



LITERATURE REVIEW:

Bridging Developmental Biology and Regenerative Endodontics: Postnatal Rat Tooth Germ as A Model for Root Dentinogenesis

Tendiendo puentes entre la biología del desarrollo y la endodoncia regenerativa: el germen dental postnatal de rata como modelo de dentinogénesis radicular

Maryam Hameed Alwan M.Sc. PhD¹ <https://orcid.org/0000-0002-1594-9126>

Jwan Arkan M. Saeed M.Sc.¹ <https://orcid.org/0009-0005-7362-6017>

¹Department of Oral Diagnostic sciences, College of Dentistry, Baghdad University, Iraq.

Correspondence to: Maryam Hameed Alwan - maryam.h@codental.uobaghdad.edu.iq

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ABSTRACT: Regenerative endodontic treatment (RET) aims to promote continued root maturation and restoration of the pulp–dentin complex through tissue-engineering principles. However, conventional infection-based experimental models introduce inflammatory and microbial confounders that may obscure the intrinsic mechanisms of physiological root dentinogenesis. This narrative review evaluates postnatal day 7-19 rat mandibular first molar root tooth germ as a biologically defined developmental model for mechanistic RET research. The literature was searched in PubMed, Scopus and Web of Science using predefined developmental, molecular and regenerative endodontic keywords. Studies addressing root development, Hertwig's epithelial root sheath (HERS), odontoblast differentiation, dentin matrix protein expression, stem cells of the apical papilla (SCAPs) and inflammatory effects on odontogenic signaling were included. Evidence indicates that the P7-P19 developmental window encompasses sequential HERS elongation, odontoblast differentiation, predentin secretion, dentin mineralization and an early cementogenesis. The model also preserves physiological signaling pathways, including Wnt/ β -catenin, BMP and TGF- β networks without the confounding influence of lipopolysaccharide-induced NF- κ B activation or matrix metalloproteinase-mediated extracellular matrix degradation. Although translational limitations between rodent and human biology remain, the reviewed evidence supports the value of this normal-tissue developmental model as a reproducible baseline for mechanistic studies in regenerative endodontics.

KEYWORDS: Rat tooth germ; Root dentinogenesis; Regenerative endodontic treatment; Hertwig's epithelial root sheath; Odontoblast differentiation; Postnatal molar development.



RESUMEN: Tratamiento endodóntico regenerativo (RET), cuyo propósito es fomentar la maduración progresiva de la raíz y la rehabilitación del complejo pulpa-dentina, empleando principios de ingeniería tisular. No obstante, los modelos experimentales convencionales basados en la infección introducen factores de confusión inflamatorios y microbianos que pueden ocultar los mecanismos intrínsecos de la dentinogénesis fisiológica de la raíz. El propósito es evaluar el germen dental de la raíz del primer molar mandibular de rata. Dicho estudio se llevará a cabo entre los días 7 y 19 posnatales, con el objetivo de establecer un modelo de desarrollo biológicamente definido para la investigación mecánica del RET. Se realizó una exhaustiva revisión bibliográfica en las bases de datos PubMed, Scopus y Web of Science, utilizando palabras clave predefinidas relacionadas con el desarrollo, la biología molecular y la endodoncia regenerativa. Se incluyeron estudios que abordaban el desarrollo radicular, la vaina epitelial de Hertwig (HERS), la diferenciación de los odontoblastos, la expresión de proteínas de la matriz dentinaria, las células madre de la papila apical (SCAP) y los efectos inflamatorios sobre la señalización odontogénica. La evidencia sugiere que el período comprendido entre las etapas P7-P19 del desarrollo de la dentición primaria abarca la elongación secuencial de la HERS, la diferenciación de los odontoblastos, la secreción de predentina, la mineralización de la dentina y una cementogénesis temprana. El modelo conserva las vías de señalización fisiológicas, incluidas las redes Wnt/ β -catenina, BMP y TGF- β , sin la influencia confundida de la activación de NF- κ B inducida por lipopolisacáridos o la degradación de la matriz extracelular mediada por metaloproteinasas de la matriz. A pesar de las limitaciones existentes en la traslación entre la biología de los roedores y la humana, la evidencia revisada respalda el valor de este modelo de desarrollo de tejido normal como referencia reproducible para estudios mecanísticos en endodoncia regenerativa.

