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Original Article

Ex Vivo Evidence of Oxygen-Induced Neoangiogenesis in Human Palatal Mucosa

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ABSTRACT

Despite the growing interest in oxygen-based medical interventions, limited evidence exists regarding the influence of Topical Oxygen Therapy (TOT) on oral mucosal tissues. This exploratory investigation sought to determine, through histological assessment, how palatal epithelial–connective tissue responds to localized oxygen exposure. Palatal tissue samples were collected from eight individuals undergoing free gingival graft (FGG) surgery. For each graft, one lateral section was randomly subjected to TOT (test), while the other remained untreated (control). Hematoxylin–eosin staining was used to assess overall tissue morphology, Picrosirius red staining evaluated collagen composition, and immunohistochemical assays identified inflammatory and angiogenic markers.

No structural abnormalities or cytotoxic effects were noted in either group, confirming that TOT did not compromise tissue integrity. Both groups predominantly exhibited type I collagen. Sparse immune infiltration, mainly of CD3+ T lymphocytes, was detected. Quantitative histomorphometric evaluation demonstrated that oxygen-treated specimens possessed a significantly larger mean vessel area (7607.95 μm² ± 3983.24 vs. 4038.42 μm² ± 1823.52), a greater number and caliber of blood vessels (49.82 ± 20.55 vs. 32.35 ± 16.64), and higher microvessel density (7.89 ± 3.25 vs. 5.13 ± 2.63 vessels/0.26 mm²). The findings suggest that topical oxygen application promotes microvascular development in palatal tissues without inducing morphological damage, indicating a potential role for TOT in accelerating oral wound repair through localized hyperoxia.

Keywords: Oral wound repair, Topical oxygen exposure, Tissue morphology, Immunohistochemical profiling, Vascular regeneration

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Introduction

Wound healing is a complex and highly regulated biological process that depends on the coordinated interaction of various cell populations and biochemical mediators. These components act in a precise temporal sequence to ensure the proper restoration of injured tissues and the quality of the final repair outcome [1].

Among the many factors influencing tissue repair, oxygen plays a fundamental role [2]. The regeneration of damaged tissue requires substantial energy, and this demand is tightly linked to oxygen availability and utilization at the wound site. When oxygen levels are reduced or absent, the healing process may slow down or even cease completely. Interestingly, the transient hypoxia that follows tissue injury serves as a key stimulus that activates several reparative mechanisms. It triggers macrophages, keratinocytes, and fibroblasts to release essential cytokines and growth factors, including platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), tumor necrosis factor-alpha (TNF-α), and endothelin-1 [3]. However, when hypoxia becomes chronic or prolonged, it hinders angiogenesis, as the energy required for cellular

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activity is insufficient, ultimately delaying wound closure and potentially leading to tissue necrosis [4].

Many wounds develop in areas already characterized by poor oxygenation. Conditions such as diabetic foot ulcers, pressure injuries, venous stasis ulcers, and arterial insufficiency ulcers are all marked by compromised perfusion and limited oxygen delivery to local tissues [5, 6].

Since the 1960s, a variety of oxygen-based interventions have been introduced to enhance wound healing. Among these, Hyperbaric Oxygen Therapy (HBOT), Topical Oxygen Therapy (TOT), and Continuous Diffusion of Oxygen Therapy (CDOT) have demonstrated promising results. HBOT remains the most established and is typically reserved for chronic or refractory wounds, requiring clinical supervision and pre-treatment assessment. TOT, in contrast, provides oxygen directly to the wound surface, thereby alleviating local hypoxia in a targeted and non-invasive manner. This approach offers multiple advantages—its application is simple, cost-effective, and free from the risks associated with systemic hyperoxia or barotrauma observed in HBOT [7–9].

TOT has been reported to significantly improve the healing of various wound types, including diabetic, pressure, and venous ulcers [10]. Evidence indicates that when used as an adjunctive therapy, TOT enhances healing rates by improving tissue oxygen tension, which promotes angiogenesis, bacterial clearance, and collagen production [11]. Oxygen supports both the inflammatory and proliferative phases of healing, fostering granulation tissue formation and vascular development. Although reports on the total healing time are variable [12, 13], nearly all clinical trials have documented a measurable reduction in wound surface area following TOT treatment [14]. Despite these findings, research exploring the effects of TOT on **oral tissues** remains limited.

Recently, ex vivo models using freshly harvested human skin explants have gained traction as valuable tools for biomedical research [15, 16]. These models preserve the native microenvironment and physiological responses of the tissue while eliminating the need for cell culture. However, their use is inherently limited by the absence of vascular perfusion, meaning the inflammatory and angiogenic responses observed reflect only local cell-mediated events rather than systemic influences.

To the best of our knowledge, the present study is the **first** to apply a human ex vivo model using epithelial–connective tissue obtained from the palate to investigate, at the histological level, the response of oral mucosa to Topical Oxygen Therapy.

