing the method should be stated. Method of standardization along with details of any universal standards used (if applicable) should also be clearly specified.	2	2	2	2	2	2	2	2	2	2	2
9	Outcome assessor details	The number of outcome assessors and details regarding training and calibration of assessor/s (inter-outcome and intra-outcome assessor reliability) should be clearly specified.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Table 3. *Cont.*

	Criterium	Details	S1 [18]	S2 [19]	S3 [20]	S4 [21]	S5 [22]	S6 [8]	S7 [23]	S8 [24]	S9 [25]	S10 [7]	S11 [26]
10	Blinding	Details regarding the blinding of operator(s), outcome assessor(s), and statistician should be clearly specified.	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	Statistical analysis	Details regarding the software program used and statistical analysis should be clearly specified.	2	2	0	0	2	2	2	2	2	2	2
12	Presentation of results	The outcome should be based on predefined aims and/or objectives. All data should be adequately tabulated with baseline data clearly specified (if applicable).	2	2	2	2	2	1	2	2	2	2	1
Total (%)	-	-	83.3	91.7	66.7	66.7	91.7	75	91.7	91.7	100	100	75
Risk of bias	-	-	Low	Low	Medium	Medium	Low	Low	Low	Low	Low	Low	Low

0 = not specified; 1 = inadequately specified; 2 = adequately specified; NA = not applicable; Green background = low risk of bias; Yellow background = medium level of bias; Red background = high risk of bias.

There was an exponential decrease in the EDC sponge mass during degradation by collagenases. Within 30 min, around half of the initial EDC mass remains; after 1 h, around 20% of the original mass was left, which decreased to about 10% at 90 min. The gelatin was nearly fully dissolved after 120 min.

The enzymatic degradation speed by collagenases for the MTG-sponge was slightly slower than for EDC-sponge. After 1 h of digestion, about 50% of the mass remained, and after 4 h, less than 20% of residual mass remained. The GP-sponge had a noticeably slower enzymolysis collagenase rate than the MTG-sponge and EDC-sponge, displaying a slow linear reduction. Just after 6 h, the whole degradation of the material was observed.

The GTA-sponge showed a slight decline for the enzymolysis collagenase curves; in the first 60 min of collagenase treatment, 70% of degradation was reached, reducing to approximately 50% in the second hour. Following a slight further decline, the final mass after 6 h remained at approximately 40%.

3.3. Study 3 (Tihan et al., 2015) [20]

The enzymatic degradation of collagen sponges (CGs) cross-linked with glutaraldehyde (GA) and loaded with chloramphenicol (CP) was evaluated. Bacterial collagenase (Clostridium histolyticum) and PBS (pH of 7.4) were used for the enzymatic degradation test. Each sponge (CG-CP; CG-CP GA 0.25%; CG-CP GA 0.50%; CG-CP GA 0.75%; CG-CP GA 1%) was immersed in PBS and incubated at 37 °C overnight. Then, collagenase was added (10 mg/mL), and the tube was placed back at 37 °C. After 1 h, 2 h, 4 h, 8 h, 24 h, and 48 h, the sponges were removed from the solution (halting the degradation process) and re-weighed. The percentual weight loss was then calculated considering the initial and final weight, and the time. The CG-CP collagen sponge showed complete enzymatic degradation after 1 h, while sponges with 0.25%, 0.50%, 0.75%, and 1% demonstrated complete degradation after 4 h, 8 h, 48 h, and 48 h, respectively.

3.4. Study 4 (Kang, 1999) [21]

The degradation of gelatin hydrogels cross-linked by glutaraldehyde was studied using a 20 U/mL collagenase solution in DPBS. Five mg of freeze-dried gelatin hydrogels were immersed in 10 mL of collagenase/DPBS. The solution was removed by centrifugation for 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 8, 12, 18, 24, and 32 h, and then rinsed with double-distilled water. The ratio of the remaining weight of the dried gels to their initial weight of 5 mg was used to calculate the degree of hydrogel degradation. The experiment was repeated independently three times. The hydrogel prepared by freezing at −20 °C took 18 h for complete degradation, while the hydrogel prepared from N2 took as long as 38 h for complete degradation. The hydrogels prepared at −20 °C broke into small pieces during degradation, while the hydrogels prepared using N2 kept their shape during the degradation process.

