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Investigation of Mast Cells in Human Gingiva Following Low-Intensity Laser Irradiation

L.B. Silveira,[1,2] R.A. Prates,[3] M.D. Novelli,[4] H.A. Marigo,[5] A.A. Garrocho,[5] J.C.F. Amorim,[2] G.R. Sousa,[2] M. Pinotti,[2] and M.S. Ribeiro, Ph.D.[1,3]

Abstract

Objective: The aims of the present study were to investigate the effect of low-intensity laser irradiation on the total number of mast cells as well as the percentage of degranulation in human gingiva. Blood vessel dilation was also evaluated. ***Background Data:*** It has been proposed that low-intensity laser irradiation can ameliorate pain, swelling, and inflammation. In periodontal tissue, mast cells may influence either the destructive events or the defense mechanism against periodontal disease via secretion of cytokines and through cellular migration to improve the healing process. Mast cells play an important role in the inflammatory process. ***Methods:*** Twenty patients with gingival enlargement indicated for gingivectomy were selected. Gingival fragments were obtained from each patient and divided into three different groups before surgery. One fragment was removed without any irradiation. The two others were submitted to punctual irradiation with an energy density of 8 J/cm^2 at an output power of 50 mW at 36 Hz for 36 sec before gingivectomy. Nondegranulated and degranulated mast cells were counted in five areas of the gingival fragment connective tissue. Major and minor diameters of the blood vessels were also measured. ***Results:*** Both red and infrared radiation promoted a significant increase in mast cell degranulation compared to controls; however, no statistically significant differences ($p > 0.05$) were observed between the irradiated groups. No significant differences among the groups were observed regarding blood vessel size. ***Conclusion:*** The results suggests that red and infrared wavelengths promote mast cell degranulation in human gingival tissue, although no dilation of blood vessels was observed. The effects of premature degranulation of mast cells in human tissue and the laser radiation protocol applied in this study encourage further investigations to extend these results into clinical practice.

Introduction

GINGIVAL ENLARGEMENT, or an increase in the size of the gingiva, can be caused by various stimuli. The most common is chronic inflammatory gingival enlargement; the clinical signs are a soft and discolored gingiva. This is due to tissue edema and infective cellular infiltration caused by prolonged exposure to bacterial biofilm, and it is treated with conventional periodontal treatment, such as scaling and root planing. Portions in which the chronic inflammatory gingival enlargement includes significant fibrotic components that do not respond to treatment and do not undergo shrinkage after scaling and root planing are surgically removed to eliminate the excess tissue, most often with a procedure known as gingivectomy.[1–3] In inflamed gingival lesions, a variety of cytokines are produced by lymphocytes and monocytes as well as by non-immune cells like fibroblasts and epithelial and endothelial cells.[4]

Low-intensity laser radiation as a therapeutic modality for acceleration of wound healing was first introduced by Mester and others in the 1960s.[5] This therapy is widely used as a tissue stimulator[6–9] and to promote anti-inflammatory and analgesic effects.[10] Despite its widespread use in clinical trials,[11,12] the biological basis for its effectiveness has not been elucidated. It is proposed that the healing acceleration may be due to a reduction in the duration of acute inflammation. Primary mechanisms that stimulate cell activity leading to enhanced mast cell recruitment and degranula-

[1]Professional Master Lasers in Dentistry, IPEN-CNEN/SP, Cidade Universitária, São Paulo, [2]Bioengineering Laboratory, Universidade Federal de Minas Gerais, Belo Horizonte, [3]Center for Lasers and Applications, IPEN-CNEN/SP, Cidade Universitária, São Paulo, [4]School of Dentistry, Universidade de São Paulo, São Paulo, and [5]School of Dentistry, Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, Brazil.