Materials and Methods

Study design

This pilot study employed a human ex vivo palatal tissue model to assess the histological effects of Topical Oxygen Therapy. Palatal epithelial–connective tissue segments were obtained during free gingival graft (FGG) surgeries. Each harvested graft was divided into three sections. The two lateral fragments, which were not required for keratinized tissue augmentation, were randomly allocated to one of two groups: a test group (treated with TOT) and a control group (untreated, without oxygen exposure). Both sets of samples were subsequently processed and analyzed histologically **(Figure 1)**.

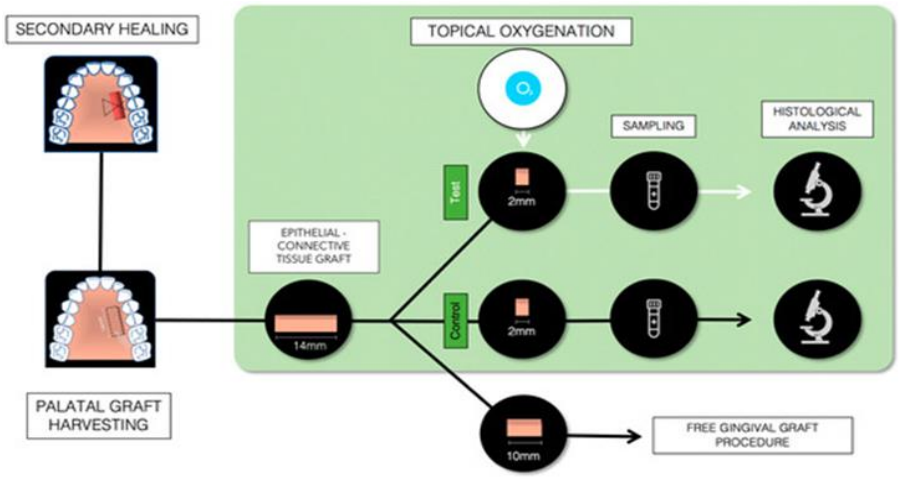


Figure 1. Flowchart of the study.

Study setting and ethical considerations

From March to July 2022, patient enrollment and treatment were carried out at the Section of Periodontics, Department of Oral and Maxillofacial Sciences, Sapienza University of Rome. Histological examinations were subsequently performed at the

Department of Radiological, Oncological and Pathological Sciences within the same university. The research protocol received approval from the Ethics Committee of Sapienza University of Rome (protocol number 0155/2022, approval date: 3 March 2022). All clinical procedures adhered to the ethical standards established in the Declaration of Helsinki and complied with international Good Clinical Practice guidelines. Written informed consent was obtained from all participants before their inclusion in the study and prior to any intervention.

Study population

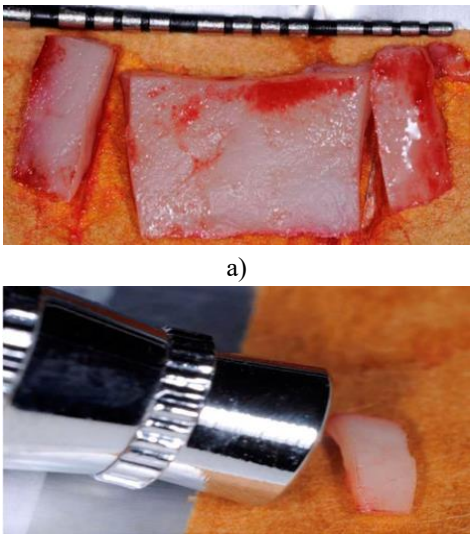
Participants were selected among patients attending the Periodontology Unit of the Department of Oral and Maxillofacial Sciences at Sapienza University of Rome. Inclusion criteria required that participants be at least 18 years old, present less than 1 mm of attached gingiva on the buccal surface of at least one mandibular incisor, and show an acceptable level of oral hygiene, indicated by a full-mouth plaque score and a full-mouth bleeding score below 15 percent. Gingival recession or a high frenulum insertion did not preclude participation. All subjects demonstrated periodontal health, either on an intact or a reduced periodontium.

Individuals were excluded if they were smokers, had a history of cancer or had received radiotherapy or chemotherapy for neoplastic conditions, were pregnant or breastfeeding within the previous five months, or were taking medications known to interfere with tissue repair, such as corticosteroids or high-dose anti-inflammatory drugs. Additional exclusion criteria included the presence of metabolic or systemic diseases capable of altering tissue metabolism and untreated periodontitis.

Tissue sampling and human ex vivo palatal tissue model

An epithelial–connective tissue graft measuring approximately 14 by 7 millimeters was obtained from the palatal mucosa located between the first premolar and the first molar, maintaining a distance of about 2 millimeters from the gingival margin. After tissue removal, the donor site was rinsed with sterile saline and sutured with 4-0 silk to stabilize the blood clot and promote healing. The graft was then refined by trimming any uneven or fatty tissue and, when required, thinned to achieve a uniform thickness of roughly 1.5 millimeters.

