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WNT Signaling Modifies Barrier Functions of Junctional and Pocket Epithelium

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Abstract

The aims of this study were 1) to determine how ligature-induced periodontitis (LIP) disrupts barrier functions of the junctional epithelium (JE); 2) to determine, using a genetic approach, the necessity of Wnt signaling for JE barrier functions; and 3) to test, using a biochemical strategy, whether a WNT therapeutic is sufficient to improve barrier functions of a pocket epithelium. In a murine model of LIP, quantitative analyses performed at multiple time points assessed epithelial apoptosis; expression of attachment proteins laminin 5 and β4 integrin, inflammation, and bone resorption. *Axin2*$^{CreERT2/+}$; *R26R*$^{mTmG/+}$ mice were used to evaluate how LIP impacted Wnt-responsive cells and their progeny, and *K14*$^{CreERT2/+}$;*Wls*$^{fl/fl}$ mice were used to determine whether Wnt signaling was required for JE barrier functions. In some cases, LIP was followed by a recovery period to assess molecular changes in pocket epithelium, and in a subset of these mice, a liposomal formulation of human WNT3A protein (L-WNT3A) was tested for its effects on early repair dynamics in pocket epithelium. LIP triggered apoptosis, significantly reduced expression of laminin 5 and β4 integrin in the JE, and disrupted the Wnt-responsive compartment; these epithelial changes were accompanied by inflammation and alveolar bone resorption. Reepithelialization occurred even with a ligature present, but this pocket epithelium had compromised barrier functions. Wntless (Wls) deletion was sufficient to convert a JE into pocket epithelium, while topical L-WNT3A treatment was sufficient to increase hemidesmosomal protein expression in pocket epithelium and reduce inflammation at early time points. LIP destroys barrier functions and thus converts a JE into pocket epithelium. Deletion of epithelial *Wls* demonstrates that this conversion is a Wnt-dependent event. L-WNT3A restores some early barrier features to pocket epithelium; future studies will focus on the durability of these effects.

Keywords: inflammation, bone loss, attachment, periodontitis, hemidesmosomes, barrier function

Introduction

The junctional epithelium (JE) is the first line of defense against periodontitis. One feature that makes the JE such an exceptional epithelial barrier is its high rate of mitosis (Sakai et al 1999; Aellos et al 2025), which eliminates keratinocytes to which oral pathogens have adhered. This continual, homeostatic regeneration of the JE is made possible by a Wnt-responsive stem cell niche that generates cells for the lifetime of an animal (Yuan et al 2020a, 2020b).

A second feature that makes the JE such a robust barrier is its adherence to the tooth. The non-keratinized JE forms a unique hemidesmosomal attachment (Marks et al 1994; Nishio et al 2010) that maintains adhesion even under continual masticatory forces (Yuan et al 2023). Components of this hemidesmosomal attachment apparatus also facilitate adherence of leukocytes to the JE and thus contribute to a third feature of this barrier: its innate immune functions (expertly reviewed in Jeon et al 2024). A fourth feature of this epithelial barrier is its selective permeability, which allows gingival crevicular fluid to egress while simultaneously preventing ingress of oral microbes.

It is generally agreed that the barrier functions of the JE are compromised in periodontitis (Stern 1981;0020Takata and Donath 1988), but the factors responsible for initiating this

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A supplemental appendix to this article is available online.

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breakdown are still poorly defined (reviewed recently in Theodoro et al 2023). After periodontal disease has compromised the JE, reestablishing a connection between connective tissue and cementum depends on first reattaching the epithelium (Stahl 1979). This reattachment does not typically occur spontaneously (Caton and Nyman 1980; Donos et al 2018), and as a result, pocket epithelium is a suboptimal barrier (Ye et al 2000).

Any conversion of pocket epithelium into a functioning JE depends upon bacterial control and resolution of inflammation, but even after these factors are controlled, there is still an open question of whether the resulting "long junctional epithelium" develops the same barrier functions as a healthy JE (Caton and Zander 1979; Magnusson et al 1983; Noguchi et al 2017).

Our goal was to understand the sequence of events involved in converting a JE into pocket epithelium, first using a ligature-induced periodontitis (LIP) model to spatially and temporally map the process of JE breakdown. We then employed a genetic loss-of-function approach to query the importance of Wnt signaling in the conversion of a JE to pocket epithelium. In final experiments, a WNT-based therapeutic strategy was tested for its ability to reestablish early barrier features to pocket epithelium.

Materials and Methods

The Appendix contains information on the distribution of mice across experiments (Table 1. Animals distribution by experiment), transgenic mice, and methods of lineage tracing; surgery details; analytic time points, L-WNT3A formulation, μ–computed tomography analyses, and quantification; sample collection; tissue preparation; quantitative immunohistochemistry (qIHC); and statistics.

Results

Ligature Placement Triggers Epithelial Apoptosis and Disrupts the JE Barrier Function

Ligatures were placed in adult male and female mice, and tissues were analyzed at multiple time points (Fig. 1A). The intact JE served as a control (Fig. 1B). For example, programmed cell death, indicated by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, is restricted to the coronal tip of a healthy JE (Fig. 1C), but within hours of ligature placement, TUNEL staining was evident throughout the damaged JE (Appendix Fig. 2A, B). The ligature continued to cause extensive soft tissue damage for the duration of its retention (n = 12; Fig. 1D–K, quantified in W; Appendix Fig. 2C–F).

LIP disrupted the JE attachment apparatus. Integration and reanalysis of 2 previously published single-cell RNA sequencing datasets (Ando et al 2024) showed that many attachment and cell–cell adhesion genes continued to be expressed or were upregulated in the epithelium following LIP (Fig. 1L), but quantitative immunohistochemistry (qIHC) analyses provided necessary spatial and temporal information. For example, laminin 5 and β4 integrin are expressed in healthy JE cells facing the tooth surface (n = 6; Fig. 1M, N), but after LIP for 3 d, protein expression in the JE was abolished (Fig. 1O, P). Instead, laminin 5 and β4 integrin were expressed in keratinocytes migrating across the wounded mucosa (Fig. 1O, P). This shift of hemidesmosomal protein expression in the wound basement membrane instead of the JE was observed from post-LIP days 5 through 15 (Fig. 1Q–V; quantified in X, Y).

Loss of the JE attachment apparatus was followed by a significant increase in $CD45^+$ leukocytes within the wound site (Appendix Fig. 3A–E). Myeloperoxidase (MPO)–positive neutrophils followed a similar trend, being abundant in an intact JE and then significantly elevated after ligature placement (Appendix Fig. 3F–J). Accompanying JE breakdown and widespread inflammation was a significant increase in tartrate-resistant acid phosphatase (TRAP)–positive osteoclast-mediated bone resorption (Appendix Fig. 3K–O), with the expected increase in ABC-CEJ (ABC: Alveolar Bone Crest, CEJ: Cement Enamel Junction) distance (Appendix Fig. 3P–R).

