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The impact of hyperbaric oxygen therapy on late irradiation injury in oral microcirculation

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Abstract

Background: Late side effects of radiotherapy in patients with head and neck cancer (HNCPs) result in decreased tissue vascularity, a compromised healing capacity and spontaneous breakdown of tissue. The aim of this study was to examine the in vivo effect of hyperbaric oxygen therapy (HBOT) on the microcirculation in irradiated oral tissue.

Methods: Using a handheld microscope, the effect of HBOT on oral mucosal microcirculation parameters was measured in 34 previously irradiated HNCPs prior to HBOT and at 4 weeks and 6 months posttreatment.

Results: A significant increase in mean buccal vessel density and decrease in buccal vessel diameter was found 6 months after HBOT compared to baseline, 22 ± 11 versus 25 ± 7 cpll/mm^2 ($p < 0.05$) and 20 ± 4 versus 16 ± 5 μm ($p < 0.05$), respectively.

Conclusion: Our results indicate that oral microcirculation histopathology associated with irradiation is able to respond to HBOT by redirecting oral microcirculation parameters towards values consistent with healthy tissue.

KEYWORDS

head and neck cancer, hyperbaric oxygen therapy, incident dark-field imaging, late irradiation injury, microcirculation

Abbreviations: ATA, atmospheres absolute; avi, audio video interleave; CC, CytoCam; CRT, chemoradiotherapy; FCD, functional capillary density; Fd, focal depth; FOV, field of view; fps, frames per second; HBOT, hyperbaric oxygen therapy; HNCP(s), patient(s) with head and neck cancer; ICC, intraclass correlation coefficient; IDFI, incident dark-field imaging; IR, irradiation; LRTI, late radiation tissue injury; MFI, microvascular flow index; $Ø_{bv}$, blood vessel diameter; ORN, osteoradionecrosis; ROI, region of interest; RT, radiotherapy; TCD, total capillary density.

1 | INTRODUCTION

Late radiation tissue injury (LRTI) is a potentially progressive complication that typically becomes clinically apparent after 6 months to years and is associated with radiotherapy (RT) in the treatment of cancer. Tissue hypoxia that results from RT-induced vascular hypoperfusion has a negative effect on the quality of tissue and prevents an adequate healing response after tissue injury or elicits spontaneous breakdown.[1] This can ultimately lead to necrosis of bone, cartilage, and soft tissues which are difficult to treat conditions. Consequently, functional and aesthetic problems can arise that have a major impact on quality of life.[2,3] It is difficult to make a prediction on whether and when late irradiation (IR) side effects will occur and evolve to clinical pathology as the degree of acute effects does not correlate with the severity of late effects.[4–6] However, fraction dose, total dose, total irradiated volume, and individual patient related factors are identified as potential risk factors.[7–9] The underlying detrimental effects of LRTI only become clinically evident when tissue breakdown occurs, with or without preceding trauma or surgery in irradiated tissue, with no tendency to heal.

Hyperbaric oxygen therapy (HBOT) is directed at improving irradiated tissue quality by increasing oxygen tension in hypovascular tissues to promote neoangiogenesis.[10,11] Although several studies show beneficial effects of HBOT on irradiated HN tissue, the mechanisms remain largely elusive and very limited evidence for preventing and managing ORN exists.[1,12] However, administering HBOT is often used curatively or prophylactically in irradiated tissues and the existing treatment protocols are still based on research performed in the 1980s.[13] In 2019 the first randomized controlled trial was published in the HN region in which data based on 100 patients showed a low occurrence rate of mandibular ORN 6 months after dentoalveolar surgery in both the HBOT group and the control group (6.4% vs. 5.7%); such results would not justify an indication of HBOT to prevent ORN after dentoalveolar surgery. However, it must be considered that patients were included in this study that received radiation doses less than 60 Gy, which minimizes the risk of ORN.[14] Furthermore, it remains uncertain whether iatrogenic trauma causes or accelerates tissue breakdown that would otherwise emerge on the long-term due to poor tissue quality as a result of IR.[15]

