In Vitro Stimulatory Effect of Diode Laser on the Secretion of Tissue Inhibitor of Matrix Metalloproteinases 1 (TIMP-1) in Human Gingival Fibroblasts: A Preliminary Report

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Purpose: A key homeostatic mechanism of gingival fibroblasts in tissue repair is maintained through their production of tissue inhibitors of matrix metalloproteinases (TIMP). The purpose of this investigation was to elucidate the influence of diode laser irradiation on the in vitro production of TIMP-1 by human gingival fibroblasts.

Materials and Methods: A human gingival fibroblast cell line (HGFB) was irradiated with a diode laser in pulsed mode at 0.5, 1, and 1.5 W for 5 s five times each with 5-s pauses in between. On the same plates in different wells, cells were then irradiated using continuous wave diode laser at 0.5 W for 5 s five consecutive times, with 5-s pauses in between. The cells were incubated overnight (18 h), and in the supernatants the concentration of free TIMP-1 was measured using a TIPM-1 ELISA.

Results: At all settings, diode laser irradiation significantly increased in vitro TIMP-1 secretion of human gingival fibroblasts ($p \leq 0.001$), measured after 18 hours of incubation. The best results were obtained using continuous wave irradiation with 0.5 W for 5 s applied five consecutive times with 5-s intervals in between. TIMP-1 secretion increased by 42.2% compared to the control.

Conclusion: Our findings show that diode laser irradiation of human gingival fibroblasts signficantly increases TIMP-1 secretion compared to control values. This could contribute to periodontal tissue healing.

Key words: human gingival fibroblast, matrix metalloproteinases, diode laser.

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Human gingival fibroblast cells are challenged frequently by pathogenic bacteria.[1] Studies about immunological factors in addition to the structural components of gingival fibroblasts have shown that these cells actively participate in immune and inflammatory events in oral diseases.[2] Since fibroblasts are the predominant cell type in soft connective tissue matrices, regulation of their proliferative, synthetic and degradative behavior is likely to be important in tissue physiology and pathology.

Fibroblasts, responsible for the assembly of the extracellular matrix, are capable of responding either directly to oral microbial challenges or indirectly, following activation of the host immune response, and can alter the composition of connective tissue in several ways: synthesis of inflammatory mediators, their

receptors or antagonists, fibroblast proliferation, collagen synthesis, phagocytosis of collagen fibrils, and synthesis of proteolytic enzymes, including matrix metalloproteinases (MMPs) and their corresponding inhibitors.[2]

Oral diseases are often characterized by the destruction of collagen fibers and other extracellular matrix components in periodontal tissues, likely to be mediated to a great extent by host-cell derived MMPs.[3] The MMPs are divided into four major subclasses, according to their substrate specificity and sequence homology: collagenases, gelatinases, stromelysins, and the membrane-type metalloproteinases.[4] Results on MMPs expression in healthy and periodontitis-affected patients are conflicting, but there is clear evidence that gingival fibroblasts are an active source of MMPs in response to periodontopathogens.[4] One key homeostatic mechanism of gingival fibroblasts in tissue repair consists of their production of tissue inhibitors of matrix metalloproteinases (TIMP). The family of TIMPs (1-4) plays a central regulatory role as inhibitors of MMPs.[5] TIMP-1, the inducible form, has been identified as a multifunctional molecule, participating in wound healing and tissue regeneration, maintaining cell morphology and cell survival. TIMP-1 helps uphold a delicate balance between physiological tissue degradation and synthesis of extracellular matrix.[6]

Among the many physiological effects of laser irradiation for therapeutic purposes, anti-inflammation and stimulation of wound healing have been reported.[7] However, little is known about the biological mechanisms of the anti-inflammatory effect of laser irradiation in oral diseases.

The aim of this investigation was to elucidate the influence of diode laser irradiation on the in vitro production of TIMP-1 by human gingival fibroblasts.