Wnt-Responsive Cells Are Lost during LIP

A Wnt lineage tracer strain $Axin2^{CreERT2/+};R26R^{mTmG/+}$ was used to follow the fates of an initial population of Wnt-responsive cells in the JE (n = 12; Appendix Fig. 4A). In an intact control, the first GFP^+ Wnt-responsive cells were positioned along the basement membrane; these GFP^+ cells divided until progeny from the initial Wnt-responsive population occupied the entire JE by day 5 (Appendix Fig. 4B–D). Stemness was demonstrated by the persistence of GFP^+ cells despite the tissue's high rate of turnover (Appendix Fig. 4E). The same lineage-tracing strategy was used to evaluate how LIP impacted the Wnt-responsive niche.

Compared to GFP staining in an intact JE (Fig. 2A), most GFP^+ cells were lost by day 3 of LIP (n = 3; Fig. 2B). Over the course of 7 d, the remaining GFP^+ cells were eliminated (Fig. 2C, D). By post-LIP day 15, no GFP^+ cells were detected in pocket epithelium (Fig. 2E).

A second series of experiments verified that descendants of the original Wnt-responsive stem cell population were lost after LIP. As before, tamoxifen was delivered and ligatures were placed, but then, 10 days later, ligatures were removed (Appendix Fig. 4F). On post-LIP recovery day 10, only a few scattered GFP^+ cells were detected in the connective tissues (Appendix Fig. 4G, H).

Reepithelization Occurs in the Presence of a Ligature

The Wnt-responsive JE compartment lost its detectable Axin2-lineage cells after LIP, despite the high rate of epithelial cell proliferation. Relative to controls, epithelial cell proliferation gradually significantly increased over the course of ligature retention (n = 3; Fig. 2F–K).

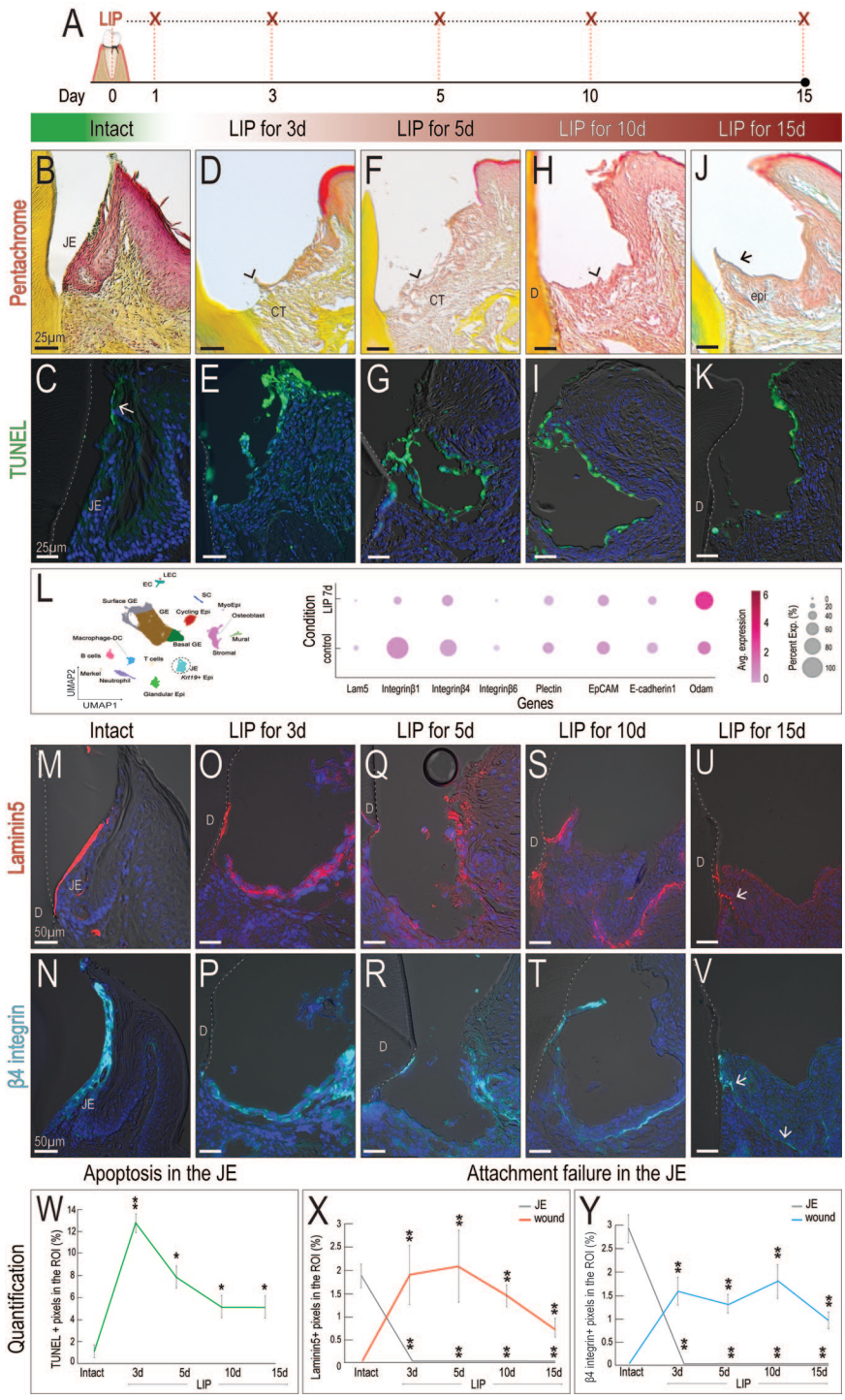

Figure 1. Ligature-induced periodontitis (LIP) causes epithelial apoptosis and disrupts the junctional epithelium (JE) barrier function. (**A**) Experimental design; tissues were collected at the time points indicated. (**B**) Pentachrome histology and (**C**) terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of intact control JEs. (**D**) Pentachrome histology and (**E**) TUNEL staining at post-LIP day 3. (**F**) Pentachrome histology and (**G**) TUNEL staining at post-LIP day 5. (**H**) Pentachrome histology and (**I**) TUNEL staining at post-LIP day 10. (**J**) Pentachrome histology and (**K**) TUNEL staining at post-LIP day 15. (**L**) Single-cell RNA sequencing analysis of attachment gene expression in controls and in samples from post-LIP day 7. Left: UMAP dimensional reduction and annotation. Right: Dot plot of average gene expression (color-coded) and percentage of cells expressing each gene (dot size). (**M**) Laminin 5 and (**N**) β4 integrin expression in intact controls. (**O**) Laminin 5 and (**P**) β4 integrin expression at post-LIP day 3. (**Q**) Laminin 5 and (**R**) β4 integrin expression at post-LIP day 5. (**S**) Laminin 5 and (**T**) β4 integrin expression at post-LIP day 10. (**U**) Laminin 5 and (**V**) β4 integrin expression at post-LIP day 15. Quantification of (**W**) apoptotic cells, (**X**) laminin 5–expressing cells, and (**Y**) β4 integrin–expressing cells, all in the JE and wound site. Arrows: wounded epithelium.

