Cross-Tissue Analysis Demonstrates Oral and Systemic Link in Chronic Periodontitis

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ABSTRACT

- **Introduction**: Despite advances in dental care and oral hygiene, the prevalence of chronic periodontitis (ChP) remains high at 47.2% in the U.S. ChP not only leads to severe gum bleeding and tooth loss but also contributes to systemic diseases, including diabetes mellitus, adverse pregnancy outcomes, and cardiovascular diseases. The mechanisms of the connection between ChP and systemic health are still not fully understood. In our phase I study, we demonstrated systemic and cell-specific immune dysfunctions in patients with ChP, which can be temporarily reversed by the local treatment of ChP. For phase II study, we aim to 1) validate our findings with larger cohorts across wider time range and 2) investigate ChP oral and systemic immunological interaction by a multiplex cross-tissue analysis.
- **Methods**: Whole-blood samples from 16 patients with ChP and 12 controls were collected at baseline (n=28), 3 weeks post-ChP treatment (n= 25), and 3 months post-ChP treatment (n= 25) in the Bell Dental Center (San Leandro, CA). The blood samples were left unstimulated or stimulated with *Porphyromonas gingivalis* lipopolysaccharide (PgLPS), tumor necrosis factor α (TNF-α), interleukin (IL-12), or a cocktail of interleukins 2/4/6 (IL-2/4/6), then will be analyzed using mass cytometry (CyTOF). The gingival tissue from ChP patients at baseline will be examined with imaging mass cytometry (IMC).
- **Future Directions**: An integrated multiomic approach will be employed to analyze the results from CyTOF and IMC.
- **Conclusion**: The results obtained from this study will empower us to explore the synergies of systemic and oral immune mechanisms that contribute to a defined ChP-driven immune milieu while underscoring the effectiveness of standard nonsurgical periodontal treatment.

OBJECTIVES

- **To validate the findings of systemic and cell-specific immune dysfunctions in patients with ChP using a larger cohort across a wider time range to enhance the robustness and generalizability of the results**
- **To investigate ChP oral and systemic immunological interaction via multiplex cross-tissue analysis along with cutting-edge multiomic technologies**

METHODS

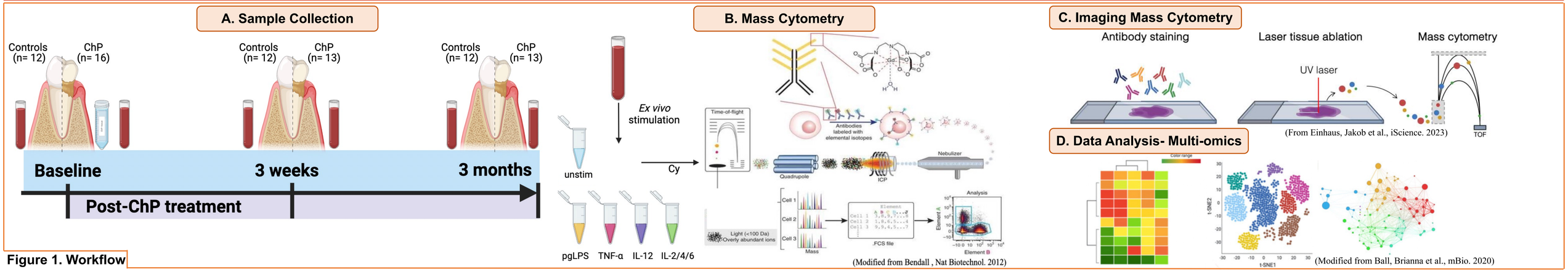

Figure 1. Workflow

RESULTS

Demographics Table

Mean±SD, *t* test.

	Control (n=12)	ChP (n=16)
Demographics		
Age (years)	32.2±5.0	31.7±5.5
Race/ethnicity		
Asian	7	4
African American	1	1
White	0	1
Hispanic	4	10
Sex		
Female	7	10
Male	5	6
Health Metrics		
BMI (kg/m^2)	26.8±6.0	29.3±6.4
DBP (mmHg)	117.4±11.0	121.3±12.3
SBP (mmHg)	82.0±8.9	78.6±11.1
HR (bpm)	73.0±10.1	74.2±11.3
Smoking History	N/A	N/A

Table 1. Patient Demographics

Lymphocytes		Myeloid cells		Structural		Signaling		Functional
IMC	CyTOF	IMC	CyTOF	IMC	CyTOF	IMC	CyTOF	IMC
CD45	CD45	CD66b	CD66b	vimentin	cRARP	pERK	pERK1/2	Granzyme B
CD20	CD19	CD14	CD14	aSMA	CD235ab	pS6	pS6	VEGF
CD45RA	CD45RA	CD209	CD123	NE	CD61	pp38	pp38	Ki67
CD4	CD4	CD16	CD16	PanCK		pSTAT1	pSTAT1	MMp-9
CD8a	CD8a	CD11b	CD11b	CD31		pMK2	pMK2	
CD3	CD3	CD11c	CD11c	Collagen1		pNFkB	pNFkB	
CD56	CD56	CD15	CD33			pCREB	pCREB	
FoxP3	FoxP3	CD163	CCR2	**Nuclear**		pSTAT3	pSTAT3	
	CD62L	HLA-DR	HLA-DR	IMC	CyTOF	pSTAT5	pSTAT5	
	CD161		FceRIα	Iridium-191	Iridium-191		pSTAT6	
	Tbet		CRTH2	Iridium-193	Iridium-193		IκB	
	TCRγδ		CXCR4	Histone H3				
	CD25		OLFM4					
	CD7							