MATERIALS AND METHODS

A human gingival fibroblast cell line (HGFB) was obtained from American Type Culture Collection (ATCC, Rockville, MD, USA). HGFB cells were cultivated in Costar 162 cm^2 flasks (Costar, Cambridge, MA, USA) in DMEM (Dulbecco's Modified Eagle Medium, Sigma, Germany) supplemented with 10% FCS (fetal calf serum, PAA Laboratories, Pasching, Austria), 1% glutamine and 1% penicillin/streptomycin at 37°C in a fully humidified air atmosphere containing 5% CO_2. They were passaged by trypsinization and used after four passages. Human gingival fibroblasts (200-ml aliquots, containing $5x10^3$ cells/well) were transferred to 96-well microtiter plates (Nunc) and irradiated with a diode laser in pulsed mode at 0.5, 1, and 1.5 W for 5 s, five times each, with 5-s pauses in between. On the same plates in different wells, cells were then irradiated using continuous wave diode laser at 0.5 W for 5 s, five consecutive times, with 5-s pauses in between. The cells were incubated overnight (18 h); the concentration of free TIMP-1 was measured in the supernatants using a TIPM-1 ELISA kit (Human biotrak TM ELISA, Amersham Biosciences, Buckinhamshire, UK). Supernatants from nonirradiated cells from the same plate served as the control.

Statistical Analysis

Groups were compared using a two-way ANOVA at a $p \leq 0.05$ level of significance. For all groups with different laser settings, the mean values (± SD) of TIMP-1 secretion were calculated (Fig 1).

RESULTS

At all settings, diode laser irradiation significantly increased in vitro TIMP-1 secretion of human gingival fibroblasts ($p \leq 0.001$) measured after 18 h of incubation. The best results were obtained using continuous wave irradiation at 0.5 W for 5 s, applied five consecutive times, with 5-s pauses in between. TIMP-1 secretion increased by 42.20% compared to the control.

At 0.5 and 1.5 W diode laser irradiation, increases of 39.93% and 39.20%, resp, in TIMP-1 secretion were obtained, compared to control values and at 1W diode laser irradiation where TIMP-1 secretion increased by 20.69% (Fig 2). Because of the small sample sizes (n = 6), further measurements are needed, but these preliminary results indicate that at all settings used, diode laser irradiation significantly stimulated TIMP-1 secretion in human gingival fibroblasts in vitro ($p \leq 0.001$).

DISCUSSION

Evidence of the role of matrix metalloproteinases (MMP) produced by resident fibroblasts or inflammatory cells in periodontal destruction is well established.[8] The activity of MMPs is strictly regulated by tissue inhibitors of matrix metalloproteinases, and the matrix is maintained by a careful balance between rates of synthesis, degradation, and connective tissue cell division.

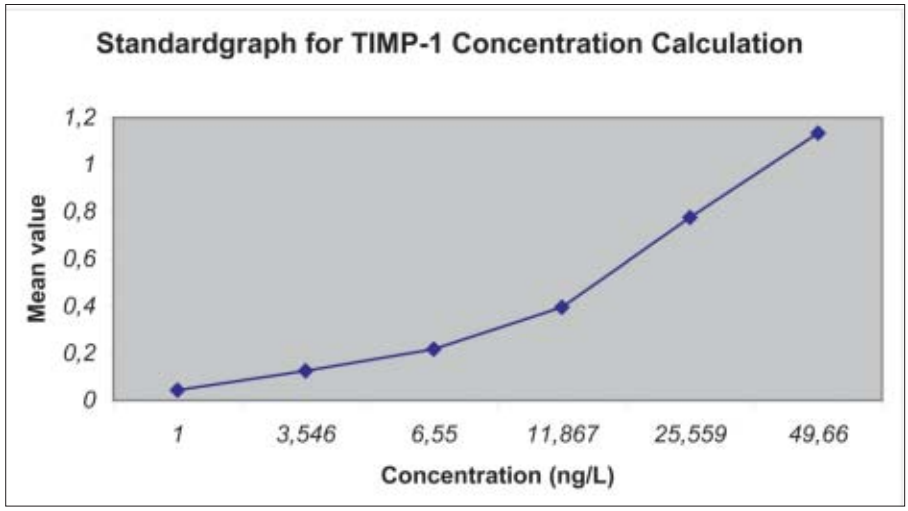

Fig 1 Standard graph for calculating TIMP-1 concentration.

