



Multimodal Single-Cell Analysis Reveals Disease-Specific Epithelial-Stromal Regulation of Inflammation in Periodontitis and Peri-implantitis

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HARVARD
School of Dental Medicine

**Multimodal Single-Cell Analysis Reveals Disease-Specific
Epithelial-Stromal Regulation of Inflammation
in Periodontitis and Peri-implantitis**

A Thesis Presented by

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to

The Faculty of Medicine

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1. Abstract

Periodontitis and peri-implantitis are chronic inflammatory diseases in which dysregulated host-microbial homeostasis compromises the oral mucosal barrier and leads to progressive soft-tissue destruction and bone loss. Although epithelial and stromal compartments are positioned to regulate barrier integrity and immune infiltration, how these non-immune cells differentially mediate inflammation in periodontitis versus peri-implantitis remains incompletely understood. The present study tested the hypothesis that distinct inflammatory profiles in periodontitis and peri-implantitis are associated with condition-specific changes in epithelial and stromal cellular states and their interactions with immune cells. Soft-tissue biopsies were collected from adults with periodontal health (n = 5 sites), periodontitis (n = 7 sites), and peri-implantitis (n = 8 sites). Single-nucleus RNA sequencing generated a cell-resolved atlas across epithelial, stromal, and immune compartments. Differential abundance, gene set enrichment, and pseudotime analyses assessed condition-specific shifts in cell proportions, biological pathways, and epithelial differentiation trajectories. Spatial transcriptomics and multiplexed immunofluorescence were used to validate candidate epithelial and endothelial niches and to visualize proximity-based interactions *in situ*. An *in vitro* endothelial assay tested cytokine inducibility of MHC class II under pro-inflammatory stimulation. The epithelial compartment resolved into seven differentiation states spanning a pseudotemporal continuum from basal repair-associated cells to suprabasal barrier-forming cells. Periodontitis was characterized by expansion of least differentiated, repair-skewed epithelial states and loss of suprabasal barrier-forming states. Peri-implantitis retained suprabasal differentiation programs but showed enrichment of stress keratins (*KRT6A/B/C* and *KRT16*). *CXCL14* expression localized predominantly to suprabasal epithelial cells and showed the strongest spatial association with immune proximity in periodontitis. In the stromal compartment, a rare HLA-DR/MHC II-positive endothelial subset was enriched in disease and localized adjacent to T cells. This phenotype was recapitulated in cultured human endothelial cells under pro-inflammatory stimulation. These results support a model in which epithelial differentiation state and endothelial immune adaptation contribute in distinct ways to inflammatory niche organization in periodontitis and peri-implantitis. By integrating high-resolution single-cell and spatial data, this work delineates cellular

niches and regulatory signatures that distinguish these diseases and identifies barrier-associated and vascular programs relevant to future host-modulatory strategies.

2. Introduction

2.1 Background

Periodontitis and peri-implantitis impose a substantial and growing global burden¹⁻³. Severe periodontitis affects more than one billion people worldwide and remains a leading cause of tooth loss¹, whereas peri-implantitis affects approximately one in five implant patients and has become an increasingly prevalent biological complication of implant therapy³. Although both conditions share a biofilm-driven inflammatory etiology and depend on host susceptibility to inflammation⁴, the tissues surrounding natural teeth and dental implants are structurally distinct⁵⁻⁷. The periodontium includes a junctional epithelium that attaches directly to the tooth through hemidesmosomes and an internal basal lamina and is supported by periodontal ligaments (PDLs) and a rich vascular network^{5,7}. In contrast, peri-implant mucosa lacks PDLs, contains collagen fibres that run largely parallel to the implant surface, and has a more limited vascular supply^{5,7}. Experimental and histopathologic studies indicate that peri-implant lesions are less well contained, extend closer to the supporting bone, exhibit larger inflammatory infiltrates than periodontal lesions, and may progress in a more accelerated and non-linear manner than periodontal lesions⁸⁻¹¹. These architectural differences may provide a biological basis for the distinct clinical presentation and progression of periodontitis and peri-implantitis.

Advances in high-resolution technologies, particularly single-cell transcriptomics, have begun to redefine the complexity of the oral mucosal barrier and its role in disease¹²⁻¹⁴. Recent studies demonstrate that structural and immune compartments act in an integrated manner to shape periodontal inflammation^{13,15-17}. In human gingiva, Williams et al. linked stromal inflammatory programs to neutrophil recruitment and to cell-specific expression of periodontitis susceptibility genes (Williams et al., 2021)¹³. Qian et al. identified HLA-DR-expressing endothelial cells, CXCL13+ fibroblasts, pro-inflammatory NLRP3+ macrophages, and amplified macrophage-lymphocyte communication in chronic periodontitis¹⁵. Easter et al. showed that sulcular and junctional keratinocyte microniches are spatially organized and associated with immune compartmentalization¹⁶, whereas Qiu et al. identified a NETs-related neutrophil subset and implicated fibroblast-neutrophil signalling through MIF-CD74/CXCR4 in human periodontitis¹⁷.

Single-cell studies of peri-implantitis remain fewer and generally smaller in sample size, but available data indicate that peri-implantitis is not directly analogous to periodontitis^{18,19}. Li et al. reported fewer stromal cells, greater immune infiltration, a pro-inflammatory CXCL13+ fibroblast subset, and ligand-receptor signatures consistent with neutrophil recruitment and monocyte/macrophage-to-osteoclast differentiation in peri-implantitis¹⁸. Mo et al. similarly described enrichment of CXCR2+ neutrophils and dominant CXCL8+ fibroblast-CXCR2+ neutrophil interactions in peri-implantitis relative to periodontitis¹⁹. While these studies defined key stromal-immune features of peri-implantitis, epithelial differentiation trajectories, epithelial chemokine gradients, and endothelial immune adaptation remain incompletely resolved across both diseases.

Within the epithelial compartment, CXCL14 is a compelling candidate mediator of barrier-immune crosstalk. CXCL14 is constitutively expressed in epithelia and contributes to antimicrobial defense, epithelial maintenance, and positioning of dendritic cell precursors, monocytes, and natural killer (NK) cells near barrier surfaces²⁰⁻²². Reduced salivary CXCL14 has also been reported in peri-implantitis, raising the possibility that altered CXCL14 signalling accompanies early barrier dysfunction²³. At the stromal interface, endothelial cells regulate leukocyte entry, adhesion, and local immune activation^{24,25}. Endothelial immunomodulation is increasingly recognized as tissue-specific and heterogeneous, and MHC class II expression can be induced by inflammatory signals such as interferon- γ (IFN- γ) even though it is not typically expressed by resting endothelium^{24,25}. HLA-DR-expressing endothelial cells were previously identified in periodontitis, but their protein-level spatial organization and possible relevance to peri-implantitis remain under-characterized¹⁵.

These considerations provided the rationale for examining how epithelial differentiation, chemokine organization, and endothelial immune adaptation differ between periodontitis and peri-implantitis.

2.2 Significance

Recent single-cell analyses of periodontal and peri-implant tissues have established that epithelial, fibroblast, endothelial, and immune populations contribute jointly to inflammatory signalling. However,

epithelial differentiation trajectories, epithelial chemokine organization, and endothelial antigen-presentation-like phenotypes have not been integrated into a unified barrier-centered framework spanning both periodontitis and peri-implantitis in human tissues. The present study addresses that gap by integrating single-nucleus RNA sequencing (snRNA-seq), spatial transcriptomics, and spatial proteomics (Fig. 1). Methodologically, the study also demonstrates the feasibility of applying snRNA-seq to cryopreserved oral gingival and peri-implant mucosal biopsies, thereby enabling profiling of low-input and structurally complex clinical specimens without requiring recovery of viable fresh whole cells.

2.3 Hypothesis & Study Aim

This study was guided by the hypothesis that distinct inflammatory profiles in periodontitis and peri-implantitis are driven, at least in part, by changes in the epithelial and stromal cellular compartments of the mucosal barrier. Specifically, the study aimed to identify disease-specific alterations in epithelial and stromal cell heterogeneity and in epithelial/stromal-immune interactions associated with periodontitis and peri-implantitis.

3. Approach

3.1 Sample Collection

Soft-tissue biopsies were collected from adults undergoing standard-of-care periodontal or peri-implant surgical procedures at the Harvard Dental Center, Harvard School of Dental Medicine, Boston, MA, USA. Twenty biopsy sites from twenty individuals were included and grouped by clinicoradiographic description as healthy periodontium (n = 5 sites), periodontitis (n = 7 sites), or peri-implantitis (n = 8 sites) (Table 1). Healthy periodontium samples were obtained from clinically healthy sites from non-periodontitis patients. Periodontitis samples were obtained from sites with advanced periodontitis (generalized Stage III or IV, Grade B or C). For improved comparability with periodontitis, peri-implantitis biopsies were restricted as far as could be determined clinically and radiographically to lesions most consistent with biofilm-induced peri-implantitis rather than dental implant failure primarily attributable to mechanical, prosthetic, or other non-biofilm causes. The study protocol was approved by the Harvard Longwood Campus Office of Regulatory Affairs and Research Compliance and Institutional Review Board (IRB22-0587).

To reduce clinical heterogeneity as much as possible, strict inclusion and exclusion criteria were applied. Inclusion criteria were age 18 years or older and the presence of either natural teeth with healthy or diseased periodontal tissues or restored titanium dental implants with diseased peri-implant tissues. Exclusion criteria included age younger than 18 years; uncontrolled systemic disease; use of medications known to affect oral tissues; antibiotic use within six months before biopsy; active smoking or a smoking history within the previous 10 years; pregnancy; secondary periodontal tissue loss (e.g., due to trauma or endodontic infection); prosthetic implant failure (e.g., due to abutment fracture or biomechanical overloading); presence of excess cement; and early implant failure occurring within three months after implant placement.

3.1.1 Case Definitions

Sites were classified according to the 2017 American Academy of Periodontology (AAP) and European Federation of Periodontology (EFP) World Workshop clinical and radiographic case definitions for periodontal health, periodontitis, and peri-implantitis^{26,27}. Healthy periodontal sites were diagnosed with clinical gingival health on and intact or reduced periodontium in non-periodontitis patients.

Periodontitis sites were classified by the extent of periodontal destruction (generalized or localized), stage and grade, and peri-implantitis sites were further described by the presence or absence of generalized or localized periodontitis when concomitantly present.

3.1.2 Participant Demographics and Site-level Parameters

The healthy periodontium group included five sites from male subjects aged 28 to 70 years (mean 43.8 years; median 33 years) with no previous history of periodontitis or peri-implantitis. Four sites represented clinical gingival health on an intact periodontium, and one site represented clinical gingival health on a reduced periodontium in a non-periodontitis patient. All periodontally healthy sites showed no radiographic alveolar bone loss and deepest probing depths of 3 mm and 4 mm in one site; samples were collected from maxillary anterior, premolar, and molar, and mandibular molar sites during distal wedge or crown lengthening procedures.