tion can occur.[13–15] The increase in mast cell degranulation in tissue may stimulate collagen synthesis and organization, and healing and migration of defensive cells that may modulate the inflammatory process.[16] Conversely, these cells participate in immune and inflammatory responses through the liberation of heparin, histamine, and eosinophil chemotactic factor of anaphylaxis,[17] and mast cells in inflamed gingiva have the potential to degrade extracellular matrix.[18] In addition, mast cells synthesize and release certain prostaglandins and leukotrienes that in concert contribute to the immediate inflammatory response. As a consequence of mast cell activation, other inflammatory cells are recruited and activated, and a cascade of inflammatory mediator production and release is set in motion.[19]

Mast cells are essential in the first line of defense against infection; the host seeks to prevent microorganisms from colonizing the tissue. If immune exclusion is ineffective, microorganisms or their products may penetrate gingival and periodontal tissues. This will induce immune elimination systems. If the host defense fails, chronic infection of the gingival or periodontal tissue will take place. Pro- and anti-inflammatory agents are kept in equilibrium and the illness fails to progress. Periodically, the equilibrium may be disturbed and progressive loss of gingival and periodontal tissue may take place. Mast cells play a crucial role in these processes, including wound healing. Mast cells may be key players in gingival homeostasis that may be important in the development of periodontitis.[20]

The therapy using low-power lasers does not involve thermal interaction.[21] Instead, the photon energy causes photochemical, photophysical, or photobiological effects in cells and tissue.[22,23] In recent years, the use of low-intensity lasers has been shown to be an adjunctive treatment to periodontal therapy.[8,24,25]

Experimental studies demonstrated that mast cells can be activated and their total number and percentage of degranulation increased by light stimulation at certain wavelengths and irradiation parameters in rat skin wounds.[16,26] While findings to date have been encouraging and typically positive, further research is still required to effectively determine the optimal parameters. Given the problems in extrapolating irradiation parameters and findings from animal research to human practice, trials in humans are crucial. In this context, the present study investigated the effect of low-intensity red and near-infrared laser radiation on the total number of mast cells as well as the percentage of degranulation in human gingiva. Blood vessel dilation was also evaluated.

Materials and Methods

The experiment protocol was reviewed and approved by the Dentistry School's Research Ethics Committee of the University of São Paulo. Twenty Caucasian ex-orthodontic patients (five males and 15 females, between 30 and 50 years of age) presenting with biofilm-induced gingival enlargement (Fig. 1a) with probing depths between 3 and 3.5 mm in the upper anterior sextant were selected. After removing the orthodontic appliances, all patients underwent conventional periodontal treatment consisting of plaque control measurements, scaling, and root planing. After the initial therapy, an excess of gingival tissue persisted in these patients, leading to discomfort and aesthetic concerns. Thus the therapeutic intent was to re-establish the contour of the gingival tissue. Gingivectomy was indicated to re-establish morphology and gingival contour, which was altered by orthodontic treatment. In addition, subjects had not taken any medication that might affect the research for at least 1 mo prior to the study. Pregnant women, smoking patients, subjects with periodontal bone loss, and systemic disease (e.g., diabetes mellitus) were also excluded from the study.

From each patient, a gingival section from the non-mineralized wall of a periodontal pocket was selected for gingivectomy. Each gingival section was divided into three sites and a Crane-Kaplan gingivectomy knife was used to delimitate the pocket apical limit. A discontinuous incision was carried out with a Bard-Parker scalpel and a curette was used to remove the first site without irradiation (control), which was immediately formaldehyde-fixed. The second site was irradiated using near-infrared laser radiation (λ = 785 nm) (Fig. 1b) and thereafter removed. The third site was also submitted to irradiation using a red laser (λ = 688 nm) prior to removal. All tissue samples were irradiated just once immediately before surgical removal. Punctual irradiation was carried out with a GaAlAs laser for 36 sec (IR 500; Laser Beam, Rio de Janeiro, Brazil). The output

