Longitudinal Cytokine Dynamics During Palatal Wound Healing After Free Gingival Graft

A Comparative Analysis of StellaLife, Chlorhexidine, and Control

Veronica Xia, DMD
Mentor: Dr. Joshi
Research Day 2026
LSUSD Periodontics

BACKGROUND

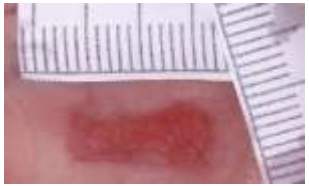
Free Gingival Graft Procedure

What Is an FGG?

A common periodontal procedure used to **increase the zone of keratinized gingiva**[1] around teeth or implants. Tissue is harvested from the palate, leaving a wound that heals via secondary intention.

Why does it matter?

Patients often experience a **significant amount of pain and discomfort during**[2] the time it takes the palatal wound to fully heal. Reducing this morbidity is a major clinical goal.



Palatal Wound Healing Phases [3,4]

1	**Hemostasis** Day 0
2	**Inflammation** Days 1–7
3	**Proliferation** Days 7–21
4	**Remodeling/Maturation** Week 3 to months

StellaLife® VEGA® Oral Care Recovery Kit

Homeopathic OTC Product

Active Ingredients

Rinse

Azadirachta (Neem), Calendula officinalis, Echinacea angustifolia, Plantago major

Spray & Gel

Arnica montana, Aconitum napellus, Calendula, Chamomilla, Echinacea, Gelsemium, Hypericum, Ignatia, Mercurius, Ruta graveolens, Hepar sulphuris

Inactive Ingredients (Rinse):

Allantoin, Citric Acid, Glycerin USP, Peppermint oil, Polysorbate 20, Potassium sorbate, Propolis, Xylitol

Inactive Ingredients (Spray/Gel):

Spray: Citric Acid, Glycerin, Potassium sorbate, Purified Water
Gel: Allantoin, Hydroxyethylcellulose, Peppermint oil, Sodium benzoate, Xylitol

Product Groups

Test Group

StellaLife VEGA Oral Care Recovery Kit

- Homeopathic, opioid-free rinse, spray, gel [5]
- 16 active plant-derived ingredients [5]
- Includes Arnica [8], Calendula, Azadirachta, Echinacea
- Claims: anti-inflammatory, analgesic, antimicrobial, accelerated healing [5,7]
- In vitro: reduced TNF-a, IL-6 vs. CHX [6]

Active Control

Chlorhexidine (CHX) 0.12% Rinse

- Gold standard antimicrobial oral rinse [11]
- Broad-spectrum antibacterial agent [11]
- Routinely prescribed after surgery
- Early wound healing, reduced plaque accumulation and gingival inflammation[12]
- Staining, alterations in taste, mucosal sloughing [16]

Negative Control

Saline (Normal Saline Rinse)

- Neutral oral rinse — no active agents
- Used as the negative control
- Represents unaided natural healing
- Establishes baseline healing pattern
- No antimicrobial or anti-inflammatory effect

Previous Studies: Do Adjunctive Rinses Alter Wound Biology?

StellaLife (In Vitro)

Fujioka-Kobayashi et al., 2020 [6]

Zhou et al., 2021 [9]

↑ Growth Factors
↑ Fibroblast viability and migration
↓ Pro-inflammatory cytokines TNF-a & IL-6

CHX (In Vitro)

Fujioka-Kobayashi et al., 2020 [6]

Mariotti & Rumpf, 1999 [10]

↓ Fibroblast proliferation
↓ Collagen (COL1) synthesis
↑ Cytotoxicity

StellaLife vs CHX (Clinical)

Aslam et al., 2025 [17]

Pardo et al., 2024 [12]

StellaLife is clinically comparable to CHX
CHX does not impair wound healing (in vitro cytotoxicity did not translate clinically)

PURPOSE

Purpose & Study Aims

Central Question: **Does the type of post-operative regimen influence inflammatory cytokine dynamics during palatal wound healing?**

Aim 1

Characterize temporal changes in inflammatory cytokines (IL-8, IL-1B, IL-10, EGF, VEGF-A, MMP-8, TIMP-1, MPO, IL-17, PDGF-BB) during healing

Aim 2

Compare concentrations of cytokine expression between StellaLife, CHX, and saline