The periodontitis group was comprised of seven sites from subjects aged 30 to 64 years (mean 51.14 years; median 56 years; four females and three males). All sites were from individuals with advanced generalized periodontitis including stages III and IV and grades B and C. Deepest probing depths ranged from 6 to 12 mm, and radiographic bone loss ranged from 20% to 75%. Sites were sampled during periodontal regeneration (i.e. guided tissue regeneration), osseous surgery, or combined regenerative (i.e. guided tissue regeneration), and osseous procedures, which followed initial periodontal therapy and periodontal re-evaluation.

The peri-implantitis group included eight implant sites from subjects aged 34 to 76 years (mean and median 59.5 years; two females and six males). Three sites were peri-implantitis without a recorded concomitant periodontitis diagnosis, four sites were peri-implantitis with concomitant generalized periodontitis, and one site was peri-implantitis with concomitant localized periodontitis. Deepest probing depths ranged from 7 to 13 mm, and radiographic marginal bone loss ranged from 25% to 100%. Sites were sampled during regenerative surgery (i.e. guided bone regeneration), resective surgery (i.e. osteoplasty) with or without implantoplasty, or implant removal.

Across all groups, subjects had no other systemic disease or with mild/controlled systemic disease. Cases were included only when the clinical and radiographic presentation was most consistent with biofilm-induced periodontitis or peri-implantitis as far as could be determined at the time of sampling.

Group/ Subject No.	Clinical Diagnosis ^{26,27}	Age (years)	Sex	ASA Class	Tooth/Implant position	Deepest probing depth (mm)	Extent of radiographic bone loss (%)	Rendered surgical procedure
Healthy Periodontium (n = 5 sites)								
1	Clinical gingival health on an intact periodontium in a non-periodontitis patient	30	Male	I	#18	3	0	Distal wedge
2	Clinical gingival health on an intact periodontium in a non-periodontitis patient	28	Male	I	#3	3	0	Functional crown lengthening
3	Clinical gingival health on a reduced periodontium in a non-periodontitis patient	70	Male	I	#2	4	0	Functional crown lengthening
4	Clinical gingival health on an intact periodontium in a non-periodontitis patient	58	Male	II	#7-11	3	0	Esthetic crown lengthening
5	Clinical gingival health on an intact periodontium in a non-periodontitis patient	33	Male	I	#5	4	0	Functional crown lengthening
Periodontitis (n = 7 sites)								
6	Generalized Stage III Grade C	30	Female	II	#14	9	70	Periodontal regeneration
7	Generalized Stage III Grade B	42	Male	I	#2, #3	6	20	Osseous surgery
8	Generalized Stage III Grade C	63	Female	II	#30	8	75	Periodontal regeneration
9	Generalized Stage IV Grade C	56	Female	I	#19	6	50	Combination of periodontal regeneration and osseous surgery
10	Generalized Stage III Grade C	57	Male	I	#30, #31	7	42	Combination of periodontal regeneration and osseous surgery
11	Generalized Stage III Grade B	46	Male	II	#15	12	68	Periodontal regeneration
12	Generalized Stage III Grade C	64	Female	II	#2, #3, #5	8	60	Osseous surgery
Peri-implantitis (n = 8 sites)								
13	Peri-implantitis with concomitant generalized periodontitis	56	Male	II	#4	10	66	Resective surgery with implantoplasty
14	Peri-implantitis with concomitant generalized periodontitis	65	Female	II	#19	13	40	Reconstructive surgery
15	Peri-implantitis	34	Male	I	#8	10	100	Implant removal
16	Peri-implantitis	76	Male	II	#12, #13	9	100	Implant removal
17	Peri-implantitis	62	Female	II	#14, #15	9	25	Reconstructive surgery
18	Peri-implantitis with concomitant localized periodontitis	57	Male	I	#30	11	60	Resective surgery
19	Peri-implantitis with concomitant generalized periodontitis	57	Male	II	#12, #14	7	40	Resective surgery with implantoplasty
20	Peri-implantitis with concomitant generalized periodontitis	69	Male	II	#30	8	50	Reconstructive surgery

Table 1. Demographic characteristics of study participants and site-level clinical and radiographic parameters for periodontal and peri-implant tissue samples for snRNA-seq analysis.

Clinical diagnoses were assigned according to the 2017 AAP/EFP World Workshop classifications^{26,27}. The table summarizes group/subject number, clinical diagnosis, age, sex, ASA class, tooth/implant position, deepest probing depth, extent of radiographic bone loss, and the surgical procedure during which the specimen was obtained. Deepest probing depth and radiographic bone loss are reported for

the sampled tooth or implant site. ASA, American Society of Anesthesiologists physical status classification; mm, millimetres.

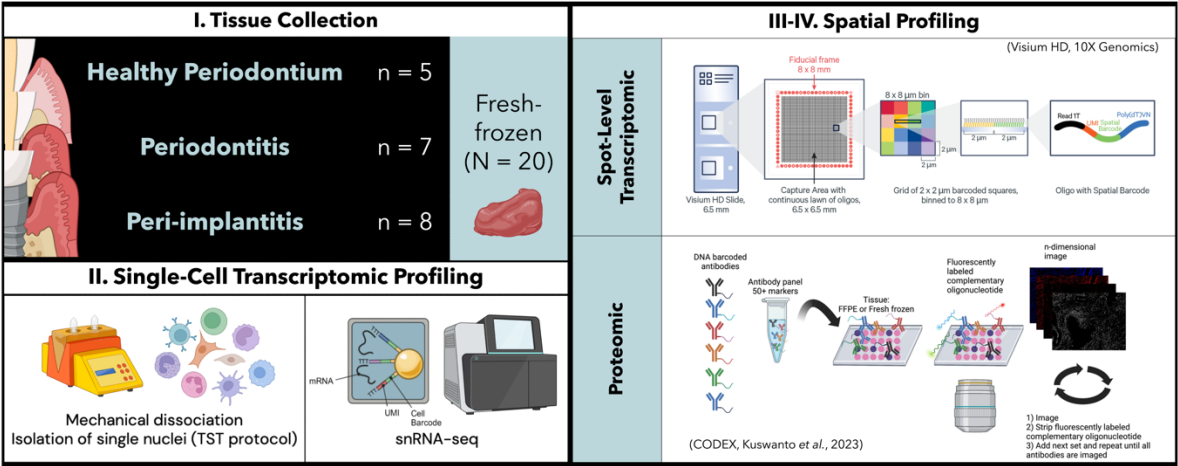


Figure 1. Experimental workflow integrating single-nucleus transcriptomics and spatial multi-omics profiling of periodontal and peri-implant tissues.

Soft tissue biopsies were collected from sites representative of healthy periodontium (n = 5), periodontitis (n = 7), and peri-implantitis (n = 8) and processed as fresh-frozen specimens. For transcriptomic profiling, tissues underwent mechanical dissociation and detergent-based nuclei isolation (Tween with salts and Tris (TST) protocol), followed by single-nucleus RNA sequencing (snRNA-seq) using the Seq-Well platform to generate a cell-resolved atlas across epithelial, stromal, and immune compartments. In parallel, spatial profiling was performed on formalin-fixed paraffin-embedded (FFPE) sections. Spatial transcriptomics was conducted using the Visium HD protocol (10X Genomics) to capture high-resolution gene expression across tissue architecture. Spatial proteomic profiling was performed using multiplexed immunofluorescence (co-detection by indexing (CODEX)) to characterize cellular phenotypes and tissue organization at the protein level. Integration of single-nucleus transcriptomics data with spatial transcriptomic and proteomic datasets enabled identification of epithelial differentiation states, stromal endothelial phenotypes, and spatially resolved epithelial- and stromal-immune interactions within periodontal and peri-implant tissues.

3.2 Transcriptomic Profiling via snRNA-seq

3.2.1 Justification for use of snRNA-seq for human gingival/mucosal tissues

In this current study, with the collaboration with Shalek Lab at Massachusetts Institute of Technology, a single nucleus RNA-sequencing method (snRNA-seq) called SeqWell was employed for transcriptomic cellular profiling^{28,29}. Because these human gingival and peri-implant mucosal specimens were limited in size and not always optimal for recovery of intact viable whole cells, snRNA-seq was employed instead of conventional single-cell RNA sequencing (scRNA-seq)²⁸. SeqWell has been validated in other tissue types in the human body, and to our knowledge, this study is the first application of single-nucleus RNA sequencing involving human oral tissues^{12,28}. Matched studies have shown that snRNA-seq can recover the same major cell types identified by scRNA-seq (Fig. 2) while reducing dissociation-related transcriptional artifacts, although it generally captures fewer genes per profile and a higher proportion of intronic or unspliced transcripts than whole-cell datasets^{30,31}.

One major advantage of snRNA-seq is the reduced dissociation bias in its profiling of cell types in a complex tissue sample. Dissociation bias can arise when processing complex tissues with enzymes to isolate cells from the extracellular matrix (ECM). For example, collagenases are commonly used for enzymatic tissue digestion in single-cell RNA sequencing of oral soft tissues³². This can increase the risk of losing cells that produce collagen at an unequal rate relative to other non-collagen producing cells. In turn, the final data may be inaccurate in its cellular representation. The Seq-Well snRNA-seq method, as used in this current study, avoids introducing this dissociation bias by replacing enzymatic digestion with detergent-based nuclei extraction²⁹. As a result, all cells comprising the tissue sample undergo equal processing, and the necessary genetic material can be extracted without bias for sequencing.

Another important advantage of snRNA-seq is that after tissue biopsy is that the specimens can be cryopreserved and archived until processing. As a result, multiple samples in parallel a time within the timeframe of months to a year following tissue collection. This is an important experimental benefit of snRNA-seq in that scRNA-seq primarily rely on fresh tissues, requiring immediate processing to ensure cell viability above 90%^{32,33}. This convenient aspect also expands the scalability of single-nucleus

analysis, potentiating multi-center studies and global collaborations which also addresses the issue of data inclusivity which is prevalent among our collective scientific community.