Each specimen was divided into three sections. The central portion, measuring 10 by 7 millimeters, was used for the free gingival graft procedure. It was carefully adapted to the recipient site, which was prepared to match the graft dimensions, and secured with 6-0 monofilament nylon sutures. The two lateral fragments, each measuring 2 by 7 millimeters, were assigned by random coin toss to one of two conditions: treatment with topical oxygen therapy (test group) or no exposure to oxygen (control group). Both samples were subsequently processed for histological examination, as illustrated in **Figure 2**.



a)

b)

Figure 2. Extra-oral topical oxygenation. (a) The graft divided into three parts. (b) Topical oxygenation treatment.

Topical oxygenation procedure

Topical oxygen therapy was delivered using an oxygen generator (VIGO-PSA-6000, CaressFlow Srl, Bologna, Italy) capable of supplying oxygen with a purity of 93 ± 3 percent at a constant flow rate of 6 liters per minute under atmospheric pressure (0.101325 MPa). The output pressure of the oxygen stream ranged between 0.04 and 0.07 MPa, remaining well below the levels typical of hyperbaric systems that operate with 100 percent oxygen in closed environments. During the procedure, oxygen was applied for a total duration of four minutes through a specialized handpiece positioned approximately two millimeters from the tissue surface.

Histological examination

Within one hour following the oxygen treatment, tissue specimens were immersed in 10 percent neutral buffered formalin and subsequently embedded in paraffin. Serial sections, each three micrometers thick, were prepared from the paraffin blocks. Sections were stained with hematoxylin and eosin to assess general tissue morphology and with Picrosirius red to visualize and differentiate collagen fibers. All samples were examined using light microscopy. Additional sections from the same tissue blocks were processed for

immunohistochemical evaluation to characterize inflammatory cell infiltration and angiogenic activity.

Picrosirius red staining was used to assess the organization and integrity of connective tissue, given that collagen constitutes a major structural component of the extracellular matrix. Type I collagen, the predominant fibrillar collagen, plays a key role in maintaining tissue strength and stability, while variations in its expression can reflect damage or ongoing remodeling processes. Under polarized light microscopy, type I collagen fibers display yellow to orange birefringence, whereas type III collagen appears green, allowing for clear differentiation between collagen types.

For immunohistochemical analysis, ready-to-use Leica antibodies were employed to identify specific cellular markers: CD3 for T lymphocytes, CD20 for B lymphocytes, CD68 for macrophages, and CD34 for endothelial cells. Staining was performed using a Leica Bond III automated immunostainer, applying the Bond Polymer Refine Detection system with 3,3′-diaminobenzidine (DAB) as the chromogen for signal visualization.

Two independent pathologists, blinded to the experimental conditions, evaluated all samples. The density of the inflammatory infiltrate was quantified in five randomly selected fields at 20× magnification and categorized according to the number of positively stained cells: absent (0), mild (1–10), moderate (10–50), or intense (>50). Data were expressed as mean ± standard deviation.

Sections labeled with the anti-CD34 antibody were used to assess vascular parameters within the subepithelial connective tissue. For each specimen, three variables were calculated: the mean number of vessels, the average vessel area (μm²), and microvessel density, expressed as the number of vessels per total examined area [17]. Immunostained slides were scanned digitally using an Aperio scanner (Leica Biosystems, Nussloch, Germany), and quantitative image analysis was performed with ImageJ software (version 1.54k). Measurements were taken in five fields at 20× magnification, corresponding to a total observed area of 6.31 mm².

Statistical analysis

This research was conceived as a pilot study aimed at exploring the feasibility and preliminary biological effects of topical oxygenation on periodontal tissues. Because of its exploratory nature, no formal sample size calculation was conducted. Quantitative data were expressed as mean values with corresponding standard error of the mean (SEM). Statistical differences between groups were evaluated using a two-tailed unpaired Student's t-test, and a p-value of less than 0.05 was considered to indicate statistical significance.

Results and Discussion

Eight patients participated in the study, including three males and five females. The mean age was 38.3 ± 8.6 years, with an age range of 29 to 51 years. Individual demographic and histological data are presented in **Table 1**.

Table 1. Demographic and histological data for each individual case.

Patient	Gender (M/F)	Age (Years)	Group	Number of Micro Vessels (in 5 Fields) (CD34)	Average Vessel Area (μm²) (CD34)	Microvessel Density (MVD) (in 1 Field) (CD34) 0.26 mm²	CD3-Positive Cells/Fields	CD20-Positive Cells/Fields	CD68-Positive Cells/Fields
1	F	51	Test	29	8.502	4.596	57	22	2
			Control	15.4	1.973	2.441	10	13	3
2	M	44	Test	65	7.591	10.301	30	34	4
			Control	15.4	1.973	2.441	13	25	5
3	F	36	Test	58	2.250	9.191	104	37	7
			Control	60	5.185	9.509	8	14	4
4	F	29	Test	79.6	15.564	12.615	201	38	7
			Control	47.8	2.679	7.575	20	13	3
5	F	36	Test	54	5.374	8.558	47	46	6
			Control	45	3.442	7.132	55	26	6
6	M	51	Test	22.6	5.404	3.582	64	18	8