Aim 3

Evaluate relationship of cytokines (IL-8, IL-1B, IL-10, EGF, VEGF-A, MMP-8, TIMP-1, MPO, IL-17, PDGF-BB) and progression of healing

Study Design

Location:	LSU Postgraduate Periodontics Clinic
Study design:	Prospective, randomized, controlled trial
Randomization:	Computer-generated randomization table
Pre-treatment:	Phase I SRP + oral hygiene instructions
Surgical template:	Standardized palatal template (6 × 15 mm donor site)
Product use:	Began day of surgery; 2× daily for 2 weeks
Follow-up visits:	Days 0, 7, 14, 21, and 28 post-surgery
Sample collection:	Days 0, 7, 14, 21, and 28

INCLUSION CRITERIA

- ✓ Age 18+
- ✓ Requires free gingival graft (FGG) procedure
- ✓ No medications affecting periodontal status within preceding 6mo
- ✓ No known allergy to test product ingredients
- ✓ Non-smoker
- ✓ No history of pregnancy or lactation
- ✓ Demonstrated good oral hygiene
- ✓ Phase I therapy (SRP + OHI) completed prior to surgery

EXCLUSION CRITERIA

- ✗ Prior surgery within 4 months of proposed re-surgery
- ✗ Donor tissue harvest from a previously harvested palatal site
- ✗ Osteoporosis
- ✗ Diabetes mellitus
- ✗ Rheumatoid arthritis
- ✗ Any systemic disease known to compromise wound healing

Follow-up visits

StellaLife Group

CHX Group

Control Group

Sample — Sample — Sample — Sample — Sample

Surgery (Day 0) — **Day 7** — **Day 14** — **Day 21** — Day 28

Palatal wound exudate collected at Days 0, 7, 14, 21, 28 using 10 sterile paper points. Only cytokine data on Days 7-21 were analyzed.

Inflammatory Mediators Analyzed

Palatal wound exudate was analyzed for these inflammatory mediators: [13,14,15]

IL-8
Pro-inflammatory chemokine
Recruits and activates neutrophils at the wound site

IL-1β
Regulator of acute inflammation
Produced by activated macrophages
Amplify cytokine production, neutrophil recruitment, and fibroblast stimulation

IL-17
Pro-inflammatory cytokine from T-helper 17 cells
Amplifies mucosal inflammation and antimicrobial responses

IL-10
Anti-inflammatory regulatory cytokine
Suppresses pro-inflammatory signaling (IL-1β, TNF-α) to resolve the inflammatory response

MMP-8
Neutrophil-derived collagenase
Degrades type I–III collagen and ECM to facilitate wound debridement and tissue remodeling

TIMP-1
Endogenous inhibitor of MMP-8
Regulates net proteolytic activity at the wound site

EGF
Mitogenic growth factor
Stimulates epithelial cell proliferation and migration to drive re-epithelialization of the wound surface

VEGF-A
Key angiogenic growth factor
Promotes new blood vessel formation

PDGF-BB
Released from platelets at wound site; promotes fibroblast recruitment, proliferation, and collagen biosynthesis

MPO
Myeloperoxidase; neutrophil granule enzyme released upon activation; marker of neutrophil-mediated oxidative activity and acute inflammatory intensity

Statistical Analysis Methods

Kruskal-Wallis Test

Applied to: Compare analyte concentrations across Days 7, 14, and 21 within each group

Dunn-Bonferroni Post-Hoc Test

Applied to: Identify which specific time point pairs differ significantly within each analyte and group (Day 7 vs 14; Day 7 vs 21; Day 14 vs 21)

Change Score Analysis (Δ = Day 21 − Day 7)

Applied to: Compare the magnitude of cytokine reduction between treatment groups; Kruskal-Wallis applied to change score distributions across groups

Spearman Rank Correlation (ρ)

Applied to:
(1) All 21 cytokine–cytokine pairs: assess network-level coordination
(2) Each analyte vs. time point: assess temporal decline relationship

Significance threshold: α = 0.05 for all tests · Correlation p-values not adjusted for multiple comparisons (exploratory)

Results: Patient Demographics

31	27	4	21–48
Total Enrolled subjects	Analyzed subjects	Attrition not included	Age Range years