3.5. Study 5 (Rusu et al., 2023) [22]

This study analyzed the degradation of enzymatically cross-linked gelatin-based hydrogels. Their enzymatic degradability by bacterial collagenase (Clostridium histolyticum) was assessed in PBS (pH 7.4, c = 0.1 mg/mL). The initial weight for each sample was 80 mg; they were incubated in Eppendorf tubes (1 mL of the solution at 37 °C). All the experiments were done in triplicate. HGel3 and HGel4, gelatin hydrogels reinforced with MAC5/PAS nanogels, were enzymatically cross-linked and compared to HGel2 (non-enzymatically cross-linked hydrogels), to compare the degradability rate. During degradation by collagenase, HGel2 was totally degraded in 4 h compared to cross-linked hydrogels, which were completely degraded in 8 h.

3.6. Study 6 (Salvatore et al., 2021) [8]

Collagen scaffolds had degradation assessed by incubating samples of approximately 6 mg in PBS (6 mL) within bacterial (Clostridium histolyticum) collagenase (0.1 mg/mL,

37 °C). After 0.5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, and 6 h, the degradation was halted to harvest the supernatant to be frozen (−40 °C). The percentual weight of each scaffold was evaluated at each period as well as the collagen solubilized using the bicinchoninic acid (BCA). While non-cross-linked samples disintegrated completely within 5 min of incubation, one hour was necessary to have a complete solubilization of DHT-treated scaffolds. The degradation kinetics were affected by the use of chemical crosslinking and DHT proportionally to the crosslinking density. The sample chemically cross-linked with genipin (GP) dissolved within 2 h. The most efficient treatments with chemical crosslinking using dimethyl suberimidate (DMS) and carbamide (EDC) induced weight losses of approximately 90% and 50% after 8 h, respectively. Aldehyde cross-linked scaffolds exhibited slower degradation, with less than 5% mass loss after 7 h for DHT + GA samples and less than 3% after 24 h for DHT + FA (formaldehyde chemical crosslinking). The results were presented in the form of half-life time in minutes, such as 12.6, 26.6, 57.7, and 173.2, corresponding to, respectively, DHT, DHT + GP, DHT + DMS, and DHT + EDC cross-linked samples.

3.7. Study 7 (Ribeiro et al., 2020) [23]

In this in vitro study, GelMA hydrogel with ciprofloxacin-eluting short nanofibers had the degradation assessed through enzymatic incubation and subsequently monitored the weight. Specifically, the four groups evaluated had 100 µL deposited and photo-cross-linked for 15 s. All samples had the initial weight registered; then, they were immersed in 2 mL of DPBS containing type A collagenase (1 U/mL) (renewed every 3 days) and incubated (37 °C, up to 7 days). Collagenase A (also named MMP1) was utilized to assess GelMA's enzymatic degradation profile. At specific time intervals, the excess PBS was gently removed, and their wet weights were recorded. There was a total degradation of 2.5% GelMA-PDS-CIP-SF and 2.5% GelMA-PDS-CIP/β-CD-IC-SF after 24 h, whereas the total degradation of 10% GelMA-PDS-CIP-SF and 10% GelMA-PDS-CIP/β-CD-IC-SF was found after 168 h.

3.8. Study 8 (Kishan et al., 2015) [24]

Gelatin networks suffered enzymatic degradation using type I collagenase (349 U/mg). Samples (non-cross-linked gelatin, gelatin 1 [32% cross-linked], gelatin 5 [61% cross-linked], and gelatin 10 [91% cross-linked]) were placed in tubes containing 2 mL of 0.02 U collagenase/mL PBS and incubated at 37 °C with agitation (solution changed every 3 days). After 1, 2, and 4 weeks, the samples were harvested, centrifuged, rinsed thrice with distilled water, then frozen overnight, lyophilized, and observed using SEM. Complete dissolution of non-cross-linked gelatin was verified after 12 h of immersion. Gelatin 1 had mechanical integrity loss within 4 days and complete degradation in 10 days; whereas gelatin 5 and gelatin 10 networks had mechanical integrity loss after 16 and 22 days, with complete degradation after 24 and 35 days, respectively.

3.9. Study 9 (Ribeiro et al., 2020) [25]

Photocrosslinkable gelatin methacryloyl hydrogel loaded with chlorhexidine had the degradation profile evaluated. Identical samples (n = 4/group) were incubated with 5 mL DPBS containing 1 U/mL collagenase type I at 37 °C (replaced every 3 days). At specific periods, the samples were washed twice in sterile DI water, blot-dried, and re-weighed on an analytical balance. HNT without CHX had faster degradation than groups with CHX-loaded nanotubes (p = 0.05). Similar degradation was observed after 7 days for groups without CHX to groups with CHX at 10% and greater than CHX at 20%. The groups without CHX groups had total degradation after 14 days. Therefore, there was no statistical difference among the remaining groups after 21 days, with most of them being completely degraded.