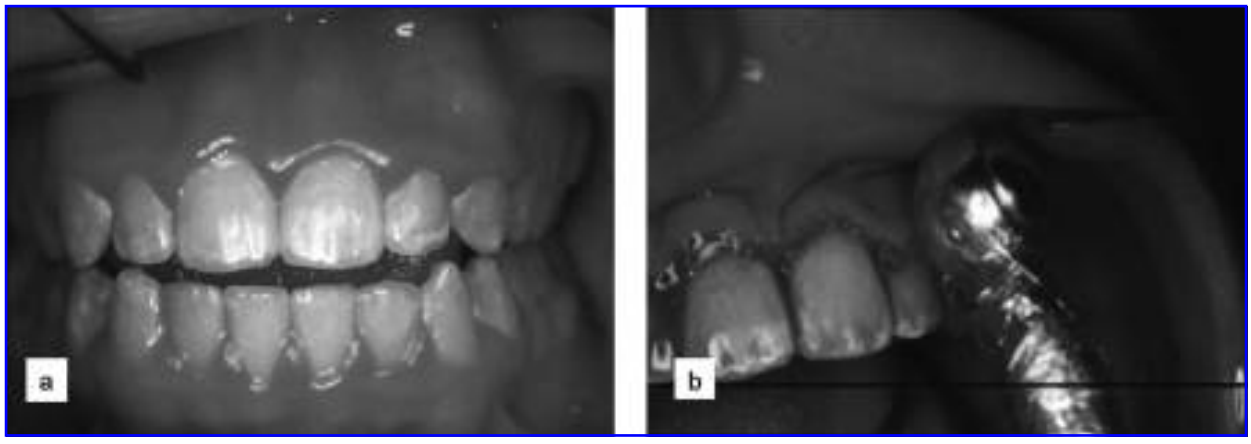

FIG. 1. (**a**) Clinical appearance of enlargement in gingival tissue with abnormal form and contour. (**b**) Infrared irradiation of the second fragment before gingivectomy.

mean power, dose, and pulse frequency were set at 50 mW, 8 J/cm^2, and 36 Hz, respectively. The control and irradiated sites were in the same areas for all patients. After gingivectomy, a periodontal dressing was placed following irrigation of the area with saline and 0.12% chlorexidine.

Control and irradiated fragments of gingiva were fixed in formaldehyde 10%, and put in small glasses that were appropriately labeled according to the study group. The specimens were then submitted to routine histological processing. Sections of each fragment were stained using toluidine