			Control	29	7.064	4.596	19	24	4
7	F	31	Test	28.8	6.090	4.564	82	30	5
			Control	20	4.670	3.17	20	19	3
8	M	29	Test	61.6	10.080	9.762	83	34	6
			Control	26.2	5.321	4.152	21	19	4

Histomorphological analysis

Microscopic evaluation of hematoxylin and eosin (H&E)–stained sections revealed no appreciable histological differences between the control and treatment groups **(Figure 3)**. In both cases, the oral mucosa exhibited a stratified squamous epithelium characterized by mild hyperkeratosis (both ortho- and parakeratotic forms), focal hypergranulosis, slight elongation of the epithelial rete ridges, and acanthosis. Importantly, none of the examined specimens showed evidence of epithelial dysplasia. The underlying subepithelial connective tissue appeared focally loose in structure.

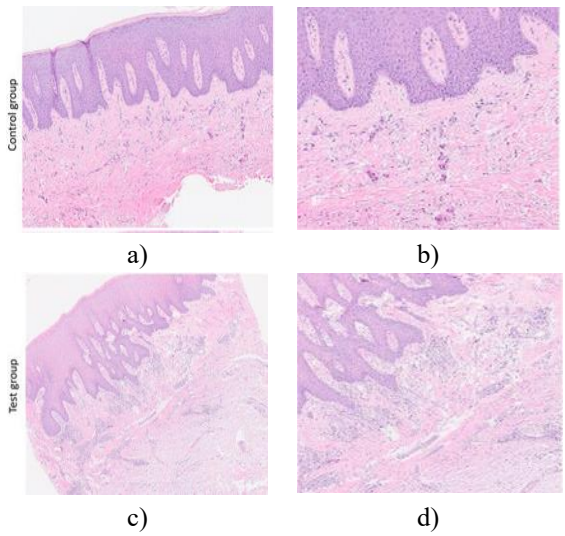


Figure 3. Representative microscopical sections of oral mucosa of control group and test group. Hematoxylin–eosin stains for each group: 10× (on left side) and 40× (on right side) magnification.

Polarized light microscopy

Under polarized light microscopy, Sirius red–stained sections demonstrated a predominant presence of type I collagen fibers, appearing orange to yellow in dark-field illumination, in both the control and treatment samples **(Figure 4)**.

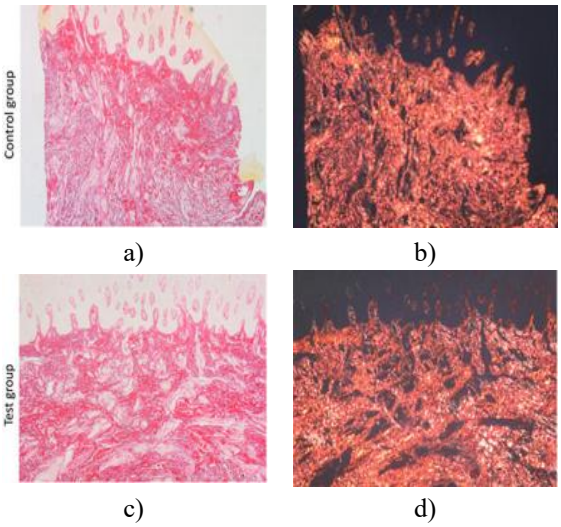
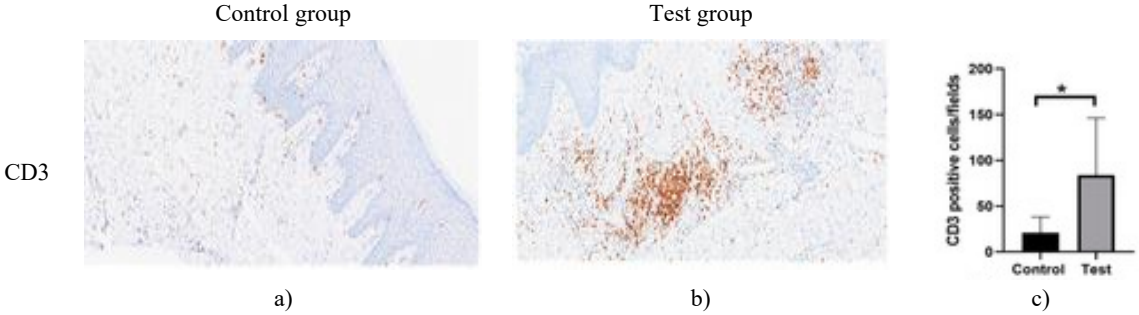


Figure 4. Microscopic view of Picrosirius-stained sections under a light optic microscope and a polarized light optic microscope (dark field) in the control group and the test group (20× magnification).