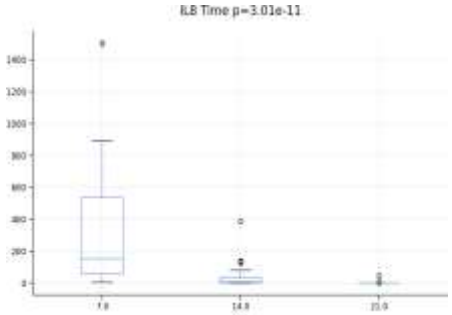
Group Distribution

Group	Total n	Day 7 n	Day 14 n	Day 21 n
StellaLife	**9**	9	9	8
CHX	**10**	10	10	10
Control	**8**	8	8	8
TOTAL	**27**	27	27	26

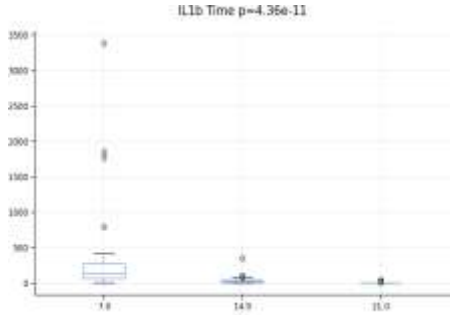
Sex Distribution	
Male	**Female**
n = 15 (56%)	**n = 12 (44%)**

Results: Temporal Distribution of Cytokines

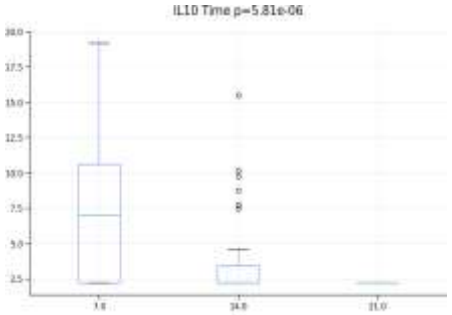
Temporal distribution of cytokines across Days 7, 14, and 21. All analytes showed significant time-dependent differences (Kruskal–Wallis p < 0.01).



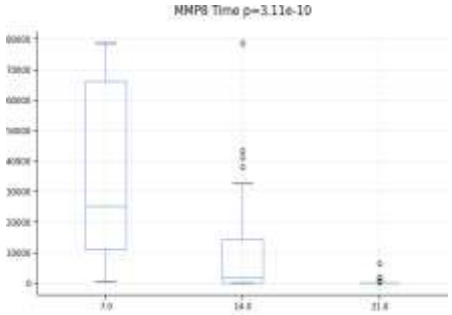
IL-8 Time p=3.01e-11 · Y-axis: Concentration (pg/mL)



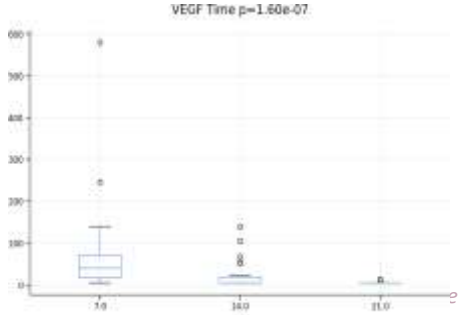
IL-1β Time p=4.36e-11 · Y-axis: Concentration (pg/mL)



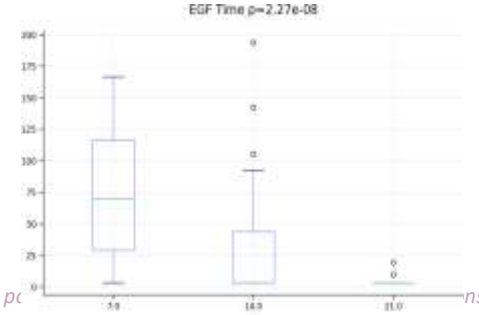
IL-10 Time p=3.53e-02 · Y-axis: Concentration (pg/mL)



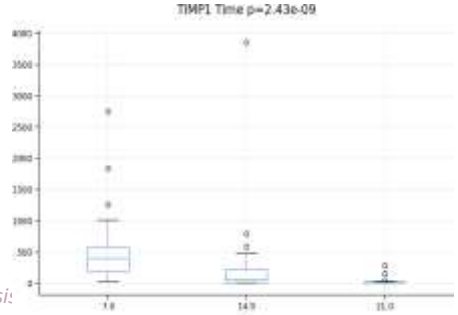
MMP-8 Time p=8.75e-05 · Y-axis: Concentration (pg/mL)



