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Research Article

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TGF-β1+ memory B cells regulate macrophage polarization in periodontitis

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Abstract: Background: Periodontitis is a chronic inflammatory disease with high incidence and prevalence, leading to periodontal inflammation and tooth mobility. Microbial infection and host immune responses are the primary causative factors of periodontal tissue damage and progression. However, the intricate inflammatory reactions and dynamic changes of cell subsets in the immune microenvironment of periodontitis have not been fully clarified. Methods: To analyze the microenvironmental characteristics of inflammatory reactions in periodontal lesions, we performed single-cell transcriptome analysis, histology, immunohistochemistry, and flow cytometry on samples from three healthy volunteers and four patients with periodontitis, mapping single cells and identifying cell subtypes and their roles in the periodontal microenvironment. Results: Single-cell transcriptomic analysis showed that memory B cells located in periodontitis lesion microenvironments highly expressed transforming growth factor-β1(TGF-β1) and modulated macrophage phenotypic transformation, thereby promoting alveolar bone loss. In addition, cell-cell crosstalk involving macrophages and B lymphocytes was strengthened within inflamed periodontal tissues, which aggravated the damage to periodontal tissue. Conclusions: Based on single-cell sequencing datasets, we constructed a refined landscape of human gingival cellular subsets and cell interaction networks. Meanwhile, we uncovered previously unrecognized pro-inflammatory cell populations closely linked to chronic periodontitis, laying a foundation for advancing host-targeted therapeutic strategies.

Key words: Single cell transcriptome; Memory B cells; TGF-β1; Macrophage polarization; Periodontitis

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1 Introduction

As a chronic tissue infection initiated by bacterial colonization and invasion, periodontitis constitutes one of the most prevalent oral disorders leading to adult tooth loss (Trindade et al., 2023). Disease onset and progression are attributed to the interplay of unique microbial ecological changes, impaired host

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immune regulation, and external environmental hazards (Baima et al., 2025). Core diagnostic features include gingival bleeding, periodontal probing depths, attachment loss and alveolar bone destruction (Wang et al., 2023). Beyond oral destruction, periodontitis has been epidemiologically linked to several systemic comorbidities, such as cardiovascular pathologies, diabetes mellitus, and neurodegenerative Alzheimer's disease (Salhi et al., 2023, Ge et al., 2024). Standard treatment regimens aim to control ongoing infection and inflammation, thereby protecting periodontal soft and hard tissues from further deterioration. Nonetheless, currently available therapeutic modalities often fail to yield sustained clinical improvement and prevent long-term recurrence.

During the progression of periodontitis, immune cells work in concert with epithelial and stromal cells to limit microbial invasion of the periodontium (Zimmerman et al., 2020). Immune cells, including macrophages (Xiao et al., 2018), B cells (Mahanonda et al., 2016), T cells (Tsukasaki et al., 2018), and plasma cells (Mahanonda et al., 2016), play a crucial role in the pathogenesis of periodontitis. Accumulating studies have verified that macrophages in the periodontal microenvironment can be divided into two distinct subsets. The severity of local inflammation is largely determined by the relative infiltration level of these two cell phenotypes. Of note, a phenotypic transition from M2 toward M1 macrophages can significantly aggravate the progression of alveolar bone loss (Yu et al., 2016, Yang et al., 2018). Following infection with *Porphyromonas gingivalis*, the body converts more macrophages to the M1 phenotype, enabling them to infiltrate the periodontal parenchyma and accelerate bone resorption (Lam et al., 2016). The polarization direction and residence behavior of macrophages are key variables in regulating the rhythm of periodontal destruction.

B lymphocytes exert context-dependent, dual functions in periodontitis, contributing to pathogen clearance and immune regulation while also promoting tissue destruction (Zouali 2017, Figueredo et al., 2019). Beyond antibody production, B cells act as antigen-presenting and cytokine-producing cells. In response to periodontitis-associated bacteria, B cells can produce IFN-γ, TNF-α, TGF-β and IL-10, indicating that they actively shape the local inflammatory milieu (Danielsen et al., 2023). B cells also participate directly in periodontal bone loss: B and T lymphocytes are major sources of RANKL within bone-resorptive periodontal lesions (Kawai et al., 2006), and B cell derived TGF-β1 suppresses osteoblast differentiation and contributes to alveolar bone loss (Chen et al., 2023). Recent studies further indicate that distinct B cell subsets can either restrain or amplify disease through macrophage interactions: B10 cells inhibit macrophage polarization and alleviate periodontal inflammation and bone loss (Cao et al., 2025), whereas, in obesity, periodontitis induces pathogenic B cell expansion and B-cell–macrophage crosstalk through the NLRP3/IL-18 axis, exacerbating glucose dysregulation (Lin et al., 2026). Nevertheless, whether TGF-β1+ memory B cells regulate macrophage polarization within local periodontal lesions remains insufficiently understood.

Single-cell RNA sequencing (scRNA-seq) enables specific analysis of cell populations at single-cell resolution, thereby enabling detailed description and analysis of different cell types and states (Lam et al., 2016, Liu et al., 2023). In this study, we used single-cell transcriptomics to comprehensively analyze and explore the effects of B cells on periodontal immune responses and macrophage polarization in vivo. We also determined the potential mechanisms by which B cells regulate the development of periodontitis through in vitro and in vivo experiments.