Immunohistochemical analysis

Immunohistochemical evaluation of the inflammatory infiltrate revealed a marked predominance of $CD3^+$ T lymphocytes, particularly in the test group, where their presence was significantly higher than in the control group ($p < 0.05$). $CD20^+$ B lymphocytes were occasionally detected in the test samples but were rarely observed in the control specimens. $CD68^+$ macrophages were sparsely distributed and exhibited minimal representation in both groups **(Figure 5)**.



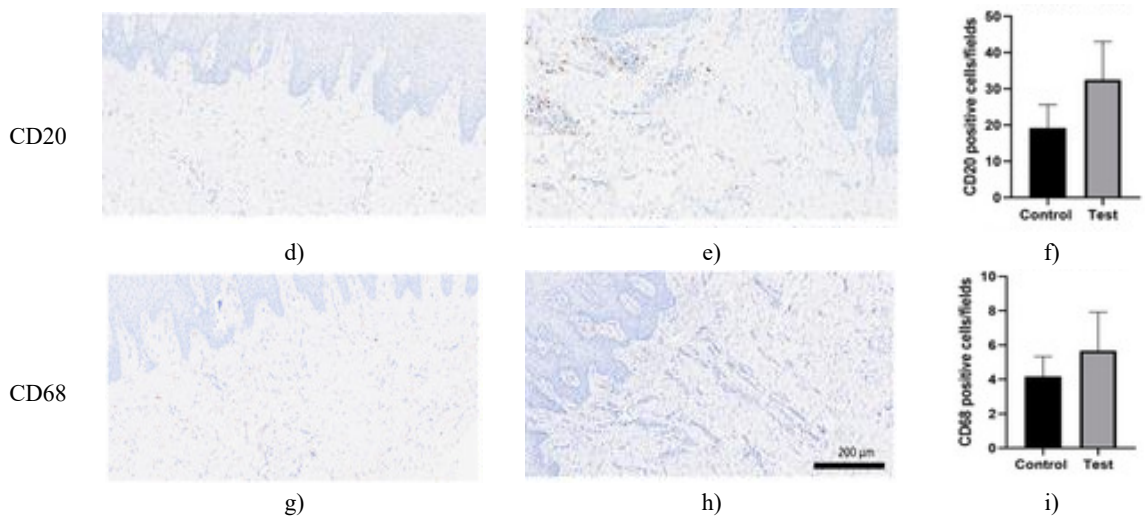


Figure 5. Immunohistochemical characterization of the inflammatory infiltrate: CD3+ T lymphocytes, CD20+ B lymphocytes, and CD68+ macrophages. (*: $p < 0.05$).

Analysis of sections stained with the anti-CD34 antibody revealed a significantly larger mean vessel area in the test group compared with the control group (7607.95 µm² ± 3983.24 versus 4038.42 µm² ± 1823.52). In addition, the test samples exhibited a greater number of vessels (49.82 ± 20.55 versus 32.35 ± 16.64) and an increased microvessel density (7.89 ± 3.25 vessels/0.26 mm² compared with 5.13 ± 2.63 vessels/0.26 mm²) relative to the control group **(Figure 6)**.

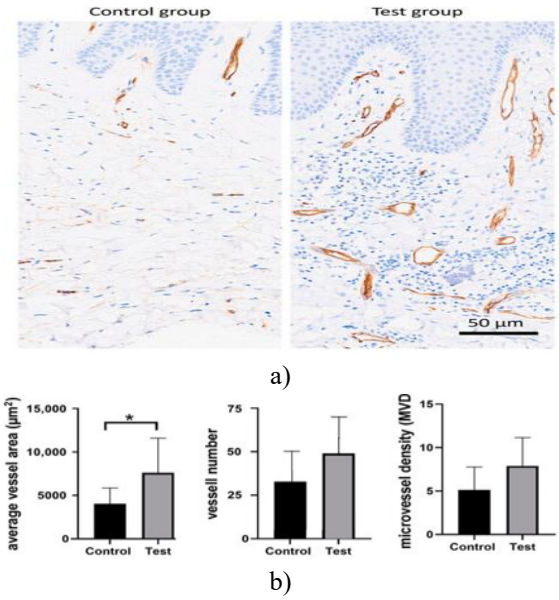


Figure 6. (**a**) Representative microscopic sections immunostained with CD34 antibody (brown stain) in the control group and test group. (**b**) Graphical representation of the vascular parameters in the control and test groups; *: $p < 0.05$.

Interest in strategies that enhance tissue oxygenation stems from the fundamental role of oxygen in the wound healing process. Elevated oxygen availability supports tissue repair by increasing cellular energy production, promoting epithelial proliferation, stimulating collagen synthesis, facilitating angiogenesis, supporting bone regeneration, and enhancing antimicrobial defenses. In contrast, hypoxic conditions impair angiogenic processes and reduce collagen deposition, partly through upregulation of collagenase-1 expression in fibroblasts, which degrades extracellular matrix components [18].

