Assessment of Vascularity in Irradiated and Nonirradiated Maxillary and Mandibular Minipig Alveolar Bone Using Laser Doppler Flowmetry

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Purpose: *The purpose of this animal study was to confirm that laser Doppler flowmetry (LDF) is a reproducible method for the assessment of maxillary and mandibular alveolar bone vascularity and that there is less vascularity in irradiated mandibular and maxillary bone compared to nonirradiated bone.* ***Materials and Methods:*** *All maxillary and mandibular premolars and molars of 6 Göttingen minipigs were extracted. After a 3-month healing period, 3 minipigs received irradiation at a total dose of 24 Gy. Three months after irradiation, 5 holes were drilled in the residual alveolar ridge of each edentulous site in each minipig. Local microvascular blood flow around all 120 holes was recorded by LDF prior to implant placement. In 1 irradiated and 1 nonirradiated minipig, an additional hole was drilled in a right maxillary site to enable repeated LDF recordings.* ***Results:*** *The alveolar bone appeared less vascularized in irradiated than in nonirradiated minipigs. The effect of radiation appeared more pronounced in the mandible than in the maxilla. LDF was demonstrated to be a reproducible method for assessing alveolar bone vascularity. However, recordings varied by edentulous site as well as by minipig.* ***Conclusion:*** *The authors' hypotheses regarding LDF and vascularity were supported. Further research validating the use of LDF in human beings, especially in those who have undergone radiation therapy for head and neck cancer, is necessary. Int J Oral Maxillofac Implants 2007;22:774–778*

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Current data suggest that osseointegration is impaired in irradiated bone.[1,2] Implant survival rates are known to be lower in irradiated bone than in nonirradiated bone, particularly if the irradiation dose exceeds 50 Gy.[3–6] Prospective studies have shown that irradiated bone becomes hypocellular and hypoxic and that the vascularity of irradiated bone decreases over time.[7] As a result, the continuous bone remodeling capacity diminishes, which explains the lower implant survival rates.

In irradiated patients, a method for assessment of vascularity of intended implant recipient sites would be of great significance in preventing early implant loss. Laser Doppler flowmetry (LDF) could be an appropriate method. Modern LDF techniques use a laser diode device to produce a beam of near-infrared laser light with an operating wavelength of 780 to 820 nm, which is beamed into human tissues by a fiber optic connector.[8,9] The photons are scattered, and light hitting moving blood cells undergoes a change in wavelength (Doppler shift), while the wavelength of the light hitting static structures is unchanged. A returning fiber in the probe picks up and carries the light back to a photo detector. The magnitude of the signal and the frequency changes is directly related to the relative number and velocity of blood cells in a recorded volume.

Wong introduced the concept of LDF as a diagnostic tool for verification of bone graft vitality following a maxillary sinus grafting procedure. Six months after the grafting procedure, detection of blood flow in all graft sites indicated successful angiogenesis.[10] In a previous study, LDF was used for recording microvascular blood flow in cancellous mandibular bone of young pigs.[11] LDF has also been shown to be useful for the assessment of bone vitality in osteomyelitis and in many other applications.[12–14]

The hypotheses of this study were that less vascularity would be found in irradiated maxillary and mandibular alveolar bone compared to nonirradiated bone and that LDF is a reproducible method for the assessment of alveolar bone vascularity.

MATERIALS AND METHODS

Six 1-year-old adult Göttingen minipigs were used for this study. The experiments were conducted in accordance with German and European Community guidelines on the protection of laboratory animals. Permission was obtained from the Animal Ethical Committee of the University of Aachen.

