Journal of
PERIODONTAL RESEARCH

J Periodont Res 2010; 45: 323–330

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2009.01239.x

Expression of heat shock proteins, Hsp70 and Hsp25, in the rat gingiva after irradiation with a CO_2 laser in coagulation mode

A. Yamasaki, H. Ito, J. Yusa, Y. Sakurai, N. Okuyama, R. Ozawa
Division of Oral Pathology, Department of Oral Medical Sciences, Ohu University School of Dentistry, Koriyama, Fukushima, Japan

Yamasaki A, Ito H, Yusa J, Sakurai Y, Okuyama N, Ozawa R. Expression of heat shock proteins, Hsp70 and Hsp25, in the rat gingiva after irradiation with a CO_2 laser in coagulation mode. J Periodont Res 2010; 45: 323–330. © 2009 John Wiley & Sons A/S

Background and Objective: The therapeutic rationale of low-energy pulsed CO_2 laser coagulation mode has not been clarified yet. We conducted this study to characterize the effect of low-energy pulsed CO_2 laser coagulation mode irradiation of the rat gingiva in terms of the expression of heat shock proteins.

Material and Methods: Laser irradiation was achieved with the parameters of 5 W, 600 μs pulse duration, and fluence of 326 J/cm^2. The gingiva dissected at different times after irradiation was processed for immunohistochemical examination of the expression of the heat shock proteins, Hsp70 and Hsp25.

Results: One hour after irradiation, the epithelial keratinocytes facing the laser wound exhibited an overexpression of Hsp70 in their nucleus. The connective tissue cells facing the laser wound, which included fibroblasts and capillary endothelial cells, showed *de novo* expression of Hsp70 at 3 h post-irradiation, the level of which peaked at 1 d and thereafter decreased. An enhanced and/or *de novo* expression of Hsp25 in the connective tissue cells facing the laser wound became evident at 3 h after irradiation, and after 1 d the Hsp25-expressing cells increased in number and spread over the wound as wound repair progressed. There was a temporospatial difference in the expression pattern between Hsp70 and Hsp25, with only a few cells appearing to co-express both heat shock proteins.

Conclusion: The CO_2 laser treatment in coagulation mode produced the expression of heat shock proteins, and the findings suggest that while Hsp70 mainly conferred cell protection, Hsp25 was involved in the progress of wound repair as well as cell protection.

Akira Yamasaki, DDS, PhD, Division of Oral Pathology, Department of Oral Medical Sciences, Ohu University School of Dentistry, 31-1 Misumido, Tomita-machi, Koriyama, Fukushima 963-8611, Japan
Tel: +81 249 32 8973
Fax: +81 249 33 7372
e-mail: a-yamasaki@den.ohu-u.ac.jp

Key words: heat shock protein; rat gingiva; CO_2 laser; wound healing

Accepted for publication May 18, 2009

Owing to effective tissue removal by evaporation, the CO_2 laser is now widely used in a variety of surgical procedures, including oral surgery, as an alternative to the traditional scalpel (1–3). Currently, the pulsed CO_2 laser set at relatively low energy level, termed the coagulation mode, has been successfully used in dental clinics for treatment of superficial lesions of the oral mucosa, such as inflammatory and/or ulcerated lesions. The therapeutic concept of this treatment modality differs from that of the traditional CO_2 laser in that the irradiation is performed not simply to ablate but also to coagulate the target tissue and thereby it is expected to offer more conservative and minimally invasive surgery for treatment of oral soft tissue disorders, including periodontal lesions.

Despite its extensive clinical use, however, this treatment modality is

still empirical, and there is no evidence-based clinical trial to substantiate the clinical benefits of this laser mode. To establish reliable laser treatment using this laser mode, we must fully understand the tissue reaction to the irradiation and the subsequent repair process. We recently conducted an experimental study using this laser mode to obtain basic data for its clinical translation and suggested that the coagulation necrosis produced in the rat gingiva *per se* did not disturb but in fact promoted the repair process and subsequent tissue remodelling (4). This result prompted us to elucidate further how the gingival tissue responded to this specific type of laser beam and how this laser approach may have influenced the subsequent repair process and tissue remodelling.

Wound healing is a complex biological process that involves an orderly series of cellular and molecular events that are temporally and spatially regulated. The thermal effects caused by the laser in coagulation mode are rather subtle and not obviously visible. Among the most sensitive biological indicators of thermal stress are heat shock proteins (Hsps), also called stress proteins. Heat shock proteins have been found in all organisms, and these proteins are expressed not only to protect damaged cells in response to a variety of environmental stresses but also to facilitate a variety of biological processes by acting as a molecular chaperone in unstressed conditions (5,6). Heat shock proteins are classified into several groups according to their molecular weight, and among the most extensively studied is the Hsp70 family (7). The Hsp70 family consists of several members, the best known of which are the heat-inducible Hsp72 and the constitutively expressed Hsp73/Hsc73. A small heat shock protein family including murine Hsp25, a homologue of the human Hsp27, is expressed in both stressed and unstressed conditions and is involved in the regulation of cell growth and differentiation (8), cytoprotection (9) and modulation of apoptosis and inflammation (10,11). Furthermore, its chaperone function also plays an important role in assembling and stabilizing actin filaments, and thereby it also functions in cell motility and migration (12). Thus, any or all of these functions of heat shock proteins are thought to be essential for the process of wound healing. The present study was undertaken to further characterize the wound made by a laser in the coagulation mode in terms of the expression of Hsp70 and Hsp25 with emphasis on their role in cell protection and promotion of repair.