3.2.2 Seq-Well platform for snRNA-seq

In this study, the collected tissue samples were flash-frozen and cryopreserved at -80°C and were dissociated via mechanical perturbation (gentleMACS™) and detergent-based tissue digestion (TST protocol) to prepare a single-nucleus suspension²⁹. For library preparation, isolated nuclei were processed using Seq-Well, a portable, low-cost, picowell-based massively parallel RNA-sequencing platform^{28,29}. 15,000 to 20,000 nuclei per specimen in 200 µl of staining buffer were loaded onto each PDMS-based Seq-Well which consisted of subnanolitre wells that co-confined a single nucleus and a uniquely barcoded oligo-dT capture bead (ChemGenes) in each well. Arrays were rested for 10 minutes to allow gravity-based loading of cells into wells before being washed with PBS followed by non-supplemented RPMI media to remove any proteins bound to the array surface and allow adequate membrane sealing. A plasma-treated semi-permeable membrane was added to each array and allowed to seal in an Agilent clamp at 37°C for 30 minutes. The semi-permeable membrane permitted buffer exchange and efficient nucleus lysis while retaining nucleic acids within each well. The arrays were then moved to lysis buffer (5 M guanidinium thiocyanate/1 mM EDTA/1% BME/0.5% sarkosyl) for 20 minutes followed by hybridization buffer (2 M NaCl/8% w/v PEG8000) for 40 minutes. After hybridization, the arrays were washed with 2M NaCl, 3 mM MgCl₂, 20 mM TrisHCl, and 8% v/v PEG8000, and the beads were removed from wells via pipette washing and manual scraping with a glass slide. The removed beads then underwent reverse transcription at 52°C overnight using Maxima H Minus Reverse Transcriptase (ThermoFisher). Excess primers were removed using Exonuclease I enzyme (New England Biolabs) and denatured with 0.1 M NaOH for 5 minutes. Second strand synthesis was performed using Klenow Fragment (3'5' exo-) (New England Biolabs), and whole transcriptome amplification was achieved via PCR with KAPA HiFi HotStart ReadyMix (Roche) within spoke-in primer for amplifying Seq-Well captured LDTs. cDNA and LDT libraries were separated during a 0.6X SPRI using AmMPure XP beads (Beckman Coulter), where the beads captured the cDNA and the supernatant contained the LDTs. cDNA libraries were prepared using Nextera XT Dual 22 Index kits (Illumina). The LDT libraries were further SPRI-cleaned and then amplified using P5 and RPI P7

primers. The libraries were quantified and then loaded onto NextSeq 2000 P3 50 cycle kits and sequenced using the read structure: 21-8-0-50 for Read1-Index1-Index2-Read-2. The resultant data were converted to FASTQ files using bcl2fastq and then aligned to the human genome (GRCh38) using drop-seq tools on Terra maintained by the Broad Institute. Collapsing the aligned reads was based on barcode and UMI sequences, and digital gene expression matrices were obtained for each array, covering 10,000 cell barcodes.

3.2.3 Data analysis

snRNA-seq data were aligned and demultiplexed using GRCh38 as the reference genome. Gene-by-cell matrices were filtered for high quality cells with at least 200 genes and mitochondrial genes of <5 %. Data were log-normalized, and 2000 most variable genes were identified. Dimensionality reduction, cell clustering, and differential gene analysis were performed in Seurat v4.0^{34,35}. For Principal Component Analysis, optimal number of principal components was manually chosen to describe a large portion of the variance in the dataset based on the elbow-method. Further dimensionality reduction was performed using Uniform Manifold Approximation and Projection (UMAP). Nuclei/cells were clustered using Louvain clustering at optimal resolution. Differentially expressed gene markers for each cluster were identified and used to annotate cell types, and cell identities were determined based on a list of known markers. Gene regulatory analysis for transcription factor activity was performed using DoRothEA to reveal distinct transcription factor activities across identified cell populations³⁶. Standard statistical analyses were used to compare periodontal health with periodontitis and periodontitis with peri-implantitis. Cellular compositions and differential gene expressions were compared using two-sided Wilcoxon rank-sum tests, and multiple testing correction for differential gene expression was performed using the false discovery rate method.

3.3 Spatial Analysis

Whole-tissue specimens were fixed in 20% neutral-buffered formalin for 24 hours and dehydrated in 70% ethanol for at least 24 hours prior to FFPE tissue block processing. The entire workflow from tissue collection to the initiation of FFPE processing was strictly completed within 5 days to ensure

consistency and optimal tissue fixation. FFPE tissue blocks were then sectioned at 5 μ m thickness onto SuperFrost glass slides.

3.3.1 Spatial proteomic profiling via co-detection by indexing (CODEX), Akoya

Tissue sections were subsequently processed either using the IN-DEPTH same-slide spatial multi-omics workflow (10 cases) or spatial transcriptomics only (2 cases)³⁷. For samples following the IN-DEPTH workflow, they were first baked in an oven at 70°C, followed by, deparaffinization in xylene and rehydration through graded ethanol washes, with final washes in nuclease-free water. Sections were then subjected to heat-induced epitope retrieval using a pH 9 target retrieval buffer at 97°C for 20 minutes, followed by equilibration in Buffer S2 and 1X TBST for a total for 20 minutes. As periodontal and peri-implant tissues contain abundant collagen-associated autofluorescence, sections were deliberately photobleached and incubated in blocking buffer for 2 hours prior to antibody staining. Spatial proteomic profiling with CODEX is achieved through iterative cycles of reporter hybridization and chemical stripping on tissues stained with oligo-conjugated antibodies, enabling multiplexed whole-tissue imaging.

Tissue sections were incubated with a 22-plex oligo-conjugated antibody panel comprising markers for major immune and structural cell types of the periodontal and peri-implant tissues. Prior to staining, the antibody panel was centrifuged to remove aggregates, filtered through a 50 kDa membrane for buffer exchange, and further filtered through a 0.1 μ m filter to eliminate any residual precipitates that could interfere with staining quality. The panel was then applied to the blocked and photobleached tissue sections and incubated for 16 hours at 4°C. Following antibody staining, tissues were washed with S2 buffer and subsequently fixed sequentially with 1.6% paraformaldehyde, ice-cold methanol, and BS3 final fixative. Slides were then mounted with flow cells and imaged on the PhenoCycler FUSION platform.

Following imaging, the multiplexed images were assessed for quality, and tissues proceeded to spatial transcriptomic profiling only upon passing initial quality control standards. The flow cell was then removed from the slides, which were subsequently washed in 2xSSC buffer prior to probe hybridization.

3.3.2 Spatial transcriptomic profiling via Visium HD, 10X Genomics

Following spatial proteomic profiling, tissues were subjected to hematoxylin and eosin (H&E) staining, scanning, and destaining with 0.1M HCl at 42°C for 15 minutes, followed by tissue digestion using Proteinase K at 40°C for 20 minutes, and subsequent fixation in 4% PFA for 1 minute and washes in Fixation Stop Buffer and 1X PBS. Tissues were then subjected to probe hybridization at 50°C for 16 hours in a thermal cycler. Subsequent steps were performed according to manufacturer's instructions, including post-hybridization washing, ligation, and probe release in the Visium CyAassist instrument, followed by library extension, elution, indexed PCR amplification, clean-up, library quality control, and paired-end sequencing.

For samples that underwent spatial transcriptomic profiling with VisiumHD alone, FFPE tissue sections were first baked at 60°C for 2 hours, followed by de-crosslinking at 80°C for 30 minutes. All subsequent steps performed were as described above.

3.3.3 Image and data processing: Spatial proteomics

Background subtraction was performed by default on the PhenoCycler Fusion platform, generating QTIFF files by subtracting blank cycles from the first and last imaging cycles, followed by stitching and alignment of all images to generate a final data file. The images were then subjected to cell segmentation, single-cell feature extraction, clustering, and annotation.

Segmentation for all tissues were performed using the CODEX images with the MESMER model of DeepCell (v0.12.2), with maxima threshold set to 0.075 and interior threshold set to 0.05. The nuclear channel input of MESMER was DAPI while the membrane channel input of MESMER was a summation of KRT14, VIM, α -SMA, CD31, CD3, CD68, and HLA-I.

For each marker, the pixel value within the area of each cell (determined by the segmentation mask) was summed and then divided by the area of each cell, and the resulting cell-size scaled sum was set as the expression value for a given marker. The extracted features were first scaled to a standardized range of [0,1], and cell phenotyping was then performed through an iterative clustering and annotating process with PhenoGraph ($k = 45$, $\text{seed} = 23$). Phenotyping markers used were Pax5, CD3, CD8, CD68, CD56, CD15, CD31, KRT14 and COL1A1, which allowed the annotation of B cell, CD4 T cell, CD8 T cell,

macrophage, NK, neutrophil, endothelial cells, epithelial cells and fibroblast. Cells that showed unclear marker enrichment patterns were annotated as “Other” cells. Junctional epithelium from each tissue was separately annotated based on the H&E images.

During the annotation process, clustering results were first visualized using a heatmap showing the Z-score of each marker within each cluster. This was used as a basis to annotate each cluster based on their marker Z-score combinations while visually inspecting the original images to confirm annotation accuracy. After an initial round of clustering with PhenoGraph was performed, clusters with clear enrichment patterns were annotated, while clusters with mixed patterns underwent additional rounds of clustering and annotation using a targeted set of phenotyping markers. This process was iterated until all identifiable cells were annotated. To visualize and confirm the assigned annotations, Mantis Viewer (v1.2.0-beta.1; Zenodo. Available from: [10.5281/zenodo.4909620](https://doi.org/10.5281/zenodo.4909620)) was utilized to overlay the annotation onto the segmentation mask and the marker image for visual inspection. Final cell-type annotations were subsequently reviewed and validated by Y.W.C. using multiplexed marker expression patterns and corresponding H&E-stained sections.

3.3.4 Data processing: Spatial transcriptomics

The demultiplexed FASTQ output files from next-generation sequencing were used to map and quantify the human probes through the 10X Genomics Space Ranger v3.1.1 count pipeline. Manual alignment and tissue detection was first performed with 10X Genomics Loupe Browser v8.0.0 using the CytAssist image and the H&E-stained microscope image. These images, together with the human transcriptome reference GRCh38, Visium probe set v2.0, and the FASTQ files, were input into the Space Ranger’s count pipeline.

3.4 *In Vitro* Cell-line Perturbation with Cytokines

Human umbilical vein endothelial cells (HUVECs; Lonza Biosciences) were cultured in EGM[®]-2 Endothelial Cell Growth Medium-2 (Lonza Biosciences) for four passages. For the cytokine stimulation, cells were stimulated with recombinant TNF- α (10 ng/ml) and recombinant IFN- γ (250 U/ml) for 72 hours. Cells were then stained with antibodies for HLA-DR (MHC class II), HLA-A/B/C (MHC class

I), and mice IgG isotype controls at a concentration of 1:100 for 1 hour at 4°C. Cells were then washed, stained for viability, and fixed for flowcytometry analysis. Cells were then analyzed on Attune NxT flow cytometer, and data were analyzed using FlowJo software.

3.5 Statistical Analysis

Statistical tests specific to each assay are described within the relevant analytic subsections.