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2 Results

2.1 scRNA-seq map landscape of human periodontal tissues

This study set out to delineate the cellular composition of human periodontal tissue at single-cell resolution. Transcriptomic sequencing was therefore applied to tissue specimens collected from three healthy participants (HCs) and four periodontitis cases (PDs). (Fig. 1a). We first processed the original sequencing data with standardized workflows and strict quality filtering, and then obtained 69,386 valid single-cell transcriptomic profiles. With the uniform manifold approximation and projection (UMAP) dimensionality reduction implemented, unsupervised clustering further classified these cells into 11 clusters. We annotated 11 cell types (Fig. 1b), according to their canonical markers: B cells (BANK1, MS4A1 and TAGLN), T cells (CD3E, TRAC and CD3G), plasma cell (MZB1, XBP1 and IGHG1), epithelial cells (KRT5, KRT14 and KRT6A), fibroblasts (LUM, FAP and DCN), macrophages (LYZ, MS4A6A and CD68), mast cells (TPSAB1, TPA3 and MS4A2), endothelial cells (ENG, VWF and PECAM1), vascular mural cells (ACTA2, RGS5 and TAGLN), neural cells (SLC35F1, SHL1 and SOX10) and neutrophil cells (CXCL8, CSF3R and FCGR3B) (Fig. 1c). Eleven predominant subclusters were stably detectable in nearly all study subjects. We further compared the proportional distribution of these subclusters between HCs and PDs. (Fig. 1d). Next, we compared the composition of each identified cell cluster between the different groups. Relative to healthy periodontal tissue, the diseased tissue had significantly reduced proportions of epithelial and fibroblast cells, while B-cell and plasma-cell populations were notably expanded. (Fig. 1e).

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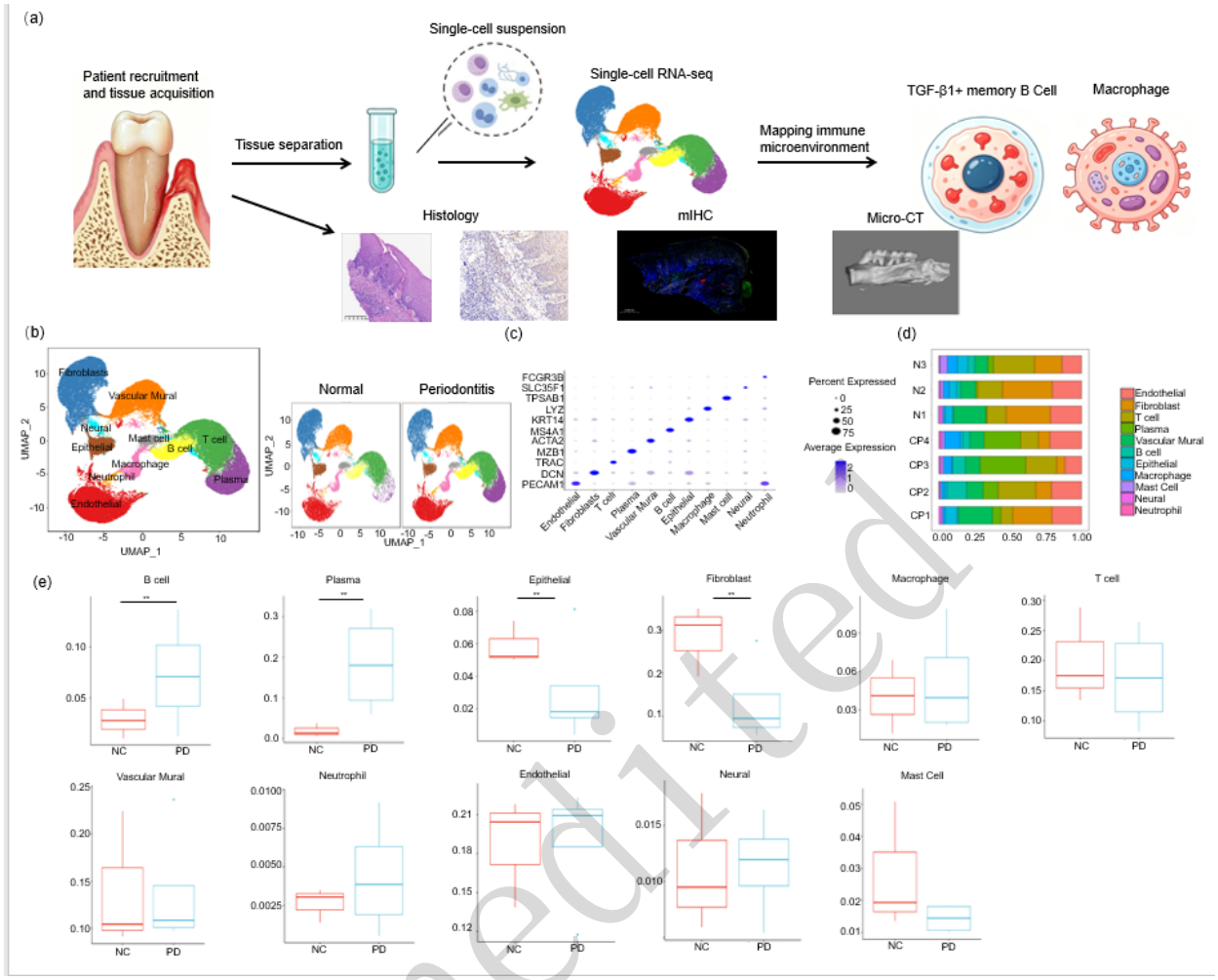


Fig. 1 Overview of the 69,386 single cells from periodontal tissues of healthy participants (HCs) and periodontitis patients (PDs).

(a) Study overview. (b) Uniform Manifold Approximation and Projection (UMAP) of the 69,386 cells, colored by cell-type annotation from left to right: the total corresponding donors and the different conditions (HCs and PDs). (c). Dotplot depicting gene expression levels and percentage of cells expressing genes associated with each cluster. (d) Proportions of the different subclusters in periodontal tissues of HCs and PDs. (e) Boxplots show the percentage of cells in each of the eleven clusters for normal and periodontitis tissues. Statistical analysis was performed using an unpaired two-tailed t test.

