COMPARISON OF FIBROBLASTIC PROLIFERATION AND COLLAGEN DEPOSITION IN RESPONSE TO CO₂ LASER SURGERY AND SCALPEL

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ABSTRACT
Introduction: CO₂ laser is the advocated treatment for the removal of various soft tissue lesions in oral cavity in recent years. A number of clinical advantages are reported by CO₂ laser as compared to scalpel.¹,² The objectives include oral tissue fibroblastic response after CO₂ laser surgery and to compare advantages and disadvantages of CO₂ laser and scalpel after an oral surgical procedure with special reference to fibrosis and collagen deposition. Place and Duration of Study: A comparative study, conducted at animal laboratory of Post Graduate Medical Institute, Lahore for about three months. Subjects and Methods: This study was conducted on 150 Sprague – Dawley Albino rats, which were divided into 3 equal groups of 50. Partial glossectomy was done with 3 watts CO₂ laser for group (A), with 6 watts CO₂ laser for group (B) and with scalpel for group (C). The fibroblastic activity and collagen deposition was observed and compared histologically, 24 hours, 3rd day, 7th day, 14th day and 28th day after surgery. Results: Histologically fibrosis and collagen deposition were highly significant in A and C groups as compared to group B. Conclusion: six watts CO₂ laser wound was much better as compared to scalpel in terms of lesser fibroblastic activity and collagen thickness hence having a good wound healing response.

Key words: CO₂ laser, Scalpel, fibroblastic proliferation, wound healing, fibrosis, collagen deposition, partial glossectomy.

INTRODUCTION
CO₂ laser is the advocated treatment for the removal of various soft tissue lesions in oral cavity in recent years. A number of clinical advantages are reported by CO₂ laser as compared to scalpel.¹,²

Using CO₂ laser, large areas in oral cavity can be excised or vaporised without the need of primary closure thus resulting in good secondary wound healing.³ The fibroblasts and synthesis of new collagen predominate in the proliferative phase of secondary wound healing and reflect directly the extent of recovery towards normal wound healing.⁴ Fibroblasts start appearing after a day in the open wound but collagen deposition starts on third day after injury. Initially type III collagen appears but by the end of first week collagen type I dominate and it becomes the major collagen of mature scar tissue.⁵

The deposition of collagen is on its peak level after the start of 3rd week, after that remodeling begins fibroblasts number dwindles and collagen begins to consolidate.⁶ Reorientation of collagen is achieved by the removal of initially deposited collagen fibers and deposition of new one according to stress forces. Maturation of wound is the last phase of repair process involving revascularization, a decrease in the rate of collagen degradation and formation of stable intermolecular and intramolecular collagen crosses linkage.⁷ These properties determine the future cosmetic and functional results of wound after healing, especially when delicate dissection such as tongue is involved.⁸ With this reference advantages CO₂ laser surgery has been reported by many authors to have less post-operative complications and better recovery.⁹–¹² While various studies have reported against it,¹³–¹⁵ Keeping in mind the existing controversies about CO₂ laser healing effects on oral tissue at different wattages as compare to scalpel, the present study was planned to evaluate healing with reference to fibroblastic activity and collagen deposition. Tongue is used for the study because it is the best representative of oral soft tissue.

SUBJECTS AND METHODS
This study was conducted at the Animal Laboratory of Postgraduate Medical Institute Lahore. One hundred and fifty albino Sprague – dawley rats of about nine weeks age, weighing 250 to 350 grams, were included in the study.¹⁶–¹⁷ Animals were divided into 3 equal groups (A, B, C) of 50 each. Group A had partial glossectomy with 3 watts CO₂ laser, Group B with 6 watts CO₂ laser and Group C with the scalpel.

Animals were kept at room temperature (22 ± 2°C), under natural conditions of humidity and light, the rats were allowed one week of acclimatisation to the laboratory conditions before being used in experimental procedure.¹⁸ Standardised surgical condi-
Partial glossectomy was planned for each group with a specially designed forceps of non reflected surface having two blades. Each blade was 10 mm X 5 mm at right angle. Partial glossectomy was planned and done by CO\textsubscript{2} Laser and scalpel as reported by Malik and Bajwa.\textsuperscript{20} The wound was left exposed in the mouth and no sutures were inserted in order to produce standard test situation as is practiced clinically.\textsuperscript{21,22} Generally 3 watt is considered as minimal and 6 watt as maximum output power of waveguide laser for oral clinical application is practised.\textsuperscript{23,24}

Animals were killed after 24 hours, 3\textsuperscript{rd}, 6\textsuperscript{th}, 13\textsuperscript{th} and 28\textsuperscript{th} day. Thirty animals on each selected day (10 from each group) by an over dose of general anesthesia and immediately fixed in 10% formalin. To maintain the tissues in correct relationship the tongue was preserved with mandible. After at least 48 hours, the tongues were dissected out.\textsuperscript{21}

