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Interpreting Histological and Immunohistochemical Findings in Animal Models of Extraction Socket Healing: A Narrative Review

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ABSTRACT

Extraction socket healing in animal models is commonly evaluated with hematoxylin–eosin (H&E) staining, collagen stains, immunohistochemistry (IHC), and micro-computed tomography (micro-CT), yet these methods measure different aspects of repair. This narrative review aims to explain how histological, and immunohistochemical findings should be interpreted together, and in relation to structural bone measurements, during socket healing. Thirteen purposively selected animal studies published between 2021 and 2026 were compared, with supporting literature providing biological and methodological context. Histology described tissue architecture, inflammatory cells, collagen-containing matrix, and new bone, whereas IHC localized proteins related to inflammation, angiogenesis, and osteogenesis. Osteogenic marker findings were often reported without a matched bone measurement, and where both were measured, the results sometimes agreed and sometimes diverged. In a periodontitis model, higher osteocalcin staining accompanied a larger woven bone area, whereas in a platelet lysate model most osteogenic genes were expressed at lower levels than in controls at day 14 while bone volume fraction was higher. Improved wound closure was reported without a significant difference in collagen density, and in diabetic rats a higher absolute bone volume at day 42 was not accompanied by a difference in bone volume fraction. Protein staining should therefore be interpreted alongside tissue measurements, sampling time, anatomical site, and experimental conditions to avoid overstating healing outcomes.

Keywords: tooth extraction; tooth socket; wound healing; histology; immunohistochemistry; animal models; bone regeneration

INTRODUCTION

Tooth extraction initiates a coordinated healing process in which a blood clot is progressively replaced by granulation tissue, collagen-rich connective tissue, and newly formed bone [1, 2]. These overlapping events occur while the alveolar ridge undergoes dimensional remodeling, which may influence the bone available for subsequent prosthetic rehabilitation or implant placement [2, 3]. The biological course of healing is not uniform: socket anatomy, local inflammation, age, and systemic conditions, particularly diabetes, can modify the timing and quality of tissue repair [4, 5]. Consequently, an apparent improvement in one tissue characteristic should not automatically be interpreted as complete socket regeneration.

Controlled animal models permit tissue collection at predefined postoperative intervals and therefore provide valuable opportunities to investigate the spatial and temporal characteristics of socket healing [6]. Hematoxylin and eosin (H&E) staining reveals cellular distribution and tissue architecture, whereas collagen-oriented stains provide additional information about extracellular matrix deposition and organization [7–9]. Immunohistochemistry (IHC) localizes selected proteins involved in inflammatory signaling, angiogenesis, and osteogenic differentiation [10–14]. When available, micro-computed tomography (micro-CT) complements these examinations by quantifying mineralized structures within a defined three-dimensional region of interest [15–17]. Importantly, protein immunoreactivity, histological bone area, and volumetric bone measurements represent distinct outcomes rather than interchangeable indicators of healing success.

Interpretation across animal studies remains challenging because experimental species, extraction sites, interventions, postoperative intervals, and outcome definitions vary substantially [18, 19]. An increase in osteogenic marker staining may indicate a biological response without demonstrating a corresponding increase in newly formed bone, and a larger measured bone volume does not necessarily establish superior bone maturity or mechanical competence [12, 14–17]. This narrative review therefore aims to explain how histological, and immunohistochemical findings should be interpreted together, and in relation to structural bone measurements, across the inflammatory, proliferative, and bone-forming phases of extraction socket healing in animal models. The review first outlines the biological sequence of socket healing, then compares findings on inflammatory cells and cytokines, growth factors and collagen, and osteogenic markers and bone formation, and finally considers the experimental factors that limit comparisons between studies and their application to human healing.

Biological Sequence of Extraction Socket Healing

- *Hemostasis and Inflammation*

Immediately after extraction, the blood clot functions as a provisional matrix that supports the recruitment of inflammatory and reparative cells. Neutrophils and macrophages contribute to debris clearance and coordinate signaling pathways involved in subsequent tissue formation [1]. The presence of inflammatory cells should therefore be evaluated against the expected postoperative phase rather than interpreted as intrinsically beneficial or harmful. Persistent inflammatory infiltration may indicate delayed resolution, but a single histological observation cannot establish the duration or functional consequences of inflammation without appropriately timed comparisons [20–22].