All maxillary and mandibular premolars and molars of the minipigs were extracted under general anesthesia induced by isoflurane 0.8% to 1.1%. Interoperatively, and for 3 days postoperatively, clindamycin was administered as an antimicrobial agent. After a 3-month alveolar bone healing period, the maxillary and mandibular bone of 3 minipigs received bilaterally 3 irradiation (cobalt) exposures up to 8 Gy, with 7-day intervals between exposures, for a total dose of 24 Gy. Each radiation field (left and right) contained half the mandible as well as half the maxilla; thus, the irradiation was evenly distributed among the arches and jaws. At 3 months after irradiation, computed tomographic (CT) scans were performed under general anesthesia. The data from these scans were used for generating stereolithographic 3D models. Subsequently, the data were imported into a software program (Simplant; Materialise, Leuven, Belgium) for preoperative planning of implant positions at the edentulous maxillary and mandibular sites in a virtual environment and for the design of accurate customized surgical templates, which were made by rapid prototyping for transfer of the planned implant positions to the minipigs. Surgical treatment of the maxillary and mandibular edentulous sites was begun with an incision on top of the alveolar crest and a release incision that sloped buccally and anteriorly. Subsequently, the periosteum was reflected gently, exposing the underlying alveolar bone. To avoid interference with the local blood flow, no anesthetic agent was administered by local infiltration. Using a customized surgical template and a pilot drill of the implant system used (Biocomp, Vught, The Netherlands), 5 initial holes were drilled in the residual alveolar ridge of each edentulous site (20 holes in each minipig). LDF recordings were carried out, and the initial holes were further widened for implant insertion. Implant placement was carried out as part of an ongoing study on the effects of irradiation on implant stability and implant survival. A total of 120 nonsubmerged Biocomp implants, 3.4 mm in diameter and 10 mm in length, were placed in the 6 minipigs. In 1 irradiated and 1 nonirradiated minipig, an additional hole was drilled in the right edentulous maxillary site to be able to perform repeated LDF recordings for determining the recording error and for validating the standardization of recordings.

Local microvascular blood flow in the surrounding alveolar bone of all 120 initial holes was recorded by LDF at a fixed depth of 6 mm, according to the protocol used by Wong.[10] The emitted laser light (780 nm) was transduced to the recording site by a special side-reading optical fiber probe with a diameter of 2.8 mm (Fig 1; PF 415-254; Periflux System, Perimed, Sweden). Before installation of the probe, the initial hole was rinsed with a saline solution to avoid contamination. The minipig was optimally stabilized during the recording to avoid disturbing movements. Disturbing movements of the pig, if any, were promptly apparent in the recording graphic. Within a few seconds after installation of the probe, the graphic stabilized and remained stable during the recording period. A 20-second noise-free period appeared sufficient for a stable and reliable recording session. In every initial hole 4 recordings were carried out with the probe perpendicularly directed to the mesial, buccal, distal, and palatal or lingual hole wall successively (Fig 2). To test the reproducibility of recordings, in the additional right maxillary hole in 1 irradiated and 1 nonirradiated minipig, 10 similar recordings were carried out, providing 40 recordings per hole.

The LDF module was connected to a personal computer for calculating the recordings. The magnitude of the signal and frequency changes was directly related to the relative number and velocity of the blood cells in the recorded volume.[15] The recordings

Fig 1 Side-reading LDF probe.

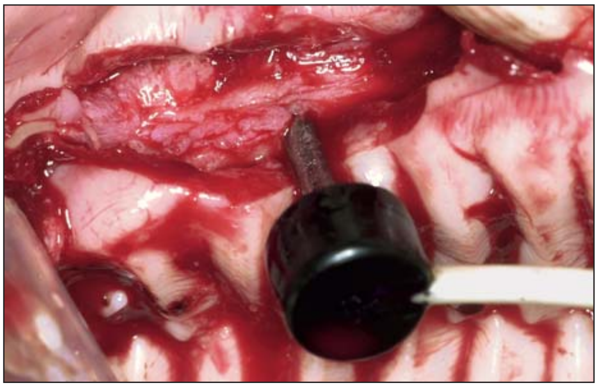

Fig 2 LDF probe placed in an initial hole drilled in the maxilla.

in the 4 directions were averaged, revealing the average blood flow per hole, expressed in perfusion units (PU). Although PU is an arbitrary unit, a linear relationship between PU and blood flow expressed in mL/min/100 g has been demonstrated.[15]

Local microvascular blood flow recordings were tested for normality of distribution by the Kolmogorov-Smirnov test.