Microcirculation monitoring can give insight in the pathophysiology of LRTI and provide functional and anatomic feedback on treatment effects of supporting therapies. In our previous study the late effect on oral microcirculation parameters was measured in irradiated oral mucosa when compared to healthy oral mucosa in a chairside setting using a CytoCam (CC) microscope system based on incident dark-field illumination imaging (IDFI). Large vessel diameters in buccal mucosa and a reduction in vessel density in mandibular gingiva was observed and morphologic observations showed telangiectasias in the oral mucosa consistent with injured microvascular endothelium.[16] The in vivo net effect of HBOT over time on reconstitution of adequate tissue perfusion and tissue vitality associated with irradiated oral tissue has not yet been elucidated on the level of individual vessels. In this study the aim was to monitor in vivo the effects of HBOT prospectively on irradiated oral microcirculation with the use of the CC microscope system. We tested the hypothesis that HBOT redirects microcirculation parameters such as functional capillary density (FCD), blood vessel diameters ($Ø_{bv}$), and angiomorphology towards levels corresponding with healthy tissue.

2 | MATERIALS AND METHODS

A prospective observational study was conducted between November 2014 and July 2017 in the Department of Hyperbaric Medicine and the Department of Oral and Maxillofacial Surgery of the Amsterdam University Medical Centre (UMC). The institutional medical ethics committee of the Amsterdam UMC/AMC (Ref. No. NL49017.018.14) approved all procedures and guidelines of the study, which were performed in accordance with the principles established in the Declaration of Helsinki (Fortaleza, October 2013).

2.1 | Patients

Patients with head and neck cancer (HNCPs), previously treated with RT, that were referred to the Department of Hyperbaric Medicine for either prophylactic or therapeutic HBOT indications were included in this study. Inclusion criteria were a minimum dose of 50 Gy RT received >6 months prior to participation. Exclusion criteria were surgery in the HN region or prior HBOT 6 months previous to the study and severe trismus preventing adequate mouth opening for measuring intraorally. Furthermore, patients that received <20 HBOT sessions in total were excluded from the study. History of general health and IR-dose was acquired from medical records after patient approval. Smoking status was based on information provided by the patient. Dentate or edentulous state was noted.

2.2 | Hyperbaric oxygen therapy

Hyperbaric treatment sessions were carried out in a multiple person hyperbaric chamber and had a total duration

of 110 min; 100% oxygen was administered for 75 min total at 2.4 ATA through an oronasal mask kept in place with a headband to prevent air leakage.[17] HBOT was planned on weekdays from Monday to Friday. Patients receiving HBOT sessions for osteoradionecrosis prophylaxis prior to extraction (20 sessions prior and 10 sessions postoperative, total of 30 sessions) and patients receiving HBOT therapeutically (≥20 sessions) were pooled.

2.3 | Microvascular imaging

To visualize the oral microcirculation a CC Microscope System (Braedius Medical, Huizen, the Netherlands) based on IDFI was used; details on this vital handheld imaging instrument are described elsewhere.[18–20] Briefly, a clear and high-resolution image (14-megapixel, 25 fps) with a field of view (FOV) equal to 1.55 × 1.16 mm (1.80 mm^2) is created of the microcirculation displaying the lumen of blood vessels filled with dark circulating erythrocytes contrasted by a bright background. A medical PC (Braedius Medical, Huizen, the Netherlands) is connected with the CC which is set with the CCTools software (CytoCamTools Camera Manager v1.7.12, Braedius Medical, Huizen, the Netherlands) for camera operation and video data processing.

2.4 | Measurement procedures

Microcirculation measurements were performed at baseline (T0), 4 weeks after start of HBOT (T4) and 24 weeks (6 months) after start of HBOT (T24). The measurement procedures followed on protocol patient instruction and microscope handling as described in a previous study of our group.[16] An allocated examination room maintained at a constant 20 ± 2°C was used for performing the microcirculation measurements at all time points. A trained investigator (RH) performed all measurements together with an assistant (NFS) who controlled the CC PC to adjust contrast, focus and focal depth (Fd; μm) and recorded the images. Four regions of interest (ROIs) were measured: the left and right cheek parallel to the upper premolar region and the left and right gingiva in the lower premolar region. In each region four different videos of 4 s each were recorded of adjacent sites. The average of the four videos represented the ROI.