3.10. Study 10 (Long et al., 2017) [7]

Gelatin sponges suffered enzymatic degradation by placing the weighed hydrated sponges in 0.1% type I collagenase (>125 collagen digestion units/mg) at 37 °C for 6 h. The resulting sponges were re-weighed at specific periods. PBS-immersed sponges were used as controls. Sponges exposed to 0.1% collagenase completely degraded in approximately 6 h, while the control group had no mass loss.

3.11. Study 11 (Borrego-González et al., 2021) [26]

The enzymatic degradation of atelocollagen sponges with different degrees of crosslinking was conducted. Samples of collagen (0.6 to 0.7 mg) were pretreated with 0.1 M glycine for 60 min and bathed three times in buffer solution (0.1 M Tris-HCl and 5 mM $CaCl_2$, pH 7.4). Then, they were immersed in tubes containing 0.5 mL of 0.5 CDU/mL type I collagenase/buffer solution at 37 °C; the solution was replaced every 48 h. At 0.5, 1.5, 3, 6, 24, 72, and 120 h, measurements were taken to quantify the detectable levels of soluble collagen. The absorption of the peptide bond at 205 nm using a droplet UV-Vis spectrophotometer (NanoDropTM 2000, Thermo Fisher Scientific Inc., Waltham, MA, USA.) was measured and quantified. All samples were measured in triplicate. The enzymatic degradation of DHT-cross-linked atelocollagen sponges (DCol-S) reached around 80% after 90 min and almost totally degraded after 3 h. Sponges were additionally treated with various concentrations of GA, as follows: DCol-S 0.0015G, DCol-S 0.015G, DCol-S 0.03G, and DCol-S 0.3Gl, which showed no significant degradation after 1.5 h; however, after 24 h, DCol-S 0.0015G exhibited approximately 8% degradation, whereas the other sponges (higher GA concentrations) had degradation by around 1.5%. After 72 h, a complete degradation was observed for DCol-S 0.0015G, whereas after 120 h, respectively, 8.4%, 7.0%, and 6.5% were found for sponges DCol-S 0.015G, DCol-S 0.03G, and DCol-S 0.3G.

4. Discussion

4.1. Desirable Characteristics of Biomaterials in In Vitro Studies

A biomaterial developed for dental applications should be studied in an environment similar to the intraoral cavity. Therefore, human saliva, with a collagenase concentration close to physiological levels, should be the preferred solution for studying material degradation.

4.1.1. Human Saliva

According to Kunrath and Dahlin [16], there are two limitations in in vitro studies using saliva. First, the choice between artificial saliva and human saliva poses a challenge. Artificial saliva cannot accurately replicate the presence of contaminants, impurities, and cells found in human saliva. Therefore, despite similarities in composition, it should not be used in studies. Second, the method of saliva collection introduces limitations. Techniques such as direct in-clinic collection without storage or treatment, salivary stimulation techniques, saliva filtration, and various storage methods exist. Concerns arise about the potential loss of certain salivary characteristics during saliva collection, which could hinder translating conclusions to clinical applications. The authors recommend direct saliva collection in a clinical setting. However, some studies have shown the stability of salivary composition at low temperatures, allowing for consideration of this alternative to facilitate laboratory studies.

4.1.2. Physiological Concentration of Collagenase

Currently, there is no known collagenase concentration corresponding to physiological levels due to a low number of studies. In healthy individuals, collagenase levels are relatively low. However, inflammatory processes, such as periodontal disease or chronic pulpitis, significantly increase collagenase concentration [27,28]. Among the studies analyzed, only studies 8 and 9 [24,25] exhibited degradation times comparable to oral degradation time. However, the concentration used in study 9 [25] was closer to that found in salivary collagenase concentration studies, reported to be around 0.14 U/mL in healthy

subjects [29]. Therefore, a 0.02 U collagenase/mL concentration might be suitable for simulating oral collagenase concentration. Studies often avoid using concentrations close to physiological levels because their primary goal is to prove material biodegradability. However, understanding a material's physiological biodegradation time is crucial, and this limitation should be addressed in future studies.

4.2. Solution Used for Degradation

4.2.1. Buffer Solution

Of the eleven studies in this systematic review, seven studies (S1–S3 [18–20], S5 [22], S6 [8], S8 [24], and S10 [7]) used phosphate-buffered saline (PBS) solution, three (S4 [21], S7 [23], and S9 [25]) used DPBS solution, and one (S11 [26]) used Tris-HCL. Only studies S3 [20], S7 [22], and S9 [25] specifically developed material for oral application. Therefore, these studies were limited by not using human saliva as the solution.

4.2.2. Collagenase

Regarding the concentration, S3 [20], S5 [22], and S6 [8] did not specify collagenase digestive units (CDUs) in the degradation solution. Additionally, there was a big variation in the collagenase concentration used in the studies, contributing to the disparity in results. It is known that a higher collagenase concentration leads to a faster degradation, hence the importance of standardizing the concentrations to be employed in future studies.