**HISTOLOGICAL OBSERVATIONS**

After tissue processing, staining with haematoxylin and eosin was done as it defines necrotic tissue most clearly and is suitable to get all sorts of measurements.\textsuperscript{13,26,27} Van Geison Staining technique was done to assess the collagen fibers.\textsuperscript{28} Histological observations were recorded at 10 X and 40 X objectives, for the assessment of wound healing after partial glossectomy. The observations like fibrosis, and collagen fibers were assessed and recorded on semi-quantitative scale of 0 to ++++. During observation 0 is taken as no activity, + for minimum, ++ for moderate, +++ for abundant and ++++ was recorded for extensive activity.\textsuperscript{11,29}

The fibroblasts were counted at high power magnification (X 40) by a counting graticule mounted on the eye piece. The graticule had 100 cubical division in 1 cm\textsuperscript{2} representing 0.25 mm\textsuperscript{2} (0.625 m\textsuperscript{2}) area at 40 X magnification. The fibroblasts were counted in the whole graticule area by placing it on the edges of the dorsal surface, ventral surface and in the mid of the tongue wound.\textsuperscript{30}

**STATISTICAL ANALYSIS**

Mean, SD were calculated and Student “t” test, were used for comparing two means. Comparison of paired observation was carried out by paired t test. The observation in the groups was compared by Chi square test and subgroups by Fisher’s exact test in different kinds of data analysis.
RESULTS
The three groups were studied and compared after partial glossectomy histologically in following manner.

Comparison of Fibrosis
Comparison of fibrosis was carried out on 3rd, 6th, 14th and 28th day as the fibrosis is apparent after 3rd day of injury. The mean ± SD degree of fibrosis on third day in group C was (2.4 ± 0.5) more than group B (1.7 ± 0.4) and group A (1.5 ± 0.5) but the difference was not significant. On the 6th day the fibrosis in group C was (2.9 ± 0.9) was significantly raised than group B (1.7 ± 0.6). Group B has less fibrosis than group A (1.9 ± 0.7) but the difference was not significant. On 14th day, group B (2.4 ± 0.5) has less fibrosis than group A (2.6 ± 0.6) and group C (2.9 ± 0.9). On 28th day, group C (2.7 ± 0.4) fibrosis was more than group B (2.1 ± 1.02) and group A (2.5 ± 0.5) but the difference was not significant (Table 1).

Comparison of collagen deposition
Comparison of collagen deposition was carried out on 6th, 14th and 28th day as it is obvious after 6th day of injury. Mean ± SD degree of collagen deposition was more in group C than group A and B but the difference was not significant. The collagen deposition on 14th day was least in group B, moderate in C and was raised in group A, the difference was not significant. On 28th day, group C has more deposition than groups A and B. However group B showed least deposition. The difference was not significant (Table 2).

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NS = Not significant

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Comparison of collagen fibers in 3 groups 28th day after surgery

Fibroblasts Proliferation
Comparison of fibroblastic proliferation was carried out after 24 hours, 3rd, 6th, 14th and 28th day as the proliferative activity starts within 24 hours after injury.

Mean ± SD count / 0.625 m² of fibroblasts after surgery were noted in each group. After 24 hours
in group C (29.0 ± 4.5) count was more than group A (12.6 ± 4.8) and B (18.7 ± 2.9) and the difference was highly significant (P < 0.001). The group B also showed significantly (P < 0.01) more count than group A. On 3rd day the count in group C (83.3 ± 27.7) was highly significant (P < 0.001) as compared to group B (43.3 ± 6.3) and group A (51.0 ± 13.1). The group A had more count than B but insignificant. On the 6th day the count in group C (105.0 ± 18.3) was raised than group A (51.0 ± 28.1) and group B (51.3 ± 21.3). The difference was highly significant (P < 0.001), while group A and B had no significant difference. On 14th day count was reduced significantly in group C (19.4 ± 7.0) s compared to group A (25.0 ± 7.23) and group B (27.6 ± 7.37). On 28th day the count increased in group C (26.0 ± 5.16) as compared to group A (23.0 ± 5.3) and group B (21.67 ± 5.47) but the difference was not significant (Table 3).

DISCUSSION
Since the development of the CO₂ laser as a surgical knife in 1970, many studies have been conducted to compare wound healing at different watts of CO₂ laser with the scalpel, but these studies have certain deficiencies because these studies were conducted on very limited animal models. Many missing histological observations like cellular (especially fibroblastic) response, limited spectrum of research (excluding collagen deposition, fibrosis) and lot of controversies in the previous studies gave us initiative to perform this study. The 3 watts CO₂ laser as minimum power and 6 watts CO₂ laser as maximum power in clinical practice have never been compared with scalpel to see the tissue response with reference to fibroblastic activity and collagen deposition after partial glossectomy.