2.2 Heterogeneity of B cells in the periodontal tissue microenvironment

Immune cells are major contributors to chronic inflammatory development, and T as well as B lymphocytes stand out as essential regulatory players. Accumulated research data indicate plasma cells occupy about 50% of periodontitis lesional cells, compared with nearly 18% for B cells. Notably, B lymphocytes exhibit a higher relative proportion than T cells within these diseased tissue sites. Therefore, we further analyzed B cells in the periodontal tissue microenvironment (Fig. 2a). Among the B cell clusters, we identified memory B cells, naive B cells, transitional B cell, and plasmablasts (Fig. 2b-c). We observed a higher proportion of memory B cells in patients with periodontitis. In addition, naive B cells and plasmablasts were also significantly more abundant than in healthy controls. (Fig. 2d). We next used Monocle

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2.0 to build pseudotime trajectories and map the dynamic differentiation process of B cells. The resulting pseudotime trajectory started from pre/pro-B cells and then divided into two major branches, with two terminally differentiated cell types—memory B cells and plasmablasts-located at opposite ends (Fig. 2e). We further characterized dynamic transcriptional changes throughout B-cell maturation. Genes driving inflammatory signaling (JUN, LYN, NFKB2) and those mediating bone immune regulation (EGR1, PRKCB, ITGB1) all exhibited an initial increase followed by a gradual reduction in expression. (Fig. 2f-g). Interestingly, gene set enrichment analysis showed that memory B cell transcriptomic profiles in periodontitis patients were enriched in periodontitis-relevant signaling pathways relative to healthy controls. These pathways included TGF-β, MAPK, and RANKL-RANK signaling cascades (Fig. 2h).

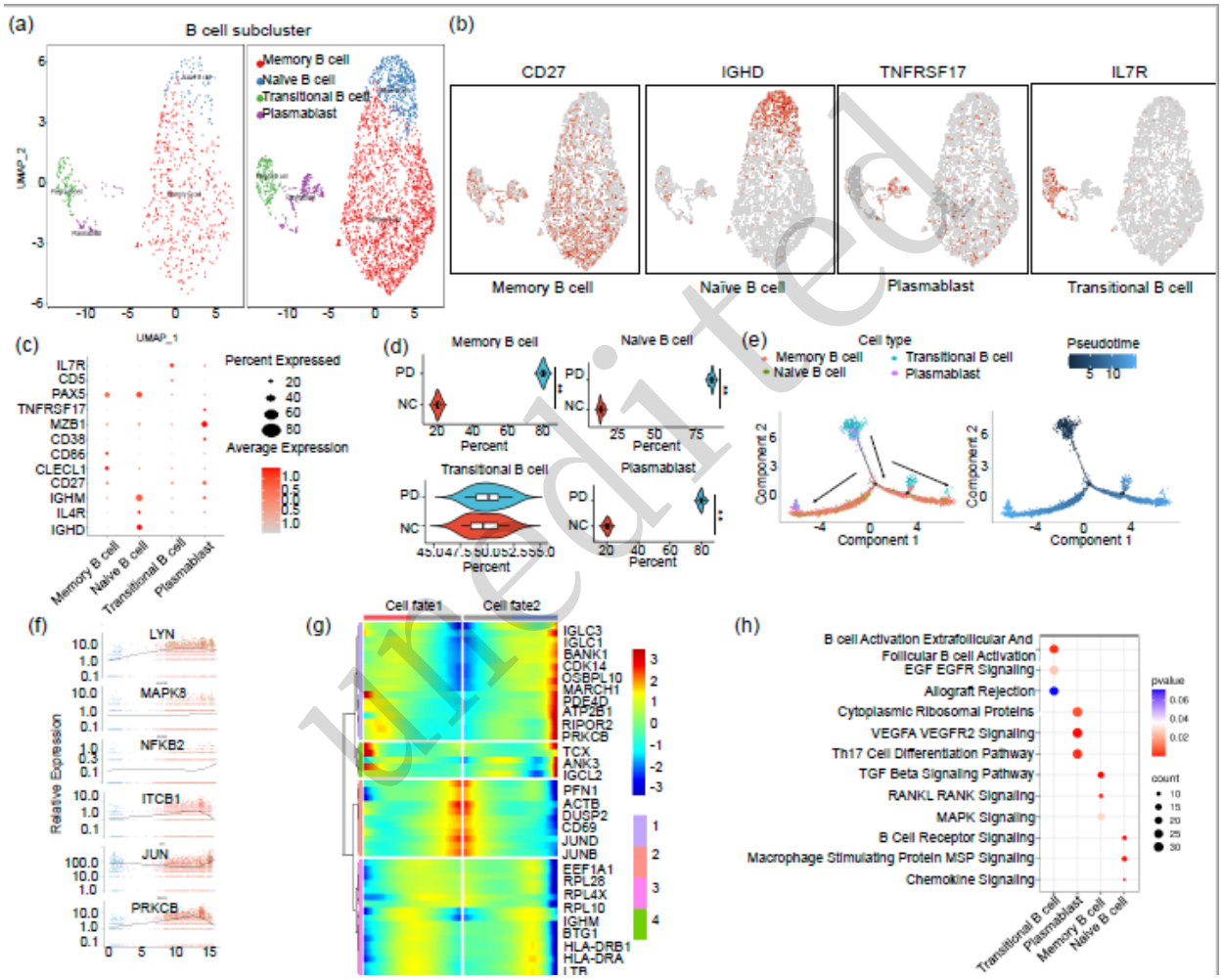


Fig. 2 The heterogeneity of B cells in periodontal microenvironment.