VEGF-A Time p=1.27e-03 · Y-axis: Concentration (pg/mL)



EGF Time p=2.86e-04 · Y-axis: Concentration (pg/mL)



TIMP-1 Time p=4.47e-05 · Y-axis: Concentration (pg/mL)

Results: Correlation Analysis

ρ = 0.97 — **IL-8 & IL-1β**
Strongest pair correlation

ρ = 0.96 — **EGF & VEGF-A**
Growth factors move together

ρ = 0.95 — **MMP-8 & IL-1β**
Strong pair correlation

ρ = −0.78 — **IL-8 vs Time**
Strongest decline over time

- Strong positive correlation between cytokines
- Strong negative correlation of all cytokines with time
- Indicates coordinated wound healing response

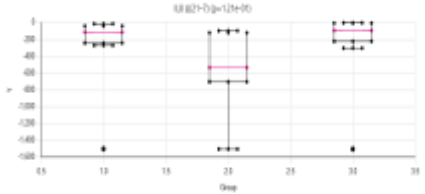
ρ (Spearman's rho) · All 7 analytes: $p < 0.001$ · All groups combined

Results: Change Score Analysis

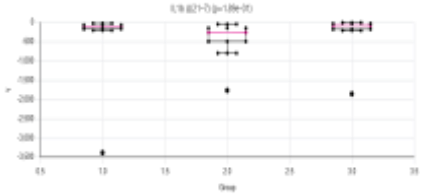
Change scores (Δ Day 21 − Day 7) across groups. No statistically significant differences between Control, CHX, and SL (p > 0.05).

Group Comparison p-values (StellaLife vs. CHX vs. Control)

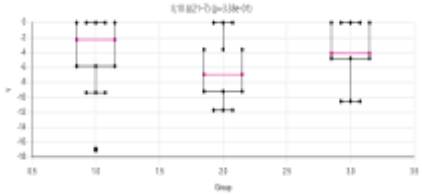
Marker	IL-8	IL-1β	IL-10	EGF	VEGF-A	MMP-8	TIMP-1
p-value (Kruskal-Wallis)	0.273	0.322	0.614	0.246	0.459	0.152	0.108



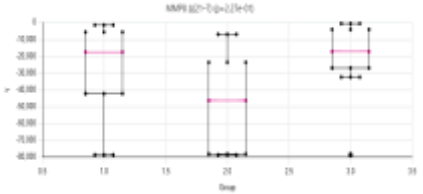
IL-8 Δ(21−7)



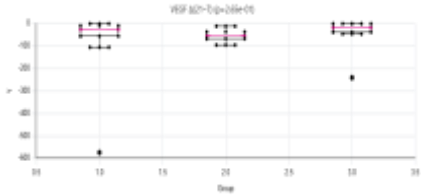
IL-1β Δ(21−7)



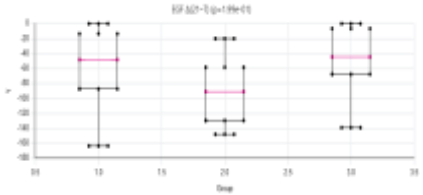
IL-10 Δ(21−7)



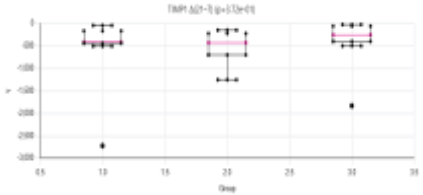
MMP-8 Δ(21−7)



VEGF-A Δ(21−7)

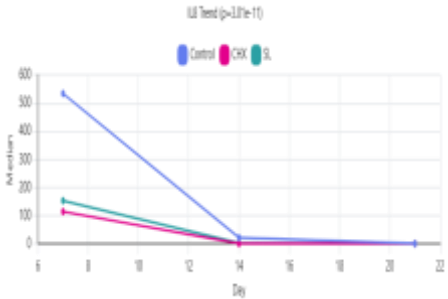


EGF Δ(21−7)