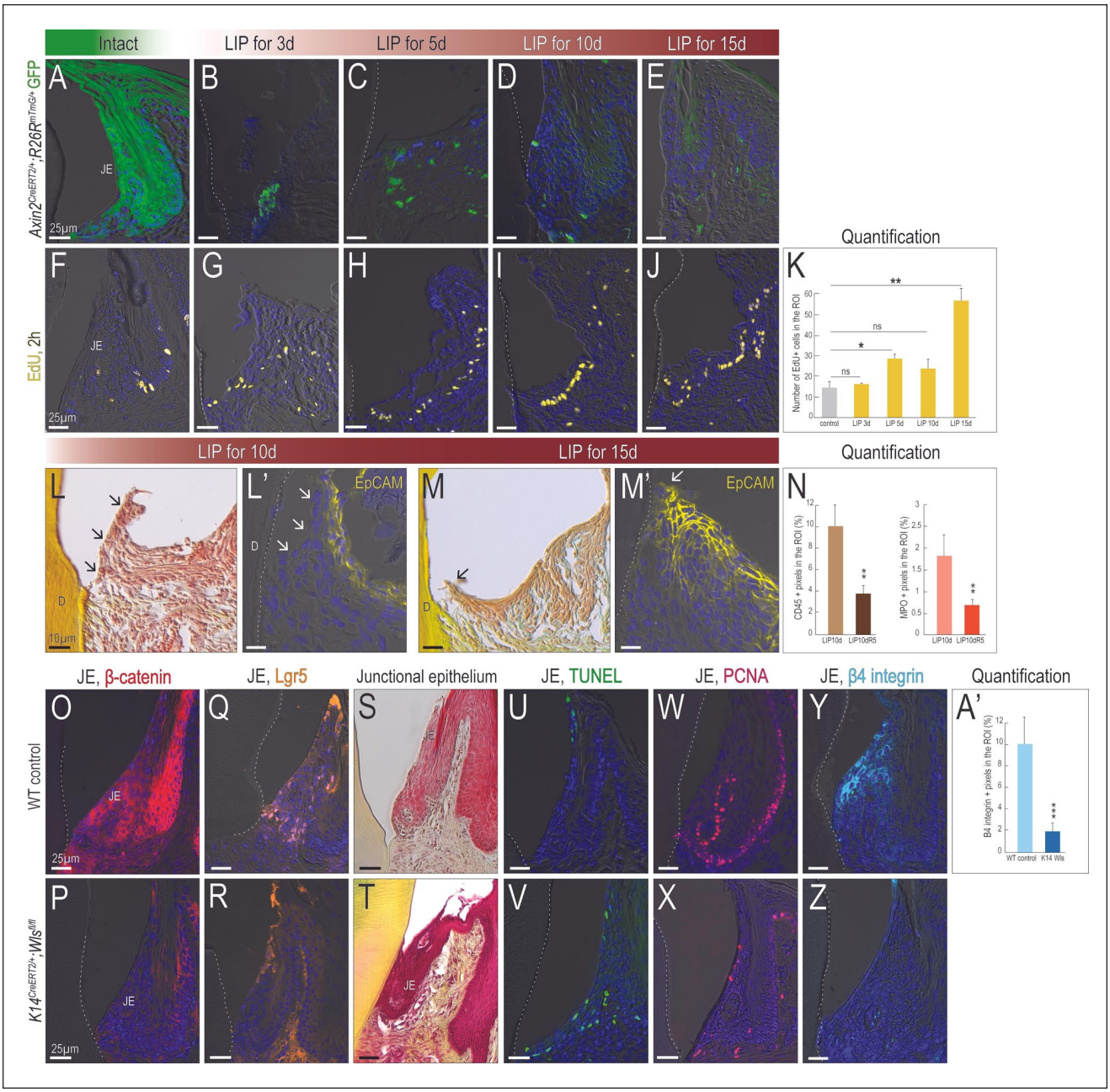

Figure 2. Wnt signaling is disrupted by ligature-induced periodontitis (LIP). *Axin2*$^{CreERT2/+}$;*R26R*$^{mTmG/+}$ mice were used to fate-map Wnt-responsive cells (**A**) in an intact junctional epithelium (JE) and (**B–E**) after LIP. Green fluorescent protein (GFP) immunostaining identified progeny of a Wnt-responsive population on post-LIP (B) day 3 (C) day 5, (D) day 10, and (E) day 15. Distribution of EdU^{+} cells after a 2-h chase in (**F**) intact JE and on post-LIP (**G**) day 3 (**H**) day 5, (**I**) day 10, and (**J**) day 15. (**K**) Quantification of EdU^{+} cell number in the region of interest (ROI). (**L**) Pentachrome histology and (**L′**) EpCAM expression at post-LIP day 10 and on (**M, M′**) post-LIP day 15. (**N**) Quantification of CD45^{+} cells (brown) and myeloperoxidase (MPO)–positive cells (red) on post-LIP day 10 versus post-LIP day 10 with 5 d of recovery. Comparison between wild-type (WT) control JE and JE from *K14*^{CreERT2}; *Wls*$^{fl/fl}$ mice analyzed for (**O, P**) β-catenin and (**Q, R**) Lgr5 expression. (**S, T**) Pentachrome staining, (**U**,**V**) terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, (**W**, **X**) proliferating cell nuclear antigen (PCNA) expression, and (**Y, Z**) β4 integrin expression. (**A′**) Quantification of β4 integrin-expressing cells in WT control JE versus *K14*^{CreERT2}; *Wls*$^{fl/fl}$ JE. D, dentin. Arrows: wounded epithelium or EpCAM^{+} cells. Statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001; ns, not significant.

Between post-LIP days 10 and 15, the ligature-induced wound site had reepithelialized, shown by histology and expression of the epithelial cell adhesion molecule, EpCAM (Fig. 2L–M). Commensurate with this reepithelialization was a significant reduction in inflammatory cells in the underlying connective tissues (Fig. 2N and Appendix Fig. 5A–C) and a

significant reduction in osteoclastic activity around the alveolar bone (Appendix Fig. 5D, E).