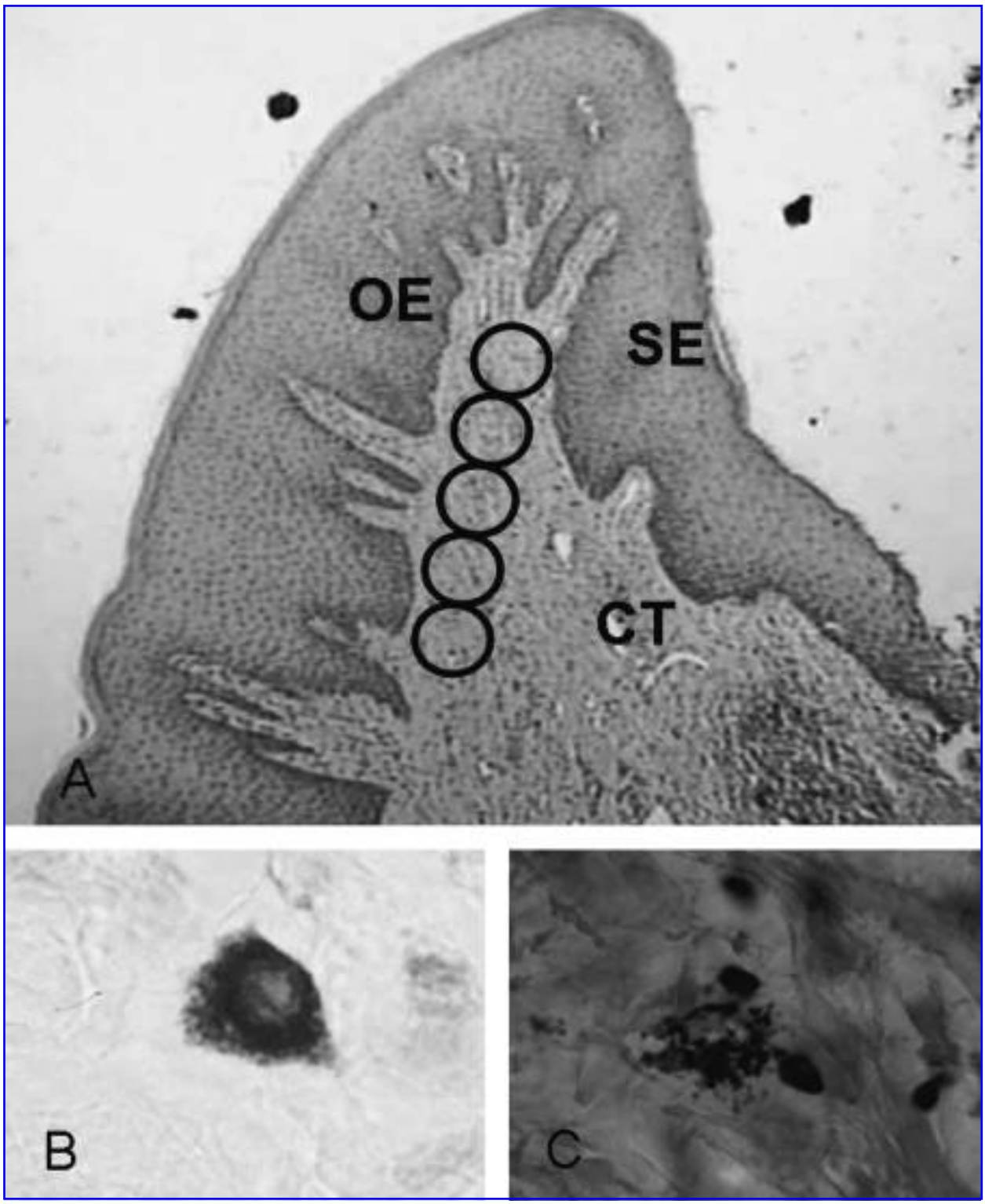

FIG. 2. (**A**) Photomicrograph of a bucco-lingual section through the gingiva. The black circles indicate where counts were made. OE, oral epithelium; SE, sulcular epithelium; CT, connective tissue. Hematoxylin and eosin 40×. (**B**) and (**C**) Photomicrographs of gingival connective tissue. **B** shows an intact mast cell. **C** shows a degranulated mast cell. Toluidine blue. ×1000.

blue, which is specific and metachromatic for mast cells, and hematoxylin and eosin.[16,26] Intact and degranulated mast cells of each specimen were counted inside five circular areas adjacent to the basal layer of the oral gingival epithelium (Fig. 2A), and thereafter added to obtain the total count for each specimen, for a total of 20 measurements for each group. Toluidine blue is a well-known staining method used to count mast cells in connective tissue and that clearly indicates if they are intact (Fig. 2B) or degranulated (Fig. 2C). The mast cells appear purple against a blue background in the sections.

The degranulation index was calculated using the following equation:

$$IDg = \frac{M_{deg} \times 100}{M_{tot}}$$

where IDg is the degranulation index, M_{deg} is the number of degranulated mast cells, and M_{tot} is the total number of mast cells (intact and degranulated mast cells).

Major and minor diameters of blood vessels were also measured (Fig. 3). Five blood vessels were randomly selected in the gingival connective tissue bordering the epithelium. Microscopic analysis was accomplished using a Zeiss Winkel (Carl Zeiss, Microimaging, Göttingen, Germany) light microscope and histological sections were examined by a pathologist. Kruskal-Wallis testing and Student's *t*-test were used for comparisons among groups. The results were considered significant when $p < 0.05$.