PALABRAS CLAVE: Germen dental de rata; Dentinogénesis radicular; Tratamiento endodóntico regenerativo; Vaina epitelial radicular de Hertwig; Diferenciación de odontoblastos; Desarrollo molar postnatal.

INTRODUCTION

Tooth root formation is a tightly regulated developmental process depend on sequential and reciprocal interaction of epithelial and mesenchymal tissue compartments (1). The root dentinogenesis involve the odontoblasts differentiation and deposition of radicular dentin along the emerging root surface which represents a process of clinical and biological significance (2). The structural integrity of the radicular dentin determines the mechanical competence of the erupted tooth and constitutes the primary biological scaffold upon

which the regenerative strategies of RET ultimately depend (3,4).

Furthermore, the regenerative endodontic treatment considered as the standard method of repair for immature permanent teeth with necrotic pulps over the past decade (5). By coupling the self-renewing and odontogenic potential of stem cells of the apical papilla (SCAPs), the RET aims to promote continued root elongation, dentinal wall thickening and the restoration of a functional pulp dentin complex (6). Despite accumulating clinical evidence of efficacy, the fundamental biological

mechanisms governing successful root dentinogenesis during RET remain incompletely characterised (7).

However, a critical methodological concern with the experimental investigation of root dentinogenesis in the RET context which is the selection of an appropriate *in vivo* or *ex vivo* model. The conventional animal models simulate the clinical scenario by inducing pulpal infection or periapical pathology through surgical inoculation. While such models preserve clinical reliability, but they certainly introduce microbial and inflammatory confounders that independently alter the molecular and cellular outcome variable relevant to root dentinogenesis (8,9).

In fact, the lipopolysaccharide (LPS) activates TLR4/NF- κ B signalling in dental pulp and periapical cells which triggering upregulation of IL-1 β , IL-6 and TNF- α (10,11). These mediators suppress the odontoblastic gene networks, including DSPP and DMP-1 transcription, through mechanisms entirely extrinsic to the physiological programme of root formation (12). Furthermore, the MMP activation degrades the non-collagenous protein scaffold of the dentinal matrix and compromising the bioavailability of growth factors of direct biological relevance to RET (13).

Nonetheless, normal-tissue model for the controlled study of root dentinogenesis in the context of RET research not commonly applied. The rat (*Rattus norvegicus*) first mandibular molar undergoes root formation during a well-defined postnatal developmental period from P7 through P19, including histologically definite and a sequentially ordered stages. Despite these characteristics, the systematic utilisation of normal rat root tooth germ tissue at these stages as a confounder free model for RET-relevant dentinogenesis has not been comprehensively reviewed in the current oral biology literature.

The objectives of this narrative review are: (i) to characterise the sequential developmental histology and molecular biology of rat root tooth germs between P7 and P19 (ii) to identify and critically evaluate the specific confounding variables introduced by infection and inflammation based models that can be eliminated by the adoption of normal tissue (iii) to appraise the practical, ethical and the translational advantages of the proposed model and (iv) to propose a conceptual and methodological framework supporting its integration into the experimental methodology of RET research.

METHODS

REVIEW DESIGN

The review adheres to the Scale for the Assessment of Narrative Review Articles (SANRA) criteria to ensure transparency, scholarly rigour and the methodological reproducibility (14).

LITERATURE SEARCH STRATEGY AND DATA SOURCES

The search was conducted across PubMed, Scopus and Web of Science to February 20, 2026, using the keywords related to tooth root development, regenerative endodontics and inflammatory confounders. ("rat tooth germ" OR "rodent molar postnatal development"), ("root dentinogenesis" OR "radicular odontoblast differentiation"), ("Hertwig's epithelial root sheath" OR "HERS"), ("stem cells of the apical papilla" OR "SCAP"), ("regenerative endodontic treatment"), ("dentin sialo phosphoprotein" OR "DSPP" OR "DMP-1"), and ("LPS dental pulp" OR "infection confounders").