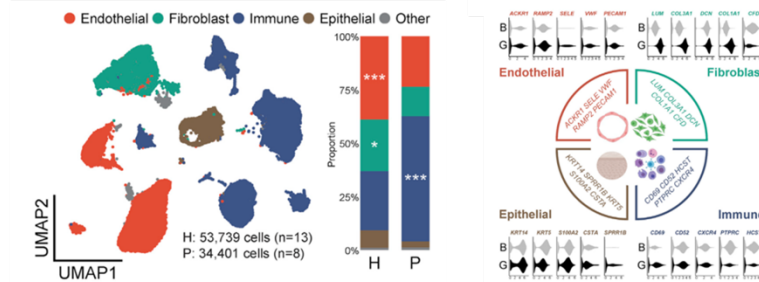
4. Results

At the broad cell-type level, the current snRNA-seq dataset recovered the major structural and immune compartments that have been consistently reported across prior human single-cell studies of gingiva and peri-implant tissues. Epithelial, fibroblast, endothelial, and immune compartments were reproducibly annotated by known cell markers across the published datasets^{13,15,19} and in the current study (Fig. 2). Within the current dataset, healthy periodontal tissue retained the largest epithelial compartment, periodontitis showed relatively greater fibroblast and endothelial representation, and peri-implantitis showed the lowest epithelial fraction with a prominent immune component. These broad trends were directionally concordant with prior human studies.

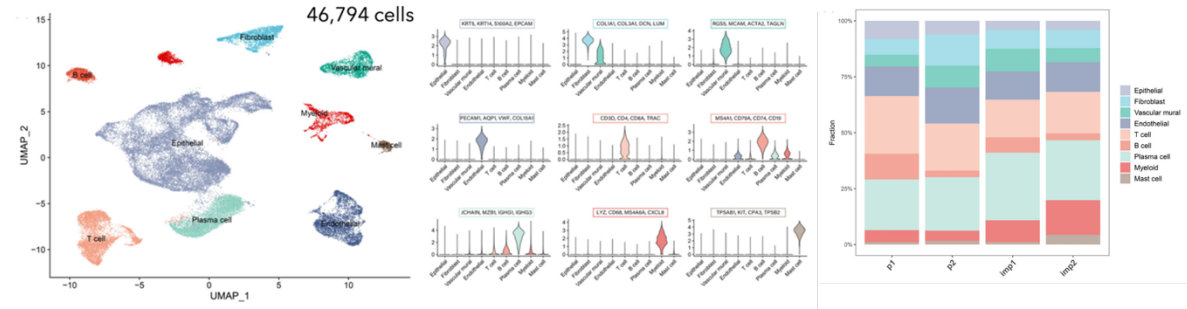
In the dataset reported by Williams et al., periodontitis was associated with a marked increase in the immune compartment with reduced fibroblast, endothelial, and epithelial proportions relative to healthy periodontium, indicating a clear inflammatory shift¹³. Qian et al. supported the same broad annotation framework through canonical marker expression¹⁵. In Mo et al., peri-implantitis displayed a more inflammatory composition than periodontitis, including relative enrichment of plasma-cell and myeloid populations and reduced epithelial representation, despite some inter-sample variability¹⁹. These cross-study comparisons support the use of the current dataset as a structural-cell focused atlas while also underscoring that the exact magnitude of compositional change likely reflected differences in tissue source, tissue processing, and cell-capture efficiency.

Immune-cell composition was then summarized descriptively at the sample level within the current study (Fig. 3). Immune-cell counts and immune-cell proportions varied across individual tissues but tended to be higher in diseased specimens, particularly peri-implantitis. Because immune-cell recovery was reduced in the nuclei-based dataset relative to structural compartments, this immune cell dataset was used primarily to supplement the contextual overview of healthy periodontal, periodontitis, and peri-implantitis tissues rather than the basis for detailed immune-subset inference.

Williams et al. (scRNA-seq; Healthy Periodontium vs. Periodontitis)



Mo & Qian et al. (scRNA-seq; Periodontitis vs. Peri-implantitis)



Current study (snRNA-seq; Healthy Periodontium, Periodontitis, Peri-implantitis)

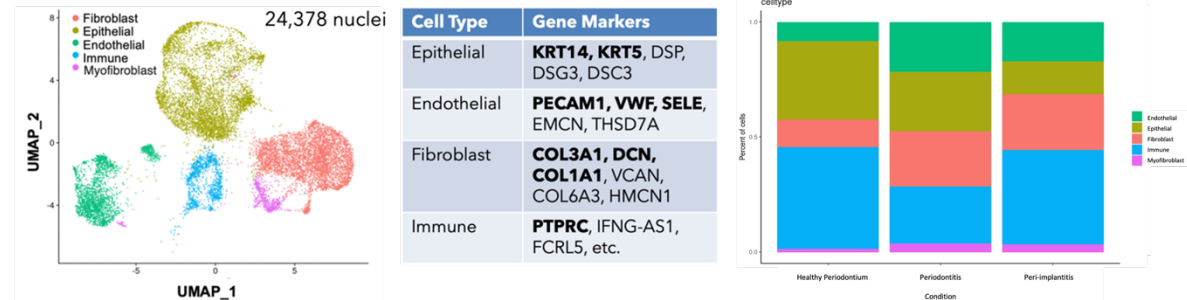


Figure 2. Cross-study comparison of broad cell-type annotation and relative cell-type proportions in periodontal and peri-implant tissues.

Comparison of the published datasets^{13,15,19} with the current study showed strong agreement in the identification of the major cell compartments with epithelial, fibroblast, endothelial, and immune populations consistently recovered across studies. The current study showed a similar overall trend that disease progression is generally accompanied by a shift away from epithelial-dominant homeostatic composition toward immune-enriched inflammatory states. The middle table lists the marker genes used for broad cell-type annotation in this current study; bolded markers denote genes overlapping with prior published studies that were also used for cell-type annotation.

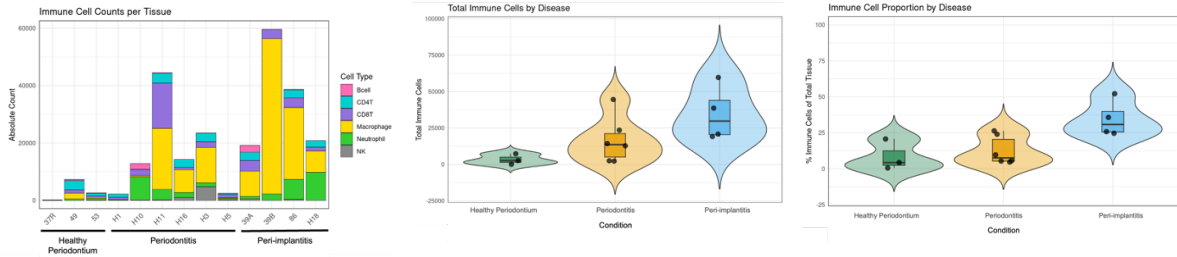


Figure 3. Broad immune-cell composition across conditions.

Stacked bars shows immune-cell counts per tissue sample (left), violin plots show total immune-cell counts by condition (middle) and adjusted immune cell density per tissue area (mm^2) by condition (right).

4.1 Epithelial Compartment

4.1.1 Imbalances in barrier-associated transcript programs

To establish the broader epithelial transcriptional signatures before examining epithelial-state structure, barrier-associated transcript programs were first compared across conditions. At the transcript-program level, the epithelial compartment showed a wide-ranging dysregulation of genes involved in differentiation, structural integrity, and barrier maintenance across the disease states (Fig. 4). In periodontal health, the dominant pattern resembled mature barrier organization with strong expression of terminal differentiation and suprabasal keratinization genes (*KRT1*, *KRT10*, *KRT76*, *CALML3*, *CALML5*), cornification/cornified-envelope genes (*FLG*, *LOR*, *IVL*, *SPRR1A/B*), and adhesion-associated genes (*DSG1*, *DSCI*) together with pathway enrichment for epidermis development and peptide crosslinking. In periodontitis, that terminal barrier program was diminished and shifted toward a reparative configuration marked by increased regenerative and regulatory transcription factors (*TP63*, *KLF4*, *IRF6*, *GRHL1/3*) and depressed late differentiation and cornification genes (*KRT1*, *KRT10*, *FLG*, *LOR*, *SPRRs*) and junctional/barrier genes including desmosomal cadherins (*DSG1*, *DSCI*) and tight-junction components (*CLDN1*, *CLDN4*, *OCN*). Gene ontology analysis periodontitis further emphasized ECM organization, collagen fibril organization, glycosaminoglycan metabolism, proteolysis, and cytoskeletal remodelling, consistent with a tissue state engaged in repair but not fully completing terminal epithelial maturation under chronic inflammation (Figs. 4A and 4B). Peri-

implantitis showed a different epithelial program from periodontitis (Figs. 4A and 4C). The most prominent transcriptional pattern in peri-implantitis included the enrichment of stress keratins (*KRT6A/B/C* and *KRT16*) and persistent deficiency in mature cornification markers, while pathway-level analysis still retained epidermal development and differentiation signatures relative to periodontitis. This pattern supported the interpretation of peri-implantitis as a stress-adapted barrier phenotype that preserves elements of suprabasal differentiation while remaining deficient in a fully mature, homeostatic cornification program.

The epithelial compartment was then examined as a differentiation hierarchy to determine how these program-level shifts were distributed across epithelial states.

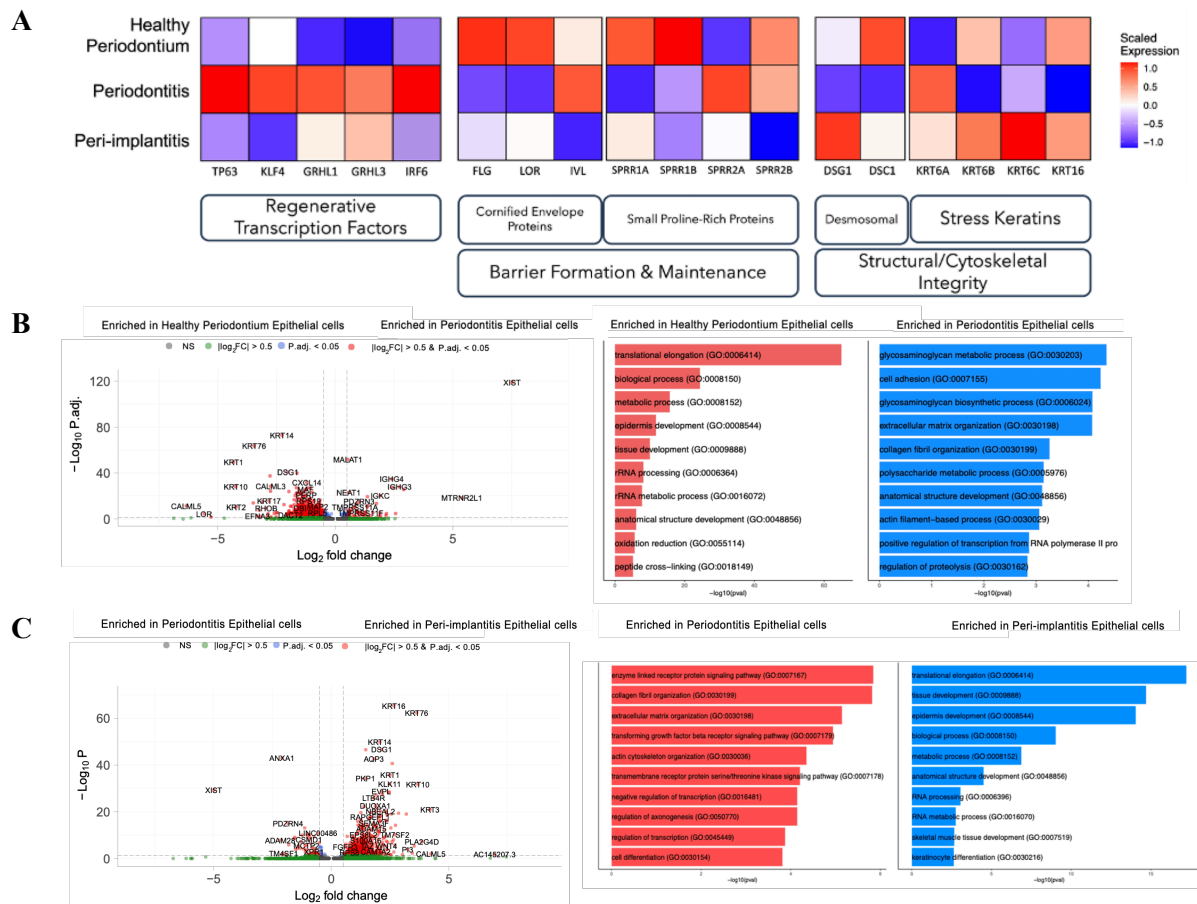


Figure 4. Comparative analyses of barrier-associated epithelial programs across conditions.