Horibe et al. identified transforming growth factor-β (TGF-β) in F4/80- and CD206-positive macrophages during early socket healing in mice and detected phosphorylated Smad3 in α-smooth muscle actin (α-SMA)-positive progenitor cells [16]. These findings support a spatial association between macrophage-associated signaling and cells participating in repair, but colocalization alone does not demonstrate causality. A subsequent macrophage depletion experiment reported impaired mucosal closure, angiogenesis, and bone formation, providing complementary functional evidence for macrophage involvement [23]. In malnourished mice, a greater proportion of M2-associated macrophages was reported together with delayed healing [24]. Together, these observations caution against equating a higher proportion of CD206-positive cells with more effective healing irrespective of the host environment.

- *Proliferation*

During the proliferative phase, fibroblast activity, vascular ingrowth, and extracellular matrix deposition provide the conditions for early woven bone formation [1]. Vascular endothelial growth factor (VEGF) and platelet-derived growth factor (PDGF) participate in signaling pathways relevant to angiogenesis and osteogenesis [25], while RUNX2 and osterix (SP7) contribute to osteoblast-lineage differentiation [26]. Nevertheless, immunostaining for these proteins identifies their distribution or relative immunoreactivity under the chosen analytical protocol; it does not independently quantify functional perfusion, collagen accumulation, or mineralized bone production. Such claims require corresponding histological, histomorphometric, vascular, or imaging outcomes.

- *Bone Maturation and Remodeling*

As healing progresses, immature woven bone undergoes remodeling and is gradually replaced by more organized bone within the socket and surrounding alveolar ridge [1, 2]. The histological sequence from clot organization to granulation tissue, coarse fibrillar bone, and more mature bone has been described in oral pathology references [27]. However, the timing of each stage varies by species, tooth type, host condition, and sampling location. Early bone formation was evident during the first postoperative week in the rodent molar models examined by Guo et al. and Horibe et al. [15, 16], whereas clinical socket biopsies are commonly obtained at substantially later intervals. Comparisons should therefore emphasize the biological stage and measured outcome rather than assuming equivalence between postoperative days across species.

Histological and Immunohistochemical Findings in Animal Studies

Table 1 summarizes the 13 animal studies published between 2021 and 2026 that were selected for this review. They were chosen purposively rather than through a systematic search, because each illustrates a distinct interpretive question: inflammatory cell identification, cytokine or growth-factor expression, collagen assessment, osteogenic marker localization, or direct measurement of bone formation. Studies that combined tissue methods with macrophage phenotyping or micro-CT were included so that marker findings could be compared with structural outcomes. The table is intended to show differences in study design and measurement, not to rank interventions or pool their effects. Most studies tested local materials or treatments, whereas others examined host-related factors or the normal course of healing [7–17, 28, 29]. Five studies combined IHC with H&E or micro-CT in the same experiment [13–17], so their marker and tissue findings can be compared directly; the other comparisons in this review are made across studies. The following subsections interpret the studies by theme and distinguish cellular or protein responses from direct observations of matrix and bone.

TABLE 1: Animal Models, Tissue Methods, And Principal Findings of the Selected Studies.