STUDY SELECTION

The initial database search identified 760 records (PubMed 132, Scopus 222 and Web of Science 406). After duplicate removal, 584 articles

remained for title and abstract screening. A total of 121 studies underwent full-text assessment for eligibility and then 41 studies were included in the narrative synthesis.

INCLUSION AND EXCLUSION CRITERIA

The included studies involve the one that related to developmental histology or molecular biology of rat/rodent tooth germs at defined postnatal stages; odontoblast differentiation or DMP expression during root formation, HERS function and molecular regulation, SCAP biology in the context of RET, the effects of infection, LPS, inflammatory cytokines on odontoblast or pulp stem cell biology. The studies that address only crown dentinogenesis, *in vitro* cell study without complementary *in vivo* data or the studies that lacking peer-reviewed status were excluded.

DATA EXTRACTION AND SYNTHESIS

The extracted data includes the study design, species, developmental stage, primary outcome measures and the key findings relevant to each stated research objective. Findings were organised under four headings: the developmental histology and molecular biology of the P7-P19 model; HERS-mediated induction and the DMP expression; confounding effects of infection and inflammation and the practical, ethical and translational advantages.

RESULTS

THE RAT FIRST MOLAR AS A MODEL SYSTEM FOR ROOT DENTINOGENESIS

In the rodent first mandibular molar, crown morphogenesis is complete around birth and root

formation initiated at the postnatal period under the orchestrating influence of HERS (15). The developmental period from P7 to P19 has been studied through multiple independent histological and molecular investigations to encompass the stage-ordered sequence of root dentinogenesis from the start of HERS elongation and radicular odontoblast induction through active predentin secretion, mineralised dentin deposition and the mineralisation progression to almost complete root formation followed by the initiation of cementogenesis (16,17).

At P7, HERS has initiated its characteristic apical elongation from the cervical loop, forming a bilayer epithelial structure whose inner epithelial cells directly contact and inductively signal to the adjacent dental papilla ectomesenchyme (18,19).

Between P7 and P10, the radicular odontoblast specification and terminal differentiation is initiated, the dental papilla progenitor cells acquire a polarised tall-columnar morphology and begin to express early odontogenic markers including nestin (20). By P14-P16, the root has elongated to encompass its mid-third, the odontoblast layer is fully established with the expression of markers including DSPP and DMP-1 at a developmental peak (21,22).

By P17-P19, root development approaches anatomical completion and the cementoblast differentiation is initiated following HERS fragmentation with the early periodontal ligament fibres become recognizable (19,23,24). Table 1. sequential developmental stages of the rat first molar root tooth germ at postnatal days: histological features, cellular events, molecular markers, and correspondence with human root development based on (19,20,22,24,25-39)

Table 1. Sequential developmental stages of the rat first molar root tooth germ at postnatal days.