(A) Heatmap of epithelial barrier-related gene expression shows preserved cornification and adhesion programs in healthy periodontium (top), loss of terminal differentiation and adhesion with increased regenerative signals in periodontitis (middle), and enrichment of stress keratins in peri-

implantitis (bottom). (B) Differential gene expression and gene ontology terms between periodontal health and periodontitis demonstrated downregulation of barrier formation pathways and enrichment of ECM-remodelling and structural reorganization in periodontitis. (C) Comparison between periodontitis and peri-implantitis showed enrichment of inflammatory and stress-related pathways in peri-implantitis, alongside relative preservation of differentiation-associated programs.

4.1.2 Structured epithelial hierarchy in periodontal and peri-implant tissues

Unbiased clustering of the captured epithelial nuclei identified seven epithelial subsets with distinct differentiation states, represented as monocle clusters arranged along a pseudotemporal continuum (Figs. 5A and 5B). Pseudotime analysis ordered these epithelial nuclei from least differentiated basal states to most differentiated states suprabasal states from inferred timepoint zero, corresponding to monocle cluster 1 (MC1) and monocle cluster 7, respectively (Fig. 5A). The differentiation gene programs across the seven clusters were transcriptionally distinct, supporting a structured epithelial hierarchy in periodontal and peri-implant tissues rather than a random distribution of epithelial states.

4.1.3 Disease-specific shifts in epithelial differentiation

Pseudotime analysis further suggested that disease alters the epithelial barrier not only through differential gene expression but also through redistribution of cells along the differentiation trajectory (Fig. 5B). Relative to periodontal health, periodontitis showed expansion of the least differentiated epithelial states and loss of highly differentiated suprabasal states. By contrast, peri-implantitis retained larger differentiated suprabasal epithelial states while showing depletion of the least differentiated pool. This pattern indicated that epithelial response to chronic inflammation differed between the two diseases.

Based on these disease-associated shifts, the two poles of the differentiation trajectory, MC1 and MC7, were selected for closer examination to determine how disease-associated changes in epithelial differentiation may influence barrier integrity and immune signalling in periodontitis and peri-implantitis (Fig. 5C). MC1, the least differentiated basal state, was enriched for ECM-remodelling and repair-associated genes and was expanded in periodontitis but depleted in peri-implantitis. In contrast, MC7, the suprabasal differentiated state associated with barrier formation, was reduced in periodontitis

yet preserved in peri-implantitis. The epithelial landscape in periodontitis therefore favoured a persistent repair-oriented program, whereas peri-implantitis preserved more suprabasal differentiation.

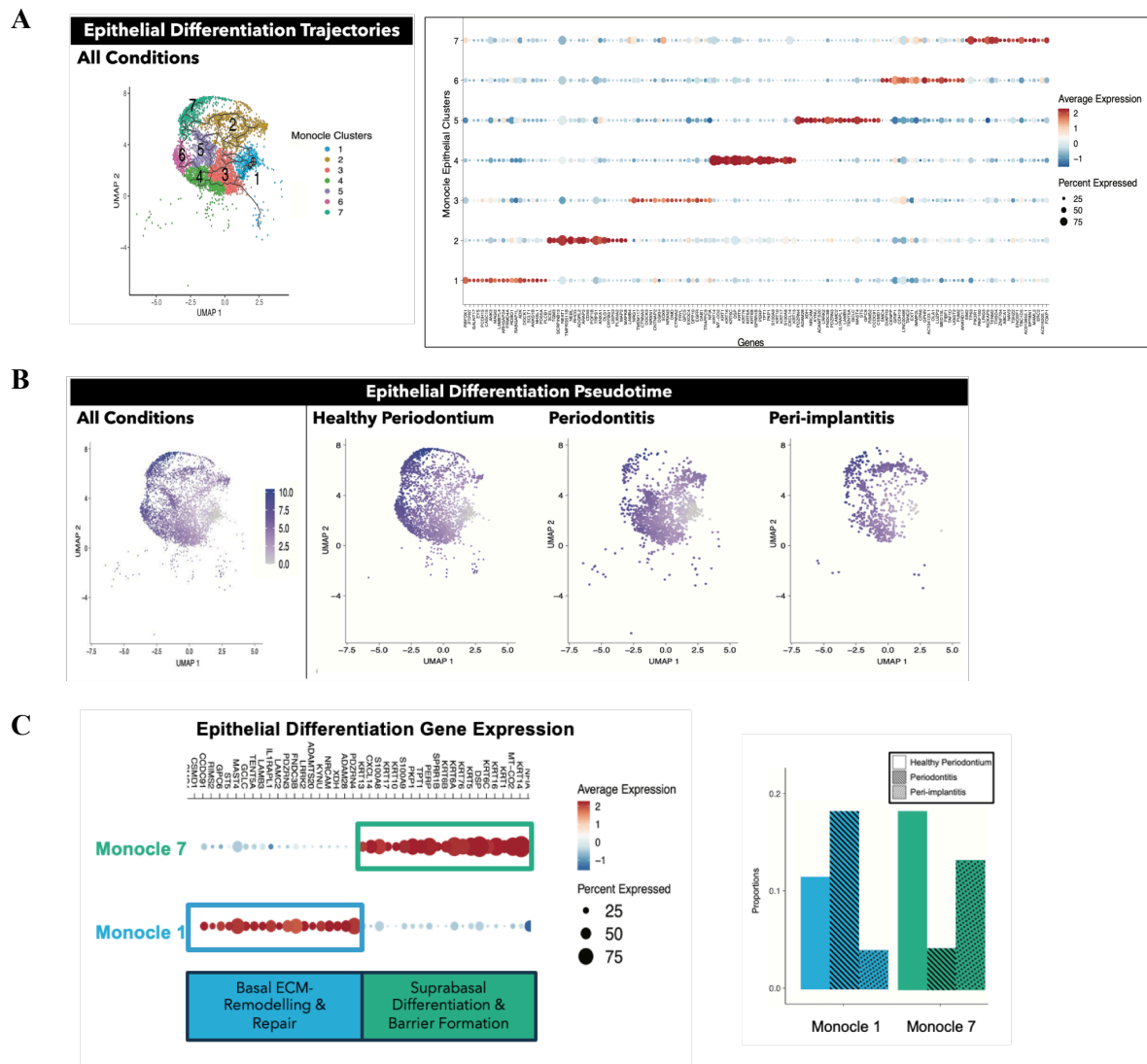


Figure 5. Characterization of epithelial heterogeneity by differentiation state.

(A) UMAP depicts seven distinct epithelial monocyte clusters (MCs) across all conditions; the black trajectory line spanning the monocyte clusters illustrates the dynamic paths of epithelial differentiation (left). Differential expression of epithelial differentiation genes supported a structured hierarchy from least- to most-differentiated states (right). (B) Pseudotime analysis revealed condition-specific trajectory differences on an inferred temporal continuum from 0 (least differentiated) to 10 (most differentiated). (C) Differential gene expression identified MC1 as a basal repair- and ECM-

remodelling state and MC7 as a suprabasal differentiation and barrier-formation state (left). Relative proportions of these two trajectory poles differed by condition (right).

4.2 Link between Suprabasal *CXCL14* Expression and Immune Proximity

To investigate how these epithelial changes related to immune-cell positioning at the mucosal-tooth/implant interface, epithelial chemokines were screened for candidates (Fig. 6A) that might account for the observed heavy immune cell infiltration into diseased lesions (Fig. 3). *CXCL14* was prioritized because it was epithelial-enriched, detectable across epithelial differentiation states, and most strongly associated with the MC7 (Fig. 6A), the differentiated suprabasal subset that differed in relative abundance between periodontitis and peri-implantitis (Fig. 5C).

Spatial transcriptomics localized *CXCL14* signal predominantly to the suprabasal epithelium rather than to basal epithelial, stromal, or immune regions (Fig. 6B). In periodontitis, *CXCL14*⁺ suprabasal keratinocytes showed the strongest negative correlation with distance to immune cells ($r = -0.298$, $p = 6.44\text{e-}122$), which was compatible with a localized chemotactic gradient. This relationship was weaker in peri-implantitis ($r = -0.136$, $p = 2.19\text{e-}27$) and least evident in periodontal health ($r = -0.081$, $p = 1.76\text{e-}14$) (Fig. 6B). The spatial pattern suggested a context-dependent role for *CXCL14*.