2.5 | Data analysis

The CCTools software was used to export image recordings in .avi file format. The following parameters were analyzed form the exported videos: Fd, classification of angioarchitecture, total and functional capillary density (TCD and FCD, respectively), microvascular flow index (MFI), and buccal blood vessel diameter ($Ø_{bv}$). Angioarchitecture was divided into three class types[21]: an array of capillary loops (score 1), both capillary loops and vascular network (score 2) and vascular network without any loops (score 3). Frame selection for capillary density assessment was based on clarity, resolution and the absence of pressure artifacts.[22] To determine TCD and FCD the capillary loops in each frame (area of 1.80 mm^2) were counted. At completion of the density dataset the results were divided by 1.80 to convert to mean number of capillaries per millimeter squared (cpll/mm^2). The type of flow per quadrant was described using the MFI scoring system: absent (score 0), intermittent (score 1), sluggish (score 2), or normal (score 3).[23] Blood vessel diameter ($Ø_{bv}$) was analyzed in Adobe Photoshop (Adobe Photoshop CC 2020, Adobe Systems Incorporated, San Jose,

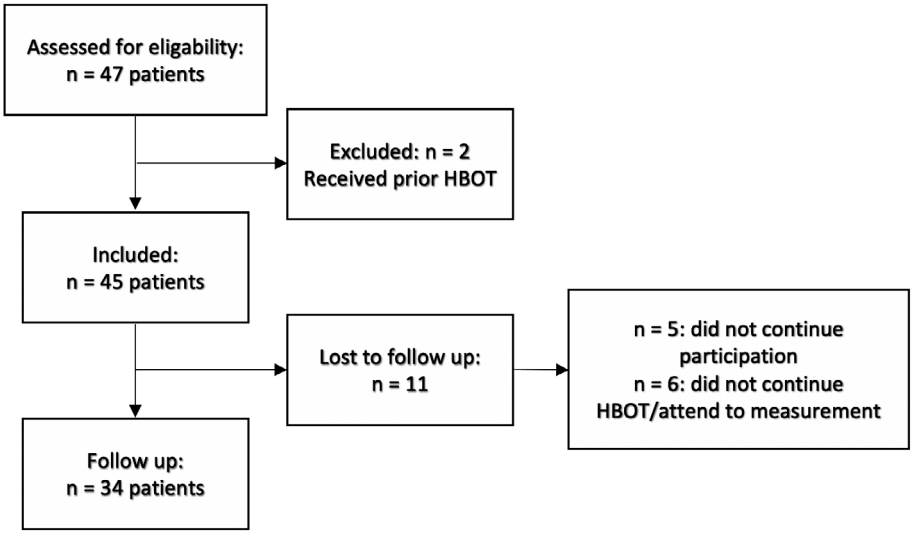

FIGURE 1 Patient selection flowchart

CA) using a digital overlay diameter measuring tool (containing magnitudes of 5, 10, 20, 30, 40, and 50 μm) that was previously described elsewhere.[16] The diameter magnitude of the five largest vessels per quadrant (20 total) were measured and averaged as $Ø_{bv}$ per frame. Vessel morphology was screened throughout all time points for IR induced alterations in the form of telangiectasias and/or disorganization of vessel loops. An intraclass correlation coefficient of 0.982 ($p < 0.001$) was computed from a sample subset of data on buccal and gingiva FCD to ascertain interrater reproducibility agreement between examiners (RH, NFS, and DMJM). Remaining datasets were randomized and analyzed at random for each parameter (Fd, angioarchitecture, MFI, and buccal $Ø_{bv}$) by the same investigator (RH).