(a) UMAP of 3531 B cell clusters, annotated and colored by the sample type of origin (HCs and PDs) and clustering. (b) UMAPs color-coded for expression (gray to red) of key cell-type markers to define the four cell groups: memory B cells, naïve B cells, prepro B cells, plasmablasts. (c) Dotplot depicting gene expression levels and percentage of cells expressing genes associated with the B cell subcluster. (d) Proportions of the different B cell subclusters in periodontal tissues of HCs and PDs. (e). Trajectory plot shows pseudotime trajectory analysis of the B cell subcluster. (f) Dot plots of dynamic expression of key genes in different pathways. (g) Heat map of differential expression genes in different cell types of the pseudotime trajectory. (h) Bubble plot showing the top 3 pathways.

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2.3 Macrophage subclusters strongly associated with immune regulation

Macrophages play important roles in periodontitis. To further explore the inflammatory landscape within gingival tissues, we examined the composition of macrophage subsets. We identified two macrophage subsets and determined which genes were differentially expressed between them (Fig. 3a-c). Compared to healthy gingival tissue, periodontitis lesions showed a clear increase in M1 macrophage proportions. (Fig. 3d). According to the pseudotime trajectory analysis described previously, as inflammation intensified, M2 macrophages were increasingly transformed into the M1 phenotype. According to their expression trends, genes were divided into four gene clusters, and KEGG pathway analysis was performed to evaluate the biological functions of each cluster. The gene expression patterns showed two distinct directions (Fig. 3e-f). GSVA revealed that M2 macrophages were associated with the TGF-β signaling pathway, the IL2-STAT5 signaling pathway, and the B-cell receptor complex. Moreover, M2 macrophages also participate in angiogenesis. M2 macrophages showed enhanced WNT signaling activity. They also had higher expression of molecules that regulate epithelial cell development and migration (Fig. 3g).

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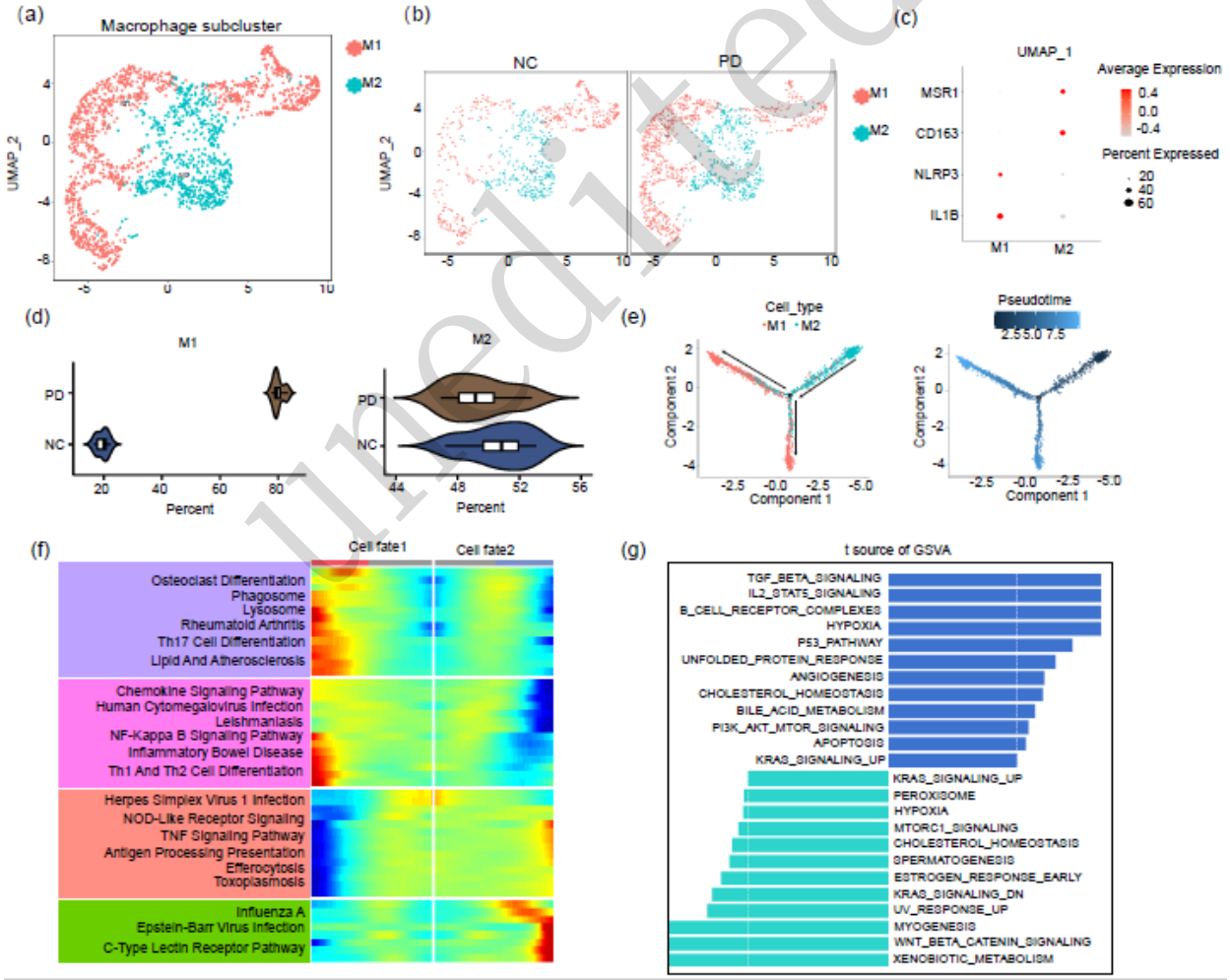


Fig. 3 The heterogeneity of macrophage cells in the periodontal microenvironment.
(a) UMAP of 1927 macrophage cell clusters, annotated and colored by the sample type of origin (HCs and PDs) and clustering. (b) Dotplot depicting gene expression levels and percentage of cells expressing genes associated with

macrophage subcluster. (c) Proportions of the different macrophage subclusters in periodontal tissues of HCs and PDs. (d) Trajectory plot showing pseudotime trajectory analysis of the macrophage subcluster. (e) Heatmap showing the scaled expression of differentially expressed genes in three branches. (f) Heat map of differential expression genes in different cell types of the pseudotime trajectory. (g) Differences in pathway activities scored per cell by GSVA between M1 and M2.