Postnatal Day	Developmental Stage	Key Histological Features	Cellular Events	Dominant Molecular Markers	Human Developmental Correspondence
P7	HERS Initiation	HERS begins apical elongation from cervical loop; bilayered epithelial structure; dental papilla ectomesenchyme undifferentiated	HERS epithelial cell proliferation; early cell-cell signalling at epithelial-mesenchymal interface; no odontoblast differentiation yet	Wnt/ β -catenin (active in HERS); BMP-2, BMP-4; E-cadherin in HERS; Ki-67 (proliferation)	Early bell stage/Cervical loop elongation; preroothinitiation in human primary tooth (approx. 14-16 wks in utero)
P8-P9	Radicular Odontoblast Specification	HERS elongation advanced; dental papilla periphery shows early columnar change; no visible predentin yet	Radicular odontoblast specification from papilla progenitors; downregulation of undifferentiated mesenchymal markers; nestin upregulation begins	Nestin; BMP-2 (HERS); TGF- β 1 (papilla mesenchyme); RUNX2; MSX2	Radicular odontoblast induction during root commencement of human permanent tooth (approx. 4-5 years post-birth for first molar)
P10-P12	Active Predentin Secretion	Tall columnar polarised odontoblasts aligned along HERS; predentinal zone clearly visible; mineralisation front not yet established	Terminal odontoblast differentiation complete; Type I collagen deposition; DSPP and DMP-1 transcription initiated; dentinal tubule formation begins	DSPP (peak on-set); DMP-1; Type I collagen; nestin; dentin matrix protein expression maximal	Active predentin secretion phase in human root dentinogenesis; equivalent to initial radicular dentin deposition
P13-P14	Mineralised Dentin Deposition	Predentinal zone well-defined; mineralisation front advancing; odontoblast layer fully established; midroot third reached	Hydroxyapatite crystallite nucleation; DMP-1-mediated mineralisation front progression; dentinal tubule elongation; odontoblastic process retraction	DMP-1 (strongly upregulated); DSPP (sustained); BSP; Type I collagen crosslinking; MAPK pathway components	Midroot mineralised dentin deposition phase in human permanent tooth root formation
P15-P16	Root Elongation and Apical Progression	Two-thirds of root length achieved; continuous mineralised dentin layer; HERS intact at apex; early HERS fenestration at midroot	Continued odontoblast secretory activity; early cementoblast specification at midroot surface following HERS fenestration; ECM remodelling upregulated	Wnt10a-Notch (furcation region; Cementum attachment protein (CAP); BSP; periostin (early expression)	Progressive root elongation phase; HERS fragmentation beginning; equivalent to late root formation in human primary tooth
P17-P19	Near-Completion and Cementogenesis	Root near anatomical completion: full thickness mineralised radicular dentin; cementoblasts differentiated at root surface; early PDL fibres identifiable	Cessation of active odontoblast elongation; cementum matrix deposition; early Sharpey fibre insertion; HERS fragmentation completes apically	Cementum-specific markers (CAP, CEMP-1); Periostin; β 1 integrin; PDL formation markers; Wnt/ β -catenin downregulated in HERS	Root completion; cementogenesis; equivalent to late permanent root formation prior to eruption in humans

HERS=Hertwig's epithelial root sheath; DMP=dentin matrix protein; DSPP=dentin sialo phosphoprotein; BMP=bone morphogenetic protein; ECM=extracellular matrix; PDL=periodontal ligament; CAP=cementum attachment protein.

CORRESPONDENCE WITH HUMAN ROOT DEVELOPMENT

The developmental sequence of root dentinogenesis in the rat at P7-P19 exhibits substantial histological, cellular and molecular correspondence with the normal process in the human permanent tooth, despite the markedly accelerated rhythm of rodent development (1,40). The cellular architecture of the odontoblast layer, the predentin–dentin boundary, the sequential elaboration of DMP species, the HERS-mediated inductive mechanism, and the temporal coupling of cementogenesis to HERS fenestration are conserved between the two species. This developmental conservation is reinforced by the shared deployment of Wnt/ β -catenin, BMP/Smad, FGF and SHH signalling pathways in orchestrating root formation in both rodents and humans (17,40).

MOLECULAR AND CELLULAR DETERMINANTS OF ROOT DENTINOGENESIS

HERS-MEDIATED THE INDUCTION

The HERS functions as the primary inductive signalling centre for the root dentinogenesis (41). Moreover, the inactivation of Wnt/ β -catenin in HERS leads to premature sheath fragmentation and interrupted root elongation which recognise the β -catenin as a determinant of HERS structural continuity (24). Additionally, Jeong et al study showed that stabilisation of β -catenin arrests

root elongation and impairs dentin mineralisation, confirming that dynamic temporal regulation of Wnt signalling within HERS is essential. Another study showed that a Wnt10a–Notch signalling axis controls HERS proliferative behaviour in the root furcation region, with Wnt10a knockout recapitulating taurodontism phenotypes in mice (26).