A Loss-of-Function Strategy Demonstrates Wnt Signaling Is Required for JE Barrier Function

These data demonstrated a correlation between loss of Wnt-responsive cells and loss of JE barrier functions, but fell short of demonstrating a causal link between Wnt signaling and JE barrier dysfunction. We therefore turned to a genetic approach to inhibit Wnt signaling in epithelial cells, including the JE, using a *K14Cre* strain of mice (Vasioukhin et al 1999).

Using $K14^{CreERT2}$; $R26R^{mTmG}$ mice, we validated that the Cre driver was specific to epithelial cells, including the JE (Appendix Fig. 6A), then generated $K14^{CreERT2};Wls^{fl/fl}$ mice carrying a conditional deletion in the chaperone gene, *Wntless* (*Wls*). Lipid-modified Wnt proteins require Wntless for their secretion (Zhong et al 2012); consequently, *Wls* inactivation blocks Wnt release. $K14^{CreERT2}$; $Wls^{fl/fl}$ mice were treated with tamoxifen, and 7 d later, the absence of β-catenin expression (Fig. 2O, P) and reduction in Lgr5 expression (Fig. 2Q, R) in the *Wls* mutant confirmed Wnt signaling inhibition in the JE. The *Wls* oral epithelium was also affected (Appendix Fig. 6B); this phenotype is being assessed separately.

Within 7 d of *Wls* deletion, the mutant JE was juxtaposed to cementum rather than the enamel space (Fig. 2S, T). Cell death was significantly elevated (Fig. 2U, V) while proliferation was significantly reduced (Fig. 2W, X) in the *Wls* mutant JE. These cellular changes were accompanied by significantly reduced expression of β4 integrin (compare Fig. 2Y, Z; quantified in A′).

A Therapeutic Strategy to Amplify Endogenous Wnt Signaling

Genetic data supported the conclusion that loss of Wnt signaling caused JE barrier dysfunction, and single-cell RNA sequencing (scRNAseq) data confirmed that LIP was associated with a reduction in expression of Wnt pathway components (Fig. 3A). These data raised the possibility that reinitiating Wnt signaling might restore some barrier functions to pocket epithelium. To test this possibility, purified WNT3A protein was stabilized with a lipid nanoparticle (Dhamdhere et al 2014). This formulation (e.g., liposomal WNT3A [L-WNT3A]) was tested in a cell-based Wnt activity assay (Liu et al 2006) to determine a starting dose of 0.2 µg/mL (Fig. 3B). The dosing interval depended on L-WNT3A stability at 37°C. Testing in the same LSL reporter assay, L-WNT3A showed no significant loss of activity over a 24-h period (Fig. 3C); consequently, a daily dosing regimen was set.

Our third step was to validate that L-WNT3A was internalized by cells via endocytosis (Fig. 3D) and, fourth, to demonstrate that L-WNT3A was capable of activating Wnt signaling. This latter step involved treating Wnt reporter cells with liposomal PBS (control) or L-WNT3A, then assaying for the expression of the target protein luciferase (Fig. 3E, F). Finally, using a Wnt reporter strain (e.g., $Axin2^{LacZ/+}$ mice; Lustig et al 2002), JEs topically treated with L-WNT3A showed significantly more $Xgal^+$ staining than PBS controls, on par with the distribution of Xgal staining in intact controls (Fig. 3H–J). These results support the conclusion that topical L-WNT3A was capable of activating endogenous canonical Wnt signaling in vivo.

L-WNT3A Treatment Restores a Subset of Early Barrier Functions to Pocket Epithelium

With these data in hand, we returned to the LIP model. Ligatures were placed for 10 d, then removed, and either topical L-WNT3A or topical L-PBS treatment was initiated. Tissues were collected for analyses on post-treatment days 5 and 7 to assess early tissue repair dynamics.

Five days into the recovery phase, pocket epithelia in both groups were juxtaposed with cementum surfaces (Fig. 4A, B), indicating damage to the attachment caused by LIP. Compared to PBS-treated cases, however, L-WNT3A–treated JEs had significantly more proliferating cells (Fig. 4C, D) and significantly higher expression of β4 integrin (Fig. 4E–H), with quantification shown in Figure 4I.

$Axin2^{CreERT2/+};R26R^{mTmG/+}$ mice were used to fate-map cells that responded to the L-WNT3A stimulus by delivering tamoxifen on the day of ligature removal. GFP immunostaining was performed 5 d later. For reference, an intact control JE was fully occupied by GFP^+ cells after a 5-d chase (Fig. 4J). In PBS-treated cases, pocket epithelium was devoid of GFP^+ cells (Fig. 4K). In L-WNT3A–treated cases, the epithelium was populated by GFP^+ cells (Fig. 4L).

If L-WNT3A restored some barrier features to pocket epithelium, we reasoned that local inflammation should be reduced. In intact controls, $CD45^+$ leukocytes are typically distributed throughout a healthy JE and in underlying connective tissues (Fig. 4M). In PBS-treated cases, a dense infiltrate of $CD45^+$ cells was evident throughout the connective tissue and pocket epithelium (Fig. 4N). L-WNT3A–treated samples showed a distribution of $CD45^+$ cells similar to the intact JE (Fig. 4O; quantified in S).

The cell–cell adhesion molecule EpCAM is strongly expressed in an intact JE (Fig. 4P); its expression was significantly reduced in PBS-treated samples that underwent LIP (Fig. 4Q; quantified in S). In L-WNT3A–treated cases, EpCAM expression was restored to levels seen in an intact JE (Fig. 4R; quantified in S). Collectively, these data supported the conclusion that daily topical delivery of L-WNT3A for 7 d restored some barrier features to pocket epithelium.