Results

Laser irradiation had no impact on recruitment of mast cells. The total number of mast cells showed no significant differences between control and laser-irradiated groups ($p > 0.05$), therefore the total number of mast cells was equal among the groups. However, comparison of the ratio of the degranulated and intact mast cells in the laser and control groups showed that laser irradiation significantly increased the percentage of mast cell degranulation ($p < 0.05$). Table 1 displays the values of mast cell counts obtained from the 20 volunteers for each investigated group.

The percentages of degranulation of mast cells following exposure to 688-nm and 785-nm laser irradiation are presented in Fig. 4. The results show that the degranulation index for the red group was increased by 27% compared to the control group. Similarly, an increase of 26% was seen in the infrared group compared to the control group. In fact, the degranulation index of the laser groups were appreciably increased compared to the controls. Conversely, no statistically significant differences in percentage of degranulation were observed between the red and infrared groups.

Regarding the major and minor blood vessel diameters (Fig. 5), no statistically significant differences were observed among the groups ($p > 0.05$).

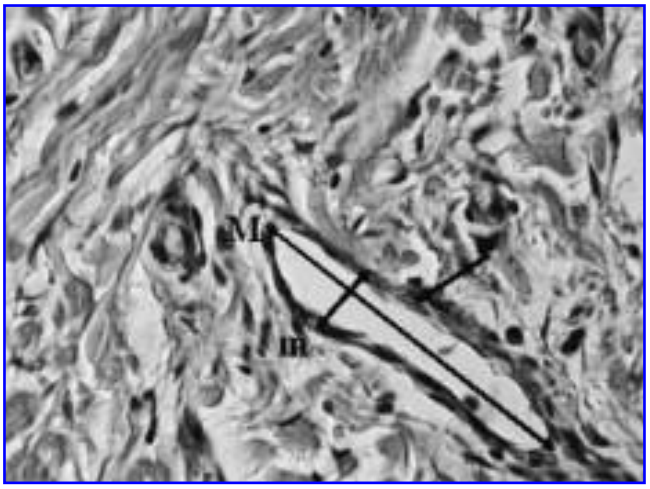

FIG. 3. Photomicrograph of gingival connective tissue. The arrow points to a blood vessel. M, major diameter; m, minor diameter. Hematoxylin and eosin 400×.

Discussion

This study used human gingival tissue obtained immediately after irradiation to evaluate the effect of laser radiation of two different wavelengths on mast cells.

Mast cells are seen scattered throughout gingival connective tissue, often in close association with endothelial cells, but are also found sub- and intra-epithelially. In inflamed and in healing gingiva, the quantity of mast cells has been found to increase.[20,27] In the present work, we investigated the number of degranulated and total mast cells detected in the area adjacent to the basal layer of the oral gingival epithelium. This region in connective tissue was selected due to its high number of mast cells near to blood vessels.[20,28]

Previous studies have emphasized the importance of choosing the correct laser parameters in order to increase mast cell number and degranulation.[26] In this study, we analyzed two wavelengths: 688 nm and 785 nm. Other laser parameters, such as output power, pulse frequency, and energy dose were unchanged. Dose and pulse frequency values were based on previous work by El-Sayed and Dyson,[16,26] since those authors wrote that laser irradiation increases the percentage of mast cell degranulation in animal models. They verified that mast cells can be activated and their total number increased by light at certain wavelengths. These authors also concluded that the number of degranulated mast cells was higher at certain pulse frequencies. To the best of our knowledge, no trials using human tissues have been reported in the literature.

Wavelength is an important parameter in the evaluation of the effect of laser irradiation, since absorption, and hence penetration depth, varies with wavelength. Infrared radiation is more readily scattered in a forward direction than red light.[22] Thus, the infrared laser was used before the red laser. The points of laser application in each gingival area were 1.0 cm apart.