Table 2. IMC and CyTOF Antibody Panel

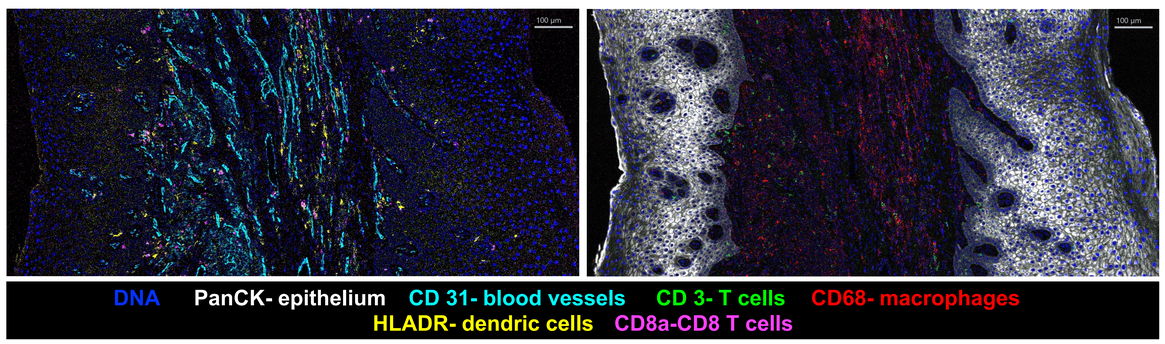

Figure 3. Represent IMC image of gingival tissue from patients with ChP collected at baseline.

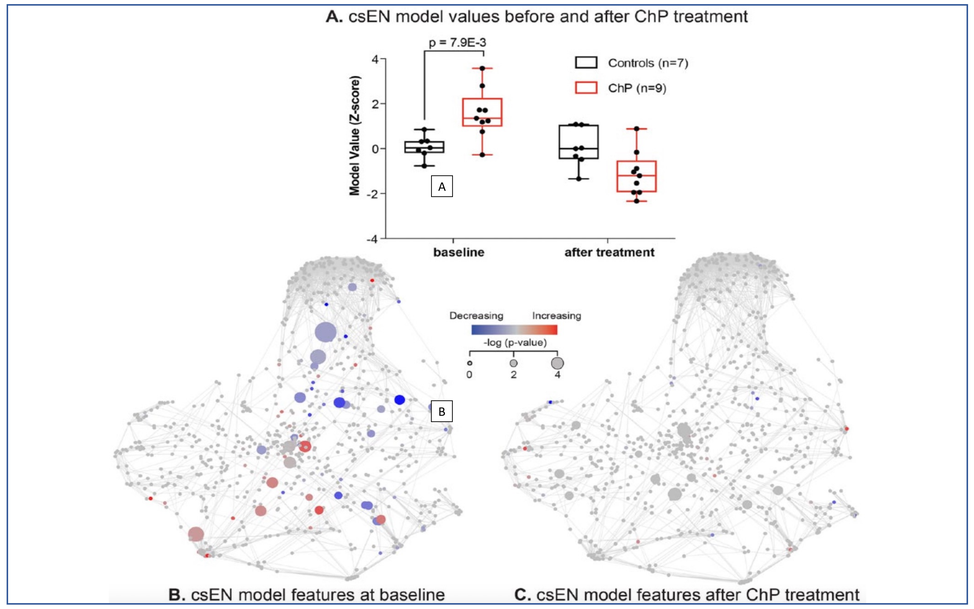

Figure 2. Systemic immune signature of chronic periodontitis (ChP) pre- and post-ChP treatment (phase I study). (A) Box plot illustrating the cell signaling–based elastic net (csEN) model values in subjects with ChP and controls, pre- and post-ChP treatment. Before ChP treatment, the csEN values increase in subjects with ChP in comparison to controls (9 patients, 7 controls, Wilcoxon rank sum test P = 7.9E-3). There is no change in the csEN values between subjects with ChP and controls post-ChP treatment. **(B, C)** csEN values overlaid on the immune signaling network for subjects who underwent ChP treatment. **(B)** Pre-treatment. **(C)** Post-treatment.

FUTURE DIRECTIONS

- Proceed with future experiments and data analysis to address the **hypothesis**: The active innate immunity in local gingival tissue and peripheral blood due to chronic periodontitis could be normalized with ChP treatment.

Future Experimental Timeline	
04/2024-06/2024	Conduct CyTOF experiment
07/2024-09/2024	Conduct IMC experiment
10/2024-03/2025	Data analysis

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Project Title

Cross-Tissue Analysis Demonstrates Oral and Systemic Link in Chronic Periodontitis

Full name(s) and class year(s) of all project collaborators

Example: Jane Smith, DDS 2022; John Smith, DDS 2022

Alice Lin DDS 2026, Nicole Thompson DDS 2026, Makaylan Tseng undergraduate

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