2.4 Cell-cell interactions in healthy and periodontitis tissues

To explore cell–cell crosstalk within the gingival microenvironment, we applied CellChat to map intercellular signaling interactions. While the overall number of communication events among major periodontal cell populations remained comparable between groups, periodontitis samples showed markedly stronger signaling interaction. Specifically, we measured stronger interactions between macrophages and B cells in the PD group (Fig. 4a-b). Moreover, we also found that B cells and macrophages regulate periodontitis inflammatory epithelial cells and fibroblasts through the TGF-β signaling pathway (Fig. 4c). Hematoxylin and eosin (H&E) staining revealed dense infiltration of mononuclear inflammatory cells, including lymphocytes, in periodontitis tissues (Fig. 4d). Next, to further clarify the expression profiles and clinical significance of B cells, macrophages and TGF-β1 in periodontitis, we examined the expression of CD27, IL-1β and TGF-β1 in periodontal tissue sections by immunohistochemistry (IHC). The expression levels of these genes were markedly higher in periodontitis lesions than in healthy gingival tissues. (Fig. 4e). In addition, we used quintuple immunofluorescence (IF) staining to analyze paraffin sections and examined the anatomical relationship between the expression of TGF-β1, CD27, IL-1β, STAT3, and BMPR2 in the periodontal tissues samples in the HC and PD groups. We observed a large number of CD27+ memory B cells and IL1B+ M1 macrophages on the alveolar bone surface of the PD group samples and a large number of epithelial cells expressing TGF-β1. In contrast, the number of these cells in the HC samples was significantly lower (Fig. 4f)

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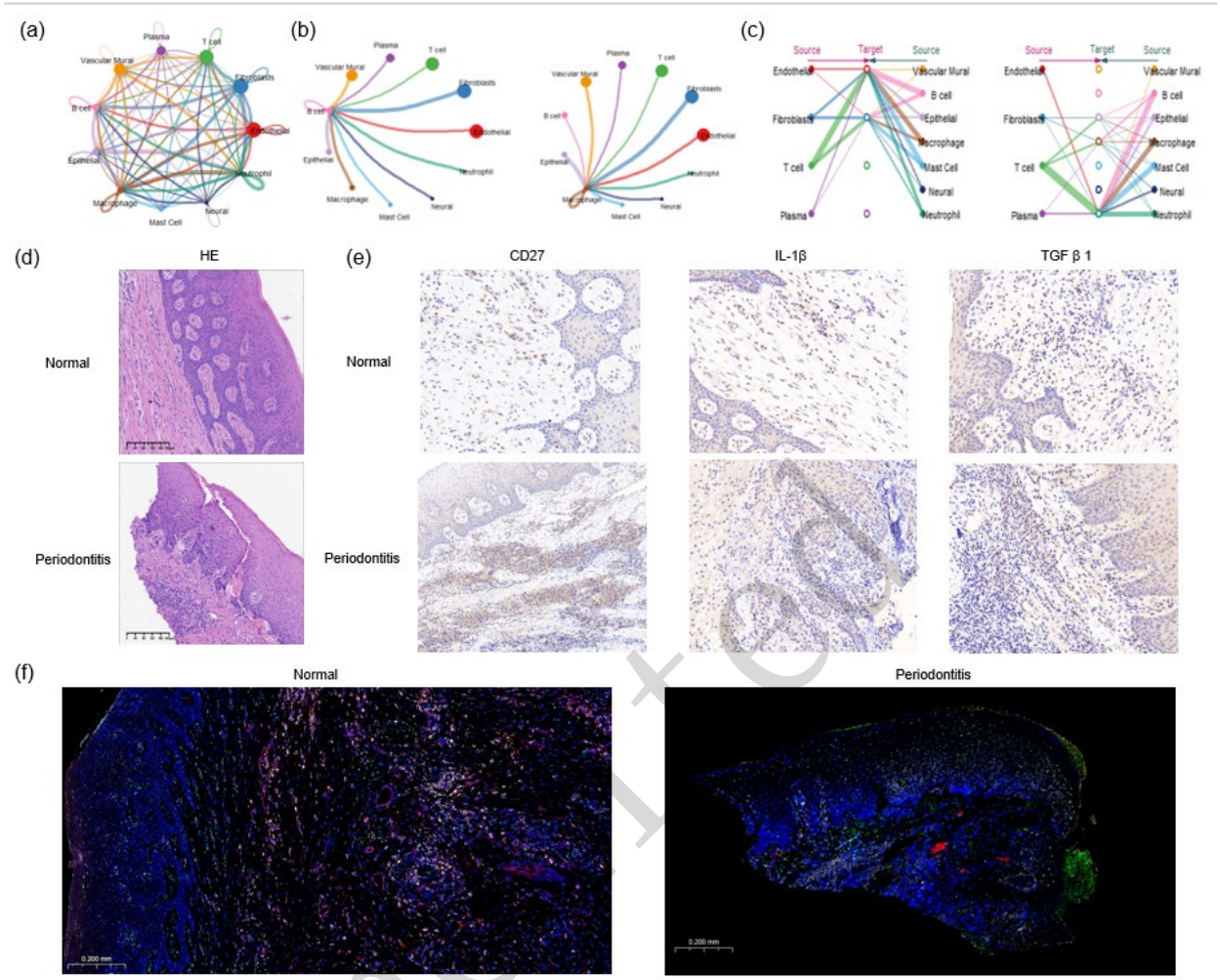