Both wavelengths (688 nm and 785 nm) promoted a considerably higher percentage of degranulation of mast cells. This finding agrees the results of El Sayed and Dyson, who reported a significant increase in mast cell degranulation, although those authors investigated injured skin in rats.[26]

Mast cells can be regarded as specialized secretory cells, which in the resting state, contain several hundreds of granules, each one surrounded by a membrane. In fact, mast cells commonly are found in the gingiva, and affect gingival and periodontal infection.[29] They are important in the first line

TABLE 1. MAST CELL RESPONSE IMMEDIATELY AFTER GINGIVECTOMY IN GINGIVAL TISSUE FOR EACH VOLUNTEER

	Total mast cells			*Degranulated mast cells*			*Degranulation index*		
Volunteer	*Control*	*IR laser*	*Red laser*	*Control*	*IR laser*	*Red laser*	*Control*	*IR laser*	*Red laser*
1	135	80	123	65	71	86	48	89	70
2	150	85	150	68	66	129	45	78	86
3	113	139	86	51	103	67	45	74	78
4	114	78	58	46	61	36	40	78	62
5	220	83	151	90	76	135	41	92	89
6	149	84	84	90	61	65	60	73	77
7	99	112	82	54	88	57	54	79	70
8	182	161	167	98	141	155	54	88	93
9	81	78	72	38	55	60	47	71	83
10	105	127	143	51	114	128	49	90	90
11	86	56	75	40	46	57	47	82	76
12	72	99	56	24	66	44	33	67	79
13	92	86	103	45	59	77	49	69	75
14	57	49	60	28	31	47	49	63	78
15	134	74	93	89	57	70	66	77	75
16	90	88	77	58	73	64	64	83	83
17	105	84	106	65	75	87	62	89	82
18	184	168	134	109	147	111	59	88	83
19	172	109	127	106	83	103	62	76	81
20	115	87	91	84	62	67	73	71	74
Mean	122.8	96.4	101.9	65.0	76.8	82.3	52.4	78.9[a]	79.2[a]
± SD	42.9	31.4	34.0	25.8	29.5	33.7	10.0	8.5	7.4

The results are the sum of five circular areas adjacent to the basal layer of the oral gingival epithelium.
[a]Significant differences were observed between irradiated and control groups ($p < 0.05$).

of protection against infection, when the host tries to avoid microorganism contamination of the tissue.[20] In the periodontium in particular, a local inflammatory response can be constantly seen due to the presence of the oral microbial biofilm; therefore, many mast cells are anticipated.[27]

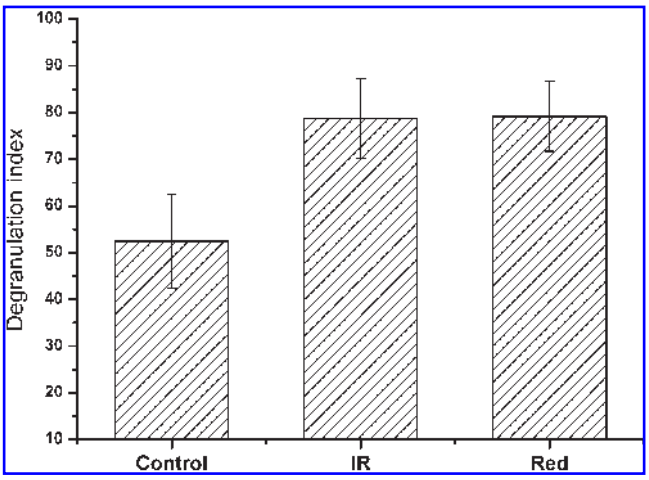

FIG. 4. Effect of red and near-infrared laser irradiation on the percentage of degranulation of mast cells in gingival tissue. Significant differences were observed between the irradiated and control groups ($p < 0.05$). No significant differences were observed between the red and near-infrared groups ($p > 0.05$). Thin bars indicate standard deviations.

Human periodontal disease and healthy gingiva were investigated by Batista et al., and they observed a higher number of mast cells in inflamed gingiva. They reported a mean of 58.3 mast cells/mm^2 in healthy samples, and more than 100 cells/mm^2 in periodontal disease.[27] In fact, recently Amorim et al. investigated gingival healing of gingivectomy wounds after red laser irradiation. These authors reported that 3 d after surgery laser-treated wounds showed better repair with regard to color and contour of the gingiva, and improved mucosal healing. These results pointed to an improvement in wound healing associated with laser treatment.[25] It was likely that a reduction in the duration of acute inflammation resulted in a more rapid entry into the proliferative stage of repair, when granulation tissue is produced, and that this accelerated wound healing.