Discussion

Conversion of Junctional Epithelium into Pocket Epithelium

A defining feature of periodontitis is the conversion of a junctional epithelium into pocket epithelium (Page et al 1978; Schroeder and Lindhe 1980). This pathological conversion has

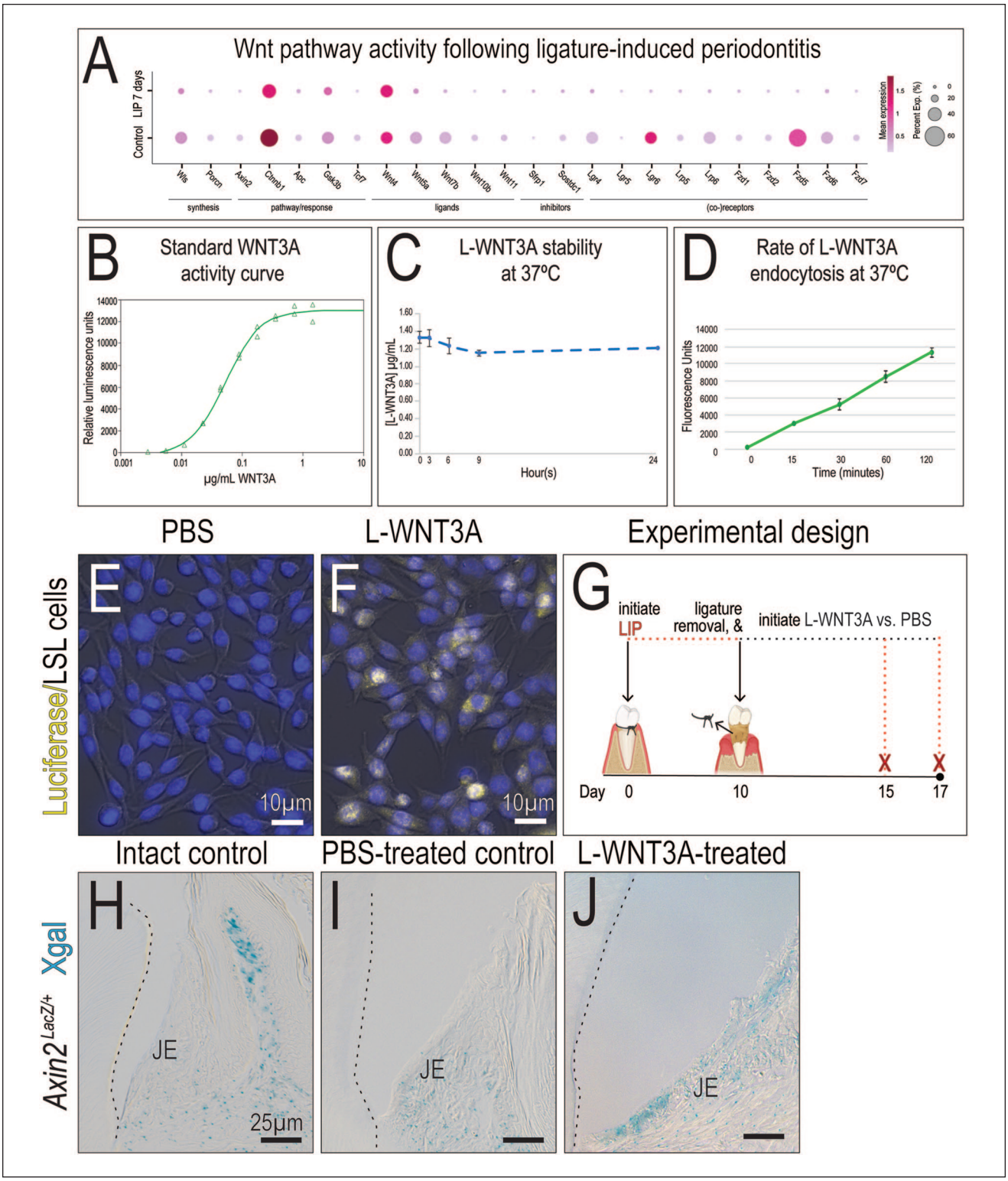

Figure 3. Wnt signaling is affected by ligature-induced periodontitis (LIP) and a biochemical method to alter Wnt signaling. (**A**) Single-cell RNA sequencing analysis of the junctional epithelium (JE) in controls and after 7 d of LIP, showing dot plots where the percentage of cells expressing each gene is reflected in dot size and average gene expression is color-coded. (**B**) Standard WNT3A activity curve. (**C**) L-WNT3A stability at 37°C. (**D**) Rate of L-WNT3A endocytosis at 37°C. LSL cells treated with (**E**) phosphate-buffered saline (PBS) or with (**F**) L-WNT3A followed by immunostaining for luciferase. (**G**) Experimental design where LIP was initiated on day 0, ligatures were removed on day 10, and daily L-WNT3A or PBS treatment was initiated; tissues were collected for analyses after 5 and 7 d of treatment. To visualize the distribution of Wnt-responsive cells, Xgal staining in *Axin2*$^{LacZ/+}$ mice was employed. Xgal staining in (**H**) untreated controls, (**I**) PBS-treated cases, and (**J**) L-WNT3A–treated cases.