Material and methods

Animals

Adult male Wistar rats, weighing 280–300 g, were used in this study. All experiments were conducted in accordance with the regulations of the Ohu University Animal Care and Use Committee.

Laser irradiation

A CO_2 laser with a wavelength of 10.6 μm (Panalus C05Σ®; Panasonic Shikoku Electronics Co., Ltd, Matsuyama, Japan) was used in this study. Prior to this study, we performed a preliminary test of the effect of various parameters on the rat gingiva and thereby determined those by which the thermal damage, i.e. coagulation necrosis, extended over the whole gingiva without surface ablation.

The irradiation was performed with the parameters of 5 W peak power and 600 μs pulse duration with a 6 ms off time. Under general anaesthesia induced and maintained by an intraperitoneal injection of sodium pentobarbital (50 mg/kg), a laser-produced wound that was 1.5 mm wide by 3.0 mm long was made on the palatal gingiva of the first molar by slowly moving the contact tip mounted on a handpiece along the tooth surface for 20 s. This contact tip produced a beam with a diameter of 1.5 mm. The calculated fluence obtained with the present parameters was 326 J/cm^2, and this irradiation caused a chalky white discoloration of the gingival surface without ablating the surface (Fig. 1).

A size-matched scalpel-made wound reaching the underlying bone and prepared at the same location as the laser-made wound, i.e. gingivectomy, was made in other rats and served for comparison.

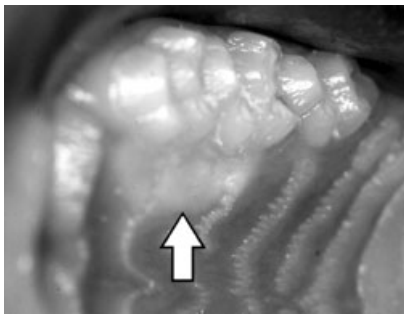

Fig. 1. Rat gingiva immediately after CO_2 laser irradiation. The irradiated gingiva shows a chalky white discoloration (arrow) but no obvious surface defect.

Tissue procurement

Animals were killed by cervical dislocation under general anaesthesia with sodium pentobarbital at different times from 1 h to 7 d after irradiation (six rats at each time point) and scalpel gingivectomy (four rats at each time point), and then the maxillae were excised and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at 4°C for 18 h. After decalcification in a 10% EDTA solution, the specimens were embedded in paraffin.

Immunohistochemistry

Deparaffinized and rehydrated sections of 4 μm thickness were incubated for 20 min in 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) to inactivate endogenous peroxide. Nonspecific protein binding was attenuated by incubation for 20 min with normal goat serum. Then individual sections were incubated with one of the following antibodies: mouse anti-human Hsp72/73 monoclonal antibody (diluted 1:100; Calbiochem, Darmstadt, Germany) or goat anti-human Hsp27 (diluted 1:750; Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature. The sections were next incubated for 30 min with the appropriate secondary antibody, i.e. biotinylated goat anti-mouse IgG antibody or biotinylated donkey anti-goat antibody (diluted 1:100;

Chemicon, Temecula, CA, USA) and then with streptavidin-conjugated horseradish peroxidase complex (Histofine SAB-PO kit; Nichirei, Tokyo, Japan) for 30 min. After development of the peroxidase reaction with diaminobenzidine, the sections were counterstained with haematoxylin.

Double immunofluorescence

In order to examine the temporospatial relationship of Hsp70 and Hsp25, double immunofluorescence was performed according to the methods described by Ito *et al.* (13). Briefly, deparaffinized sections were first incubated with anti-human Hsp27 goat antibody, followed by incubation with biotinylated donkey anti-goat antibody, and then with Alexa Fluor 488-conjugated streptavidin (diluted 1:250; Invitrogen, Carlsbad, CA, USA). Next, the sections were incubated with mouse anti-human Hsp72/73 antibody, followed by labelling with Cy3-conjugated (Cy_3 is another name of indocarbocyanine) goat anti-mouse antibody (diluted 1:500; Jackson ImmunoResearch, West Grove, PA, USA). After having been washed in PBS followed by distilled water, the slides were mounted with ProLong Antifade Kit (Molecular Probes, Eugene, OR, USA) and examined with an Axiovert 2000M microscope equipped with a LSM510 laser confocal microscopy system (Carl Zeiss, Oberkochen, Germany).

Results

Histological findings on the healing of the laser-made wound

Histological findings on the wound healing of the rat gingiva after irradiation with CO_2 laser in coagulation mode were described in our previous study (4), but for the sake of understanding, a brief description of them is presented again here. While the gingival epithelium at the irradiation site showed a distinct feature of coagulation necrosis and shortly after was sloughed off, the lamina propria did not show any visible histological alteration by 1 d post-irradiation (Fig. 2A). After 1 d, although the irradiated lamina propria retained its connective tissue architecture, it was gradually invaded by newly formed fibroblasts and blood vessels from the adjacent connective tissue. The regenerating epithelium migrated along the surface of the coagulated lamina propria (Fig. 2B). By 7 d post-irradiation, the re-epithelization was complete, and the irradiated lamina propria had been mostly replaced by the regenerating connective tissue cells (Fig. 2C).