Study	Model and comparison	Tissue methods and time points	Principal findings	Interpretation
Budhy et al. (2023) [7]	Male Wistar rats; mandibular left incisors; n = 20. Three doses of mahogany seed extract versus untreated sockets.	H&E; day 3.	Highest mean macrophage count at the lowest dose; the highest dose did not differ from control.	Early cell abundance; macrophage phenotype was not assessed.
Salim et al. (2022) [10]	Male guinea pigs; mandibular left incisors; n = 24. Crab-shell hydroxyapatite and gelatin scaffold versus untreated sockets.	IHC (TNF-α, osterix); days 7 and 14.	Lower TNF-α and higher osterix with the scaffold; inverse correlation (r = −0.78).	Concurrent marker changes do not establish causation or greater bone volume.
Kardikadewi et al. (2023) [11]	Male Wistar rats; mandibular left incisors; n = 24. *Pangasius djambal* gelatin versus untreated sockets.	IHC (PDGF, VEGF, FGF-2, EGF); days 3, 5, and 7.	Higher expression of all four growth factors with gelatin, highest at day 7.	Protein localization; vessels, matrix, and bone were not measured.
Irmawati et al. (2024) [8]	Male Wistar rats; mandibular incisors; n = 28. Swimming exercise before extraction versus water immersion without swimming.	Masson's trichrome, ordinal score 0 to 4; days 3 and 7.	Highest collagen score in the exercise group at day 7.	Host conditioning before injury; an ordinal score does not measure collagen type or bone.
Rahayu et al. (2024) [9]	Male Wistar rats; left mandibular first molars; n = 24. Cocoa pod husk extract gel versus CMC-Na gel and Alvogyl.	Masson's trichrome; days 7 and 14.	Better wound gap closure; no significant difference in collagen density at day 14.	Wound closure and collagen density are distinct outcomes.
Kresnoadi et al. (2022) [12]	Male guinea pigs; mandibular left incisors; n = 56. Propolis plus bovine bone graft versus single-component and PEG controls.	IHC (RUNX2, ALP), with illustrative H&E images; days 14 and 30.	Highest RUNX2 and ALP expression with the combination at day 30.	Osteogenic protein expression; bone quantity was not measured.
Kresnoadi et al. (2023) [28]	Male guinea pigs; mandibular left incisors; n = 56. *Moringa oleifera* extract plus xenograft versus single-component and PEG controls.	H&E, trabecular area; days 7 and 30.	Largest trabecular area with the combination at day 30.	Two-dimensional bone area within the sampled sections.
Widjaja et al. (2021) [13]	Male Wistar rats; mandibular left incisors; n = 50. Zinc oxide and turmeric extract gel (10%, 20%, 40%) versus zinc oxide-eugenol gel and untreated sockets.	IHC (RUNX2, ALP) and H&E; days 3 and 7.	Highest RUNX2 and ALP expression with the 40% gel at day 7; no significant difference from zinc oxide-eugenol.	Early osteogenic protein expression; H&E findings were reported briefly and bone quantity was not measured.
Indrawati et al. (2026) [14]	Male Wistar rats with *Porphyromonas gingivalis*-induced periodontitis; left mandibular first molars; n = 36. Hyaluronic acid with bovine tooth graft versus hyaluronic acid alone and untreated sockets.	H&E for woven bone area and IHC for IL-6 and OCN; days 14 and 21.	Lowest IL-6, highest OCN, and largest woven bone area with the combined graft; no woven bone in control or hyaluronic acid groups.	Protein and tissue measurements in the same sockets; the Results also mention a graft-only group not listed in the Methods.
Horibe et al. (2021) [16]	Male mice; maxillary first molars. Descriptive healing model.	IHC and micro-CT; days 1, 3, and 7.	F4/80-positive and CD206-positive macrophages contained TGF-β; phosphorylated Smad3 in α-SMA-positive progenitor cells; abundant new bone by day 7.	Spatial association between macrophages and progenitor cells; function was inferred rather than tested.

Study	Model and comparison	Tissue methods and time points	Principal findings	Interpretation
Guo et al. (2022) [15]	Male Sprague–Dawley rats; maxillary first molars; n = 36, plus 9 donors. Platelet lysate versus saline, both on gelatin sponge.	H&E and IHC (OPG, RANKL, IL-6, TNF-α) at days 7, 14, and 28; real-time PCR; micro-CT at day 14.	Higher BV/TV, Tb.N, and Tb.Th at day 14; transient IL-6 increase at day 7; osteogenic mRNA lower than control at day 14.	Tissue and imaging measurements used the left maxilla. PCR used the right maxilla.
Shin et al. (2026) [17]	Male Sprague–Dawley rats; mandibular molars; n = 17. Age groups of 2, 10, and 18 months.	H&E, IHC (ALP, TRAP), micro-CT, and serum markers; week 2.	Lowest mean BV and Tb.Th at 18 months; empty sockets with inflammatory cells; weakest ALP staining.	Age comparison using a tooth-based region of interest; IHC was descriptive.
Paini et al. (2026) [29]	Male Wistar rats with STZ-induced diabetes; right maxillary incisors; initial n = 48. Fibrin biopolymer versus blood clot.	H&E, histomorphometry, picrosirius red, and micro-CT; days 7, 14, and 42.	BV 31.86% higher than blood clot at day 42 ($p < 0.05$); no group difference in BV/TV.	Closure differed (sutures vs biopolymer alone); n = 5 per group analyzed at day 42.