The mechanisms behind laser therapy are not completely understood, and it seems likely that there is not a single means of action.[7,21] Some studies have attempted to explain the effect of laser irradiation on biological systems through its effects on mitochondrial respiratory processes, and this may be one of the most important mechanisms.[30–32] Recent literature suggests that cytochrome c acting on the Krebs cycle plays an important role in laser action.[33] In addition, more than one cellular function (e.g., replicative, motile, and secretory functions) can be influenced by light. Even more interesting is that when one of these cellular functions is altered by irradiation, a preferential mechanism is triggered in the cell, and it uses the light energy to affect cellular activity.[32]

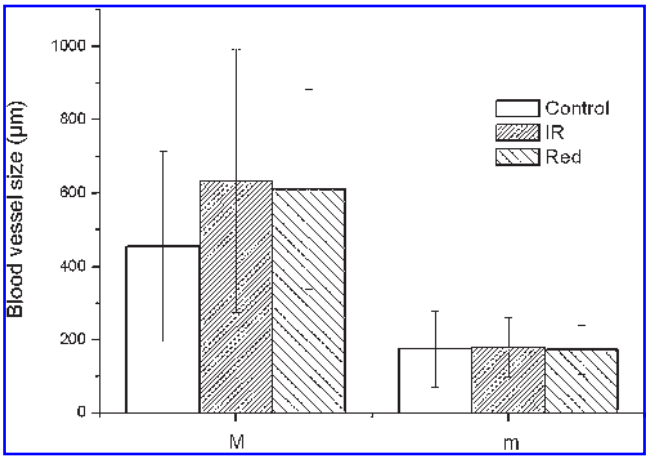

FIG. 5. Effect of red and near-infrared laser irradiation on the dilation of blood vessels. No statistically significant differences were observed among the groups ($p > 0.05$). Thin bars indicate standard deviations. M, major diameter; m, minor diameter.

The biological processes behind wound healing have been widely discussed, among them mast cell recruitment and degranulation,[16,26] which are necessary parts of the inflammatory response. The inhibition of the inflammatory process may be important for the improvement of wound healing.[30] Particularly in human periodontal disease, an increase in mast cell degranulation may be participating either in the destructive events, or in the defense mechanism against periodontal disease via secretion of cytokines, cellular migration, and healing processes.[27]

The mast cells produce and store vasoactive amines; the effect of increased blood flow induced by histamine occurs around 180 sec after mast cell degranulation.[34] In this study, minor and major diameters of blood vessels were measured. Even though enlarged vessels were seen in the red and infrared groups in relation to the control group, no significant differences were found among groups. This finding is probably due to the fact that when the gingival tissue was removed, the histamine did not have time to take effect.[34]

Though a complete understanding of the effects of laser irradiation on different types of human cells has yet to be attained, according to our results one effect of laser irradiation with red and near-infrared wavelengths appears to be the disruption of mast cell membrane equilibrium, leading to increased degranulation. This information may be significant in a more complete understanding of the effects of laser irradiation on the inflammatory process.

Conclusion

The study design and laser radiation protocol used in this work showed that red and near-infrared wavelengths promote mast cell degranulation in human gingival tissue, although no increase was seen in blood vessel diameter. Taking into account the effects of degranulation of mast cells on gingival tissue, and the role of mast cells in periodontal disease, further investigation is necessary to extend these results and to fully understand their clinical usefulness.

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Address reprint requests to:
Martha Ribeiro, Ph.D.
Center for Lasers and Applications
IPEN-CNEN/SP
Av. Prof. Lineu Prestes, 2242
Cidade Universitária
São Paulo, SP Brazil

E-mail: marthasr@usp.br