2.6 | Statistical analysis

Previous capillary density mean comparisons between healthy and irradiated oral mucosa demonstrated a large effect size (r) of 0.86.[16] In the present study comparative analysis of capillary density between each time point was performed using generalized linear model approach. With a total sample size of 32, a single-group repeated measures analysis of variance (ANOVA) with a 5% significance level has an 80% power to detect an effect size of 0.22. Finally, 34 patients were included in this study (Figure 1). Two patients were excluded from the study due to discontinuation of the HBOT before 20 sessions (at T4) were received. Nine patients were lost to follow up due to logistical or personal reasons (i.e., could not wait for measurement due to arranged transport, switch to different HBO treatment session were no measurement was available or discontinuation at request of patient with no given reason). Normality distribution of all data parameters (FCD, TCD, $Ø_{bv}$, Fd) was assessed by the Shapiro–Wilk test. Based on the outcome of the Shapiro–Wilk test, repeated measures ANOVA or Friedman test was used to detect overall time effects. Significant main effects were subsequently analyzed using paired samples t test with Bonferroni correction. A linear regression analysis was performed to detect whether (previous) smoking, chemotherapy or dentition state (dentate vs. edentulous) had a significant association on the outcome of microcirculation parameters. McNemar test was used to check whether proportion of healthy tissue (class I type angioarchitecture) was significant different over time. Descriptive and statistical analyses were performed using the SPSS Statistics software package (IBM SPSS Statistics version 27, IBM Corp., Armonk, NY). All data are presented as mean ± standard deviation (SD). A p-value of <0.05 was considered statistically significant.

3 | RESULTS

The noninvasive oral microcirculation measurements were well tolerated by all patients and no pain or discomfort was experienced or reported. Radiation dose, indications for HBOT (prophylactic or therapeutic) and patient demographics are presented in Table 1. Microcirculation image quality recorded from the buccal and gingival tissue with the CC was excellent with sharp high-resolution and sufficient contrast (CC brightness = 450 in all measurements) to observe the tissue microvasculature and luminal blood flow. 16 videos were acquired from each patient, 4 videos per ROI. A total of 1632 videos were

TABLE 1 Patient demographics

Patient demographics, $N = 34$	Mean ± SD
Age (years)	59 ± 12
Sex (F:M)	12:22
Dental status (edentulous:dentate)	10:14
Comorbidities, N (%)	
Diabetes	1 (3%)
Hypertension	2 (6%)
COPD	2 (6%)
Intoxication	
Smoking history	4 (12%)
Smoker	8 (24%)
Cancer therapy	
CRT	14 (41%)
Only RT	20 (59%)
Site of radiation	
Oral cavity	12 (35%)
Oropharynx	12 (35%)
Hypopharynx	0 (0%)
Nasopharynx	4 (12%)
Larynx	2 (6%)
Other	4 (12%)
Oral lesion presence	9 (26%)
RT	
Mean RT dosage buccal mucosa (Gy)	38 ± 13
Mean RT dosage gingiva (Gy)	51 ± 12
Time since RT (years)	4 ± 5
HBOT, N (%)	
Prophylactic indication	16 (47%)
Therapeutic indication	18 (53%)
Number of sessions	32 ± 7

Abbreviations: CRT, chemoradiotherapy; COPD, chronic obstructive pulmonary disease; HBOT, hyperbaric oxygen therapy; RT, radiotherapy; SD, standard deviation.

	Buccal angioarchitecture			Gingival angioarchitecture		
	T0	T4	T24	T0	T4	T24
Focus depth (μm)	136 ± 74	146 ± 70	141 ± 53	130 ± 52	116 ± 57	84 ± 95

Note: Data are presented in means ± SD.

TABLE 2 Focus depth during CytoCam microcirculation measurement

	Buccal mucosa			Mandibular gingiva		
	T0 (%)	T4 (%)	T24 (%)	T0 (%)	T4 (%)	T24 (%)
Class I	59	74	76	79	69	81
Class II	37	26	24	17	31	19
Class III	3	0	0	4	0	1

TABLE 3 Classification of angioarchitecture of the buccal mucosa and mandibular gingiva in HNCP; class 1 (only capillary loops), class 2 (both capillary loops and vascular network), and class 3 (vascular network without any loops)

