ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)
IJDSIR : Dental Publication Service
Available Online at: www.ijdsir.com
Volume – 4, Issue – 6, November - 2021, Page No. : 104 - 111

Assessment of healing in periodontitis after treatment with microsurgical and conventional flap surgery by Histopathology and Scanning electron microscopy: A controlled clinical trial

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Citation of this Article: Dr. Bebika Thoudam, H.N. Santosh, Aditi Bose, Dr. Moirangthem Niteshore Singh,"Assessment of healing in periodontitis after treatment with microsurgical and conventional flap surgery by Histopathology and Scanning electron microscopy: A controlled clinical trial", IJDSIR- November - 2021, Vol. – 4, Issue - 6, P. No. 104 – 111.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Objective: The primary objective of the study is to assess and compare the periodontal treatment outcomes and healing patterns in patients with chronic periodontitis using open flap debridement approach by microsurgical and conventional surgical methods by using histopathology & immunohistochemistry. The study objective was also to assess and compare the root surface alterations & presence of residual calculus in the teeth treated by both the methods using scanning electron microscopy (SEM).

Method: In the clinical study; a total of 15 patients were selected. 30 sites in these 15 patients with persistence of ≥5mm pocket depth in two or more than two teeth in each quadrant were selected and randomly microsurgical open flap debridement and control sites were treated with conventional open flap debridement. The clinical parameters gingival indexes, probing pocket depth, relative attachment level, were recorded at the selected sites at baseline, 1 month, 3 months and 6 months. Primary healing following flap approximation was assessed before surgery, immediately after surgery, at 1day, 8day, 1month, 3months & 6months postoperatively. The in vitro study consisted of a parallel study design, in which 5 patients having teeth with grade III mobility on contralateral sites indicated for extraction were selected and periodontal flap surgeries were performed . Seven days following surgery, the concerned teeth were extracted with a margin of healing soft tissue and hard tissue changes such as residual calculus and loss of tooth substance were assessed using SEM. Healing granulation

tissue was examined using H&E staining and immunohistochemistry.

Result: A statistically significant improvement ($p<0.05$) in gingival index, probing pocket depth, relative attachment level in both the microsurgery and conventional sites at all the time intervals throughout the study was observed. SEM examination revealed residual calculus in both the micro surgically treated and conventionally treated tooth specimens. The H&E staining examination revealed more areas of haemorrhage in the conventionally treated groups than in the microsurgical groups.

Conclusion: Both the procedures resulted in improvement of clinical parameters equally. Loss of tooth structure was more in the conventional procedure than in microsurgical procedure. The healing was better in the microsurgical procedure compared to conventional flap procedure.

Keywords: Chronic periodontitis, Conventional Flap Surgery, Microsurgical Flap Surgery, Healing.

Introduction

Periodontitis is one of the most common oral inflammatory infectious diseases & the leading cause of tooth loss and is characterized by the destruction of tooth-supporting tissues.[1] The ultimate goal of periodontal treatment is the surgical elimination of anatomic deformities by restoring the lost supporting tissues. The main aim of surgical intervention is no longer only the survival of the patient's teeth but the effort is to preserve a maximum amount of function and to improve patient comfort and the demands.[2]

Microsurgical periodontal flap surgery has many perceived advantages in periodontal surgery related to the enhanced visual acuity associated with magnification and better soft tissue manipulation. These play an important role in determining the surgical outcome.[3,4,5,6]

Several studies have documented the use of microsurgery in various root coverage procedures, interdental papillae preservation techniques and periodontal regeneration procedures in intra bony defects. Till date, no clinical studies have documented the use and possible advantages of operating microsurgical loupes in periodontal flap debridement surgery.

This study attempts to compare the clinical outcomes following open flap debridement with and without magnifying loupes. In addition, the study also intends to evaluate and compare the primary flap closure, healing outcomes and hard tissue surface changes that are loss of tooth substance and presence of residual calculus in both the approaches.