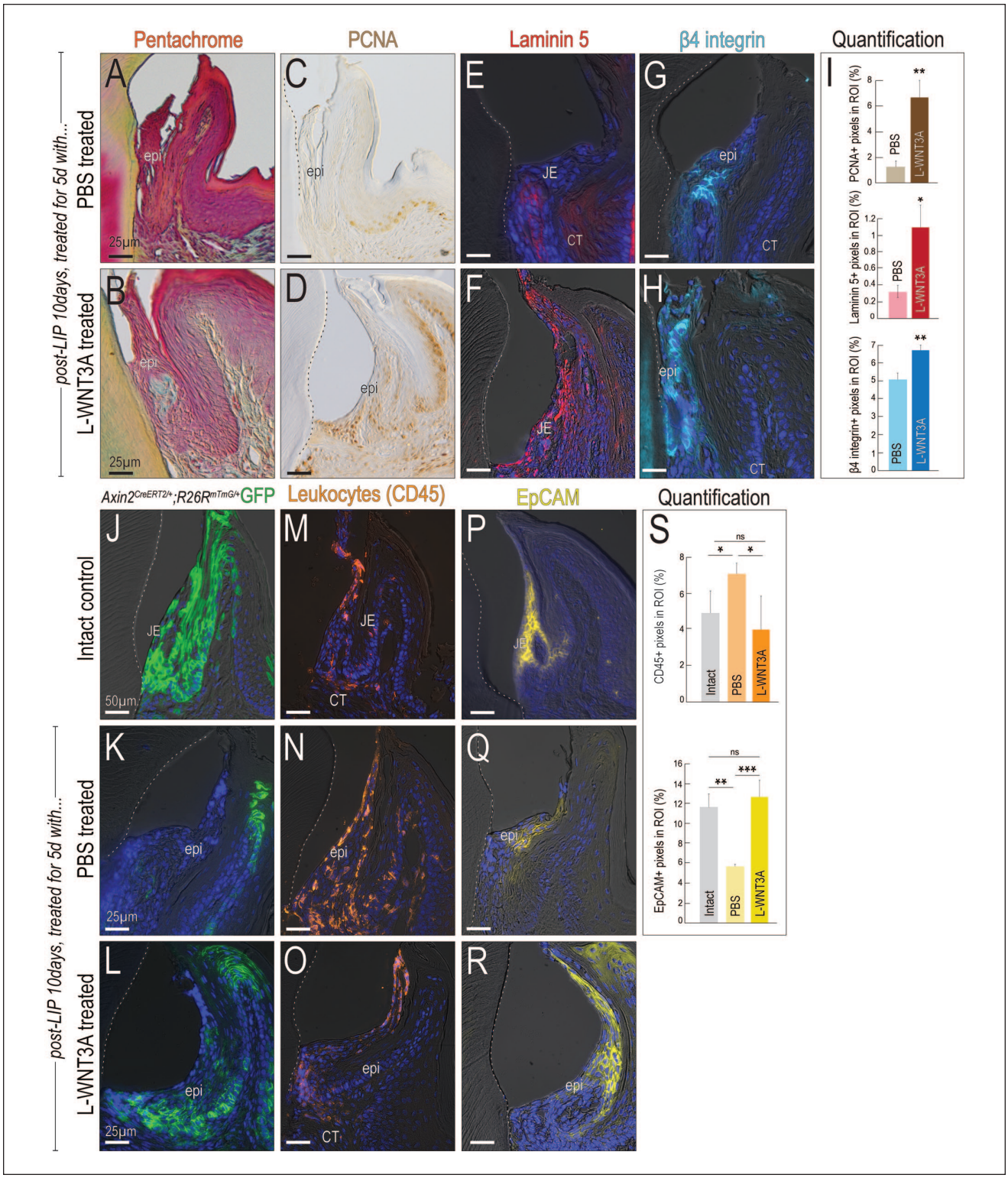

Figure 4. L-WNT3A restores some early barrier functions to pocket epithelium. (**A, B**) Pentachrome histology. (**C, D**) Proliferating cell nuclear antigen (PCNA) expression. (**E, F**) Laminin 5 expression. (**G, H**) β4 Integrin expression in phosphate-buffered saline (PBS)–treated and L-WNT3A–treated cases. (**I**) Quantification of PCNA$^+$ cells, laminin 5$^+$ cells, and β4 integrin$^+$ cells in PBS-treated versus L-WNT3A–treated cases. GFP immunostaining in tissues from *Axin2*$^{CreERT2/+}$;*R26R*$^{mTmG/+}$ mice that were (**J**) untreated, intact controls or from (**K**) PBS-treated versus (**L**) L-WNT3A–treated groups. CD45 immunostaining in (**M**) untreated, intact controls or (**N**) PBS-treated versus (**O**) L-WNT3A–treated groups. EpCAM immunostaining in (**P**) untreated, intact controls or (**Q**) PBS-treated versus (**R**) L-WNT3A–treated groups. (**S**) Quantification of CD45$^+$ cells and EpCAM$^+$ cells across intact, PBS-treated, and L-WNT3A–treated conditions. CT, connective tissue; epi, epithelium; JE, junctional epithelium. Statistical significance: **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns, not significant.

been the subject of investigation for decades (Becks 1929; Takata and Donath 1988); consequently, there are multiple hypotheses regarding the factor(s) that induce this conversion (reviewed recently in Theodoro et al 2023). Here, we made use of an LIP model in mice to gain new insights into this transformation.

Widespread Apoptosis Is Coupled with Proliferation in Pocket Epithelium

In an LIP model, one of the first steps of a JE transitioning into pocket epithelium was widespread apoptosis (Fig. 1). Normally, programmed cell death in the JE is restricted to the coronal tip (Yuan et al 2020b), but in response to ligature placement, apoptotic epithelial cells were abundant (Fig. 1). Apoptosis was accompanied by extensive epithelial cell proliferation (Figs. 1, 2). This mitotic activity culminated in reepithelization of the wound, even in the presence of the ligature (Fig. 2). Thus, in our LIP model, pocket epithelium was generated by post-LIP day 10 (Fig. 2). Similar results have been reported in canine models of LIP, where evidence of reepithelialization prompted the authors to speculate that a periodontal pocket may harbor a repair potential (Schroeder and Lindhe 1980). Similar findings of apoptosis coupled with proliferation have also been reported in patients, where apoptotic cells were found near a periodontally involved JE, and proliferating keratinocytes were identified in the basal layers of epithelium (Jarnbring et al 2002).

Molecular Features of an Attachment Apparatus Are Missing in Pocket Epithelium

Within days of ligature placement, expression of hemidesmosomal proteins shifted from the JE to the basement membrane damaged by the ligature (Fig. 1). This spatial shift in expression domains, together with extensive apoptosis, suggested that the attachment apparatus was destroyed (Fig. 1). An extensive inflammatory infiltrate in the underlying connective tissue supported this interpretation (Appendix Fig. 3).

In silico studies have also reported on changes in adhesion gene expression after LIP (Gursoy et al 2016; Kondo et al 2023). Indeed, our own analyses of these publicly available databases confirm changes in attachment gene expression levels following LIP (Fig. 1). These transcriptomics data, however, should be interpreted with caution since the same genes/proteins mediating adherence of epithelial cells to a wounded basement membrane (Martin 1997) are also involved in mediating adherence of JE cells to a tooth surface (Hormia et al 1998). In the conversion of a JE to pocket epithelium, these spatial shifts in expression domains represent an alteration in the tissue's barrier functions, but this shift cannot be easily distinguished by transcriptomics data alone.

Pocket Epithelium Is Apically Positioned along the Root Surface

As periodontitis develops, pocket epithelial cells are positioned more apically along the root surface (Page et al 1978; Takata and Donath 1988). In a mouse model, this meant pocket epithelium juxtaposed cementum, as compared to a JE that attaches to enamel (Figs. 1, 2 and reviewed in Donos et al 2018). Apical displacement of pocket epithelium appears to be a passive process: increased matrix metalloproteinase activity (Sorsa et al 2016) is thought to weaken the supracrestal connective tissue compartment, which allows pocket epithelial cells to be displaced or migrate apically. MMPs are also expressed in a healthy JE (Liu et al 2025), however, so the presence or absence of MMPs should not be considered a defining feature of pocket epithelium.

Loss of Wnt Signaling and Conversion of a JE into Pocket Epithelium

An LIP model allowed us to establish a longitudinal characterization of JE breakdown, beginning with extensive epithelial apoptosis, downregulation of attachment proteins, and loss of Wnt-responsive cells and their progeny. Breakdown was followed by repair: previously, we showed that LIP triggers epithelial-to-mesenchymal transition in the JE, a physiologic process mediated by BMP and Wnt signaling (Liu et al 2025) that contributes to reepithelialization of the wound.