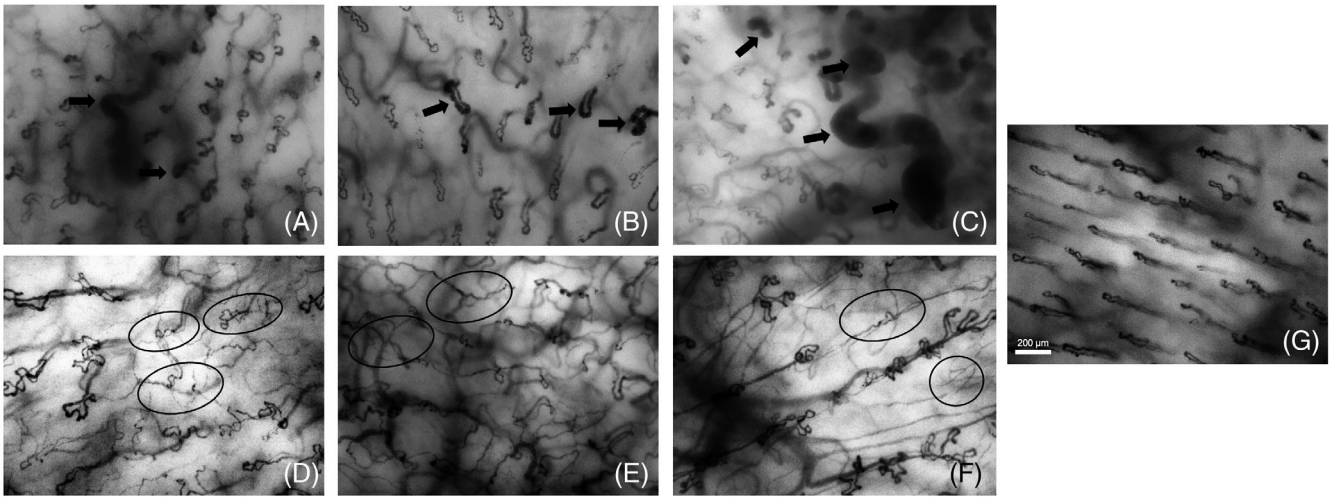

FIGURE 2 CytoCam images illustrating microvascular alterations in IR buccal mucosa observed across all time points: morphologic alterations in the form of telangiectasias or "ballooning" of vessels (black arrows) at T0 For Peer Review (A), T4 (B) and T24 (C), disorganization, malformation and loss of vessel loops (encircled) at T0 (D), T4 (E) and T24 (F). As a comparison, similar to healthy buccal mucosa,16 an example of organized vessel loops of the buccal mucosa observed at T24 weeks (6 months) after start of HBOT (G)

analyzed. No significant difference in Fd was observed between the time points (Table 2).

3.1 | Angioarchitecture

Temporal classification of buccal and gingival tissue angioarchitecture is presented in Table 3. Overall, the proportion of class 1 angioarchitecture increases overtime although no significant difference was detected ($p > 0.05$).

3.2 | Vessel morphology

Altered vessel morphology associated with IR, i.e. telangiectasias in the form of distortion or "ballooning" of vessels loops, was observed at all time points in both buccal and gingival mucosa. Furthermore, disorganization of vessel loops remained present at T4 and T24. Figure 2 illustrates images of these microvascular alterations in irradiated buccal mucosa. Normal (continuous; MFI = 3) blood flow was present in all observed vessels.

3.3 | Capillary density and blood vessel diameters

An overview of capillary density and buccal $Ø_{bv}$ is presented in Table 4. Mean TCD and FCD was the same in all patients and no difference in MFI was observed. A statistically significant decrease in mean $Ø_{bv}$ was found between T0 and T24 (20 ± 4 vs. 16 ± 5 μm) ($p < 0.001$) and T4 and T24 (20 ± 6 vs. 16 ± 5 μm) ($p < 0.05$). No significant difference was detected in mean FCD of the mandibular gingiva between the time points T0, T4, and T24 (respectively 33 ± 16, 46 ± 36, and 27 ± 36 cpll/

TABLE 4 Summary of microcirculation measurement in irradiated oral tissue (HNCP)

	Buccal mucosa			Mandibular gingival mucosa		
	T0	T4	T24	T0	T4	T24
FCD (cpll/mm^2)	20 ± 11	24 ± 10	25 ± 7	33 ± 16	46 ± 36	26 ± 36
MFI (AU)	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0	3 ± 0
$Ø_{BV}$ (μm)	20 ± 4	20 ± 6	16 ± 5*,**			

Note: Data are presented in means ± SD.
Abbreviations: FCD, functional capillary density; MFI, microvascular flow index; $Ø_{bv}$, blood vessel diameter.
*$p < 0.05$ vs. T4; **$p < 0.001$ vs. T0.