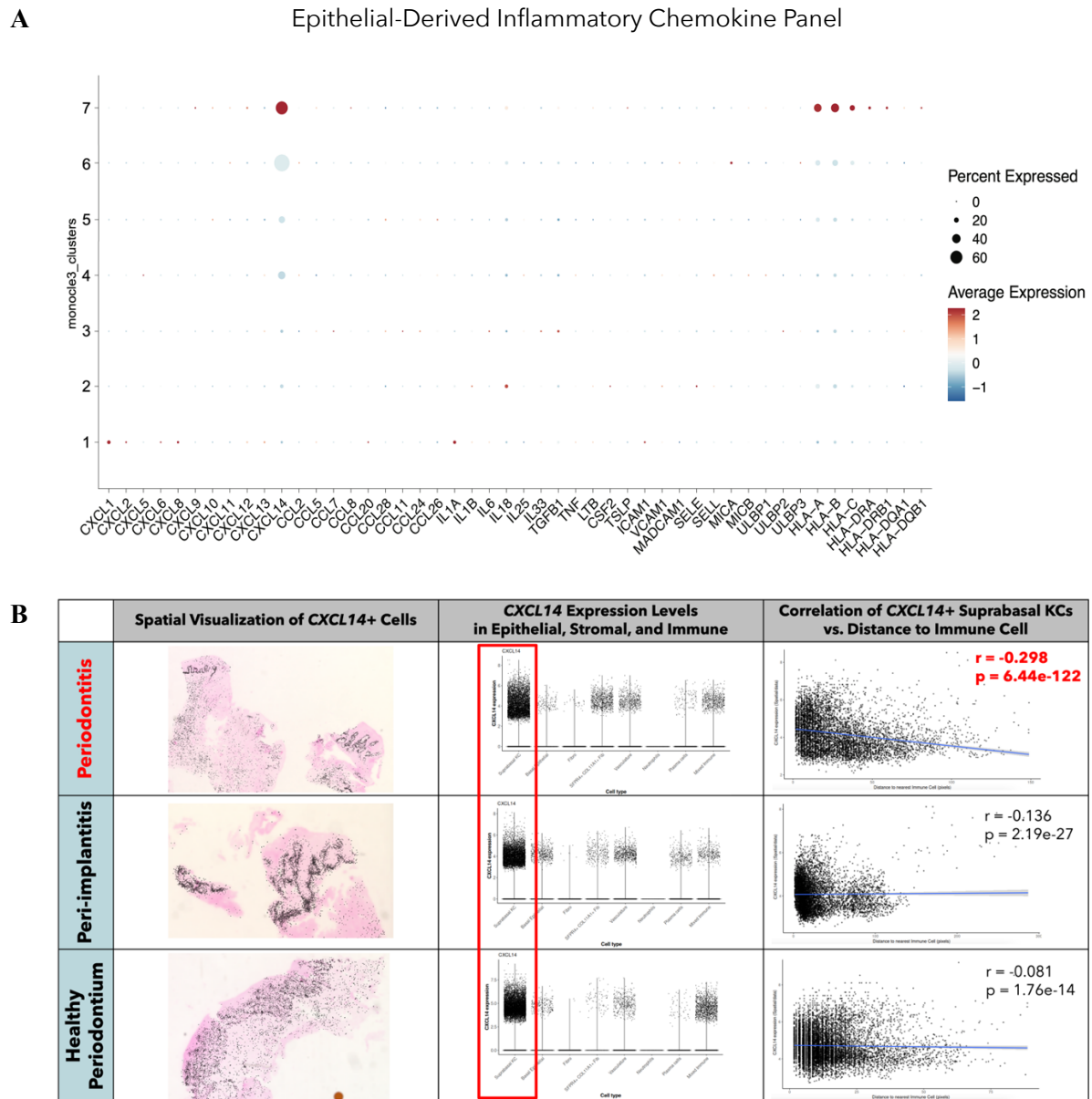


Figure 6. CXCL14-associated epithelial programs and spatial immune organization.

(A) Epithelial-derived inflammatory cytokine panel highlighted enrichment of *CXCL14* expression across all identified monocluster clusters and most significantly in MC7. (B) Spatial transcriptomics localized CXCL14-expressing regions (represented as black dots on H&E images) predominantly to suprabasal epithelium (red box) than other epithelial, stromal, and immune regions. *CXCL14*-expressing suprabasal epithelial cells showed the strongest negative correlation with immune-cell distance in periodontitis, consistent with a localized chemotactic gradient (right).

4.3 Endothelial Compartment

Although the epithelial compartment appeared to help organize immune proximity at the tissue surface, immune infiltration into periodontal and peri-implant lesions also depends on vascular entry. The endothelial compartment was therefore profiled to determine whether stromal vascular programs contribute to inflammatory niche formation.

4.3.1 Detection of a rare HLA-DR/MHC II-positive endothelial subset

Subclustering of the captured endothelial nuclei identified a rare HLA-DR/MHC II-positive endothelial subset, annotated as Endo 1.1, that was more abundant in the diseased conditions of periodontitis and peri-implantitis than in periodontal health (Figs. 7A and 7C). Pathway analysis of this endothelial subset showed enrichment for immune signalling, cytokine responsiveness, leukocyte-associated endothelial activation, and interspecies response, raising the possibility that the vascular participates more directly in pathogen-associated inflammatory response than would be expected for resting endothelium (Fig. 7B). This observation was notable because MHC class II is typically expressed on professional antigen-presenting cells and is not generally considered a canonical marker of resting endothelium^{15,24,25}. The presence of a disease-enriched HLA-DR/MHC II-positive endothelial subset raised the possibility that chronic inflammation in periodontal and peri-implant tissues promotes an endothelial phenotype with enhanced immunologic capability.

4.3.2 *In situ* localization of MHC II+ endothelium near T cells

Multiplex spatial proteomic profiling provided protein-level support for the interpretation by confirming HLA-DR/MHC-II expression on CD31+ endothelial cells in diseased tissues (Fig. 7C). In both periodontitis and peri-implantitis, these endothelial cells were positioned near CD3-positive T cells, whereas this pattern was not evident in healthy periodontal tissue (Fig. 7C). The transcriptional and spatial data were compatible with local immune modulation or semi-professional antigen-presentation-like activity by endothelium under chronic inflammatory conditions, although these data alone do not establish professional antigen presentation or direct T-cell activation.

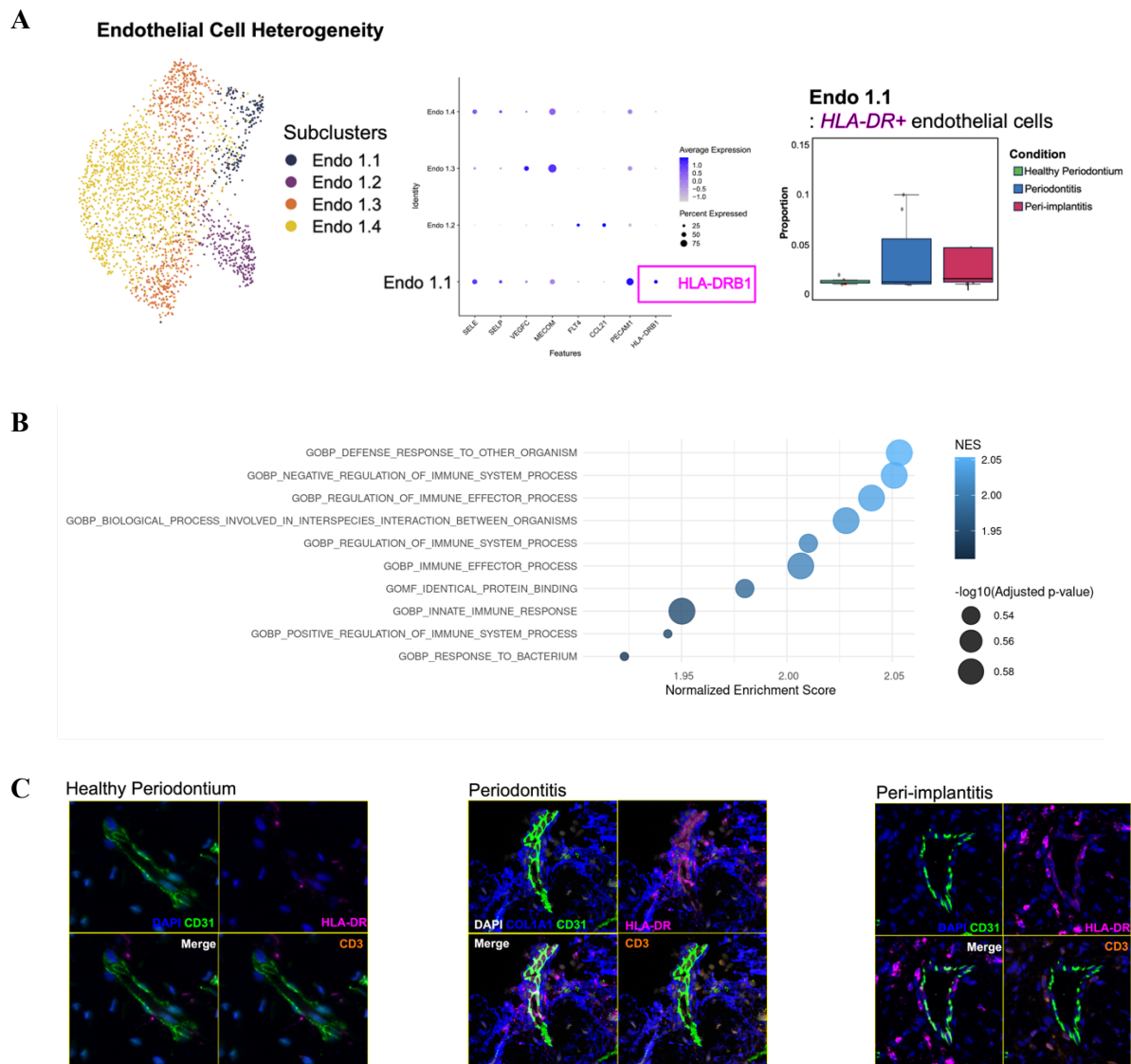
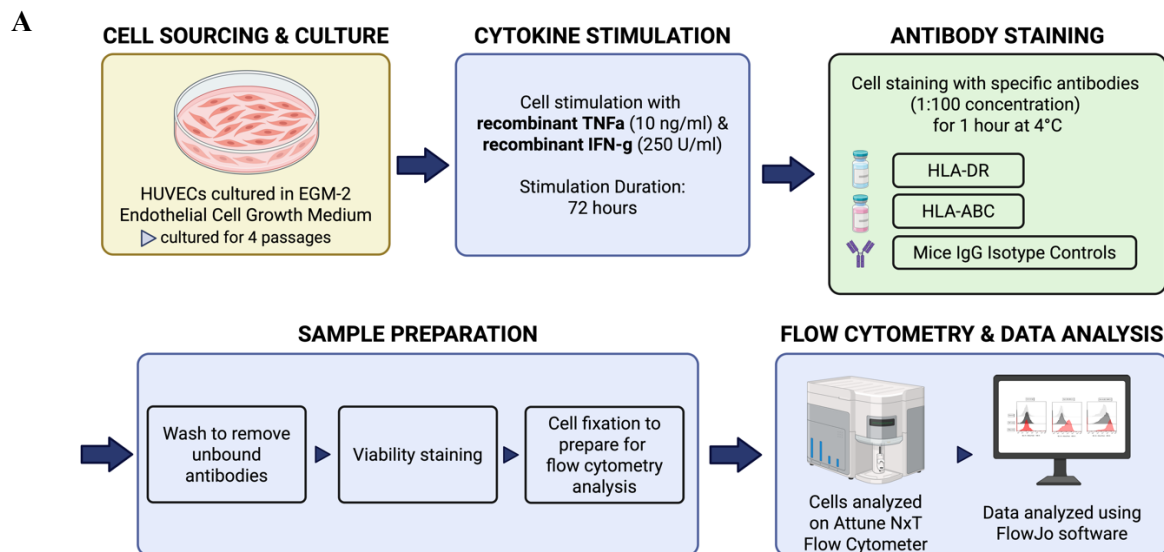


Figure 7. Characterization of endothelial heterogeneity.