Methodology

This was a single blinded randomized controlled clinical trial with a split mouth study design. The study included 15 patients with chronic localized or generalized periodontitis. Male & female patients aged between 25-55 years were selected for the study. Patients with true supra bony pockets periodontal pockets having probing pocket depth ≥ 5mm in two or more than two teeth and in minimum two or more than two quadrants with a minimum number of teeth present ≥ 20 were selected for the procedure. Patients with complex medical condition, habits like tobacco chewing were excluded. Patients were screened for periodontal disease using Gingival index (Loe and Sillness 1963), Probing pocket depth (PPD) and Relative attachment level (RAL) (using an acrylic stent). In the presurgical therapy phase, patients satisfying the inclusion and exclusion criteria were recruited for the study based on the initial screening procedures. Oral hygiene instructions were given and scaling and root planing was performed. Periodontal evaluation was performed 4 weeks after Phase I therapy to confirm the suitability of sites for periodontal surgery. Persistence of

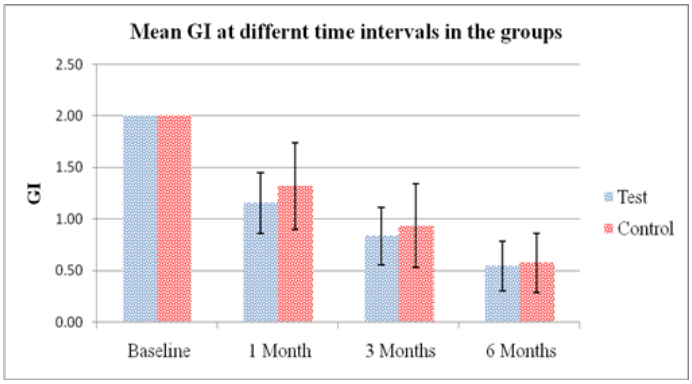
≥5mm pocket depth in two or more than two teeth and in minimum two or more than two quadrants were considered for flap surgery. The selected patients were randomly divided into two groups following the split mouth study design. The test group was treated by open flap procedure using microsurgical technique and the control group was treated by open flap procedure using conventional surgical technique. The microsurgical procedure was performed on the test site. The patients were asked to rinse the mouth with 10 ml of 0.2% chlorhexidine digluconate solution as a pre-procedural rinse. The surgical procedure was performed using 3X optimal magnification dental loupe. The surgical field was anesthetized using local anaesthetic agent. After administration of local anaesthesia, intrasulcular incisions were given with microsurgical blade, and then using a periosteal elevator, full thickness buccal and palatal/ lingual flaps were elevated. Granulation tissue was removed using curettes to provide full access and visibility to the root surfaces. Root planing using curettes was done to get a clean smooth hard surface. The flaps were approximated to the original level and secured with 5-0 mersilk sutures. Postoperative instructions were given. Patients were prescribed antibiotics and non steroidal antiinflammatory drugs for post operative pain management. Patients were instructed to rinse with 0.2% chlorhexidine solution for 1 minute twice a day for 2 weeks. Removal of sutures was done after 7 days. The clinical parameters like Gingival Index, PPD & RAL were evaluated at baseline (at the day of surgery) and at 1, 3 and 6 months after surgery with the help of a Williams periodontal probe. For the control site, similar protocol was followed. But a conventional flap surgery was done. Intrasulcular incisions were given with a no.15 blade, and then using a periosteal elevator, full thickness buccal and palatal/ lingual flaps were elevated. Granulation tissue was removed using Gracey curettes to provide full access and visibility to the root surfaces. Root planing using curettes was done to get a clean smooth hard surface. The flaps were approximated to the original level and secured with 3-0 mersilk sutures. In a parallel in vitro model, 5 patients in whom extraction was indicated in teeth in two or more quadrants owing to severe form of periodontitis were selected. The same exclusion criteria followed in the clinical study was maintained. The teeth in these selected patients were randomly divided into 2 groups (test and control) and the same procedure carried out in the clinical study following the spilt mouth design was done with the test quadrant being treated by microsurgical open flap debridement and the control quadrant by conventional open flap debridement. One week following this treatment, the concerned teeth were extracted with a margin of healing soft granulation tissue around them. They were evaluated for Contents of the healing soft tissue using histopathology &immunohistochemistry and presence of residual calculus and loss of tooth substance using Scanning Electron Microscopy (SEM).