Reepithelialization does not equate with regeneration of the original attachment apparatus (Fig. 2). Following periodontal treatment, healing often results in a long junctional epithelium, which represents epithelial repair rather than regeneration of the original connective tissue attachment (Stahl 1979; Susin et al 2015). Whether this reparative epithelial attachment has the same durability and barrier properties as a native JE remains incompletely understood (Garnick 1977; Renvert and Persson 2004). This distinction is clinically important: a long junctional epithelium may provide a stable repaired attachment, but it should not be equated with true periodontal regeneration. By contrast, residual periodontal pockets lined by inflamed pocket epithelium remain at increased risk for further breakdown (Matuliene et al 2008).

In a mouse model, one can use a genetic approach to establish a causal relationship between Wnt signaling and JE homeostasis. When an epithelial-specific source of Wnt signaling was blocked, multiple hallmarks of pocket epithelium replaced normal JE barrier functions (Fig. 2). The precise feature(s) of pocket epithelium versus long JE versus healthy JE remain to be elucidated, but the fact that a molecular mechanism can control barrier features of an epithelium provides a direction for therapeutic interventions.

Can Pocket Epithelium Be Reverted Back into a Functioning Junctional Epithelium?

We speculated that pocket epithelium might gain some of its lost barrier functions via manipulation of the same biological signaling pathway involved in the transformation of a JE into pocket epithelium. Daily exposure to a topical L-WNT3A (Fig. 3) stimulated Wnt responsiveness in pocket epithelial cells and underlying connective tissue (Figs. 3, 4) and upregulated expression of attachment proteins laminin 5 and β4

integrin (Fig. 4). Whether additional proteins involved in the attachment apparatus are also expressed in L-WNT3A–treated samples remains to be determined, and longer-term studies will be required to assess the durability of these changes.

Finally, the restoration of hemidesmosomal protein expression alone does not support claims of a functional reconstruction of the epithelial barrier; if L-WNT3A meaningfully restored barrier integrity, then corresponding reductions in inflammatory cell infiltration and neutrophil accumulation would be expected. We did observe a significant reduction in leukocyte infiltration in the L-WNT3A–treated samples compared to the PBS treatment (Fig. 4). Consequently, it remains to be seen whether L-WNT3A treatment alone is sufficient to return inflammatory marker levels back to those seen in an intact JE.

Limitations of the Current Study

In choosing a preclinical model for this study, the following criteria were used: biological similarities between the animal and human periodontal soft tissues, the availability of genetic models, ethical considerations related to the use of companion animals, and cost, which directly influences sample sizes and thus the reproducibility and reliability of results. Although no single preclinical model met all criteria, a mouse model was selected after careful consideration.

There are inherent limitations in using animals to study human diseases (Donos et al 2018) and with the LIP model itself. Placement of a ligature causes acute, traumatic ulceration of the JE (Rovin et al 1966); consequently, other factors involved in initial JE breakdown are challenging to identify. Nonetheless, the model also has an advantage: the onset of periodontal breakdown is unambiguous, meaning that molecular and cellular steps in JE conversion to pocket epithelium can be monitored longitudinally.

There are limitations to L-WNT3A experiments. First, only 2 endpoints were analyzed, and both focused on early repair dynamics. To support a claim that L-WNT3A treatment results in durable restoration of barrier functions, longer endpoints are required. Second, topical L-WNT3A increases the number of Wnt-responsive cells, but the source(s) of these cells were not identified. Mechanistic insights into the potential therapeutic effects of L-WNT3A on pocket epithelium will require a better understanding of this important issue.

Conclusions

Using an LIP model, a sequence of molecular and cellular events that convert a JE into a pocket epithelium was characterized. One event included the loss of detectable Axin2-lineage cells within the Wnt-responsive JE compartment, suggesting that disrupted Wnt signaling might contribute to loss of JE barrier functions. A genetic model established a causal link between loss of Wnt signaling from JE cells and loss of JE barrier functions. A WNT protein-based strategy was tested for its ability to rebuild barrier features absent from pocket epithelium. Early tissue repair dynamics were promising, but longer time points will be required before concluding that barrier functions can be fully restored using a WNT therapeutic.

Author Contributions

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Declaration of Conflicting Interests

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Data Availability

The raw data underlying all figures and analyses in this article, including original data from all experimental repeats, have been deposited in the Zenodo repository at https://doi.org/10.5281/zenodo.18487493. These data are provided in a format that enables reproduction of the analyses and figures presented in the article. The same datasets are also provided as Appendix files.

References

Aellos F et al. 2025. 5-FU weakens defensive functions of the junctional epithelium. J Periodontal Res. 61(1):66–75. https://doi.org/10.1111/jre.13375

Ando Y et al. 2024. The neutrophil-osteogenic cell axis promotes bone destruction in periodontitis. Int J Oral Sci. 16(1):18. https://doi.org/10.1038/s41368-023-00275-8

Becks HM. 1929. Normal and pathologic pocket formation. J Am Dent Assoc. 16(12):2167–2188. https://doi.org/10.14219/jada.archive.1929.0328

Caton J, Nyman S. 1980. Histometric evaluation of periodontal surgery. I. The modified Widman flap procedure. J Clin Periodontol. 7(3):212–223. https://doi.org/10.1111/j.1600-051x.1980.tb01964.x

Caton JG, Zander HA. 1979. The attachment between tooth and gingival tissues after periodic root planing and soft tissue curettage. J Periodontol. 50(9):462–466. https://doi.org/10.1902/jop.1979.50.9.462

Dhamdhere GR et al. 2014. Drugging a stem cell compartment using Wnt3a protein as a therapeutic. PLoS One. 9(1):e83650. https://doi.org/10.1371/journal.pone.0083650

Donos N, Park JC, Vajgel A, de Carvalho Farias B, Dereka X. 2018. Description of the periodontal pocket in preclinical models: limitations and considerations. Periodontol 2000. 76(1):16–34. https://doi.org/10.1111/prd.12155

Garnick JJ. 1977. Long junctional epithelium: epithelial reattachment in the rat. J Periodontol. 48(11):722–729. https://doi.org/10.1902/jop.1977.48.11.722

Gursoy UK et al. 2016. Analyses of gingival adhesion molecules in periodontitis: theoretical in silico, comparative in vivo, and explanatory in vitro models. J Periodontol. 87(2):193–202. https://doi.org/10.1902/jop.2015.150361

Hormia M, Sahlberg C, Thesleff I, Airenne T. 1998. The epithelium-tooth interface—a basal lamina rich in laminin-5 and lacking other known laminin isoforms. J Dent Res. 77(7):1479–1485. https://doi.org/10.1177/00220345980770070201

Jarnbring F, Somogyi E, Dalton J, Gustafsson A, Klinge B. 2002. Quantitative assessment of apoptotic and proliferative gingival keratinocytes in oral and sulcular epithelium in patients with gingivitis and periodontitis. J Clin Periodontol. 29(12):1065–1071.