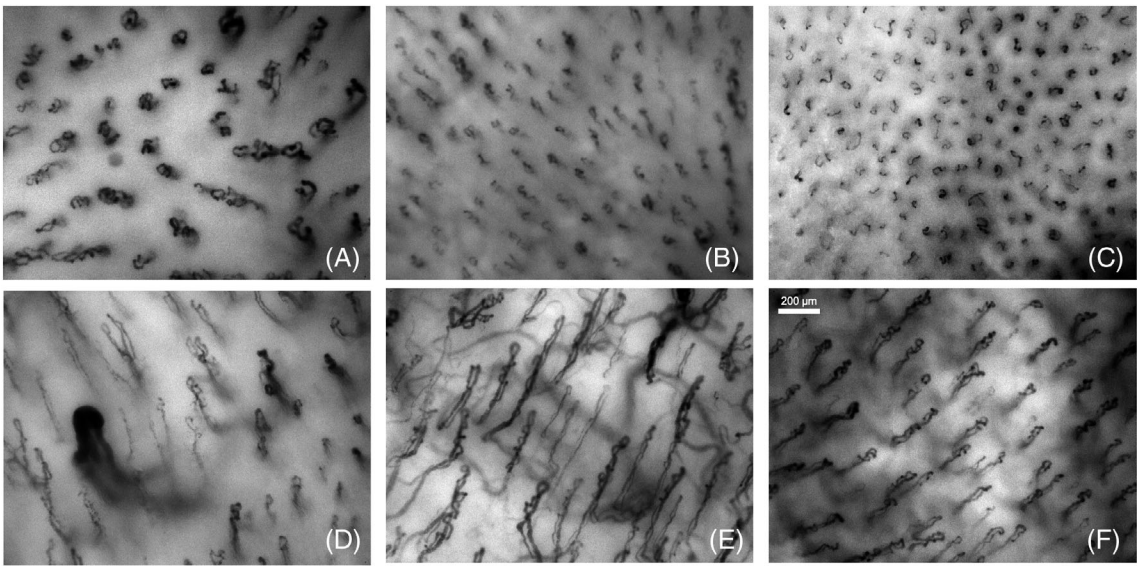

FIGURE 3 CytoCam images illustrating mandibular gingival mucosa at T0 (A), T4 (B), and T24 (C) and buccal mucosa at T0 (D), T4 (E), and T24 (F)

mm^2). An overall statistically significant increase in mean buccal mucosa FCD was found overtime (T0: 20 ± 11 cpll/mm^2, T4: 24 ± 10 cpll/mm^2, and T24: 25 ± 07 cpll/mm^2) ($p < 0.05$). However, the difference between all the time points was not significant in the post hoc analysis based on the Bonferroni correction. Sample images of buccal and gingiva microcirculation at baseline (T0), after 4 weeks (T4) and after 24 weeks (6 months; T24) are presented in Figure 3. The difference in $Ø_{bv}$ and FCD overtime was not statistically significant different between the following groups: (previous) smokers and nonsmokers, patients that received CRT and patients that only received RT and between edentulous and dentate patients. An exception to this was a significantly higher $Ø_{bv}$ at T4 that was observed in previous smokers compared to nonsmokers (22 ± 6 vs. 18 ± 5 μm) ($p < 0.05$). Having a history of smoking showed a significant increasing effect ($p < 0.05$) on the change of $Ø_{bv}$ between T0 and T4 (unstandardized coefficient B; B = 3.88). MFI for both buccal and gingival microcirculations was scored as 3 (normal, continuous blood flow) throughout all time points.