Fig. 4 Cell-cell communication analysis

(a) Bar plot showing the number and strength of intercellular interactions in the periodontal microenvironment. (b) Bar plot showing the number and strength of intercellular interactions in B cells and macrophages. (c) Bubble plots of the significant differentially expressed ligand–receptor pairs in the TGF pathway. (d) H&E staining of the representative gingival tissue from healthy (left) and periodontitis (right) sections. (e) Immunohistochemistry results showing negative expression of CD27, IL-1β and TGF-β1 protein in periodontal tissues. (f) Results of immunofluorescence assay in the periodontal microenvironment (TGF-β1, CD27, IL-1β, STAT3, and BMPR2).

2.5 Inhibition of TGF-β expression reduce the inflammatory response in experiment periodontitis

To verify the role of TGF-β signaling in periodontitis, we established a periodontitis rat model. Inhibition of TGF-β1expression significantly reduced the CEJ-ABC distance and the destruction of tissue in rats with periodontitis (Fig.5a). Immunofluorescence revealed that after inhibition of TGF-β1 expression, RANKL expression in the periodontal tissues was also significantly reduced (Fig. 5b). Flow cytometry analysis showed that neutralization of TGF-β1 significantly reduced the M1 macrophage population (reduced CD86) and increased the M2 macrophage population (increased CD206) compared to the periodontitis group (Fig. 5c). To assess the potential changes in biological functions induced by targets of the TGF signaling pathway, we performed virtual knockout (KO) experiments in B cells and macrophages. The results showed that knockout of TGF-β1 and Smad3 perturbed 475 and 500 genes, respectively (Fig.

5d-e). We performed KEGG pathway enrichment to explore the functional roles of genes regulated by TGF-β1 and Smad3. These target genes were steadily enriched in a series of signaling cascades closely driving periodontitis progression, containing IL-17, TNF, NF-κB pathways and the differentiation of Th17, Th1 and Th2 cells. (Fig. 5g-h). KEGG pathway enrichment revealed 10 significantly enriched pathways after knocking out TGF-β1 and SMAD3 ($p < 1.0 \times 10^{-8}$). For knocking out TGF-β1, the cytokine-cytokine receptor interaction pathway was the most significantly enriched (46/296 genes, $p=1.90 \times 10^{-23}$), followed by the viral protein-cytokine and cytokine receptor interaction pathway (32/99 genes, $p=4.19 \times 10^{-27}$) and the rheumatoid arthritis pathway (27/92 genes, $p=8.51 \times 10^{-22}$). For knocking out SMAD3, the toll-like receptor signaling pathway was the most significantly enriched (12/109 genes, $p=1.82 \times 10^{-5}$). Furthermore, other immune and inflammation-related pathways, such as the IL-17, chemokine, and asthma pathways, were also significantly enriched. Network analysis showed close interrelationships among these pathways, indicating a synergistic role in regulating inflammation and immunity (Fig. 5f-i). Subsequently, we constructed a co-culture system of macrophages and B cells using Transwell chambers, with B cells in the upper layer and macrophages in the lower layer. The control group received no TGF-β1 neutralizing antibody, while the blank control group received TGF-β1 neutralizing antibody. Real-time qPCR and western blot results showed that inhibiting TGF-β1 expression in B cells significantly reduced the expression of INOS, IL1β, and IL6 in macrophages, indicating that macrophage inflammation was suppressed (Figs. S1).