Oxygen contributes directly to the suppression of anaerobic pathogens and indirectly by augmenting the activity of leukocytes, thereby supporting local immune defense mechanisms [19]. As a substrate, oxygen also drives the generation of reactive oxygen species (ROS), which exert bactericidal effects [20]. Efficient bacterial clearance depends on a high local oxygen tension, which triggers the neutrophil respiratory burst. Activation of NADPH oxidase in neutrophils generates superoxide, which reacts with oxygen to produce ROS that are essential for microbial killing [21].

Regenerative processes are further supported by enhanced vascularization and increased capillary formation. Oxygen promotes collagen synthesis, stimulates fibroblast proliferation, and improves bone metabolism and turnover [22]. Exposure to hyperoxic conditions can induce a robust and sustained angiogenic response, supporting wound healing through subsequent stages of tissue repair [23]. In a preclinical model of excisional dermal wounds in pigs, Fries and colleagues demonstrated that topical oxygen therapy markedly increased tissue oxygen partial pressure—fourfold compared with controls after four minutes of application—and accelerated wound closure following repeated treatments. Histological

analyses of treated wounds revealed higher VEGF expression, improved angiogenesis, and more advanced tissue organization with better-quality collagen compared with untreated controls [24].

Although previous studies have documented the efficacy of topical oxygen therapy in extra-oral wound models [12–14], its effects on oral tissues remain largely unexplored. Schlagenhauf *et al*. evaluated repeated subgingival oxygen irrigations in untreated deep periodontal pockets and reported significant improvements in clinical and microbiological parameters relative to controls [25]. Similarly, Gaggi and colleagues showed that adjunctive oxygen therapy facilitated early elimination of pathogenic anaerobes and reduced periodontal tissue destruction in cases of acute necrotizing periodontal disease [26].

Most research on oxygen therapies in dentistry, however, has focused on hyperbaric oxygen treatment. In an experimental periodontitis model in rats, Gajendrareddy *et al*. applied hyperbaric oxygen for two hours twice daily over 28 days. Treated animals displayed increased expression and more uniform deposition of type I collagen fibers, whereas the inflammatory response remained unchanged [27]. Clinical studies of hyperbaric oxygen in patients with periodontitis have similarly reported reduced microbial load in periodontal pockets, with the combination of hyperbaric oxygen and conventional scaling and root planing yielding superior outcomes [28–30]. In an additional study, Shennon *et al*. treated guinea pigs with wedge-shaped gingival excisions under varying oxygen concentrations and pressures. The group exposed to 100% oxygen at 2.4 atmospheres showed more advanced healing and earlier wound maturation, while normobaric oxygen-treated animals exhibited prolonged junctional epithelium formation [31].

The present pilot study is the first to evaluate the histological impact of topical oxygen therapy in a human ex vivo palatal tissue model. The methodology was chosen because epithelial and connective tissues maintain cellular vitality and responsiveness for a limited period after excision, allowing for meaningful histological and immunological assessments even outside the organism. The study specifically examined cells most likely to respond to oxygenation, including endothelial and immune cell populations, as well as potential morphological changes such as vessel number, caliber, and density.

The results were notable. Histomorphological evaluation of H&E-stained epithelial–connective samples revealed no detectable differences between the test and control groups, indicating that topical oxygen treatment followed by formalin fixation did not induce tissue damage. Immunohistochemical characterization of the inflammatory infiltrate showed a low density of immune cells, predominantly $CD3^+$ T lymphocytes. $CD20^+$ B lymphocytes were infrequent, and $CD68^+$ macrophages were scarce. These cells were localized in their typical positions beneath the epithelium and surrounding capillary structures.

The most notable observations of this study were related to vascular parameters. Analysis of vessel number and caliber revealed a significant increase in microvessel density in the test group relative to the controls, aligning with previous findings reported by Fries and colleagues [24]. Interestingly, the test samples also exhibited the highest levels of $CD3^+$ T lymphocytes, which coincided with the greatest number and density of microvessels. This pattern suggests that the enhanced microvascularity may result not only from the direct effects of local hyperoxia but also from angiogenic signaling mediated by $CD3^+$ T lymphocytes, likely through the upregulation of VEGF [32, 33]. An alternative explanation is that localized oxygenation may induce the dilation of pre-existing small vessels, rendering previously imperceptible capillaries visible under microscopic examination.

These results have potential clinical relevance. Successful healing following mucogingival surgery depends critically on the rate and quality of revascularization, as well as the maintenance and restoration of the microvascular network within gingival tissues [34]. Altered perfusion after soft tissue grafting can affect the wound healing trajectory and influence long-term clinical outcomes. Assessing blood volume at grafted sites could also provide insights into how modifications in tissue phenotype impact peri-implant health, given that inflammation is closely linked to changes in tissue perfusion [35]. After harvesting an epithelial–connective tissue graft, healing at the recipient site relies on the reestablishment of collateral circulation from the periosteum and underlying connective tissue. Initial blood supply originates from superficial capillaries within the graft approximately 24 hours post-surgery and continues to develop over four to five days. Inadequate perfusion during this critical window can lead to ischemia, jeopardizing graft survival. A functional vascular connection between donor and recipient sites typically forms within 8 to 10 days [36]. In free gingival graft procedures, insufficient blood supply can result in vertical and horizontal graft shrinkage, potentially compromising the acquisition of adequate keratinized tissue [37]. Topical oxygen therapy may help preserve and stimulate the gingival microvasculature, reducing tissue contraction and

improving the outcomes of FGG procedures. It could also benefit a range of periodontal surgical interventions by enhancing local vascularization, which is particularly advantageous for secondary healing at the palatal donor site.