Abbreviations: *ALP, alkaline phosphatase; α-SMA, α-smooth muscle actin; BV, bone volume; BV/TV, bone volume fraction; CMC-Na, sodium carboxymethylcellulose; EGF, epidermal growth factor; FGF-2, fibroblast growth factor-2; H&E, hematoxylin–eosin; IHC, immunohistochemistry; IL-6, interleukin-6; micro-CT, micro-computed tomography; OCN, osteocalcin; OPG, osteoprotegerin; PDGF, platelet-derived growth factor; PEG, polyethylene glycol; RANKL, receptor activator of nuclear factor-κB ligand; RUNX2, runt-related transcription factor 2; STZ, streptozotocin; Tb.N, trabecular number; Tb.Th, trabecular thickness; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; TRAP, tartrate-resistant acid phosphatase; VEGF, vascular endothelial growth factor. n refers to animals in the extraction experiment; initial and endpoint numbers may differ.*

- *Inflammatory Cells and Cytokines*

Budhy et al. quantified cells identified morphologically as macrophages in H&E-stained sections collected on postoperative day 3 and reported the highest mean count at the lowest tested dose of mahogany seed extract [7]. This result indicates a difference in cell abundance at the sampled time point, not necessarily a shift toward a reparative macrophage phenotype. By contrast, Horibe et al. used IHC to localize CD206 and TGF-β in macrophage-associated populations during early healing [16]. The distinction is methodologically important: routine morphology can support a cell count, whereas phenotype-specific labeling provides additional biological characterization. Neither approach alone establishes the functional contribution of a given macrophage population to subsequent bone regeneration.

Salim et al. observed reduced tumor necrosis factor-α (TNF-α) immunoreactivity and increased osterix staining following scaffold placement in guinea pig incisor sockets, together with an inverse correlation between the two outcomes [10]. This association is compatible with concurrent changes in inflammatory and osteogenic signaling, but it does not establish that reduced TNF-α caused increased osteoblast differentiation. In the platelet lysate study in rat molar sockets, TNF-α staining did not differ between groups at any time point [15], showing that the same cytokine may respond differently across species, socket types, materials, and sampling days. Interleukin-6 (IL-6) illustrates the importance of timing and host condition. Indrawati et al. reported lower IL-6 staining on days 14 and 21 in treated sockets of rats with induced periodontitis [14], whereas Guo et al. reported a transient increase in IL-6 staining on day 7, with no difference on days 14 and 28, alongside earlier trabecular formation in the treated group [15]. These patterns are not necessarily contradictory because they were obtained at different phases and under different baseline inflammatory conditions. The meaning of a cytokine signal therefore depends on the model, the postoperative day, and the accompanying tissue findings.

- *Growth Factors and Collagen*

Kardikadewi et al. reported increased immunoreactivity for PDGF, VEGF, fibroblast growth factor-2 (FGF-2), and epidermal growth factor (EGF) in sockets treated with *Pangasius djambal* gelatin [11]. These observations indicate altered localization or expression of repair-associated proteins within the examined tissue. However, greater growth-factor staining should not be presented as direct proof of increased functional vessel density, collagen production, or bone volume, because these endpoints require distinct assessments. A stronger interpretation would combine marker localization with quantified vascular profiles, extracellular matrix measurements, and bone outcomes obtained from anatomically comparable regions.