Result

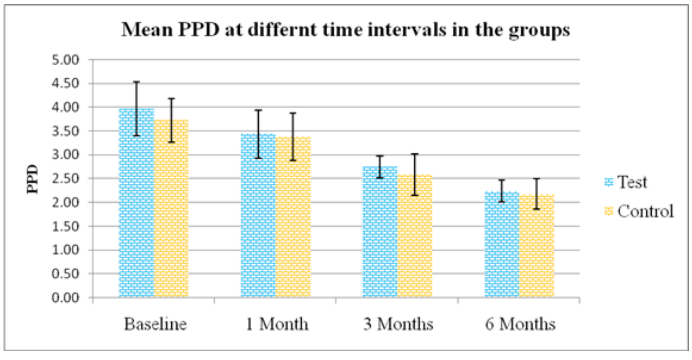
Descriptive statistical analysis was done. Paired t – test was performed to compare post treatment changes from baseline within each group. For comparison between the intergroup variations t – test was performed. No statistical analysis was performed in the in – vitro study parameters, only the observations were reported. The Gingival Index scores were significantly reduced in the individual group after the periodontal therapy with microsurgical procedure and conventional flap surgery. The Gingival index scores depleted from 2.00 to as low as 0.57 in span of 6 months in both test and control group (Graph 1). But on comparison between the two groups, statistically significant results did not appear. The comparison of the mean GI scores between the test and control group from

baseline to 1 month was 0.85 ± 0.30 and 0.65 ± 0.42 respectively, from baseline to 3 Months was 1.17 ± 0.28 and 1.06 ± 0.41 respectively, and from baseline to 6months was 1.46 ± 0.29 and 1.43 ± 0.24 respectively. In all three scenarios, the results were not statistically significant (p>0.05). The probing pocket depth significantly reduced in the individual group (Graph 2). For instance in the test group, it reduced from 3.96mm to 2.24 mm and in the control group it reduced from 3.73mm to 2.18. But on comparing the two groups, there was no significant difference. The relative attachment level showed significant improvement in the test and control group (Graph 3). It reduced from 7.74mm to 6.28 mm in test group and 7.63mm to 6.59mm in control group in the period of 6 months. However, in the inter group comparison, there was no significant difference.

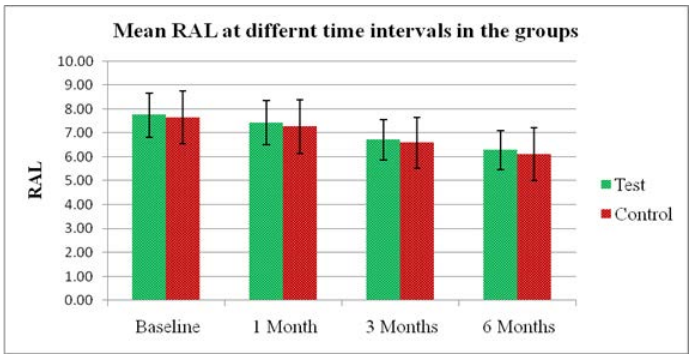
Graph 1: Showing the mean gingival index among the study groups at various time intervals.



Graph 2: Showing the mean probing pocket depth among the study groups at various time intervals.



Graph 3: Showing the mean relative attachment level among the study groups at various time intervals



The SEM findings were encouraging. Residual calculus was seen in both the test [Fig:1E] and control tooth specimens [Fig: 1F]. Tooth specimens of test site showed visible circular mounds of cementum which indicated minimal loss of cementum (tooth substance) during root planing [Fig:1E]. On other hand, tooth specimens from the control site showed a smooth surface indicating increased removal of cementum (tooth substance) during root planing [Fig: 1F].

The histopathological findings showed significant differences in the test and control group. In the H & E section of the test group (Fig:2E) all 5 specimens collected from the healing granulation tissues under histopathological examination showed hyperplastic, parakeratinized and stratified squamous epithelium. The underlying connective tissue was fibrous along with proliferating blood vessels and chronic inflammatory cells. Areas of haemorrhage were observed, but the quality and quantity of fibroblasts could not be assessed in all the sections. In the H& E section from the control group (Fig:2F) showed hyperplastic, parakeratinized and stratified squamous epithelium. The underlying connective tissue was fibrous along with proliferating blood vessels and chronic inflammatory cells. Areas of haemorrhage were observed, but the quality and quantity of fibroblasts could not be assessed in all the sections.

There were comparatively more areas of haemorrhage in the control group than in the test group specimens.