Nevertheless, this study has limitations. The ex vivo model cannot fully replicate in vivo conditions, as it lacks systemic blood flow, immune interactions, and complex tissue dynamics. The small sample size may also limit the generalizability of the findings, although it was considered sufficient for preliminary evaluation. Another limitation is the absence of an air-flow control in place of the oxygen generator, which might have provided a more rigorous comparison. The short-term nature of the study precludes assessment of long-term benefits of topical oxygenation, and histological analysis captures only a static view of tissue characteristics, potentially overlooking dynamic healing processes.

Conclusion

Within the constraints of this pilot investigation, topical oxygen therapy did not induce an increased immune response or cause tissue damage. The observed enhancement of microvascularization in oxygen-treated tissues suggests that TOT may support more efficient wound healing and optimize tissue repair.

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Conflict of Interest: None

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Ethics Statement: None

References

1. Rojas MA, Ceccarelli S, Gerini G, Vescarelli E, Marini L, Marchese C, et al. Gene expression profiles of oral soft tissue-derived fibroblast from healing wounds: Correlation with clinical outcome, autophagy activation and fibrotic markers expression. J Clin Periodontol. 2021;48(5):705–20.
2. DiPietro LA. Angiogenesis and wound repair: When enough is enough. J Leukoc Biol. 2016;100(5):979–84.
3. Bishop A. Role of oxygen in wound healing. J Wound Care. 2008;17(9):399–402.
4. Younis I. Role of oxygen in wound healing. J Wound Care. 2020;29(Suppl 5b):S4–10.
5. Mayrovitz HN, Wong S, Mancuso C. Venous, arterial, and neuropathic leg ulcers with emphasis on the geriatric population. Cureus. 2023;15(3):e38123.
6. Ehrenreich J, Bette M, Schmidt A, Roeßler M, Bakowsky U, Geisthoff UW, et al. Evaluation of digital image analysis as a supportive tool for the stratification of head and neck vascular anomalies. Eur Arch Otorhinolaryngol. 2020;277(10):2893–906.
7. Howard MA, Asmis R, Evans KK, Mustoe TA. Oxygen and wound care: A review of current therapeutic modalities and future direction. Wound Repair Regen. 2013;21(4):503–11.
8. Gottrup F, Dissemond J, Baines C, Frykberg R, Jensen PØ, Kot J, et al. Use of oxygen therapies in wound healing. J Wound Care. 2017;26(Suppl 5):S1–43.
9. Rao C, Xiao L, Liu H, Li S, Lu J, Li J, et al. Effects of topical oxygen therapy on ischemic wound healing. J Phys Ther Sci. 2016;28(1):118–23.
10. Putri IL, Alyssa A, Aisyah IF, Permatasari AAIY, Pramanasari R, Wungu CDK. The efficacy of topical oxygen therapy for wound healing: A meta-analysis of randomized controlled trials and observational studies. Int Wound J. 2024;21(4):e14960.
11. Nagarsheth K, Kankaria A, Marsella J, Dunlap E, Hawkins S, Ucuzian A, et al. Systematic review of the effects of topical oxygen therapy on wound healing. JVS Vasc Insights. 2024;2(1):100051.
12. He S, Liang C, Yi C, Wu M. Therapeutic effect of continuous diffusion of oxygen therapy combined with traditional moist wound dressing therapy in the treatment of diabetic foot ulcers. Diabetes Res Clin Pract. 2021;174(1):108743.
13. Frykberg RG, Franks PJ, Edmonds M, Brantley JN, Téot L, Wild T, et al. A multinational, multicenter, randomized, double-blinded, placebo-controlled trial to evaluate the efficacy of cyclical topical wound oxygen (TWO2) therapy in the treatment of chronic diabetic foot ulcers: The TWO2 study. Diabetes Care. 2020;43(3):616–24.
14. Tang TY, Mak MYQ, Yap CJQ, Boey JEC, Chan SL, Soon SXY, et al. An observational clinical trial examining the effect of topical oxygen therapy (Natrox™) on the rates of healing of chronic diabetic foot ulcers (OTONAL trial). Int J Low Extrem Wounds. 2024;23(3):326–37.
15. Hofmann E, Fink J, Eberl A, Prugger EM, Kolb D, Luze H, et al. A novel human ex vivo skin model to study early local responses to burn injuries. Sci Rep. 2021;11(1):364.