At 1 d after scalpel gingivectomy, the wound was filled with an admixture of blood coagulum, purulent exudates and tissue debris (Fig. 2D). At 3 d post-scalpel gingivectomy, a small amount of newly formed connective tissue was observed at the wound edge, but the wound was still largely occupied by fibrinopurulent exudates and tissue debris. The regenerating epithelium (RE) showed downward

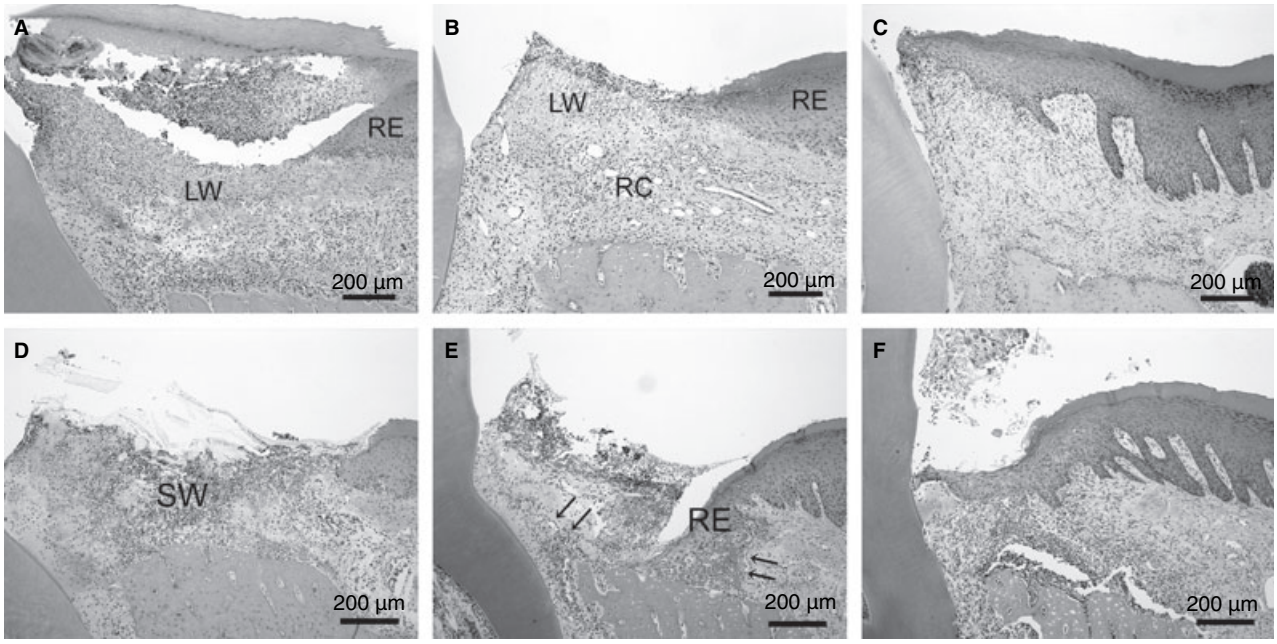

Fig. 2. Histological features of laser-irradiated rat gingiva compared with scalpel gingivectomy. (A) One day after irradiation. The gingival epithelium shows a feature of necrosis but the lamina propria does not show any visible histological alteration. LW, laser wound; RE, regenerating epithelium. (B) Three days after irradiation. Both re-epithelization and replacement by regenerating connective tissue (RC) have progressed. (C) Seven days after irradiation. Re-epithelization is mostly complete, and regenerated gingiva has recovered its original contour. (D) One day after scalpel gingivectomy. The scalpel wound (SW) is filled with an admixture of blood coagulum, purulent exudates and necrotic tissue debris. (E) Three days after scalpel gingivectomy. A small amount of new connective tissue has formed at the wound edge (arrows). Regenerating epithelium has migrated towards the wound bottom. (F) Seven days after scalpel gingivectomy. The re-epithelization is mostly complete but the regeneration of connective tissue is insufficient to recover the original gingival contour.

migration along the wound edge (Fig. 2E). At 7 d post-scalpel gingivectomy, the re-epithelization was almost complete but the connective tissue regeneration was insufficient to recover the original gingival contour (Fig. 2F).

Immunohistochemical expression of heat shock proteins

Expression of Hsp70 — In the non-irradiated normal gingiva, although all layers of the epithelium showed a weak and diffuse cytoplasmic labelling of Hsp70, no labelling was detected in the gingival lamina propria. At 1 h after irradiation, an increased labelling of Hsp70 was noted in both the nucleus and the cytoplasm of the epithelial keratinocytes adjacent to the necrotizing ones (Fig. 3A). At 3 h, connective tissue cells at the border zone of the gingiva and periodontal ligament, which included fibroblasts, osteoblasts, cementoblasts and capillary endothelial cells, appeared to exhibit labelling of Hsp70 (Fig. 3B), and thereafter their labelling intensity increased. At 1 d, not only the nucleus but also the cytoplasm of those cells showed an intense labelling (Fig. 3C,D). The labelling in the epithelium was transient and mostly disappeared at 1 d post-irradiation (Fig. 3C). The level of Hsp70 expression in the connective tissue cells peaked at 1 d post-irradiation and thereafter rapidly became reduced, and only a few labelled cells were detected after day 3 (Fig. 3E).