The collagen-related studies illustrate why outcome definitions must remain explicit. Irmawati et al. evaluated collagen using an ordinal histological score after moderate-intensity swimming before extraction [8], whereas Rahayu et al. examined wound gap closure and collagen density following topical treatment [9]. Although the latter study reported improved closure, collagen density did not differ significantly between groups on day 14 [9]. This is not inherently inconsistent: closure reflects the approximation or coverage of the wound surface, whereas collagen density describes the amount of stained matrix within a selected microscopic area. An ordinal score also cannot be interpreted as equivalen

to a continuous percentage of collagen area without additional validation.

Picrosirius red viewed under polarized light can provide useful information about collagen fiber orientation, organization, and birefringence. Nevertheless, polarization colors alone do not reliably identify collagen types I and III. López De Padilla et al. demonstrated this limitation in tendon specimens using type-specific immunolabeling as a comparator [30]. Because that validation concerns tendon rather than extraction sockets, its relevance should be framed as a methodological caution rather than direct evidence about socket collagen composition. Descriptions of fiber thickness and organization in socket sections [29] may support an interpretation of matrix remodeling, but collagen subtype assignment requires appropriately validated type-specific methods.

- *Osteogenic Markers and Bone Formation*

The two experiments by Kresnoadi et al. measured different outcomes and should be read separately. In the first, a combination of propolis extract and bovine bone graft produced the highest RUNX2 and alkaline phosphatase (ALP) immunostaining at day 30, but bone quantity was not measured [12]. In the second, which used *Moringa oleifera* leaf extract and a xenograft, the combination produced the largest trabecular bone area on H&E at day 30, but osteogenic markers were not assessed [28]. Because the marker findings and the bone area came from different experiments with different materials, they cannot be treated as paired evidence from the same specimens. Widjaja et al. reported the highest RUNX2 and ALP staining with a 40% zinc oxide–turmeric gel on day 7, although the difference from zinc oxide–eugenol was not significant [13]. RUNX2 immunoreactivity is consistent with osteogenic differentiation, and ALP immunostaining shows where the ALP protein is located; neither quantifies mineralized bone, and ALP staining does not measure the enzymatic activity of ALP. Conclusions about treatment efficacy therefore require separate structural endpoints.

Indrawati et al. measured woven bone area on H&E sections and osteocalcin (OCN) immunoreactivity in osteoblasts in the same periodontitis-associated sockets on days 14 and 21 [14]. Reading the two outcomes together adds information that neither provides alone. The hyaluronic acid–bovine tooth graft group had the highest OCN staining and the largest woven bone area, so the marker and the tissue outcome agreed. In the hyaluronic acid group, mean OCN staining was higher than in controls, but no woven bone was detected in either group; a higher marker signal therefore did not correspond to measurable new bone. Woven bone was observed only in groups that received the graft. The Results also mention a graft-only group that the Methods and abstract do not list, so the comparisons here use only the groups named in the Methods. Woven bone area also reflects immature bone within sampled sections rather than complete maturation. In an age-comparison model, Shin et al. described weaker ALP staining in the oldest rats alongside lower mean bone volume and trabecular thickness on micro-CT [17]. These observations suggest agreement between biological and structural measures, but the descriptive nature of the staining limits claims about the magnitude of the association.

Guo et al. collected socket tissue from the right maxilla for real-time PCR and used the left maxilla for histology and micro-CT [15]. At day 14, bone volume fraction on micro-CT was higher in the platelet lysate group, whereas the expression of most assessed osteogenic genes was lower than in controls; most of these genes had been expressed at higher levels than in controls at day 7 and were again higher at day 28. The findings are not necessarily contradictory: micro-CT measures mineralized tissue that has accumulated up to the time of sampling, whereas PCR captures gene transcription at that moment. Because the two measurements came from opposite sides of the jaw, a direct specimen-level correspondence also cannot be assumed.