(A) UMAP depicts the distinct clusters of endothelial subtypes (left). The endothelial subclusters were annotated using their differential gene expression (middle). Relative proportions of the identified endothelial subtypes were notably different for Endo 1.1 which showed significantly higher proportions in disease states (right). (B) Biological processes enriched in Endo 1.1 subset. (C) CODEX spatial proteomic imaging demonstrated co-localization of the endothelial marker CD31 and HLA-DR/MHC-II in endothelial cells in diseased tissues. CD3+ T cells were also observed in proximity to these HLA-DR/MHC II-positive endothelial cells.

4.3.3 *In vitro* induction of endothelial MHC class II expression under inflammatory stimulation

To test whether inflammatory cues could induce endothelial MHC II phenotype, human umbilical vein endothelial cells (HUVECs) were stimulated with TNF- α and IFN- γ for 72 hours and analyzed by flow cytometry (Fig. 8). These cytokines were selected to assess whether the endothelial MHC II expression was more consistent with lymphocyte-associated or innate-dominant inflammatory input. IFN- γ is produced predominantly by activated T cells and NK cells and is best-established inducer of HLA class II transcription in endothelial cells^{38,39}. TNF- α is classically associated with activated monocytes/macrophages and other innate inflammatory responses, although it can also be produced by lymphocytes and non-immune cells. Under the assay conditions used here, surface MHC class II expression increased after cytokine stimulation with a greater increase observed after IFN- γ than after TNF- α alone. Surface MHC class I expression also increased after cytokine stimulation, consistent with a broader endothelial activation program in an inflammatory milieu. These findings supported inducibility of the phenotype in an IFN-rich inflammatory environment than with TNF- α alone.



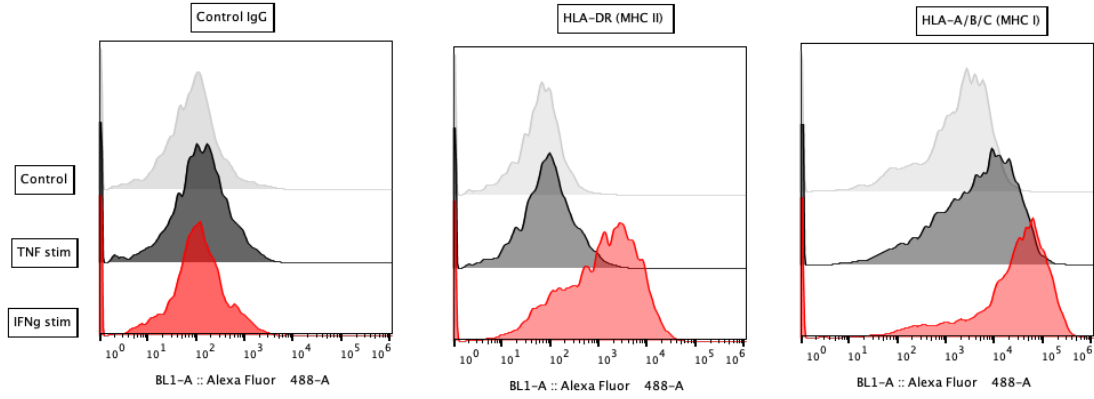
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Figure 8. *In vitro* Induction of Endothelial MHC II under Inflammatory Stimulation.

(A) Experimental workflow for the HUVEC cytokine-stimulation assay as detailed in Section 3.4.

(B) Representative flow-cytometry histograms showing control IgG, HLA-DR (MHC class II), and HLA-A/B/C (MHC class I) staining in unstimulated control, TNF- α -stimulated, and IFN- γ -stimulated HUVECs. Surface HLA-DR/MHC class II expression increased after inflammatory stimulation, with a more pronounced increase after IFN- γ . Surface HLA-A/B/C/MHC class I expression also increased following cytokine stimulation.

5. Discussion

5.1 Summary of Findings

This study refines the interpretation of chronic periodontal and peri-implant inflammation by placing epithelial differentiation state and endothelial immune adaptation within a barrier-centered framework (Fig. 8). The epithelial data were most consistent with a repair-skewed state in periodontitis, characterized by expansion of less differentiated basal and ECM-remodelling programs and depletion of suprabasal barrier-forming states, whereas peri-implantitis retained more differentiated suprabasal states but shifted toward a stress-adapted epithelial program enriched for KRT6/KRT16-family keratins. *CXCL14* expression localized predominantly to suprabasal epithelial cells and showed its strongest spatial association with immune proximity in periodontitis. In parallel, a rare HLA-DR/MHC II-positive endothelial state was enriched in disease, localized near T cells *in situ*, and was inducible under pro-inflammatory conditions *in vitro*. These results support that structural cells are not merely passive barriers in periodontal and peri-implant tissues but may help organize inflammatory niches in disease-specific ways.

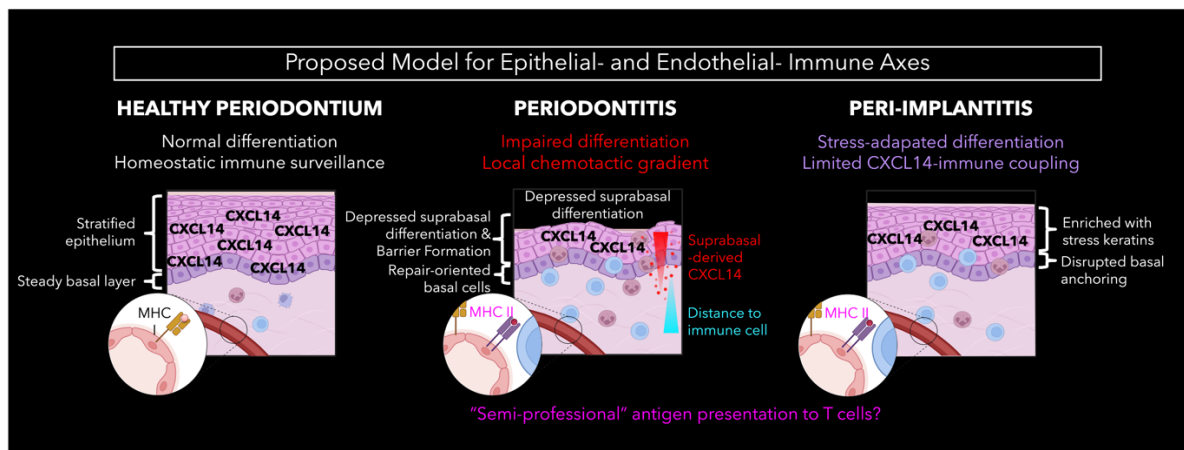


Figure 7: Summary of the proposed model for epithelial- and endothelial-immune axes.

5.2 Comparison with Prior Human Single-cell Studies of Periodontitis

When placed in the context of prior human single-cell studies, the present findings fit into a growing literature that has progressively resolved the structural cellular landscape of the human gingiva^{13–17}. Caetano and colleagues first demonstrated the mesenchymal and epithelial heterogeneity in human oral inflammatory disease and highlighted the value of resolving stromal and epithelial states at single-cell

resolution¹⁴. Williams et al. then constructed a human oral mucosa atlas linking stromal inflammatory programs to neutrophil recruitment and to cell-specific expression of periodontitis susceptibility genes¹³. Qian et al. identified inflammatory cell subsets in chronic periodontitis, including HLA-DR-expressing endothelial cells, CXCL13+ fibroblasts, and NLRP3+ macrophages in chronic periodontitis¹⁵. More recently, Easter et al. added single-cell, spatial, and microbial context by defining sulcular and junctional keratinocyte associated with distinct immune neighbourhoods, showing altered keratinocyte differentiation states in periodontitis, and identifying bacterial reads with cell-specific tropism¹⁶. Qiu et al. further emphasized the stromal-immune cell axis by identifying a NETs-related neutrophil subset coupled to fibroblast signalling through MIF-CD74/CXCR4 in human gingiva¹⁷.

The current study extends the human gingival single-cell literature by framing the epithelial response as a differentiation continuum with redistribution toward repair and remodelling programs in periodontitis and relative retention of suprabasal differentiation with stress adaptation in peri-implantitis. It also integrates epithelial-state information with spatially resolved *CXCL14* localization and an endothelial HLA-DR/MHC-II phenotype, linking epithelial differentiation and vascular adaptation to immune positioning within chronic oral inflammatory lesions.

5.3 Comparison with Prior Human Single-cell Studies of Peri-implantitis

Human single-cell studies of peri-implantitis remain fewer than those of periodontitis, but the available literature consistently indicates that peri-implantitis is not simply an analogue of periodontitis around a dental implant. Published single-cell studies of peri-implantitis have emphasized stromal-immune circuits, especially neutrophil recruitment and osteoclastogenic myeloid programs^{18,19}. Li et al. described fewer stromal cells, increased immune infiltration, a pro-inflammatory CXCL13+ fibroblast subset, and ligand-receptor signatures consistent with neutrophil recruitment and monocyte/macrophage-to-osteoclast differentiation¹⁸. Mo et al. similarly reported enrichment of CXCR2+ neutrophils and dominant CXCL8+ fibroblast-CXCR2+ neutrophil interactions relative to periodontitis¹⁹. More recently, Dionigi et al. employed spatial transcriptomics to profile human peri-implant lesions and further underscored the spatial specificity of transcriptomic signals in regions of

immune cell infiltration. The present epithelial results complement the existing literature by indicating that peri-implantitis preserves a differentiated suprabasal compartment while acquiring a stress-keratin program that differs from the repair-skewed epithelial response observed in periodontitis.

5.4 Structural Interpretation of the Epithelial Programs

These disease-specific epithelial programs are consistent with the known structural differences between periodontal and peri-implant mucosa⁵⁻⁷. Around teeth, the junctional epithelium is integrated with a PDL-supported connective-tissue apparatus and a richer cellular and vascular network. Around implants, the epithelial seal forms with fewer attachment structures, collagen fibres run parallel to the implant rather than insert directly onto tooth, and the blood supply is more limited because PDL-derived vascular anastomoses are absent. Preclinical comparative studies also show lower attachment protein expression, greater proliferative activity, and more chronically inflamed characteristics in peri-implant mucosa than in tooth-associated junctional epithelium^{40,41}. These differences likely influence how tissues respond to mechanical stress, maintain attachment, and respond to microbial challenge. The present data suggest that peri-implantitis retains suprabasal differentiation while acquiring stress-adaptive barrier features, a pattern that may help explain why peri-implantitis progresses differently from periodontitis despite overlapping microbial and inflammatory triggers.