No labelling was detected in the connective tissue around the scalpel-made wound throughout the experimental period (Fig. 3F).

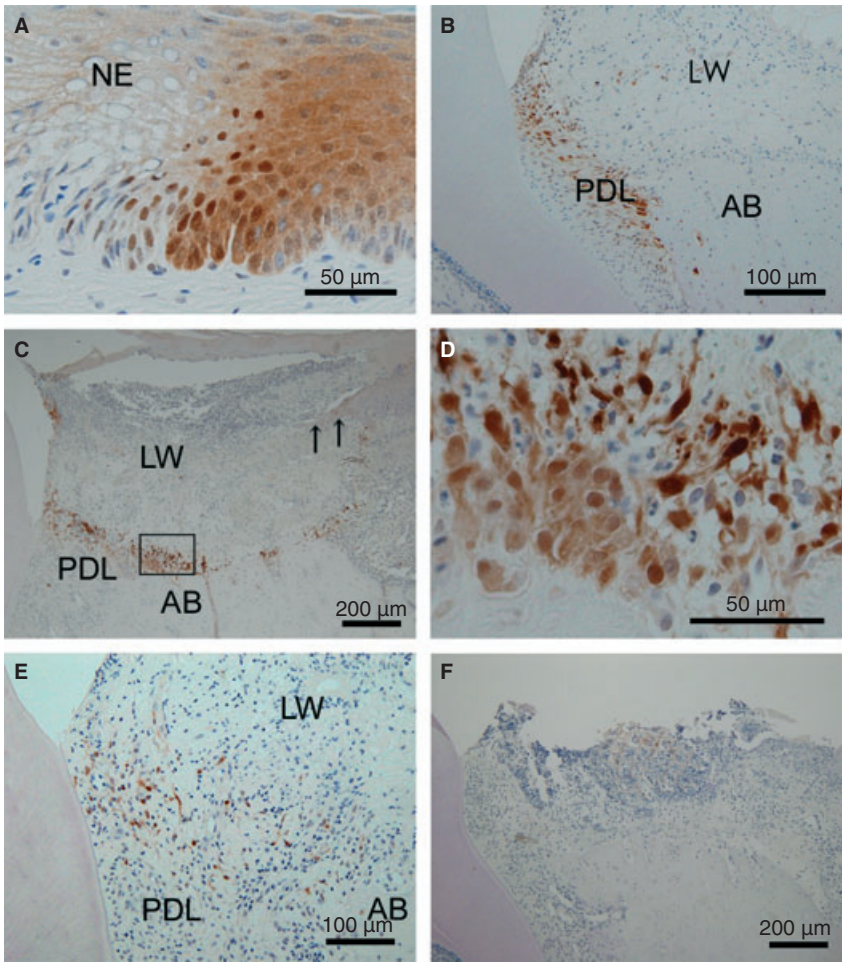

Fig. 3. Expression of Hsp70. (A) One hour after irradiation. Epithelial keratinocytes facing the necrotizing epithelium (NE) show nuclear and cytoplasmic labelling. (B) Three hours after irradiation. Connective tissue cells facing the laser wound (LW) show an intense labelling. PDL, periodontal ligament; AB, alveolar bone. (C) One day after irradiation. An increased labelling is observed at the gingiva–periodontal ligament (PDL) junction and the surface of alveolar bone but no labelling is observed in the regenerating epithelium (arrows). (D) High-power view of boxed area in C. Connective tissue cells, including osteoblasts, show nuclear and cytoplasmic labelling. (E) Three days after irradiation. A weak labelling is found at the gingiva–periodontal ligament junction. (F) One day after scalpel gingivectomy. No labelling is noted.

Expression of Hsp25 — In the non-irradiated rat gingiva, all layers of the gingival epithelium except for the stratum corneum were positive for Hsp25. In the lamina propria, many, but not all, of the vascular endothelial cells constitutively expressed Hsp25, whereas the labelling was absent in most of the fibroblasts. The labelling in both epithelial keratinocytes and connective tissue cells was localized exclusively in the cytoplasm.

At 6 h after irradiation, the connective tissue cells facing the laser wound, which included fibroblasts as well as capillary endothelial cells, appeared to show an intense nuclear labelling of Hsp25 (Fig. 4A,B), whereas the labelling had disappeared from the cells within the wound. On and after 1 d post-irradiation, Hsp25-positive capillaries and fibroblasts increased in number and labelling intensity and spread over the wound, prominent among which were tiny capillaries (Fig. 4C,D). Keratinocytes migrating on the surface of the laser wound exhibited an intense nuclear and cytoplasmic labelling (Fig. 4C). At day 7, a significant number of fibroblasts filling the healing laser wound exhibited a definite cytoplasmic labelling of Hsp25 (Fig. 4E,F).