Jeon HH et al. 2024. Inflammation and mechanical force-induced bone remodeling. Periodontol 2000. [published online ahead of print]. https://doi.org/10.1111/prd.12619

Kondo T, Gleason A, Okawa H, Hokugo A, Nishimura I. 2023. Mouse gingival single-cell transcriptomic atlas identified a novel fibroblast subpopulation activated to guide oral barrier immunity in periodontitis. Elife. 12:RP88183. https://doi.org/10.7554/elife.88183

Liu H, Thurig S, Mohamed O, Dufort D, Wallace VA. 2006. Mapping canonical Wnt signaling in the developing and adult retina. Invest Ophthalmol Vis Sci. 47(11):5088–5097. https://doi.org/10.1167/iovs.06-0403

Liu B et al. 2025. BMP/WNT-dependent mechanisms of junctional epithelium turnover. J Dent Res. 105(4):527–536. https://doi.org/10.1177/00220345251364162

Lustig B et al. 2002. Negative feedback loop of Wnt signaling through upregulation of conductin/axin2 in colorectal and liver tumors. Mol Cell Biol. 22(4):1184–1193. https://doi.org/10.1128/mcb.22.4.1184-1193.2002

Magnusson I, Runstad L, Nyman S, Lindhe J. 1983. A long junctional epithelium—a locus minoris resistentiae in plaque infection? J Clin Periodontol. 10(3):333–340. https://doi.org/10.1111/j.1600-051x.1983.tb01282.x

Marks SC Jr, McKee MD, Zalzal S, Nanci A. 1994. The epithelial attachment and the dental junctional epithelium: ultrastructural features in porcine molars. Anat Rec. 238(1):1–14. https://doi.org/10.1002/ar.1092380102

Martin P. 1997. Wound healing—aiming for perfect skin regeneration. Science. 276(5309):75–81. https://doi.org/10.1126/science.276.5309.75

Matuliene G et al. 2008. Influence of residual pockets on progression of periodontitis and tooth loss: results after 11 years of maintenance. J Clin Periodontol. 35(8):685–695. https://doi.org/10.1111/j.1600-051x.2008.01245.x

Nishio C, Wazen R, Kuroda S, Moffatt P, Nanci A. 2010. Expression pattern of odontogenic ameloblast-associated and amelotin during formation and regeneration of the junctional epithelium. Eur Cell Mater. 20:393–402. https://doi.org/10.22203/ecm.v020a32

Noguchi S et al. 2017. The histopathological comparison on the destruction of the periodontal tissue between normal junctional epithelium and long junctional epithelium. J Periodontal Res. 52(1):74–82. https://doi.org/10.1111/jre.12370

Page RC, Engel LD, Narayanan AS, Clagett JA. 1978. Chronic inflammatory gingival and periodontal disease. JAMA. 240(6):545–550.

Renvert S, Persson GR. 2004. Supportive periodontal therapy. Periodontol 2000. 36:179–195. https://doi.org/10.1111/j.1600-0757.2004.03680.x

Rovin S, Costich ER, Gordon HA. 1966. The influence of bacteria and irritation in the initiation of periodontal disease in germfree and conventional rats. J Periodontal Res. 1(3):193–204. https://doi.org/10.1111/j.1600-0765.1966.tb01860.x

Sakai T et al. 1999. Age-dependent changes in the distribution of BrdU- and TUNEL-positive cells in the murine gingival tissue. J Periodontol. 70(9):973–981. https://doi.org/10.1902/jop.1999.70.9.973

Schroeder HE, Lindhe J. 1980. Conditions and pathological features of rapidly destructive, experimental periodontitis in dogs. J Periodontol. 51(1):6–19. https://doi.org/10.1902/jop.1980.51.1.6

Sorsa T et al. 2016. Analysis of matrix metalloproteinases, especially MMP-8, in gingival creviclular fluid, mouthrinse and saliva for monitoring periodontal diseases. Periodontol 2000. 70(1):142–163. https://doi.org/10.1111/prd.12101

Stahl SS. 1979. Repair or regeneration following periodontal therapy? J Clin Periodontol. 6(6):389–396. https://doi.org/10.1111/j.1600-051x.1979.tb01937.x

Stern IB. 1981. Current concepts of the dentogingival junction: the epithelial and connective tissue attachments to the tooth. J Periodontol. 52(9):465–476. https://doi.org/10.1902/jop.1981.52.9.465

Susin C et al. 2015. Wound healing following surgical and regenerative periodontal therapy. Periodontol 2000. 68(1):83–98. https://doi.org/10.1111/prd.12057

Takata T, Donath K. 1988. The mechanism of pocket formation. A light microscopic study on undecalcified human material. J Periodontol. 59(4):215–221. https://doi.org/10.1902/jop.1988.59.4.215

Theodoro LH et al. 2023. Role of junctional epithelium in maintaining dentogingival adhesion and periodontal health. Front Dent Med. 4:1144537. https://doi.org/10.3389/fdmed.2023.1144537

Vasioukhin V, Degenstein L, Wise B, Fuchs E. 1999. The magical touch: genome targeting in epidermal stem cells induced by tamoxifen application to mouse skin. Proc Natl Acad Sci U S A. 96(15):8551–8556. https://doi.org/10.1073/pnas.96.15.8551

Ye P, Chapple CC, Kumar RK, Hunter N. 2000. Expression patterns of e-cadherin, involucrin, and connexin gap junction proteins in the lining epithelia of inflamed gingiva. J Pathol. 192(1):58–66. https://doi.org/10.1002/1096-9896(2000)9999:9999%3C::aid-path673%3E3.0.co;2-t

Yuan X et al. 2020a. The junctional epithelium is maintained by a stem cell population. J Dent Res. 100(2):209–216. https://doi.org/10.1177/0022034520960125

Yuan X et al. 2020b. Formation and regeneration of a Wnt-responsive junctional epithelium. J Clin Periodontol. 47(12):1476–1484. https://doi.org/10.1111/jcpe.13371

Yuan X et al. 2023. Linking the mechanics of chewing to biology of the junctional epithelium. J Dent Res. 102(11):1252–1260. https://doi.org/10.1177/00220345231185288

Zhong Z et al. 2012. Wntless functions in mature osteoblasts to regulate bone mass. Proc Natl Acad Sci U S A. 109(33):E2197–2204. https://doi.org/10.1073/pnas.1120407109