An additional structural feature with biological relevance is the presence of PDL-resident mesenchymal stem/progenitor cells around natural teeth and the absence of an analogous PDL-associated compartment around osseointegrated dental implants. The PDL not only is a suspensory collagenous tissue but also harbours mesenchymal stem cells (MSCs) that contribute to periodontal tissue maintenance, repair, and regeneration by supporting ECM turnover, cementum and PDL-like tissue formation, osteogenic differentiation, vascular-associated stromal niches, and immunomodulatory responses at the tooth-bone interface⁴²⁻⁴⁴. Recent single-cell work has further resolved CD55+ MSCs, pre-osteoblasts, osteoblasts, and MSC-like pericytes within human periodontal tissues, supporting that the periodontium contains resident and perivascular progenitor populations with osteogenic and regenerative potential^{42,45,46}. By contrast, peri-implant tissues comprise of direct bone-to-implant

contact and lack a PDL-cementum attachment apparatus⁵⁻⁷. Therefore, peri-implant stromal progenitor activity would be expected to arise from periosteal, bone marrow, vascular, or mucosal stromal sources rather than from a PDL-derived progenitor niche. This structural distinction may help explain the differences in tissue repair capacity, inflammatory containment, bone-remodelling dynamics, and the pathogenesis and progression of periodontitis versus peri-implantitis.

5.4.1 Stress keratins and CXCL14 as context-dependent epithelial signals

The KRT6A/B/C and KRT16 signals are informative because these keratins are not generic epithelial differentiation markers. In skin and oral epithelia, KRT6 and KRT16 are induced by injury, chronic inflammation, and wound repair and function as stress keratins or barrier alarmins to support keratinocyte migration, mechanical resilience, innate immune signalling, and rapid barrier adaptation during injury and repair⁴⁷⁻⁴⁹. In relation to the present data in this study, the suprabasal enrichment with stress keratins *KRT6A/B/C* and *KRT16* in peri-implantitis may represent an attempt to stabilize a mechanically and biologically vulnerable peri-implant soft-tissue seal rather than restoration of a mature homeostatic barrier. An unresolved question is whether similar stress-keratin programs are already present in clinically healthy peri-implant mucosa, which would help distinguish baseline implant-associated adaptation from disease-associated stress responses.

The microbial context can provide additional biologic explanation for these epithelial differences, although this current study does not report the site-related microbial features. Periodontitis and peri-implantitis both arise in the setting of dysbiotic biofilms, but microbial challenge is encountered within distinct tissue environments^{4,16}. Easter et al. demonstrated that periodontitis-associated keratinocytes can harbour bacterial signatures with apparent cell-type tropism and that bacterial 16S and messenger RNA signals align spatially with proinflammatory keratinocyte phenotypes *in situ*¹⁶. Additionally, at implant sites, microbiome-biomaterial interactions, implant surface characteristics, and other local implant-level features may further influence host sensing and tissue response.

CXCL14 provided a complementary link between epithelial differentiation and immune organization. Prior work has associated CXCL14 with epithelial antimicrobial defense, homeostatic immune surveillance, and altered salivary levels in peri-implantitis²¹⁻²³. In the present study, *CXCL14* localized

predominantly to differentiated suprabasal epithelial states across all conditions and showed its strongest spatial coupling with immune proximity in periodontitis. One interpretation is that CXCL14 has a context-dependent role in healthy and diseased oral mucosal tissues. In periodontal health, CXCL14 appeared compatible with steady-state epithelial surveillance. In periodontitis, CXCL14 aligned with localized immune positioning at a compromised barrier, potentially reflecting a compensatory adaptation to reduced suprabasal epithelial differentiation. In peri-implantitis, CXCL14 was retained in differentiated suprabasal cells but showed weaker spatial coupling with immune cells, suggesting a more differentiation-linked than chemotactic role. This model remains provisional, but it provides a biologically plausible bridge between epithelial state and immune organization.

5.5 Endothelial MHC class II expression and vascular immune adaptation

The endothelial results expand an emerging literature on immunomodulatory endothelium. Amersfoort et al. described endothelial cells as heterogeneous immune partners capable of regulating leukocyte trafficking, activation, and tissue-specific inflammatory programs²⁴, and earlier work from Pober and colleagues showed that human endothelial cells can acquire MHC II and interact with T cells under inflammatory conditions²⁵. In periodontal tissue, Qian et al. reported HLA-DR-expressing endothelial cells in chronic periodontitis, but their spatial organization and relevance to peri-implantitis remained unresolved¹⁵. The current study extends that observation by providing protein-level spatial validation in both periodontitis and peri-implantitis and by showing cytokine inducibility of endothelial MHC II *in vitro*. The combined transcriptomic, spatial, and *in vitro* data indicate that the vascular compartment is immunologically adapted in disease and should be considered as active participants in chronic oral inflammation alongside epithelium and fibroblasts.

However, interpretation of the endothelial data requires caution. While the spatial observation of MHC II⁺ endothelium near T cells is compatible with antigen presentation or other immunomodulatory behaviour, spatial proximity alone does not establish functional T-cell activation. The HLA-DR/MHC II-positive endothelial cells may provide partial immune activation cues, may influence immune cell retention or transmigration, or may reflect a general IFN- γ -rich inflammatory environment without

functioning as professional antigen-presenting cells. The value of the current studies lies in demonstrating that this non-canonical endothelial phenotype exists in periodontal and peri-implant tissues, is enriched in chronic oral inflammatory conditions of periodontitis and peri-implantitis and is experimentally inducible under pro-inflammatory stimulation.

5.6 Conceptual, Methodological, and Clinical Significance

The main conceptual contribution of this thesis is to reframe periodontitis and peri-implantitis as diseases of barrier programs in which epithelial differentiation state and stromal endothelial immune adaptation help organize inflammatory niches. This epithelial-stromal barrier-centered model emphasizes epithelial and stromal barrier programs at the periodontal or peri-implant mucosal interface play an essential role in shaping inflammation. In this model, structural cells actively organize inflammation, with the epithelial differentiation state and endothelial immune adaptation shaping immune positioning and persistence within diseased tissue.

Methodologically, the study also demonstrates the utility of integrating single-nucleus RNA sequencing with spatial transcriptomics and multiplexed proteomics in human chronic oral mucosal inflammatory diseases. This integrative approach enabled recovery of epithelial and stromal information at high resolution which would have been difficult to resolve in bulk tissue. Use of snRNA-seq on the Seq-Well platform was particularly advantageous for archived, low-input, structurally complex, clinical oral gingival/mucosal tissues^{28,29}. The multimodal approach linked cell states to tissue geography and provided orthogonal support for rare cellular populations such as HLA-DR/MHC II-positive endothelial cells.

Clinically, the data identify two local tissue axes that may inform future host-modulatory strategies. The first is an epithelial-barrier axis defined by differentiation state, stress keratins, and chemokine organization such as CXCL14. The second is an endothelial-immune axis marked by HLA-DR/MHC-II expression and proximity to T cells. Although the present study is not sufficient for immediate therapeutic translation, these results support further exploration of locally targeted interventions with less systemic exposure than broad immunosuppression.

5.7 Study Limitations

Several limitations should be acknowledged. First, the study was cross-sectional and based on modest cohort sizes, which limits inference about temporal progression and causality. Second, the absence of a healthy peri-implant mucosal control group constrained the ability to distinguish baseline implant-associated biology from disease-associated change. Third, the groups were not age- or sex-matched and disease extents and severities were not matched between periodontitis and peri-implantitis. This may be relevant because periodontitis prevalence and severity vary with age and sex at the population level^{1,50}, and demographic or disease extent/severity differences may influence tissue composition and host-response programs even when age and sex are not consistent independent risk indicators across all peri-implant datasets³. Fourth, pseudotime analysis infers differentiation trajectories from static transcriptomes rather than observing lineage transitions directly. Fifth, the study provided limited ability to interrogate host-microbe interactions. No paired microbiome profiling, spatial microbial mapping, or direct assessment of intracellular bacterial localization was performed, so microbial contributions to epithelial and endothelial states could only be inferred indirectly from clinical observations and prior literature. Sixth, spatial proximity between *CXCL14*⁺ epithelial cells, HLA-DR/MHC-II-expressing endothelial cells, and immune cells does not establish direct chemotaxis or antigen presentation. Seventh, although the HUVEC assay supported inducibility of endothelial MHC class II, it did not test antigen-specific T-cell activation and does not fully recapitulate the phenotype of oral microvascular endothelial cells.

An additional limitation was reduced immune-cell capture in the single-nucleus RNA sequencing dataset. Relative under-representation of immune cells, particularly fragile or low-RNA populations, is a recognized feature of snRNA-seq and has also been reported in some scRNA-seq studies of dense or inflamed tissues^{33,51,52}. This likely reflected both technical features of nuclei-based profiling and the challenges of processing structurally complex gingival/mucosal biopsies. As a result, the current dataset did not support robust cell-cell interaction analyses across the full immune compartment and also constrained more direct evaluation of host-microbe interactions.

5.8 Future Directions

Future work should expand cohort size, include healthy peri-implant mucosa where feasible, and incorporate longitudinal sampling before and after therapy. These additions would help determine whether epithelial and endothelial states track disease activity, treatment response, or disease relapse. On the epithelial side, perturbation of CXCL14 and stress-keratin programs in organotypic oral mucosal models could test whether these signals directly influence immune positioning or instead mark barrier state. On the endothelial side, coculture with oral pathogens and antigen-presentation assays will be required to define the functional role of HLA-DR/MHC II-positive endothelial cells more precisely. Future single-cell studies should also aim to improve immune-cell recovery so that epithelial, stromal, and immune interaction networks can be resolved at comparable depth.

A second priority is broader systems integration. Pairing host single-cell and spatial data with microbiome profiling and spatial microbial readouts could clarify whether the epithelial and endothelial states identified here are linked to specific microbial ecologies at tooth and implant sites. Integration of implant-surface features, biomechanical loading, and patient-level clinical variables will also be important for defining why the periodontal and peri-implant barriers adopt different inflammatory programs and for informing future clinical decision-making.

6. Conclusion

This study supports the hypothesis that distinct inflammatory profiles in periodontitis and peri-implantitis are associated with condition-specific changes in epithelial and endothelial compartments of the oral mucosal barrier. Periodontitis was characterized by expansion of repair-skewed basal epithelial states, loss of suprabasal barrier-forming states, and stronger CXCL14-linked immune proximity at the epithelial interface. Peri-implantitis retained differentiated suprabasal epithelial programs but displayed stress-adapted barrier features, including enrichment of *KRT6A/B/C* and *KRT16*, and weaker spatial coupling between CXCL14 and nearby immune cells. In parallel, both diseases contained a rare HLA-DR/MHC II-positive endothelial state positioned near T cells and inducible by inflammatory stimulation, primarily by IFN- γ , *in vitro*. Together, these findings refine current understanding of how epithelial and stromal cells may participate in inflammation in periodontitis and peri-implantitis and provide a foundation for future barrier-targeted and vascular-targeted host-modulatory strategies.

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