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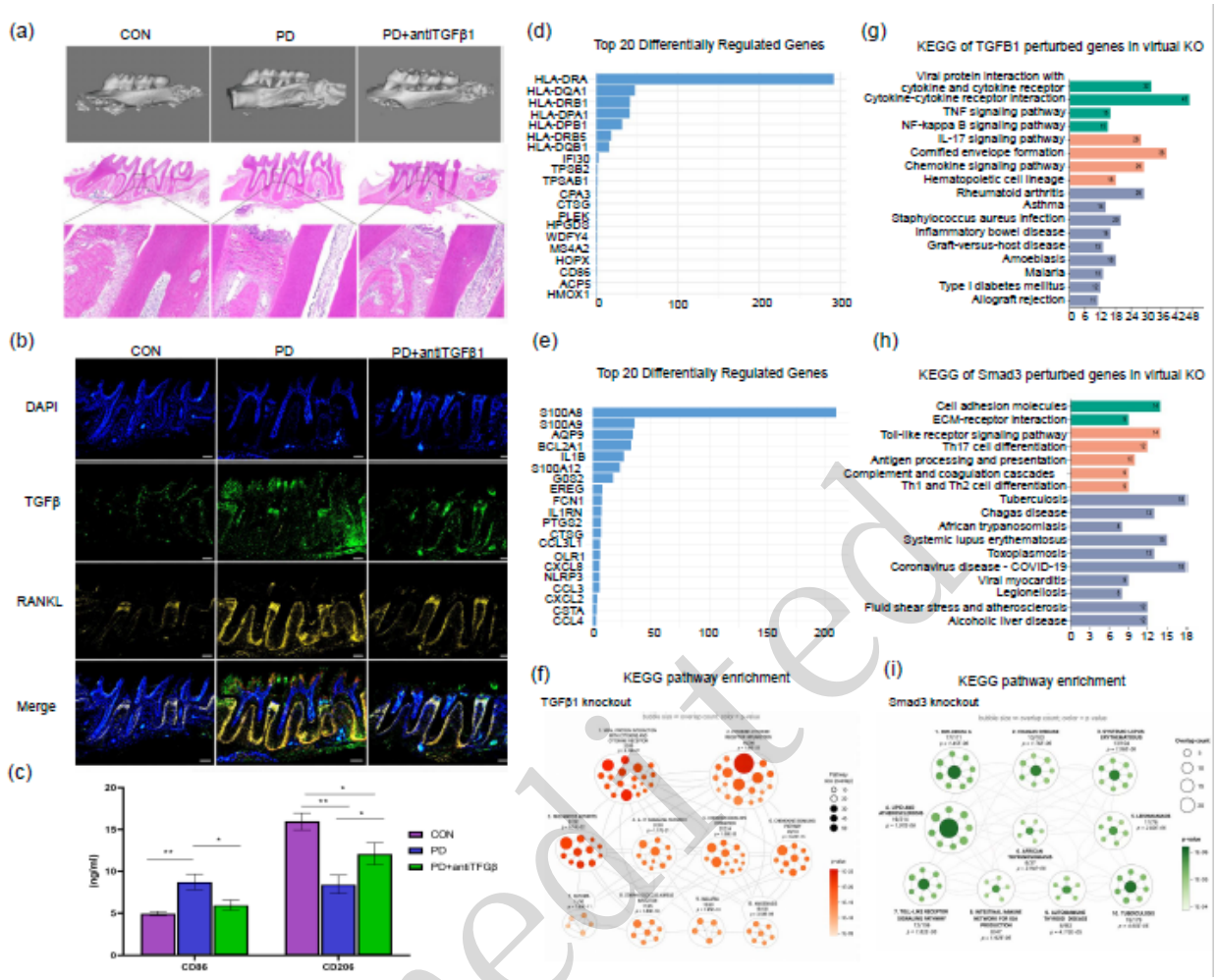


Fig. 5 The involvement of periodontitis (PD) B cells in alveolar bone loss possibly by secretion of TGF-β1.
(a) Representative 3-dimensional (3D) micro–computed tomography (CT) reconstructed sections along the longitudinal direction of the maxillae. (b) Representative images of hematoxylin and eosin (H&E)–stained paraffin sections. (c) Flow cytometry screening of macrophages. (d) Bar chart showing the changes in the top 20 genes after virtual knock out of TGF-β1 in B cells. (e) Bar chart showing the changes in the top 20 genes after virtual knockout of Smad3 in macrophages. (f) KEGG pathway enrichment network analysis of gene sets after TGF-β1 knockout (g) KEGG gene bar graph after virtual knockout of TGF-β1 in B cells. (h) KEGG gene bar graph after virtual knockout of Smad3 macrophages. (i) KEGG pathway enrichment network analysis of gene sets after Smad3 knockout.

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3 Discussion

Periodontitis is a chronic inflammatory condition that damages tooth-supporting structures, including the gingiva, periodontal ligament, and alveolar bone (Simpson et al., 2022). To better understand the periodontal immune microenvironment, we performed single-cell transcriptomic analysis on human periodontal tissue samples. We identified different cell subsets in a systematic manner, assessed how these cells communicate with each other, and clarified the abnormal changes in gene transcription and signaling pathways under disease conditions. Together, our findings improve our understanding of the biological mechanisms of periodontitis and provide a basis for developing more effective diagnostic

methods and treatment strategies (Steinsvoll et al., 1999, Chen et al., 2023). Within the dental endothelial microenvironment, hematopoietic stem cells differentiate into B lymphocytes, and these cells are closely involved in the complex regulatory processes of periodontitis (Kfoury et al., 2015, Lin et al., 2026). Nevertheless, it remained unclear how B cells regulate macrophage behavior during periodontitis progression. Our results confirmed that inflamed periodontal tissues have a much larger population of memory B cells, and these cells can secrete high levels of TGF-β1. In addition, cells expressing TGF-β1+ are also greatly accumulated within the periodontitis microenvironment (Grafe et al., 2018). We then performed a comparative analysis of TGF-β1 expression in three key cell subsets: B cells, T cells and macrophages. Notably, B cells residing in periodontitis lesions showed the strongest TGF-β1 synthesis compared with other local cells. TGF-β1 functions upstream of Smad3 to restrain Runx2 activity, subsequently lowering RUNX2/OCN expression and inhibiting bone formation. Furthermore, TGF-β1 drives macrophage M1 polarization via the RANKL-RANK axis, further inhibiting bone formation (Hu et al., 2023). Similarly, our data suggest that TGF-β1 signaling promotes macrophage M1 transformation through the RANKL-RANK pathway, thereby inhibiting osteogenic differentiation. Few studies have used B cell-deficient mice to understand the role of B cells in periodontitis, and the results have been inconsistent (Cao et al., 2026). Abe et al. (2015) verified the involvement of B cells in promoting periodontal bone resorption. Baker et al. (2009) showed that the absence of either IgD or IgM attenuates bone destruction. Compared with wild-type controls, IgM-deficient mice show reduced alveolar bone resorption under periodontitis conditions (Oliver-Bell et al., 2015). Furthermore, μMT mice, a commonly used transgenic strain lacking B cells, are protected from alveolar bone loss induced by *P. gingivalis*. Conversely, μMT mice developed more severe inflammatory injury and bone destruction in the ligature-induced periodontitis model (Zeng et al., 2021). In addition, recent research has shown that B cell depletion effectively alleviates alveolar bone destruction in ligature-triggered periodontitis. This therapeutic strategy eliminates mainly CD20-positive mature B cells while preserving long-term humoral immune memory, and protects bone tissue in rheumatoid arthritis patients (Kolomansky et al., 2020, Fang et al., 2025). Studies have revealed that pro-osteogenic implants indirectly restrain local B cell maturation. This effect is mediated by the neutrophil-derived CD52–SiglecG axis, which further inactivates the downstream ERK1/2 and NF-κB signaling cascades (Xu et al., 2026). Therefore, we infer that the memory B cells subset exerts a prominent regulatory role in alveolar bone destruction, which needs further investigation.