In streptozotocin-induced diabetic rats, Paini et al. reported that absolute bone volume (BV) at day 42 was 31.86% higher in sockets filled with fibrin biopolymer than in sockets left to heal with a blood clot ($p < 0.05$) [29]. Bone volume fraction (BV/TV) increased over time in both groups but did not differ between them. BV represents the amount of mineralized bone within the selected region, whereas BV/TV expresses that amount relative to the region volume; the two outcomes answer different questions and should not be presented as equivalent evidence of enhanced regeneration. Two limitations reported by the authors further restrict interpretation. Blood clot sockets were closed with sutures, whereas biopolymer sockets were closed with the biopolymer alone, so the difference cannot be attributed to the material alone. In addition, after exclusions based on blood glucose, five animals per group were analyzed at day 42, fewer than the eight per group required by the sample size calculation. The result therefore supports a specific structural difference under the reported conditions rather than a general improvement in socket healing.

Factors Affecting Comparisons Between Animal Studies

- *Species, Extraction Site, and Host Factors*

Eight studies summarized in Table 1 used rat or guinea pig incisor extraction models [7, 8, 10–13, 28, 29]. Because rodent incisors grow continuously, their anatomy and biological context differ from those of permanent adult human teeth [31]. Molar models involve different root configurations, surrounding bone anatomy, and socket geometry [9, 14–17, 32]. In addition, aging, diabetes, periodontitis, and nutritional status can alter inflammatory and reparative responses [4, 5, 14, 17, 24, 29]. Thus, differences between experimental outcomes may reflect model characteristics as well as treatment effects, and cross-study comparisons should avoid implying direct equivalence.

- *Timing of Observation*

Postoperative sampling time determines which stage of healing is represented by an observation. In the platelet lysate study, the direction of differences in osteogenic gene expression changed across sampling days [15], whereas the day-42 bone outcomes in diabetic rats captured a later phase of repair [29]. Treatment timing is another source of heterogeneity: pre-extraction exercise modifies host physiology before injury, whereas topical biomaterials act locally after extraction [8, 10–12]. A narrative comparison should therefore identify both when an intervention was delivered and when its effects were assessed before interpreting differences in inflammatory, matrix, or bone-related endpoints.

- *Sampling, Quantification, and Observer Bias*

Tissue processing and analytical decisions can materially influence the interpretation of histological and immunohistochemical outcomes. Fixation, decalcification, section orientation, antigen retrieval, and staining conditions affect tissue preservation and the visibility of target structures [33]. Quantification also depends on the selected region of interest, field-selection procedure, image threshold, and denominator used for reporting. Salim et al. assessed immunopositive cells across 20 microscopic fields [10], whereas Shin et al. defined a tooth-based region of interest for micro-CT analysis [17]. Multiple fields from one animal improve within-animal sampling but do not automatically constitute independent biological replicates; the experimental unit must be identified from the study design. Transparent image-analysis procedures and observer masking are important for limiting measurement bias [34, 35].

Reporting should specify the experimental unit, animal numbers at each time point, group allocation, exclusions, blinding, and the exact definition of each measured outcome, consistent with ARRIVE 2.0 principles [34]. These details are particularly important when histological fields are nested within animals or when tissue and imaging assessments are performed on different anatomical sites. Previous systematic reviews have identified reporting limitations in animal studies of extraction socket healing [18, 19]. Consequently, the strength of a narrative interpretation depends not only on the direction of reported findings but also on whether the underlying methods allow the comparison being made.

Relevance to Human Socket Healing

Clinical translation requires caution because the animal experiments reviewed here largely address early biological responses, whereas human studies commonly assess healing at later postoperative intervals. Keil et al. reported no significant histomorphometric difference between grafted and ungrafted extraction sites at approximately 8–12 weeks [36], while Gabay et al. examined tissue composition and ridge dimensions after six months [37]. These studies assess clinically relevant tissue outcomes but cannot be treated as direct validation of specific early immunohistochemical patterns observed in animals. Systemic factors, including diabetes, further complicate translation [4, 5]. Accordingly, animal IHC findings are best viewed as mechanistic or contextual evidence, whereas claims of clinical treatment benefit require appropriately designed human studies.

Interpreting and Reporting Tissue Outcomes

Table 2 summarizes, for each commonly used assessment, the question it answers, the limit of what it can show, and the complementary measurement needed for broader claims. It is derived from the studies in Table 1 and supporting methodological literature and does not present new experimental data. For IHC, for example, the number of immunopositive cells, the percentage of stained tissue, and staining intensity quantify different aspects of the result [35].