The results of the immunohistochemistry (IHC) in test group (Fig: 3E) and control group (Fig:3F) have been summarised as follows:

Sn.	Parameter	Control group	Test group
1	Smooth muscle actin around endothelial cells	Positive in 1 patient out of 5 patients	Positive in 4 patient out of 5 patients
2	Stains for myofibroblasts in the collagen fibres	Negative	Negative

SEM findings

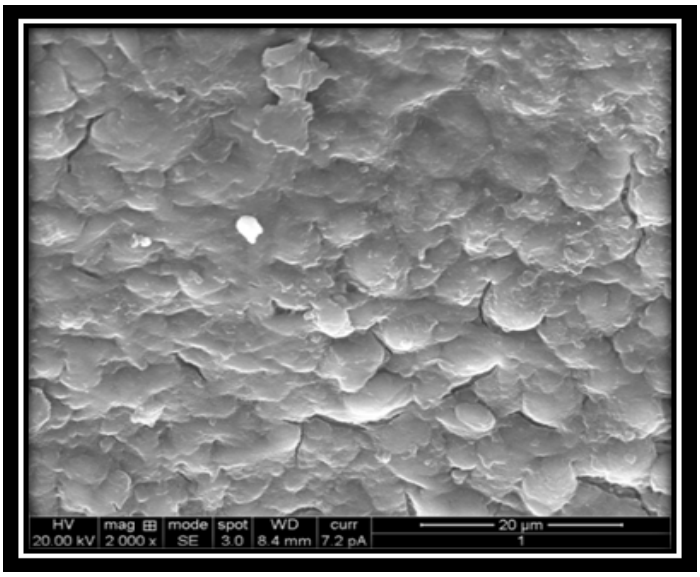


Figure: 1E (Test group)

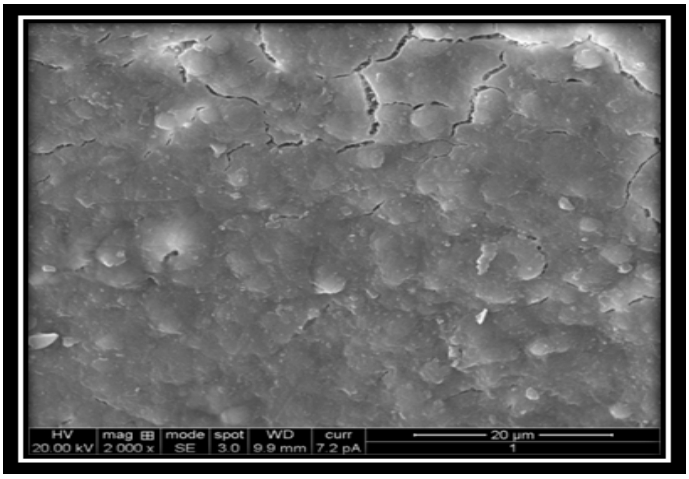


Figure: 1F (Control group)

Histopathological findings

H & E Staining Observations

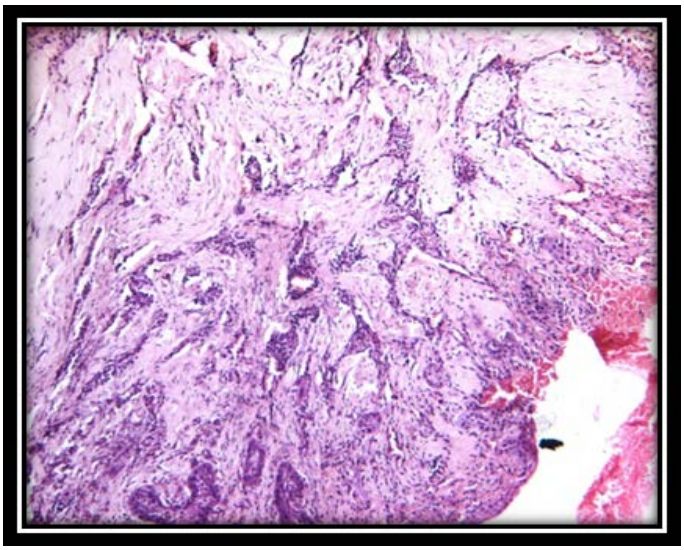


Figure: 2E (Test group)