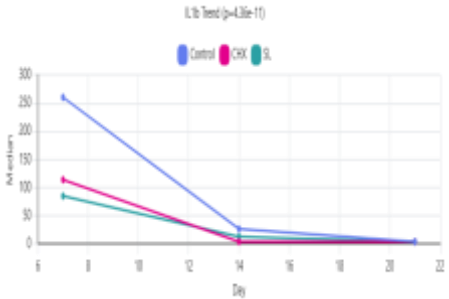


TIMP-1 Δ(21−7)

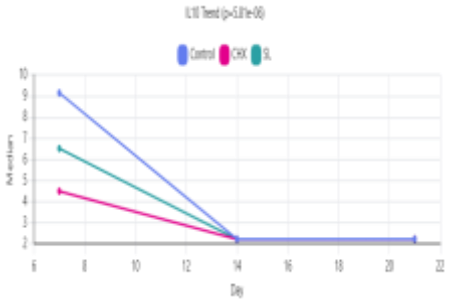
Results: Longitudinal Trajectories



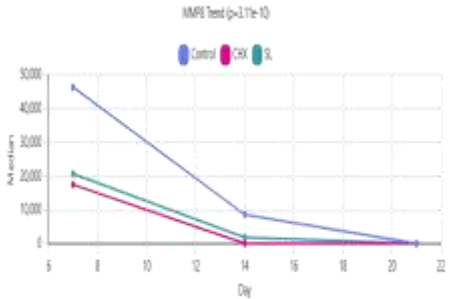
IL-8 Trend p=3.01e-11 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



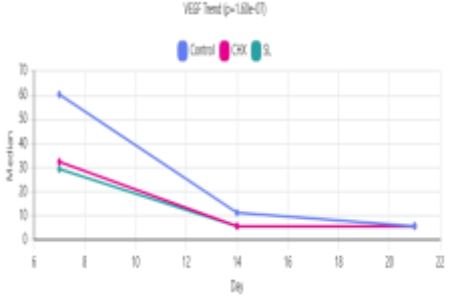
IL-1β Trend p=4.36e-11 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



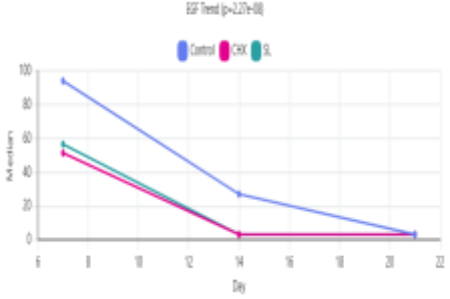
IL-10 Trend p=5.81e-06 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



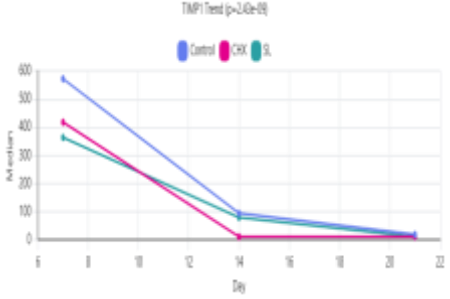
MMP-8 Trend p=3.11e-10 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



VEGF-A Trend p=1.60e-07 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



EGF Trend p=2.27e-08 · X-axis: Day · Y-axis: Median Concentration (pg/mL)



TIMP-1 Trend p=2.43e-09 · X-axis: Day · Y-axis: Median Concentration (pg/mL)

Longitudinal trajectories demonstrate progressive decline from Day 7 to Day 21 across all groups. No treatment-specific separation observed.

Discussion

Time Dependent Cytokine Expression

Cytokine expressions shows a strong temporal dependency in cytokine dynamics, with minimal influence of adjunctive treatment modalities
Cytokine profiles align with phases of secondary intention wound healing

Effect of Treatment Modality

There was absence of significant differences between treatment groups possibly due to dominance of intrinsic healing mechanisms and coordinated cytokine response

Biological Findings vs Patient Reported Outcomes

Although significant differences in cytokine concentrations were not seen between treatment modalities and were seen to be time dependent, a previous study conducted in conjunction by Dr. Amber Kreko revealed that patient reported pain, discomfort, and function were significantly improved in the StellaLife and CHX group

Limitations

Small sample size (<30)
Originally planned for Day 3 follow up
Limited bioavailability of topical agents