DENTIN MATRIX PROTEIN EXPRESSION

The temporal profile of expression reveals a tightly regulated, stage-specific molecular cascade governing rodent tooth root development from P7 to P19. Dentin matrix proteins (DSPP, DSP, DPP, DMP-1, and Type I Collagen) are low or absent at P7, rise sharply between P10 and P14 and remain elevated through P15-P16, reflecting the onset of root dentin mineralization and active odontoblast secretory function (42,43,44). DMP-1 uniquely declines at P17-19, consistent with its role in early mineralization and subsequent downregulation in mature odontoblasts (21,43,45,46). Transcription factors (OSX, NFIC) peak at P10-14, coinciding with the differentiation window of odontoblasts, then decline (43,47,48). The BMP-2/4 peaks early then drop, while TGF- β 1 peaks at P10-12, suggesting distinct signalling phases (30,49,50). Collectively, the data define a sequential activation of extracellular matrix production, transcription factor networks and signalling pathways that precisely orchestrate odontoblast differentiation, dentin mineralization and periodontal tissue formation during rodent root development (Figure 1).

Key molecular markers during rodent tooth root development

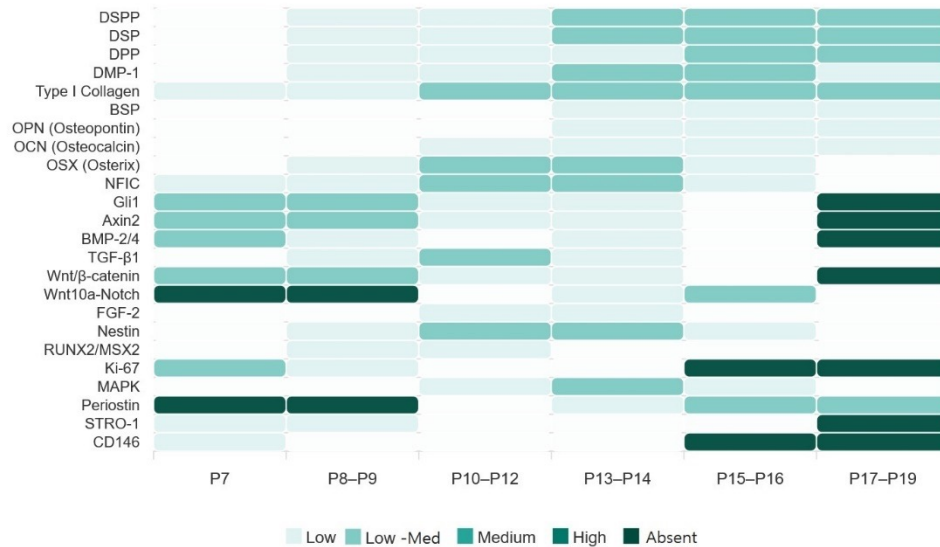


Figure 1. Temporal expression profile of key molecular markers during rodent tooth root development across postnatal days.

SCAP-ANALOGOUS PROGENITOR POPULATIONS

The apical papilla of the rat first molar at P7-P19 is a progenitor cell population with a functional and phenotypic parallels to the human SCAPs (51). However, for the proposed normal tissue model this progenitor cells are accessible in their native, uninflamed tissue microenvironment which enabling baseline characterisation without the confounding alterations in proliferative capacity, differentiation bias and the TLR expression profiles imposed by microbial and inflammatory stimuli. The TLR3 activation significantly suppresses SCAP odontogenic differentiation an effect entirely absents from the normal tissue situation (52,53).