The rat tissues contain higher percentage of fibroblasts than other cells and beginning of fibroblastic reaction in wound after one day was observed by Mondain et al. In our study fibroblasts count was less after 24 hours. The fibroblasts dominated in group C than group A and B and the difference was highly significant (P < 0.001). Group B had
more fibroblasts count than group A and was statistically significant (P < 0.01). This reflects the slow migration of fibroblasts in laser wounds.

On 3rd day, the mean ± SD degree of fibrosis was more in group C as compare to A and B. The group B fibrosis was slightly more than group A (Table 1). This was probably due to more fibroblast infiltration in group B than group A. This reflect better healing tendency in group B. In group C, the count was raised highly significant (P < 0.001) than group A, significantly (P < 0.01) more in group B than A. More fibroblast count on 3rd day in group B and C, shows the intensity towards better wound healing and same was observed by Mondain and Rayn,32 Haukipuro et al 33, Vardaxis et al.34

The rate of collagen synthesis is maximum on 6th day after injury and collagen remodeling depends upon its synthesis and degradation.41,42 Haukipuro et al35 noted that the concentration of type I collagen in wound fluid was maximum after 6th day. Madden et al.43 mentioned less collagen deposition in laser group and same was observed in our study. The mean ± SD degree of fibrosis and collagen in our study was more in scalpel group as compared to laser groups, this was also observed by Fisher and Frame.48 The fibers were most obviously dispersed in wound area but gradually compact in muscle layers. The group B fibrosis and collagen was slightly more than group A (Table 1, 2) which may be due to more fibroblasts infiltration in group B than A, hence reflecting better healing tendency in group B. Fibroblasts appearance is numerous after 6th day in wound.45 In our study the count was prominent in group C and it was high (P < 0.001) as compared to group A and B. They generally differentiate at this time to myofibroblast,36 these cells are responsible for wound contraction, due to their adhesion and pulling property. Hence the remodeling of newly formed extracellular matrix results.46,47,48 On 6th day, it was more obvious in scalpel group in our study and resulted in more wound contraction and ugly looking scar as compare to CO2 Laser groups. These were also noted in the studies by Luomanen et al49 Zeinoun38 and J Ishii.39

On 14th day, the degree of fibrosis and collagen was more in scalpel group as compared to laser groups. The mean ± SD degree of fibrosis was more in group C as compare to A and B groups. Vardaxis et al44 stated that the remodeling phase response begin with the reduction of fibroblasts numbers and consolidation of collagen. But we noticed fibrosis relatively in less number of animals of group A as compare to group B which reflects the better healing tendency in group B. Collagen bundle in our study were found more loosely and randomly arranged in laser groups as compare to scalpel and same was observed by Dimitriu40 and Fisher and Frame.14 The mean ± SD fibroblasts was reduced after 14th day in all wounds. In our study the fibroblasts were mixed with inflammatory cells and were scattered in all directions which can be easily distinguished, same observation was noted by Doillon et al.36 Scalpel wounds fibroblasts were organized well as compared to laser group in our study. It was reduced in group C significantly (P < 0.01) than group A and (P < 0.05) B. This favors the start of early remodeling phase in scalpel group as compared to laser groups. There was no significant difference between group A and B. More collagen in scalpel group gives another indication of speedy healing (Table 2).

The degree of fibrosis and collagen on 28th day was more in scalpel group as compared to laser group. The mean ± SD degree of fibrosis was more in group C. Trott42 and Messadi41 mentioned the maximum collagen deposition after 3rd week and due to this deposition the wound gained 25 – 35% of original tensile strength in 4th week. Remodeling phase response begins with the reduction of fibroblasts numbers and consolidation of collagen.34 Group A has slightly more collagen deposition and behave like burn wound where there is loss of collagen and then increase in deposition.41,42 We found that collagen was aggregated into bundles mostly in scalpel group while they were more loosely and randomly arranged in laser groups. This was also reported by Fisher and Frame43 and Dimitriu.40 Fibrosis was noted relatively less in group A but less number of fibroblasts reflect better healing in group B. Scalpel group wound favor the regulation of fibroblasts and active remodeling of wound. The mean ± SD (0.0625 m²) cellular count of fibroblasts were reduced in all laser groups and same was observed by Evrard et al.,45 Alster,46 and Grbavac.47 These observation leads towards the conclusion of better healing response in 6 watt Laser group on the basis of fibrosis and collagen deposition.

In conclusion partial glossectomy by scalpel results in more fibrosis wound contraction as compare to CO2 created wounds. CO2 laser is an effective surgical knife if used at least at 6 watts, at this power there is less fibrosis with minimum contraction of the wound. Reduced power of CO2 laser does not produce better wound healing because it takes more cutting time which results in more diffusion of heat to deeper tissue hence more fibrosis and contracture.

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