Conclusions

1. All inflammatory mediators (IL-8, IL-1β, IL-10, EGF, VEGF-A, MMP-8, TIMP-1) demonstrated statistically significant time-dependent decreases across the 21-day healing period

2. Inflammatory mediator concentrations peaked at Day 7 and declined towards Days 14 and 21, with no significant differences between Days 14 and 21 — indicating that the majority of inflammatory resolution occurs within the first two weeks post-FGG

3. No statistically significant differences in inflammatory mediator concentrations were detected between the StellaLife, chlorhexidine, and saline control groups at any time point, suggesting that adjunctive topical therapy does not significantly alter the cytokine biological environment of the healing palatal wound

4. Healing followed a consistent temporal pattern across all groups, indicating a response governed by a highly coordinated network-level and time dependent inflammatory response rather than treatment modality

Adjunctive rinses did not significantly alter cytokine dynamics during palatal wound healing

References

1 Sanz M, Simion M. Surgical techniques on periodontal plastic surgery and soft tissue regeneration. J Clin Periodontol. 2014;41 Suppl 15:S92–7.

2 Tavelli L, Barootchi S, Stefanini M, et al. Wound healing dynamics, morbidity, and complications of palatal soft-tissue harvesting. Periodontol 2000. 2023;92(1):90–119.

3 Clark RA. Wound repair — overview and general considerations. In: Clark RA, ed. The Molecular and Cellular Biology of Wound Repair. Plenum Press; 1996:3–50.

4 Harper D, Young A, McNaught C-E. The physiology of wound healing. Surgery. 2014;32:445–450.

5 Estrin NE, Romanos GE, Tatch W, Pikos M, Miron RJ. Biological characterization, properties, and clinical use of a novel homeopathic antiseptic oral recovery kit. Oral Health Prev Dent. 2022;20(1):485–499.

6 Fujioka-Kobayashi M, Schaller B, Pikos MA, Sculean A, Miron RJ. Cytotoxicity and gene expression changes of a novel homeopathic antiseptic oral rinse in comparison to chlorhexidine in gingival fibroblasts. Materials (Basel). 2020;13(14):3190.

7 Lee C, Suzuki J. The efficacy of preemptive analgesia using a non-opioid alternative therapy regimen on postoperative analgesia following block bone graft surgery. Clin J Pharmacol Pharmacother. 2019;1(2):1006.

8 Sharma S, Arif M, Nirala RK, et al. Cumulative therapeutic effects of phytochemicals in Arnica montana flower extract alleviated collagen-induced arthritis. J Sci Food Agric. 2016;96(5):1500–10.

9 Zhou P, Chrepa V, Karoussis I, Pikos MA, Kotsakis GA. Cytocompatibility properties of an herbal compound solution support in vitro wound healing. Front Physiol. 2021;12:653661.

10 Mariotti AJ, Rumpf DA. Chlorhexidine-induced changes to human gingival fibroblast collagen and non-collagen protein production. J Periodontol. 1999;70(12):1443–8.

11 Müller HD, Eick S, Moritz A, Lussi A, Gruber R. Cytotoxicity and antimicrobial activity of oral rinses in vitro. Biomed Res Int. 2017;2017:4019723.

12 Pardo C, Sanz M, Herrera D, et al. Early periodontal wound healing after chlorhexidine rinsing: a randomized clinical trial. Clin Oral Investig. 2024;28(6).

13 Yadav VS, Makker K, Tewari N, et al. Expression of wound healing markers in GCF following root-coverage procedures: A systematic review of RCTs. Arch Oral Biol. 2024;166:106035.

14 Trombelli L, Farina R, Marzola A, et al. Early healing events after periodontal surgery: observations on soft tissue healing, microcirculation, and wound fluid cytokine levels. Int J Mol Sci. 2017;18(2):283.

15 Keceli HG, Ercan N, Atilla G, et al. Cytokine and MMP levels in GCF after use of platelet-rich fibrin or connective tissue graft in treatment of gingival recessions. J Periodontal Res. 2015;50(6):761–71.

16 James P, Worthington HV, Parnell C, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017;3:CD008676.

17 Aslam Z, Wu J, Wang Z, Anderson NK, Estrin NE, Romanos GE. Efficacy of herbal vs. chlorhexidine mouthwash in experimental gingivitis: a cross-over clinical and microbiological study. Dent J. 2025;13(12):608.

Thank you!