CONFOUNDING EFFECTS OF INFECTION AND INFLAMMATION IN CONVENTIONAL ENDODONTIC MODELS

LPS, TLR4/NF-κB ACTIVATION AND SUPPRESSION OF ODONTOGENIC GENES

The LPS from Gram-negative bacteria activates TLR4 on odontoblasts and the dental pulp cells which triggering NF-κB nuclear translocation and release the IL-1β, IL-6, IL-8, TNF-α and the CCL2 (54,55). Moreover, the TLR2 engagement by lipoteichoic acid (LTA) from the Gram-positive species reduces Type I collagen and DSPP gene expression which directly impairing predentin matrix synthesis at the transcriptional level (56).

TNF- α , IL-1 β , AND SUPPRESSION OF SCAP ODONTOGENIC DIFFERENTIATION

An experimental study provide evidence that DSPP mRNA is upregulated 19.58-fold and DMP-1 6.94-fold in carious versus healthy dental pulp cells alongside simultaneous an elevation of TLR-2, -4, -9, TNF- α , IL-6, IL-8, NF- κ B and the MAPK1 transcripts (57). Besides, the TNF- α stimulation of DPSCs activates NF- κ B and C5aR-mediated cascades that alter DSPP and DMP-1 expression kinetics in a dose-dependent manner (58). Furthermore, the prolonged root canal infection significantly reduces tissue formation quality post-RET (59,60).

MATRIX METALLOPROTEINASE ACTIVATION AND GROWTH FACTOR DEGRADATION

In infected models the LPS-driven MMP-2, MMP-3 and the MMP-9 activation degrades both the collagenous scaffold and the non-collagenous

protein of the dentinal matrix which reducing the growth factor including TGF- β 1, BMP-2, FGF-2 and the VEGF and cause the dentin matrix biologically inert and inhibit its regenerative signalling function (4,56). Additionally, studies investigating dentin matrix-mediated growth factor bioavailability in disease models are therefore essentially confounded by the extent of MMP-mediated degradation, a variable entirely absent from normal tissue models. Table 2 provides a systematic comparison of all relevant parameters between the proposed normal tissue model and conventional disease-based approaches. The Normal tissue values (centre column) represent baseline physiological conditions; disease model values (right column) represent conditions in infection or inflammation induced endodontic animal models. LPS=lipopolysaccharide; LTA=lipoteichoic acid; MMP=matrix metalloproteinase; NF- κ B=nuclear factor kappa B; SCAP=stem cells of the apical papilla; 3Rs=Replacement, Reduction, Refinement.

Table 2. Systematic comparison of the proposed normal rat root tooth germ model versus conventional infection/inflammation-based disease models.