At 6 h after scalpel gingivectomy, neither up-regulation nor nuclear translocation of Hsp25 was noted at the wound edge, although Hsp25-positive capillaries were sporadically found (Fig. 5A,B). At day 3, in addition to capillary endothelial cells, a few fibroblasts showed cytoplasmic labelling of Hsp25 in the regenerating connective tissue (Fig. 5C). By day 7, Hsp25-positive fibroblasts had increased in number,

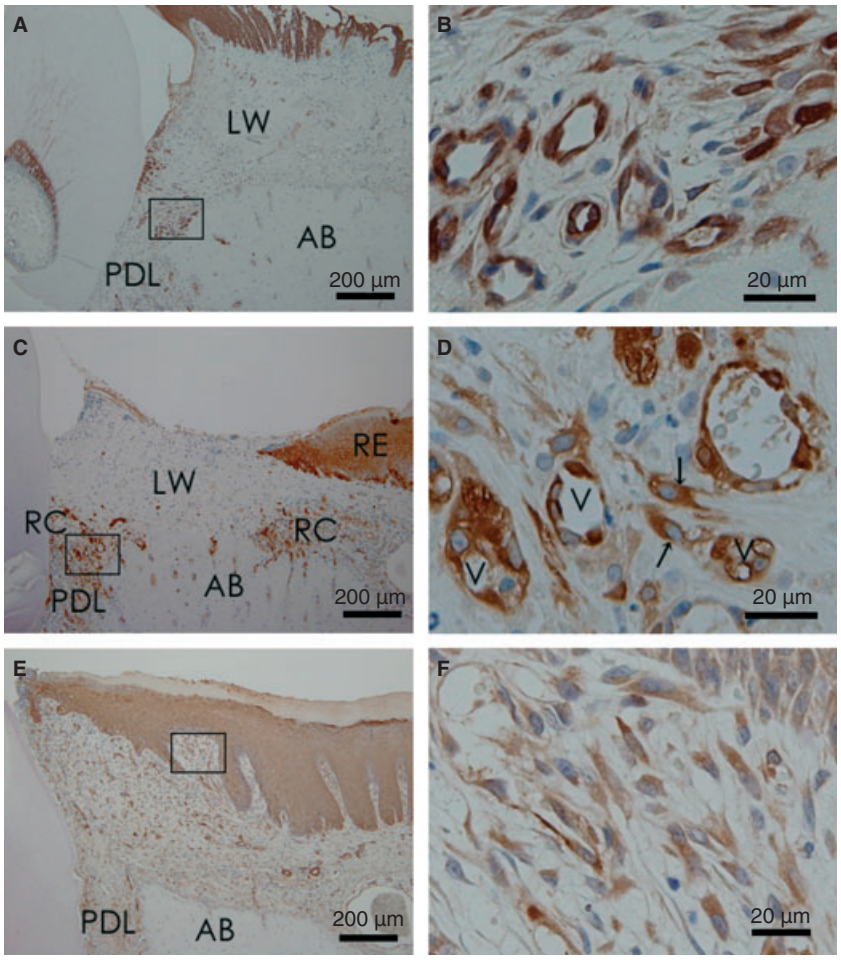

Fig. 4. Expression of Hsp25 in the laser wound. (A) Six hours after irradiation. Periodontal ligament (PDL) cells show enhanced labelling of Hsp25. AB, alveolar bone; LW, laser wound. (B) High-power view of boxed area in A. Fibroblasts, osteoblasts and capillary endothelial cells show intense nuclear labelling. (C) Two days after irradiation. Regenerating connective tissue cells (RC) have grown into the laser wound. Regenerating epithelium (RE) displays an intense nuclear and cytoplasmic labelling. (D) High-power view of boxed area in C. The labelling has disappeared from the nuclei of fibroblasts (arrows) and capillary endothelial cells. Notable are tiny capillaries (V) exhibiting intense cytoplasmic labelling. (E) Seven days after irradiation. The laser wound has been replaced by newly formed connective tissue. (F) High-power view of boxed area in E. Many fibroblasts show cytoplasmic labelling.

but they were fewer and lower in labelling intensity compared with those in the laser wound (Fig. 5D).

Double-immunofluorescence staining for Hsp70 and Hsp25 — To examine the temporospatial relationship between Hsp70 and Hsp25, we observed double-immunofluorescence-stained sections by confocal laser microscopy. At 6 h post-irradiation, the Hsp25-labelling zone was located behind the Hsp70-labelling zone, and a few cells showed the co-labelling of Hsp70 and Hsp25 (Fig. 6A). At day 1, the two zones appeared to overlap each other, and a number of cells showed co-labelling of the two heat shock proteins, with the remaining cells being positive for either Hsp70 or Hsp25 (Fig. 6B). The co-labelling was found predominantly in capillary endothelial cells and less frequently in fibroblasts. After day 3, Hsp25-positive cells increased in number and invaded into the laser wound beyond the original wound margin, whereas the Hsp70-positive cells decreased in number (Fig. 6C). Co-labelled cells were no longer observed.

The double-immunofluorescence staining was not carried out for the scalpel wounds because the expression of Hsp70 was totally absent in the connective tissue.