4 | DISCUSSION

The aim of this study was to monitor (over time) the influence of HBOT on irradiated oral mucosal microcirculation in HNCPs using a vital handheld microscope system. The results indicate that HBOT may initiate changes to the irradiated oral mucosal microcirculation that shift in the direction of healthy tissue microcirculation values by raising vessel density and reducing vessel diameter. A general increase in FCD and a decrease in $Ø_{bv}$ was observed in buccal mucosa 6 months after the start of HBOT. Interestingly, no increase in FCD in mandibular gingival microcirculation was observed. Furthermore, morphological aspects associated with irradiated microvasculature (telangiectasias, disorganization, and loss of capillary loops) remained present after HBOT. Based on these findings, the hypothesis that HBOT redirects the examined microcirculation parameters towards levels corresponding to healthy tissue could only be partially supported.

Late IR changes in oral microcirculation of HNCP show an increased vessel diameter in irradiated buccal

mucosa compared to that of healthy, age-matched control.[16] In the present study a significant decrease of irradiated buccal mucosal $Ø_{bv}$ was observed 6 months after HBOT, which indicates a trend consistent with previous measurements of $Ø_{bv}$ in healthy buccal tissue.[16] Additionally, the marginally increased percentage of buccal mucosal angioarchitecture to a class 1 was observed 6 months after HBOT which represents a gain of capillary loops akin to normal subepithelial anatomy. Unfortunately, the overall observed expression of class 1 angioarchitecture was not found to be significant after 6 months, nor was the difference in Fd. In our previous study, a described lower mean mandibular gingiva FCD in irradiated tissue compared to healthy tissue might drive the hypothesis of an increase in FCD after HBOT because of its reported angiogenic potential.[16] The data of the present study did not show a rise of FCD in the mandibular gingiva of HNCP in a follow up period of 6 months after start of HBOT. IR dose history showed that the mandibular mucosa received a higher mean dose compared to the mean dose received on the buccal mucosa. In the view of this finding, the microcirculation in the mandibular mucosa may have been injured to a greater extent and therefore be more difficult to resuscitate with HBOT. Furthermore, there is a great variability in individual response to HBOT of the mandibular tissue shown by the large standard deviations in FCD. Although previous microcirculation measurements could not detect a significant difference in mean buccal FCD between irradiated and healthy tissue,[16] the present study does show a significant increase in mean buccal FCD measured 6 months after HBOT.

Angiopathies such as telangiectasias has frequently been described in tissue that was subject to IR.[5,16,24–27] Altered capillary loops, presenting as telangiectasia-like (ballooning) vessels, were observed at all time points after HBOT. Therefore, the present data could not demonstrate that HBOT is able to restore capillary loop vascular morphology and reverse pathology associated with microvascular endothelium. However, the degree of "ballooning" of vessels was effectively decreased after HBOT as represented by the measured decreased $Ø_{bv}$. This could suggest an adaptation in flow dynamics and intravascular pressures in which bulging would be limited in the oral microcirculation.

HBOT has shown to induce neoangiogenesis and increase vascularity in wound healing models and compromised tissues.[28–31] Marx et al. described an eight to ninefold increase in microangiographically determined vascular density after hyperbaric oxygen (20 sessions, 2.4 ATA) compared to both normobaric oxygen and air-breathing controls in an irradiated rabbit jaw model.[11] Earlier, he described increased microvascular density when obtained and assessed before and after receiving HBOT (20 sessions, 2.4 ATA) in tissue biopsies of 50 irradiated HNCP.[31] In another clinical study looking at buccal mucosal biopsies (3–4 mm depth) of 20 irradiated HNCP, blood vessel density was increased by HBOT after 6 months.[32] Svalestad et al. also reported significant increase in blood flow after heat provocation at 3 and 6 months after HBOT when compared to controls. Furthermore, an increased oxygen tension was observed in facial skin after HBOT using transcutaneous oximetry.[33] Transmucosal oximetry in patients with ORN demonstrated an increase in gingival oxygen tension values from a mean of 50% to a mean of 86% of the oxygen tension of healthy controls after HBOT (30 sessions, 2.4 ATA).[34] In the present study the assessment of multiple microcirculation parameters (i.e., vascular function [flow], vascular morphology, angioarchitecture, and vascular density) were captured using IDFI and the results revealed an increased mean FCD in irradiated buccal mucosa 6 months after HBOT.