Periodontitis is a chronic infectious disease caused by bacterial stimulation. Macrophages, as an important component of host immunity, have strong plasticity and play a vital role in inflammatory responses. Optimal phenotypic conversion of macrophages from the pro-inflammatory M1 state to anti-inflammatory M2 cells is indispensable for achieving favorable periodontitis treatment outcomes. (Cui et al., 2023). Regulating macrophage polarization could be a promising therapeutic approach for inhibiting the inflammatory response in periodontitis. Our findings indicate that macrophage functional status and polarization phenotype are key contributors to alveolar bone loss in periodontitis. TGF-β1 secreted by memory B cells can increase the abundance of M1 macrophages and boost osteoclast activity, thereby accelerating alveolar bone destruction (Yang et al., 2024). Moreover, treatment with TGF-β1 inhibitors markedly lowered M1 macrophage counts relative to the control group. This was consistent with our findings in humans, even using single-cell RNA sequencing analysis. TGF-β1-producing memory B cells construct a paracrine signaling circuit with local macrophages to remodel the immune landscape of

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periodontal lesions. Stimulated by periodontal microbial ligands, gingival unswitched memory B cells synthesize abundant TGF-β1, which binds macrophage TGFBR2 and activates canonical Smad2/3 cascades (Chen et al., 2023, Yu et al., 2026). This signaling suppresses M1 macrophage markers (TNF-α, IL-6, iNOS) and drives M2 polarization featured by CD206, CD163 and Arg-1. Although M2 macrophages mitigate excessive inflammatory infiltration, they upregulate RANKL while reducing OPG secretion, disrupting the RANKL/OPG ratio and accelerating osteoclastogenesis and alveolar bone loss (Chen et al., 2023). Notably, this immunomodulatory axis operates independently of B-cell-derived antibodies, verifying that gingival B cells mediate periodontal bone destruction mainly via cytokine secretion rather than humoral immunity (Yu et al., 2026).

This molecular pathway supplies translational clues for host-targeted periodontal therapy. Selective inhibition of memory B cell-derived TGF-β1 rectifies pathological M1/M2 imbalance and restores bone homeostasis, without compromising plasma cell-derived protective antibacterial humoral immunity. B cell-specific TGF-β1 neutralizing antibodies and Smad signaling inhibitors serve as promising candidates for preclinical validation, facilitating the development of precise host-modulating therapies for progressive periodontitis. These observations indicate that macrophage polarization also contributes to periodontitis-associated bone loss. Following conventional periodontal therapy to eliminate bacterial biofilms and relieve inflammation, subsequent research will focus on modulating macrophage phenotypes and exploring their roles in alveolar bone regeneration.

This study had several limitations that require clarification. First, the systemic neutralization of TGF-β1 using diammonium dinitrate in a mouse periodontitis model does not definitively prove that B cells are the pathogenic source of TGF-β1, as TGF-β1 is also produced by epithelial cells, fibroblasts, and macrophages. A mouse model with B cell-specific conditional knockout of the TGF-β1 gene (e.g., Cd19-Cre; TGF-β1-flox mice) is needed to formally establish B cells as the key pathogenic source. Second, the virtual knockout analysis provides supporting computational evidence but does not constitute functional evidence. These predictions need to be validated in vitro using CRISPR-based knockout or conditional knockout animal models. Third, the relatively small human sample size in this study (3 healthy controls and 4 periodontitis patients) may limit the general applicability of the results. Therefore, the mechanistic conclusions drawn in this study should be interpreted with caution and require further experimental validation.

4 Conclusions

By analyzing clinical human specimens and rat periodontitis models, this study confirmed that B cells regulate macrophage polarization through a TGF-β1-dependent mechanism. Our work clarifies a key pathway whereby B cells worsen alveolar bone loss in the inflamed periodontal microenvironment. Importantly, these results offer new insights for developing targeted treatments: selectively targeting detrimental B cell populations and inhibiting TGF-β1 activity may help prevent alveolar bone damage.

Materials and methods

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The full materials and methods are provided in the electronic supplement of this paper.

Data availability statement

The data presented in this study are available from the corresponding author upon reasonable request.

Acknowledgments

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Author contributions

Jing ZHOU and Jinru WENG, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. Minran HUANG, contributed to conception, design, data acquisition and analysis, critically revised the manuscript. Jie NI and Shenglai LI, contributed to data acquisition, critically revised the manuscript. Xiaoyan CHEN and Qianming CHEN, contributed to conception, design, data acquisition and analysis, drafted and critically revised the manuscript. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Jing ZHOU, Jinru WENG, Minran HUANG, Jie NI, Shenglai LI, Xiaoyan CHEN and Qianming CHEN declare that they have no conflicts of interest.

The study was approved by the Ethics Review Committee of the Zhejiang Stomatology Hospital, Zhejiang University(2022-083(R)-X1). The experiments were in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parent.

Declaration on the use of generative AI tools

The authors declare that DeepSeek was used to refine the language of this manuscript during its preparation. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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