Discussion

The CO_2 laser used for the present study delivers a defocused beam emitted by lower peak power with a longer off time compared with the conventional pulsed CO_2 laser. Owing to this property, the irradiated tissue does not evaporate but undergoes coagulation necrosis. Our previous study revealed that when the rat gingiva was irradiated with this laser mode, the surrounding tissue promptly responded with activation of cell proliferation; thereafter, the coagulated tissue was replaced by newly formed connective tissue (4). The present study has shown that such tissue responses are preceded by the overexpression of Hsp70 and Hsp25, suggesting their participation in the progress of wound repair and tissue remodelling.

The overexpression of Hsp70 was noted in the nucleus of epithelial keratinocytes adjacent to the necrotizing ones as early as 1 h after irradiation, and *de novo* expression of Hsp70 was found in the connective tissue at 3 h. Heat shock protein 70 has been shown to play a significant role in rescuing stressed cells by helping damaged proteins refold or by participating in the synthesis of new proteins to replace damaged proteins (14,15). Thereby, the stressed cells can withstand an injury that would otherwise be lethal. The nuclear translocation of Hsp70 has been demonstrated to promote resolubilization of heat-induced protein aggregates by reactivation of thermally denatured enzymes in the nuclei of recovering cells (16,17). Thus, the Hsp70-positive zone can be interpreted as a front line of tissue survival against heat stress generated by the laser beam. The finding that non-thermal stress caused by the scalpel did not bring about any detectable expression of

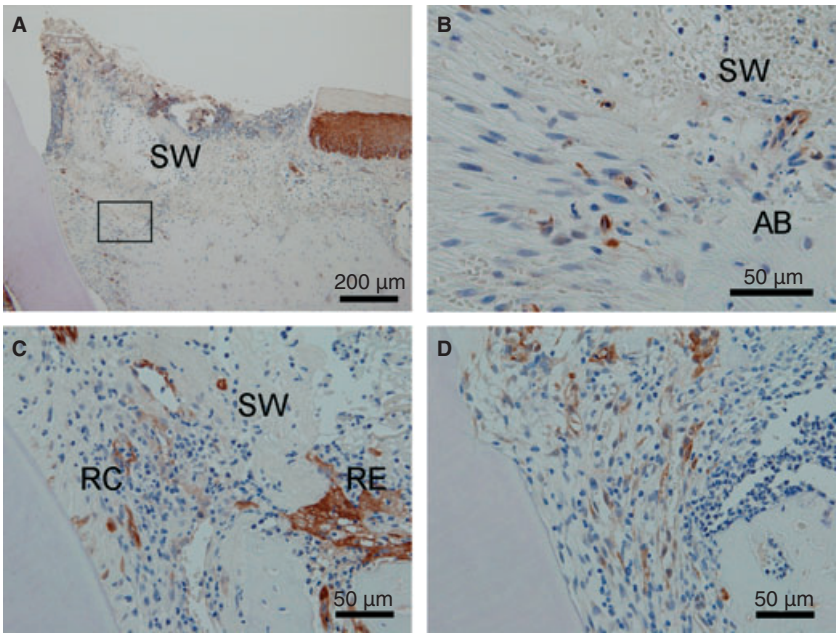

Fig. 5. Expression of Hsp25 in the scalpel wound. (A) Six hours after scalpel gingivectomy. No up-regulation of Hsp25 is evident at the wound edge. SW, scalpel wound. (B) High-power view of boxed area in A. Only a few capillary endothelial cells show cytoplasmic labelling but there is no nuclear labelling. AB, alveolar bone. (C) Three days after scalpel gingivectomy. A number of capillaries and a few fibroblasts in the regenerating connective tissue (RC) show labelling with weak to moderate intensity. RE, regenerating epithelium. (D) Seven days after scalpel gingivectomy. A significant number of fibroblasts in the regenerating connective tissue show labelling, but the intensity is not as high as in the laser wound.