Contradicting results were published by an experimental study that did not observe an effect of HBOT on histological vascular density in the irradiated mandibular skin of rats after different HBOT regimes (18 and 30 sessions, 2.5 ATA) over a period of 12 weeks.[35] Furthermore, an increased vessel diameter was noted after irradiation that did not change after HBOT. The results of the present study did show a reversible effect of HBOT on vessel diameter in buccal mucosa with a significant decrease towards healthy tissue values that appear consistent with datasets described in our previous study.[16] It is possible that a delayed effect of HBOT is not detected in studies with a shorter follow up period. However, another experimental study did show histologically increased vascular density in irradiated salivary gland in a mouse model in a short period of 10 weeks after HBOT (20 sessions, 2.4 ATA).[36]

There are some points of consideration concerning the present study. HNCP often have known comorbidities such as hypertension, diabetes mellitus and often are (former) smokers. Awareness of the influence of these comorbidities and their compromising effect on the microvascular state and tissue perfusion ahead of enduring (chemo-) radiotherapy (CRT) should be considered and interpreted in context with clinical outcomes and potential supportive care. Measurable effects of (former) smoking and diabetes on oral microcirculation were previously described.[37,38] The potential resuscitating effect associated with HBOT on late IR injury of the microcirculation could therefore be altered or even latent. The percentage of former smokers in this study was notably low compared with other reports and therefore might concern an underreported observation.[39,40] However, our results

did not show an association of former smoking and the effect of HBOT on microvascular IR injury over a period of 6 months. Additionally, no significant difference in patients with CRT compared with RT alone was found between the time points for all microcirculation parameters. Another point of consideration is that the number of patients in this study is not sufficient to divide into subgroups based on comorbidities to extract potential subtle differences in adverse effects on the microcirculation. Furthermore, the maxillary gingiva was excluded from observation as prevalence of substantial IR on the maxilla was scarce in the HNCP that were referred for HBOT. A further explanation indicates that the highest predilection of squamous cell carcinoma in the HN region is on the tongue and floor of the mouth.[41] Finally, although accumulation of plaque could hypothetically influence observed gingival capillary density, no significant association with edentulism and effect of HBOT on gingival FCD was detected in our study.

The present study reports a measurable effect of HBOT in the oral microcirculation of previously irradiated tissue. Future studies should be directed at correlating microvascular parameters to clinical appearance of IR tissue injury. Stages of emerging oral tissue necrosis linked to the state of the microcirculation should be identified and scored. Chairside appraisal of the oral microcirculation in an individual patient could determine in vivo the severity of vascular IR injury that is present, monitor the efficacy of HBOT and consequently direct timing and duration specified to response of the oral tissue requiring supportive care or treatment. Therefore, monitoring microcirculation provides an opportunity to detect emerging pathophysiology in an early state so that supportive therapies could prevent progression of late IR tissue injury to the mutilating and difficult to treat ORN in the HN region. Time-consuming and (relatively) costly HBOT (~€5000–€10 000 per patient; average of 30 sessions) could only be committed to selected cases instead of the current potential overtreatment of the general patient population. At the same time a firm decrease in patient quality of life could be avoided by timely clinical monitoring and intervention.

In conclusion, the results of this study indicate that HBOT partially redirects irradiated oral mucosal microcirculation parameters towards healthy tissue state. This could ameliorate the healing capacity of the oral cavity and its surrounding tissues.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

AUTHOR CONTRIBUTIONS

Study design: Renée Helmers and Dan M. J. Milstein. *Conduct of study*: Renée Helmers and Dan M. J. Milstein. *Collection of data*: Renée Helmers, Nina F. Straat, and Arash Navran. *Analysis and management of data*: Renée Helmers, Nina F. Straat, and Arash Navran). *Interpretation of data and preparation of manuscript*: Renée Helmers. *Intellectual content review and approval of final manuscript draft*: Renée Helmers, Dan M. J. Milstein, Nina F. Straat, Arash Navran, David N. Teguh, Robert A. van Hulst, Ludi E. Smeele, and Jan de Lange).

DATA AVAILABILITY STATEMENT

Data are available on request from the authors.

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