Data analysis was first performed on the 80 recordings of the additional maxillary hole in 1 irradiated and 1 nonirradiated minipig. The recording error or reproducibility of local microvascular blood flow recordings was estimated by variance components analysis using multifactor repeated-measures analysis of variance (ANOVA). "Minipigs" (P) and "recordings" (R) were considered random factors, with 2 (irradiated and nonirradiated) and 10 categories (10 recordings in 1 direction), respectively. "Direction" (D) was the fixed factor with 4 categories. A mean-direction intraclass correlation coefficient (ICC) for 1 direction was calculated from the ratio of the total of the estimated variance components of P and of P × D divided by the sum of estimated coefficients of P, P × D, P × R, and P × R × D. Beforehand, it was decided that the ICC should be at least 0.90 for sufficiently reproducible microvascular blood flow recordings. Separate similar analyses by repeated-measures ANOVA were performed to estimate the reproducibility of recordings in each of the 4 recording directions.

Subsequently, data analysis was performed on the recordings in the 120 initial implant holes, to determine the irradiation effect on bone vascularity. Fixed between-factor in the analysis was irradiation with 2 categories (yes-no) and fixed within-factors were jaw (maxillary-mandibular), side (left-right) and within jaw implant position (1 to 5). If associations were statistically significant, an analysis was carried out using repeated-measures ANOVA or a Student *t* test on partial data. $P = .05$ was determined as the level of significance for all comparisons. Reproducibility data were analyzed by the GENOVA program of Crick & Brennan. Irradiation effect data were analyzed by SPSS version 12.0 (SPSS, Chicago, IL).

RESULTS

The normality of the distribution of the recordings of microvascular blood flow was at an acceptable level (Kolmogorov-Smirnov test; $P = .051$). The data of the 80 recordings of the additional maxillary holes are presented in Table 1.

The 10 recordings in each direction were consistent, but there were distinct differences between the recordings for the 4 directions in each hole. In irradiated alveolar bone the recordings were consistently lower compared to the recordings in nonirradiated alveolar bone.

The overall ICC was 0.944. Separate analyses for the 4 different directions revealed ICCs of 0.981 (mesial), 0.978 (buccal), 0.894 (distal), and 0.878 (palatal). The overall F ratio for irradiation was 22.43 by 1 and 4 df ($P = .009$). The association between jaw, side, and irradiation was statistically significant (F = 23.80 by 1 and 4 df; $P = .008$). Irradiation had a maximal effect in the left part of the mandible (t = 7.47; $P = .002$; overall mandible, t = 10.62; $P < .001$; overall maxilla, t = 2.52; $P = .065$). The irradiation effect was statistically significant at the maxillary left side (t = 5.96, $P = .004$), but not at the maxillary right side (t = 1.85, $P = .139$). Table 2 lists means and standard deviations of alveolar bone vascularity of the 6 minipigs.

Table 1 Recordings (n = 80) in the Additional Maxillary Holes in an Irradiated and a Nonirradiated Minipig

	Irradiated				Nonirradiated			
Recording no.	M	B	D	P	M	B	D	P
1	12.84	4.51	9.23	19.47	28.85	13.74	12.21	34.63
2	11.38	4.43	8.30	18.01	30.44	14.44	9.01	34.83
3	11.16	4.89	8.47	17.61	33.69	12.05	10.99	27.82
4	13.67	4.55	8.89	22.29	33.94	15.39	11.34	28.78
5	12.29	4.16	8.31	19.97	29.57	13.85	10.85	25.66
6	13.46	4.26	7.85	18.17	28.09	15.01	11.09	25.23
7	13.84	4.31	8.49	18.07	31.94	11.58	12.27	34.11
8	11.23	4.23	9.28	17.77	28.24	15.47	12.50	32.29
9	11.35	4.66	8.25	17.61	25.93	15.63	11.85	29.12
10	11.87	3.99	8.42	17.46	28.61	14.39	12.86	26.64
Mean recording	12.31	4.40	8.55	18.64	29.93	14.16	11.49	29.91

The probe was perpendicularly directed to the mesial, buccal, distal, and palatal walls of the hole. M = mesial, B = buccal, D = distal, P = palatal.