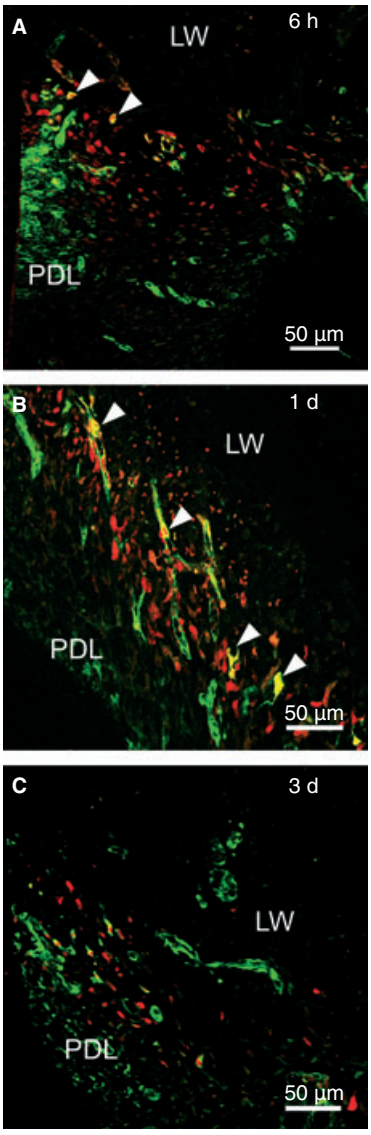

Fig. 6. Merged images of confocal immunofluorescence microscopy. Heat shock protein 70-positive cells are stained red and Hsp25-positive cells are stained green. (A) Six hours after irradiation. Most of the Hsp70-positive cells are localized ahead of the Hsp25-positive zone. A few cells stained yellow (arrowheads), indicating co-labelling of Hsp70 and Hsp25. PDL, periodontal ligament; LW, laser wound. (B) One day after irradiation. Heat shock protein 70-positive cells (red) and Hsp25-positive cells (green) are localized in overlapping areas, and some fibroblasts and capillaries show co-labelling (yellow, arrowheads). (C) Three days after irradiation. Heat shock protein 25-positive cells, probably capillary endothelial cells, invade into the laser wound. Heat shock protein 70-positive cells are decreased. No definite co-labelling is observed.

Hsp70 in the cells around the cut edge seems to support this interpretation.

The connective tissue cells facing the laser wound also showed the up-regulation and nuclear translocation of Hsp25 at 6 h post-irradiation. In contrast, the scalpel incision did not result in any notable changes in the expression pattern of Hsp25 at the same time point. Thus, as in the case of Hsp70, we consider the early up-regulation of Hsp25 to have been induced in response to the laser-generated heat stress and to have conferred the cell protection. Heat shock protein 25 had been implicated in protecting cells through the stabilization and/or restoration of cytoskeletal elements in stressed cells (18,19). Nuclear translocation of Hsp25 can prevent the irreversible denaturation/aggregation of heat-unfolded nuclear proteins (19).

When the temperature elevation is slight, as in the laser wound margin, heat shock may appear to kill cells not by irreversible damage of critical structures of the cells but via activation of a programmed cascade of events leading to cell death, i.e. apoptosis (10). Heat shock protein 70 has been shown to confer cell protection from apoptosis through inhibition of mitochondria-dependent apoptosis signalling, suppression of c-Jun N-terminal kinase (JNK) activation and activation of Bcl-2 family members, whereas Hsp25 blocks Fas/APO-1-mediated cell death and also interferes with the JNK–mitochondria pathway at different points from Hsp70 (10,20). Thus, it seems likely that the expression of both Hsp70 and Hsp25 at the wound margin indicates their anti-apoptotic function.

Heat shock protein 70 and Hsp25 showed temporospatially different expression patterns in response to the laser irradiation. Heat shock protein 70 was more rapidly expressed than Hsp25. The co-expression of the two heat shock proteins depicted by double immunofluorescence was unexpectedly less frequent, and even when

the localization of the two heat shock proteins appeared to overlap, many cells expressed either Hsp70 or Hsp25. The co-expression was predominantly found in capillary endothelial cells and less frequently in fibroblasts. Although both Hsp70 and Hsp25 have been implicated in protecting cells, the exact role of each may differ depending on the cell type and context. The diversity may be attributed to the difference in the chaperone machinery for cell protection. Members of the Hsp70 family facilitate chaperone function in an ATP-dependent manner, whereas Hsp25 can act as an ATP-independent chaperone through phosphorylation of itself by the mitogen activated protein kinase (MAPK) cascade.

Heat shock protein 25-expressing cells increased in number with time after laser irradiation and invaded into the laser wound after day 2, notable among which were the cells of tiny capillaries, presumed to be sprouting capillaries. A significant number of fibroblasts consisting of the regenerated gingival connective tissue exhibited a definite cytoplasmic expression of Hsp25. A strong cytoplasmic expression of Hsp25 was also noted in the keratinocytes migrating on the wound surface. We speculate from these findings that Hsp25 plays a significant role in the onset and advance of tissue repair that occurs after laser irradiation. Supporting this speculation, cells expressing Hsp25 were fewer and the expression intensity was lower in the scalpel wound than in the laser wound, and this expression pattern of Hsp25 seemed to be closely correlated with the less active and delayed connective tissue regeneration in the scalpel wound.

Heat shock protein 25 has been shown to modulate microfilament dynamics in a manner dependent on its phosphorylation, and thereby to regulate cell adhesion, elongation, migration and matrix contraction (12), which are essential for wound healing. In this regard, it is of interest to note the translocation of Hsp25. Found in the cytoplasm of both keratinocytes and connective tissue cells in the non-irradiated gingiva, Hsp25 was rapidly translocated into the nucleus after irradiation. When the tissue regeneration commenced, after day 2, it was relocated into the cytoplasm. Studies have reported that nuclear Hsp25 contributes to an increased chaperone capacity of cells by binding unfolded nuclear protein to protect them from stress (21), whereas cytoplasmic Hsp25 acts as a molecular chaperone to stabilize the cytoskeleton and thereby facilitates the healing process (9). Thus, we speculate that Hsp25 plays a dual role in the laser wound, i.e. cell protection and promotion of repair, and that its translocation is indicative of this role shift. Heat shock protein 70 has also been reported to participate in improvement of wound healing (22,23). In the laser wound, however, the expression of Hsp70 was transient; it peaked at 1 d after irradiation and thereafter rapidly decreased. Thus, it is more likely that Hsp70 in the laser wound plays a role mainly in protecting cells from the heat stress.