Parameter/Variable	Normal Tissue Model (Rat Root Germ P7–P19)	Infection / Inflammation-Based Disease Model
Experimental design		
Tissue condition at harvest	Physiologically normal; disease-free; immunologically quiescent	Infected, inflamed, or post-surgical; variable degree of pathology
Developmental stage control	Precisely defined by postnatal day; highly reproducible histological phenotype	Variable; pathology stage difficult to standardise; dependent on inoculation duration
Inter-individual variability	Low; developmental stage is predictable and strain-consistent	High; polymicrobial inoculum composition, infection severity, and immune response vary substantially
Experimental timeline	Short; tissue harvest at defined postnatal day without surgical preparation	Extended; requires inoculation surgery, infection establishment phase, and post-infection recovery period
Molecular outcome variables		
DSPP / DMP-1 expression	Physiological, stage-specific expression trajectory; unperturbed normative baseline	Altered by LPS (TLR2/TLR4-mediated suppression); dysregulated beyond physiological range
Type I collagen synthesis	Orderly; temporally coupled to odontoblast secretory phase	Partially degraded by MMP-2, MMP-3, and MMP-9 activated through NF- κ B pathway
Bioavailability of dentin-bound growth factors (TGF- β 1, BMP-2, FGF-2)	Growth factors intact; available for physiological or experimental liberation	Partially or substantially degraded by LPS-driven MMP activation and bacterial proteases
Odontoblast polarisation and morphology	Reproducible tall columnar polarised odontoblasts; standard histological phenotype	Disrupted morphology; vacuolisation, apoptosis, or dedifferentiation possible depending on infection severity
Progenitor cell biology		
SCAP-analogous progenitor phenotype	Baseline multipotent phenotype; unperturbed surface marker expression (STRO-1, CD146, CD73)	TLR expression upregulated; TLR3 activation by poly(I:C) suppresses odontogenic differentiation
Progenitor cell odontogenic differentiation capacity	Full intrinsic differentiation capacity; assessable against normative baseline	Altered by bacterial antigens; TNF- α /C5L2-mediated gene expression changes compromise differentiation fidelity
Stem cell proliferative kinetics	Physiological; stage-dependent proliferative activity measurable by Ki-67 / BrdU	Dysregulated; NF- κ B and hypoxia-inducible factor pathways alter proliferation in an unpredictable, dose-dependent manner
Confounders and data interpretability		
Microbial virulence factors (LPS, LTA, proteases)	Absent	Present; confound every molecular assay of odontoblast and progenitor cell biology
Pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6, PGE2)	Physiological baseline levels only	Supraphysiological; independently suppress BMP-2/Smad signalling and DSPP transcription
Matrix metalloproteinase activation	Physiological, tightly regulated; MMP activity within developmental norms	Pathological; MMP-2, -3, -9 upregulated; progressive dentin matrix degradation
Confound-free molecular attribution	Yes, experimental results attributable to defined stimulus	No; results potentially attributable to background infection/inflammation, not experimental variable
Practical and ethical considerations		
Animal procedures required	Postnatal day harvest; no surgical inoculation; minimal distress	Multi-stage surgical inoculation; chronic infection induction; greater animal distress and welfare impact
3Rs compliance	High; Reduction (bilateral tissue yield), Refinement (minimal distress)	Lower; extended procedures, greater suffering; higher total animal numbers often required
Compatibility with advanced omics (scRNA-seq, proteomics)	High; clean tissue enables clear signal; ideal for spatiotemporal single-cell analysis	Low; inflammatory transcriptional programmes overwhelm developmental dentinogenic signal in bulk or single-cell assays
Translational relevance to RET	Direct; recapitulates the developmental programme that RET aims to restore	Indirect; clinically relevant context but mechanistic data confounded by pathological variables

ADVANTAGES OF THE NORMAL TISSUE MODEL

REPRODUCIBILITY, STANDARDISATION AND EXPERIMENTAL CONTROL

The developmental stages of the rat tooth germs at defined postnatal days are reproducible across standard strains including Sprague-Dawley and Wistar rats and the histological phenotype at each stage is well documented (28,61). This standardisation represents a fundamental methodological requirement for the generation of mechanistically interpretable molecular data and comparison to the infection-based disease models which subject to substantial inter-individual variability in microbiological and inflammatory response.

APPLICATION OF SINGLE-CELL TRANSCRIPTOMICS TO NORMAL TISSUE

The accessibility of the morphologically intact, immunologically normal tooth germ tissue at defined postnatal stages is a high-resolution transcriptomic technology. The spatiotemporal single-cell transcriptomic analysis of normal tooth germ tissue enables single-cell resolution mapping of neural crest lineage variation and cellular function during tooth morphogenesis (18). Significantly, the application of equivalent approaches to normal rat root tooth germs at P7-P19 would enable construction of a comprehensive transcriptomic atlas of root dentinogenesis a task not feasible with infected tissue, in which inflammatory transcriptional programmes would overwhelm the developmental signal of interest.

ALIGNMENT WITH THE 3RS ETHICAL FRAMEWORK

The normal tissue sampling from postnatal rats at P7-P19 aligns comprehensively with the 3Rs principles. Postnatal rats can be humanely euthanised with minimal procedural distress; no preparatory surgical inoculation is required, and bilateral tissue yield per animal reduces total animal

numbers required to achieve statistical power. This ethical profile compares favourably with disease models, which necessitate multi-stage surgical procedures, sustained infectious pathology induction and longer experimental timelines (62,63).