Regarding the type of collagenase, none of the selected studies specified the matrix metalloproteinase (MMP) used, even though three (MMP-1, MMP-8, and MMP-13) correspond to collagenase. According to Helling et al. [30], most studies use MMP-1, although MMP-8, which is prevalent in healing and more efficient in degradation, should be employed. Study 2 [19] specified using collagenase I, II, and IV; study 1 [18] used collagenase I and II; and studies 9 [25], 10 [7], and 11 [26] only used collagenase I. Study 7 [23] used collagenase type A, and the remaining studies did not specify the collagenase used.

4.3. Evaluation Interval

The selected time intervals for degradation assessment varied in all studies. Additionally, except for studies 8 and 9, no other study presented a degradation time close to physiological levels. Given that collagen and gelatin biomaterials in sponge, scaffold, and hydrogel formats have physiological degradation times between 21 days and 12 weeks, assessing them at 24 h intervals until complete degradation is recommended for reliable results.

4.4. Degradation Measurement Methods

All studies used gravimetric analysis to evaluate the degradation. Other common methods available include measuring degradation through hydroxyproline release in the degradation solution, micro-CT, and ICP-OES. Studies comparing the different measurement methods have concluded that there was no statistically significant difference between them, with none being superior to the others [31].

4.5. Comparison of Degradation Test Results

Since degradation tests differed between studies in sample origin, size, treatment, test protocol, observation periods, and measurement methods, factors influencing the results must be considered before making comparisons.

Studies 2 [19] and 10 [7] examined the degradation of gelatin sponges. They employed a concentration of 0.1% collagenase, and both registered the total degradation time to be 6 h. Studies 3 [20] and 11 [26] examined collagen sponge degradation. However, due to differing methodologies, results significantly varied despite using the same gravimetric measurement. Study 3 [20] utilized a higher collagenase concentration, leading to complete degradation of the non-cross-linked sample in 1 h and the highest cross-linked sample in 48 h, while in Study 1 [18], the lowest cross-linked sample degraded in 3 h and the highest in 120 h.

Studies 4 and 5 [21,22] investigated gelatin hydrogel degradation. The collagenase concentration was different, resulting in the highest total degradation time for study 4 [21] being 38 h and 8 h in study 5 [22].

Studies 7 [23] and 9 [25] assessed GelMA hydrogels and were conducted by the same main researcher. The collagenase concentration used was the same for both studies, yet the results varied, with the highest total degradation time for Study 7 [23] being 168 h and 21 days for study 9 [25]. This can likely be attributed to the different crosslinking methods used. Since only study 1 [18] examined collagen hydrogels, only study 6 [8] studied collagen scaffolds, and only study 8 [24] studied gelatin scaffolds; no comparisons can be made for these materials.

The degradation parameter has paramount importance directly related to clinical application. Collagen has a greater quantity of amino acids than gelatin, which favors a greater period intrabody for its total degradation; this fact has a fundamental importance in regenerative cases, with a preference for collagen use. Moreover, the existence of crosslinks increases the degradation time, favoring yet more the regenerative process. Otherwise, the use of gelatin can be preferable in cases that require a faster degradation, as in cases of hemostasis.

4.6. Limitations of This Study

The high heterogeneity for the methodologies found among studies impaired a direct robust comparison of the results. Even though the number of databases was satisfactory, expanding the search to include more databases, in an attempt to include more articles, is suggested.

5. Conclusions

This systematic review identified a gap in the literature, highlighting the absence of in vitro studies using human saliva and a collagenase concentration close to the physiological levels to simulate oral dynamics. However, based on the existing literature, the mechanisms associated with collagen enzymatic degradation in collagen and gelatin biomaterials were comprehensively understood, answering the first research question postulated. Considering the various points discussed throughout the study, it is suggested that future in vitro studies on the biodegradation of biomaterials use a concentration of 0.02 U collagenase (MMP-8)/mL human saliva at 37 °C. Evaluation periods should be set at 24 h intervals until a complete degradation occurs. Regarding degradation measurement methods, although different methods influence results, asserting one's superiority over others is not possible. Therefore, gravimetric analysis, due to its accessibility, could be employed.

In response to the second research question, the main shortcomings identified in the laboratory evaluation of mechanisms associated with collagen enzymatic degradation in collagen and gelatin biomaterials included a lack of standardization in degradation test protocols; this limited inter-study comparisons, which increased heterogeneity. Additionally, variations in collagenase concentrations and types influenced collagen degradation rates, and inappropriate evaluation intervals hindered the identification of total degradation time.

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