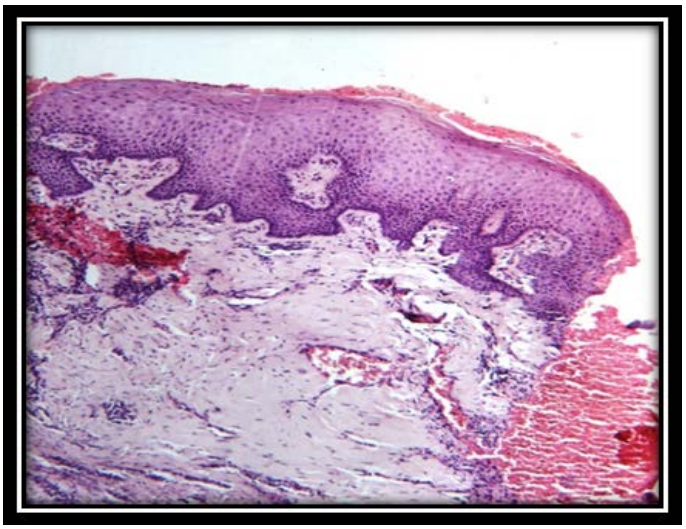


Figure: 2E (Control group)

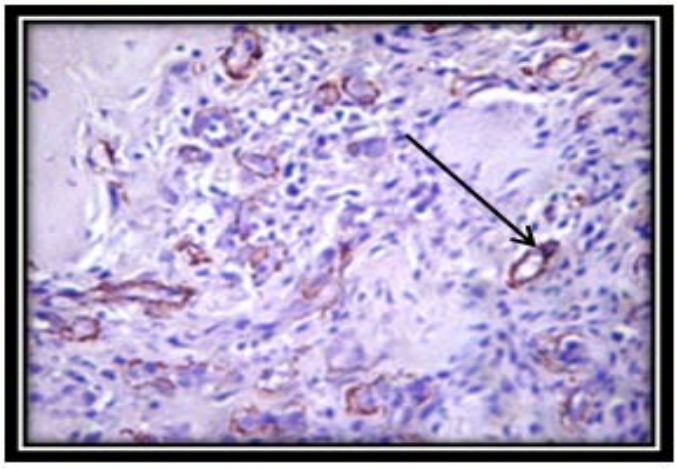


Figure: 3E (Test group)

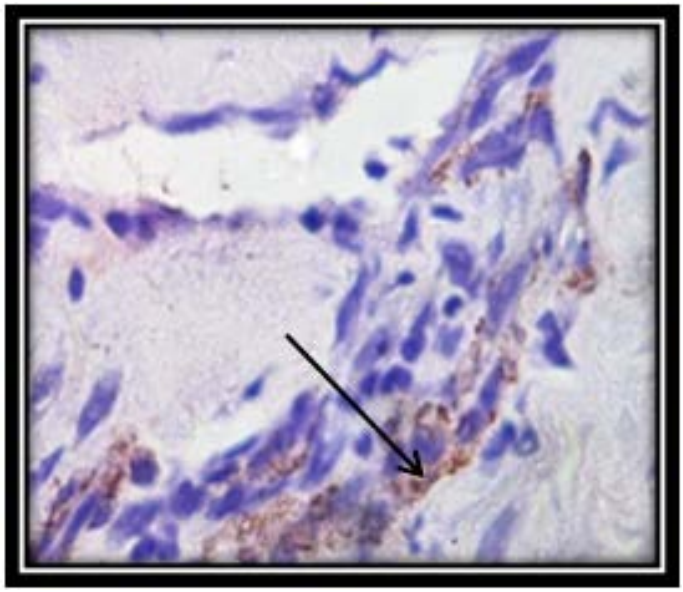


Figure:3F (Test group)