DISCUSSION

The reviewed evidence suggests that the normal rat root tooth germ tissue may provide a biologically controlled and experimentally reproducible developmental model for mechanistic RET research. The postnatal day seven to postnatal day nineteen developmental window captures the complete biological events of the root dentinogenesis in a molecularly intact and pathology-free tissue environment (19,22). Fundamentally, the four pillars of successful RET as stem cells, biomaterials, growth factors and their synergistic interactions. The mechanistic study of each pillar in isolation requires a reference tissue environment free from bacterial virulence factors and inflammatory cytokines. The normal rat tooth germ tissue provides precisely this environment (3).

The Previous histological studies of postnatal rodent tooth germ development have primarily characterised crown dentinogenesis and early odontoblast differentiation at or shortly after birth. While these investigations established a standardizing histological framework they were not explicitly positioned within the context of RET research and left the root formation arc comparatively under characterised. Nevertheless, the studies employing disease models have provided important information regarding the immune biology of apical periodontitis (4,5), but for the specific mechanistic purpose to understanding the HERS induction, DMP temporal regulation and the SCAP analogous progenitor response to regenerative stimuli the questions most relevant to RET biology disease models provide an inherently confounded situation. The present review formally bridges this methodological gap.

In fact, by the establishment of the normal rat root tooth germ tissue as the reference standard for dentinogenesis research, the field gains a biologically defined developmental baseline against which the quality and phenotype of RET regenerated tissue can be evaluating. This is of considerable practical relevance given the ongoing histological debate regarding the nature of tissue formed following clinical RET (5). Practically, the model offers a tractable platform for the evaluation of candidate bioactive molecules including TGF- β 1, BMP-2, FGF-2 and the VEGF whose effects can be assessed against a well-characterised normative baseline, without confounding by background inflammatory signalling. Importantly, the need for biologically reliable preclinical models that accurately recapitulate the developmental programme of pulp–dentin complex formation can be dependent on the normal rat model.

LIMITATIONS OF THE PRESENT REVIEW

This narrative review carries several limitations. Primary, the inter-species differences in developmental time, root anatomy and the immunological organisation require that translational from rodent data to clinical human RET protocols be made with appropriate caution. Moreover, the review is primarily theoretical; it requires direct experimental validation through controlled comparative studies measuring DMP expression, progenitor cell biology and mineralisation outcomes in matched normal and infected rat tissue at P7-P19.

FUTURE RESEARCH DIRECTIONS

The prospective controlled comparative studies directly quantifying DSPP, DMP-1, Type I collagen, STRO-1, CD146, TGF- β 1, BMP-2 and MMP activity in normal versus an infected rat root tooth germ tissue at P7-P19 would provide the validation to this review. Another is the application of spatiotemporal single-cell RNA sequencing as

to normal P7-P19 rat root germs would provide a high-resolution transcriptomic atlas of root dentinogenesis applicable globally as a reference dataset for RET research. Moreover, the organotypic three-dimensional culture systems derived from normal P7-P19 rat root tooth germs would provide a tractable platform for pharmacological intervention in root dentinogenesis under fully controllable conditions.

CONCLUSIONS

The postnatal P7-P19 rat mandibular molar developmental window represents a structured and experimentally accessible model for investigating physiological root dentinogenesis. The reviewed evidence indicates that this model may reduce several inflammatory and microbial confounders inherent to infection-based systems and may therefore improve mechanistic interpretation in regenerative endodontic research. Nevertheless, further comparative and translational validation studies remain necessary before broad standardization can be recommended.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT: The data that support the findings of this study are available on request from the corresponding author.

ETHICS STATEMENT: This article is based exclusively on the analysis of previously published studies. No new animal or human experiments were conducted by the authors.

AUTHOR CONTRIBUTIONS STATEMENT: Conceptualization, Methodology, Software: M.H.A.; Data curation, Writing-Original draft preparation: J.A.M.S. and M.H.A.; Visualization, Investigation: J.A.M.S.; Supervision, Validation, Writing- Reviewing and Editing: M.H.A and J.A.M.S.

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