In conclusion, the rat gingival tissue promptly responded to the irradiation of the CO_2 laser in the coagulation mode by expressing Hsp70 and Hsp25. We suggest that while Hsp70 mainly confers cell protection, Hsp25 plays a dual role, cell protection and promotion of repair.

References

1. Thomson PJ, Wylie J. Interventional laser surgery: an effective surgical and diagnostic tool in oral precancer management. *Int J Oral Maxillofac Surg* 2002;**31:**145–153.
2. Ishii J, Fujita K, Komori T. Laser surgery as a treatment for oral leukoplakia. *Oral Oncol* 2003;**39:**759–769.
3. Sharon-Buller A, Sela M. CO_2-laser treatment of ulcerative lesions. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004;**97:**332–334.
4. Yamasaki A, Tamamura K, Yusa J, Sakurai Y, Okuyama N, Ito H. Remodeling of the rat gingiva induced by CO_2 laser coagulation mode. *Lasers Surg Med* 2008; **40:**695–703.
5. Beckman PR, Mizzen LA, Welch WJ. Interaction of hsp70 with newly synthesized proteins: implications for folding and assembly. *Science* 1990;**248:**850–854.
6. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress protein, and implication for medicine and disease. *Physiol Rev* 1992; **72:**1063–1081.
7. Tavaria M, Gabriele T, Kola I, Anderson RL. A hitchhiker's guide to the human Hsp70 family. *Cell Stress Chaperones* 1996;**1:**23–28.
8. Leonardi R, Barbato E, Paganelli C, Lo Muzio L. Immunolocalization of heat shock protein 27 in developing jaw bones and tooth germs of human fetus. *Calcif Tissue Int* 2004;**75:**509–516.
9. Lavoie JN, Gingra-Breton G, Tanguay RM, Landry J. Induction of Chinese hamster HSP27 gene expression in mouse cell confers resistance to heat shock. *J Biol Chem* 1993;**268:**3420–3429.
10. Gabai VL, Sherman MY. Invited review: interplay between molecular chaperones and signaling pathways in survival of heat shock. *J Appl Physiol* 2002;**92:**1743–1748.
11. Sur R, Lyte PA, Southall MD. Hsp27 regulates pro-inflammatory mediator release in keratinocytes by modulating NF-κB signaling. *J Invest Dermatol* 2008; **128:**1116–1122.
12. Hirano S, Shelden EA, Gilmont RR. Hsp27 regulates fibroblast adhesion, motility, and matrix contraction. *Cell Stress Chaperones* 2004;**9:**29–37.
13. Ito H, Takekoshi T, Miyauchi M *et al*. Three-dimendional appearance of Langerhans cells in human gingival epithelium as revealed by confocal laser scanning microscopy. *Archs Oral Biol* 1998;**43:**741–744.
14. Hartl FU, Hayer-Hartl M. Molecular chaperones in the cytosole: from nascent chain to folded protein. *Science* 2002; **295:**1852–1858.
15. Bukau B, Weissman J, Horwich A. Molecular chaperones and protein quality control. *Cell* 2006;**125:**443–451.
16. Nollen EA, Salomons FA, Brunsting JF, Want JJ, Sibon OC, Konings AW. Dynamic changes in the localization of thermally unfolded nuclear proteins associated with chaperone-dependent protein. *Proc Natl Acad Sci USA* 2001;**98:**12038–12043.
17. Stage GJ, Li GC, Kampinga HH, Konings AW. On the role of hsp72 in heat-induced intranuclear protein aggregation. *Int J Hyperthermia* 1994;**10:**659–674.
18. Welch MJ, Gaestel M. Small heat-shock protein family: function in health and disease. *Ann N Y Acad Sci* 1998;**851:**28–35.
19. Bryantsev AL, Loktionova SA, Ilyinskaya OP, Tararak EM, Kampinga HH, Kabakov AE. Distribution, phosphorylation, and activities of Hsp25 in heat-stressed H9c2 myoblasts: a functional link to cytoprotection. *Cell Stress Chaperones* 2002;**7:**146–155.
20. Giffard RG, Han R-Q, Emery JF, Duan MD, Pittet JF. Regulation of apoptotic

and inflammatory cell signaling in cerebral ischemia – the complex roles of heat shock protein 70. *Anesthesiology* 2008;**109:**339–348.

21. Bryantsev AL, Kurchashova SY, Golyshev SA *et al*. Regulation of stress-induced intracellular sorting and chaperone function of Hsp27 (HspB1) in mammalian cells. *Biochem J* 2007;**407:**407–417.
22. Oberringer M, Baum HP, Jung V *et al*. Differential expression of heat shock protein 70 in well healing and chronic human wound tissue. *Biochem Biophys Res Commun* 1995;**214:**1009–1014.
23. Kovalchin J, Wang R, Wagh MS, Azoulay J, Sanders M, Chandawarkar RY. In vivo delivery of heat shock protein 70 accelerates wound healing by up-regulating macrophage-mediated phagocytosis. *Wound Rep Reg* 2006;**14:**129–137.