Discussion

The aim of conventional periodontal therapy; either surgical or nonsurgical is to stop and possibly reverse the loss of periodontal attachment resulting from this disease. Reduction of probing pocket depths, maintenance or improvement of clinical attachment levels, and reduction in bleeding on probing are the most common outcome measures used to determine whether treatment is successful. In order to treat moderate to advanced periodontitis, surgical intervention is often indicated7 which often results in greater pocket reduction and clinical attachment gain.8 Among the various advances in technology available today, periodontal microsurgery is fast gaining popularity. Of prime importance to make any surgical procedure more acceptable are gentle handling of tissues and passive wound closure aiding in primary uneventful healing. A treatment modality addressing both biologic and esthetic demands is today most often required from the periodontal care provider. However, the successful use of the surgical microscope in periodontal surgery is scarcely documented as only a few studies address the advantages of the application of magnification to periodontal surgery.9,10,11 Therefore this study was carried out to evaluate and compare the healing outcome following open flap debridement with microsurgical technique and conventional technique. Patients with chronic periodontitis, in general good health and presenting pockets on the contralateral sites were recruited in the study and randomly designated into test and control groups. A split mouth design was used as this excludes the influence of individual patient characteristics and obtains a more powerful estimate of treatment effect with a smaller size. 12 The sample size used in this study was in accordance with the vast majority of clinical periodontal regenerative studies in humans.13 The microsurgical approach was intended to increase the ability of the clinician to control each single step of the surgical procedure required to treat periodontal defects while preserving as much periodontal tissue as possible as evidenced in literature.14 It involves delicate handling of tissues and precise wound closure which accounts for the favourable early wound healing as reported from studies (Wachtel et al.200314, Fickl S et al.200915). The sharper and finer surgical blades together with finer suture material used in the microsurgical approach account for the reduced tissue damage along with primary closure of the wound. The improved vascularization after microsurgical approach also enhances the wound healing.16 The clinical study was a 6 month follow up study which assessed the clinical variables gingival index (GI), probing pocket depth (PPD), relative attachment level (RAL) at baseline, 1, 3 & 6 months postoperatively. The results showed that there was significant reduction in the mean gingival index scores within the microsurgical and conventional treated groups from baseline to 1,3 & 6months respectively which is in accordance with the results of the study by Wachtel H et al (2003) & Fickl S et al (2009) where they found significant improvement in GI scores at 6 months and 12 months in microsurgery

treated sites, and also with the results reported by Gunsolley et al (1994)where there was a reduction in GI scores at 3months , by Pihlstrom et al (1981) who observed reduction at 6 months and by Heitz – Mayfield et al (2002) who found improvement at 12 months following open flap debridement. Relative attachment level (RAL) and probing pocket depth (PPD) measurements are commonly used to assess and monitor the periodontal status. Pocket depth resolution is not only a desirable outcome of periodontal therapy, but may be the most important parameter in patient care for the clinician, since it directly impacts his or her ability to instrument a treated area during the maintenance appointments. There was marked reduction in probing depth within both microsurgical and conventional treated sites from baseline, 1, 3 and 6months which is in accordance with the studies by Wachtel H et al (2003) & Fickl S et al (2009) wherein there was significant improvement in PPD score at 6 months and 12 months in microsurgery treated sites, and the results reported by Gunsolley et al (1994), Pihlstrom et al (1981) and Heitz – Mayfield et al (2002) who found reduction in PPD at 3, 6 and 12 months respectively following open flap debridement. Both the microsurgically treated and conventional surgery treated sites showed significant improvement in relative attachment level (RAL) within their respectives groups at 1, 3 & 6months. This suggests that there was a statistically significant attachment gain from baseline to 1, 3 & 6months within both microsurgical and conventional surgically treated sites which is consistent with the findings of Wachtel H et al (2003)14 & Fickl S et al (2009)15, where it has been shown that microsurgical access flap surgery resulted in improvement of these parameters over a period of 6 & 12 months, and also with the results reported by Pihlstrom et al (1981)17 and Heitz – Mayfield et al (2002) who found gain in attachment at 6 months & 12 months respectively following open flap debridement. In contrast, Gunsolley et al (1994)18 found no gain in attachment at 3 months following conventional open flap debridement. Interestingly, no difference was observed between both the groups with regard to the clinical parameters at the various time intervals of the study. This could be attributed to the beneficial effect of microsurgery only being limited to the early healing parameters rather than the long-term effects.

Conclusion

The Microsurgical technique revealed better clinical outcomes. In order to obtain better results more sophisticated healing assessment methods like fluorescein angiogram, flow cytometry using markers for various cells and cytokines; and also immunohistochemistry evaluation of healing using various MMPs as markers could be done to establish the enhanced healing outcomes with microsurgical approaches. Besides, larger sample size involving a larger cross section of the population needs to be carried out.

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