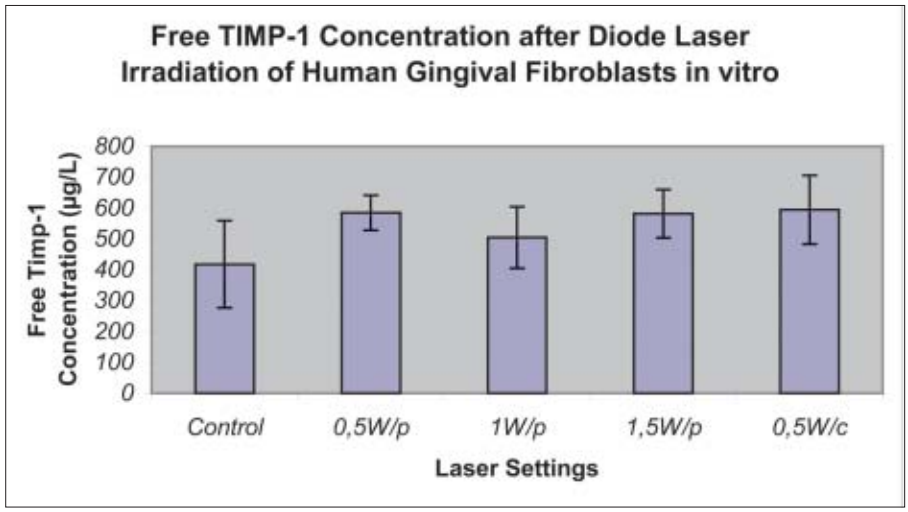

Fig 2 Free TIMP-1 concentration after laser irradiation.

An imbalance between activated MMPs and their endogenous inhibitors is followed by pathologic breakdown of the extracellular matrix. The activated inflammatory cells recruited by bacterial plaque produce inflammatory mediators, which stimulate the production of MMPs from fibroblasts, epithelial cells, and polymorphnuclear neutrophils (PMN). These gingival fibroblasts can also secrete TIMPs, and successive active phases in periodontal disease may be explained by the imbalance between MMPs and their inhibitors (TIMPs).[9]

In our in vitro experiment, diode laser irradiation of gingival fibroblasts significantly increased TIMP-1 production at all laser settings ($p \leq 0.001$). The highest TIMP-1 production rate, increased by 42%, was measured after 0.5 W continuous wave application of the diode laser, which is currently used for periodontal therapy. This secretion increase of TIMP was twice as high as after 1W diode laser irradiation in a pulsed manner. Using 0.5 W and 1.5 W pulsed diode laser irradiation, similar results were obtained, with increases of 39.93% and 39.20%, respectively.

Other authors compared the effects of low-level laser therapy on cultured human gingival fibroblasts proliferation, using different irradiance and the same fluence.[10] When irradiated, fibroblasts in nutritional deficit presented cell growth similar to or higher than that of the control cells grown in normal culture conditions. Lasers of equal power output produced similar effects on cell growth, independent of their wave-

lengths. Low-level laser therapy acts by improving the in vitro fibroblast proliferation, and a smaller laser exposure time results in higher proliferation. A similar study demonstrated a considerably higher proliferation activity of low-level laser irradiated cells.[11] The differences were highly significant 24 h after irradiation, but decreased in an energy-dependent manner after 48 and 72 h. These findings might be clinically relevant, indicating that repeated treatments are necessary to achieve a positive laser effect in clinical applications.

Our findings show that diode laser irradiation of human gingival fibroblasts is followed by a TIMP-1 secretion increase of up to 42% compared to control values. These preliminary results must be confirmed by further measurements, and the balance between TIMP-MMPs secretion implicated in matrix degradation must be investigated after diode laser application.

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