16. Wang EHC, Barresi-Thornton R, Chen LC, Senna MM, Liao IC, Chen Y, et al. The development of human ex vivo models of inflammatory skin conditions. Int J Mol Sci. 2023;24(15):17255.
17. Castoldi G, di Gioia CR, Bombardi C, Maestroni S, Carletti R, Steckelings UM, et al. Prevention of diabetic nephropathy by compound 21, selective agonist of angiotensin type 2 receptors, in Zucker diabetic fatty rats. Am J Physiol Renal Physiol. 2014;307(10):F1123–31.
18. Memar MY, Yekani M, Alizadeh N, Baghi HB. Hyperbaric oxygen therapy: Antimicrobial mechanisms and clinical application for infections. Biomed Pharmacother. 2019;109(1):440–7.
19. Schönberger T, Jakobs M, Friedel AL, Hörbelt-Grünheidt T, Tebbe B, Witzke O, Schedlet al. Exposure to normobaric hypoxia shapes the acute inflammatory response in human whole blood cells in vivo. Pflügers Arch. 2024;476(9):1369–81.
20. Alfei S, Schito GC, Schito AM, Zuccari G. Reactive oxygen species (ROS)-mediated antibacterial oxidative therapies: Available methods to generate ROS and a novel option proposal. Int J Mol Sci. 2024;25(12):7182.
21. Nguyen GT, Green ER, Mecsas J. Neutrophils to the ROScue: Mechanisms of NADPH oxidase activation and bacterial resistance. Front Cell Infect Microbiol. 2017;7(1):373.
22. Zoneff E, Wang Y, Jackson C, Smith O, Duchi S, Onofrillo C, et al. Controlled oxygen delivery to power tissue regeneration. Nat Commun. 2024;15(1):4361.
23. Zhao Y, Xiong W, Li C, Zhao R, Lu H, Song S, et al. Hypoxia-induced signaling in the cardiovascular system: Pathogenesis and therapeutic targets. Signal Transduct Target Ther. 2023;8(1):431.
24. Fries RB, Wallace WA, Roy S, Kuppusamy P, Bergdall V, Gordillo GM, et al. Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen. Mutat Res. 2005;579(1–2):172–81.
25. Schlagenhauf U, Horlacher V, Netuschil L, Brecx M. Repeated subgingival oxygen irrigations in untreated periodontal patients. J Clin Periodontol. 1994;21(1):48–50.
26. Gaggl AJ, Rainer H, Grund E, Chiari FM. Local oxygen therapy for treating acute necrotizing periodontal disease in smokers. J Periodontol. 2006;77(1):31–8.
27. Gajendrareddy PK, Junges R, Cygan G, Zhao Y, Marucha PT, Engeland CG. Increased oxygen exposure alters collagen expression and tissue architecture during ligature-induced periodontitis. J Periodontal Res. 2017;52(4):644–9.
28. Chen T, Zhou Y, Liu J, Xu B, Wu Z, Li D. Biological effects of hyperbaric oxygen on human severe periodontitis. Undersea Hyperb Med. 2002;29(3):159–66.
29. Nogueira-Filho GR, Rosa BT, David-Neto JR. Effects of hyperbaric oxygen therapy on the treatment of severe cases of periodontitis. Undersea Hyperb Med. 2010;37(2):107–14.
30. Signoretto C, Bianchi F, Burlacchini G, Canepari P. Microbiological evaluation of the effects of hyperbaric oxygen on periodontal disease. New Microbiol. 2007;30(4):431–7.
31. Shannon MD, Hallmon WW, Mills MP, Lane JJ, Newell DH. Periodontal wound healing responses to varying oxygen concentrations and atmospheric pressures. J Clin Periodontol. 1988;15(4):222–6.
32. Freeman MR, Schneck FX, Gagnon ML, Corless C, Soker S, Niknejad K, et al. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: A potential role for T cells in angiogenesis. Cancer Res. 1995;55(18):4140–5.
33. Mor F, Quintana FJ, Cohen IR. Angiogenesis-inflammation cross-talk: Vascular endothelial growth factor is secreted by activated T cells and induces Th1 polarization. J Immunol. 2004;172(8):4618–23.
34. Tatarakis N, Gkranias N, Darbar U, Donos N. Blood flow changes using a 3D xenogeneic collagen matrix or a subepithelial connective tissue graft for root coverage procedures: A pilot study. Clin Oral Investig. 2018;22(4):1697–705.
35. Tavelli L, Barootchi S, Majzoub J, Chan HL, Giannobile WV, Wang HL, et al. Ultrasonographic tissue perfusion analysis at implant and palatal donor sites following soft tissue augmentation: A clinical pilot study. J Clin Periodontol. 2021;48(5):602–14.
36. Cairo F. Periodontal plastic surgery of gingival recessions at single and multiple teeth. Periodontol 2000. 2017;75(1):296–316.
37. Silva CO, Ribeiro Edel P, Sallum AW, Tatakis DN. Free gingival grafts: Graft shrinkage and donor-site healing in smokers and non-smokers. J Periodontol. 2010;81(5):692–701.