DISCUSSION

The results presented in this study suggest that LDF can be used for the assessment of alveolar bone vascularity in pilot holes before implant placement. However, recordings varied by edentulous site as well as by minipig. In order to be useful in human beings, normal values of alveolar bone vascularity of the various alveolar sites of both the maxilla and mandible should be determined. These values may vary not only from person to person but also depending on the individual amount of local residual alveolar bone. Therefore, further research validating the use of LDF in human beings, especially in those who have undergone radiation therapy for head and neck cancer, is necessary. Standardization of instrument and measuring method is required for comparing results between different laser Doppler users. Probes and equipment parameters must be consistent, and the instrument must be calibrated according to the manufacturer's instructions.

The variations in recordings for the 4 different directions were presumably caused by the nonhomogeneous calcified and trabecular alveolar bone structure around the holes. As a consequence, recordings in different directions are needed for determining the average alveolar bone vascularity around 1 hole. The 10 recordings in the additional right maxillary holes provided consistent values. Consequently, in future research projects, using 1 recording in each of the 4 directions will be suitable. The overall ICC was 0.944; thus, the reproducibility of the recordings was sufficient.

In general, in irradiated patients at least a 6-month interval is recommended between tooth extraction and implant placement to allow for bone healing.[16] In this study, a similar interval was used: a 3-month interval between extractions and radiation therapy and another 3-month interval until implant placement. Three months after irradiation, the edentulous alveolar bone appeared less vascularized in irradiated than in nonirradiated minipigs. Whether this observation can be transferred to human beings needs to be demonstrated, but the results of this study are in accordance with the results of previous studies.[17,18]

Table 2 Means and SDs of Alveolar Bone Vascularity of the 6 Minipigs

Jaw/side/irradiation	Mean	SD
Maxilla		
Right		
No	22.136	10.475
Yes	9.762	4.998
Left		
No	16.085	1.938
Yes	9.221	0.468
Mandible		
Right		
No	15.147	4.130
Yes	5.392	3.263
Left		
No	17.755	3.128
Yes	3.544	1.030

Three minipigs received 3 irradiation (cobalt) exposures up to 8 Gy with 7-day intervals, for a total dose of 24 Gy administered to the bone. Using an α/β ratio of 2.5, this dose is biologically equivalent to approximately 56 Gy given in 28 fractions of 2 Gy each. A better research design would have been the split-mouth design: unilateral irradiation in all minipigs. An advantage of this method would have been reduction of the variability between the minipigs.

Furthermore, each minipig would have been serving as his own control. However, unilateral irradiation of the maxilla and the mandible without any exposure of the contralateral side is not practical technically.

A distinct difference in blood perfusion (LDF recordings) was seen not only between irradiated and nonirradiated bone but also between maxillary and mandibular alveolar bone (Table 2). The effect of irradiation was more pronounced in the mandible than in the maxilla. This phenomenon was in accordance with the authors' expectations because the spongious maxillary bone is known to be better vascularized compared with the more dense mandibular bone.

Irradiation has a significant negative effect on bone vascularity, which has important clinical implications. Since reduced bone vascularity impairs oral implant osseointegration in patients who have undergone head and/or neck radiotherapy, recording bone vascularity prior to implant placement could be of significance in the decision-making process while preparing a treatment plan for prosthetic reconstructive therapy.

In this animal study, LDF was demonstrated to be a reproducible method for the assessment of alveolar bone vascularity. Hence, it may be used clinically to increase the predictability of implant treatments and even decrease the risk of osteoradionecrosis by avoiding implant insertion in poorly vascularized bone. Research is needed to determine whether LDF could be used to determine a minimum level of vascularity necessary to facilitate reliable implant placement. Another area for future research with LDF is the use of hyperbaric oxygen therapy in cases of osteoradionecrosis; the effectiveness of this therapy, which is based on increasing the bone vascularity, may also be demonstrated by LDF. The authors' future research will focus on determining a human standard of bone vascularity in the nonirradiated maxilla and mandible using LDF.

CONCLUSION

The hypothesis that less vascularity would be observed in irradiated maxillary and mandibular alveolar bone compared to nonirradiated bone was confirmed. Furthermore, it was confirmed that LDF is a reproducible method for the assessment of bone vascularity.

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