Routine H&E staining provides information about cellular composition and tissue organization, while Masson's trichrome helps characterize collagen-containing matrix [33, 38]. Where feasible, conclusions about osteogenesis should integrate marker localization with matched histomorphometric or volumetric outcomes from the same anatomical region and sampling interval.

TABLE 2: Interpreting Common Histological, Immunohistochemical, and Structural Outcomes.

Assessment	Question answered	Limit of interpretation	Complementary assessment
H&E cell counts [7, 38]	How many morphologically identified cells are present, and where, at the time of sampling.	Cell phenotype or function.	Phenotype-specific IHC on serial sections; later time points.
Macrophage phenotype IHC [16, 23]	Which macrophage populations are present and where they are located.	Function, unless it is tested experimentally.	Depletion or other functional studies; a time course.
Cytokine IHC [10, 14, 15]	Where an inflammatory mediator is expressed and how strongly.	Whether inflammation is resolving, or whether the mediator causes the tissue outcome.	Healing stage on H&E; wound closure; matrix or bone outcomes.
Growth factor IHC [11, 25]	Where proteins involved in repair are expressed.	Vessel function or the formation of new matrix.	Vessel density, collagen assessment, and bone measurement.

Assessment	Question answered	Limit of interpretation	Complementary assessment
Masson's trichrome [8, 9, 38]	Distribution of collagen-containing tissue.	Collagen type; ordinal scores and area measurements are not interchangeable.	Defined fields, image analysis, and type-specific labeling.
Polarized picrosirius red [29, 30]	Collagen fiber organization and birefringence.	Collagen type from color alone.	Consistent section orientation and type-specific IHC.
Osteogenic IHC: RUNX2, osterix, ALP, OCN [10, 12–14, 17, 26]	Cells expressing markers of the osteoblast lineage.	The amount or quality of bone formed; for ALP, enzymatic activity.	Histomorphometry and micro-CT in a matched region.
Histomorphometry and micro-CT [14, 15, 17, 28, 29]	Bone area in sections, or mineralized bone volume in three dimensions.	The cellular or molecular basis of the change.	Histology and IHC at comparable sites and times. Define the region and report BV and BV/TV separately.

Abbreviations as in Table 1. The table is a guide to interpreting the reported outcomes.

Limitations

This narrative review is based on a purposive, non-exhaustive selection of 13 animal studies. This approach supports focused methodological interpretation but may introduce selection bias and does not establish the completeness of the available evidence. Only five of the studies combined IHC with H&E or micro-CT in the same experiment, so many comparisons in this review are made across studies. Heterogeneity in species, tooth type, host condition, intervention, postoperative timing, and outcome definitions prevents direct comparison of effect magnitudes. For one study, the information used was limited to the abstract. No formal risk-of-bias assessment or meta-analysis was undertaken. In addition, the caution concerning picrosirius red color interpretation draws on tendon rather than socket tissue [30]. The conclusions should therefore be understood as principles for interpreting experimental findings, not as pooled estimates of treatment efficacy or proof of clinical benefit.

CONCLUSIONS

Histological and immunohistochemical examinations provide complementary but non-interchangeable evidence of extraction socket healing in animal models. Histology characterizes tissue architecture and directly observed changes in inflammatory infiltrates, collagen-containing matrix, and new bone, whereas IHC localizes proteins associated with the underlying biological processes. Interpretation is strongest when marker findings are considered alongside tissue-level or volumetric outcomes obtained at comparable sites and postoperative stages. When both are measured in the same sockets, agreement or disagreement between marker and tissue findings becomes visible, which cannot be established by comparing separate studies. The reviewed studies also demonstrate that wound closure, collagen density, osteogenic immunoreactivity, absolute bone volume, and bone volume fraction may follow different patterns. Clear outcome definitions, transparent sampling procedures, and recognition of model-specific limitations are therefore essential for avoiding overinterpretation. These principles can improve the reporting of preclinical socket-healing research while leaving clinical efficacy to be established through human studies.

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