THE EFFECT OF REGEN-D 60 GEL ON THE RATE OF HEALING OF GINGIVAL EPITHELIUM: A RANDOMIZED CLINICAL TRIAL

INTRODUCTION

Esthetics has become an important aspect of dentistry and clinicians have to face the challenge of achieving acceptable gingival contour, along with addressing the biological and functional challenges. Pigmentation is a discolouration of the oral mucosa due to a wide variety of lesions and conditions. Oral pigmentation has been associated with a variety of exogenous and endogenous etiologic factors. Gingival depigmentation is an aesthetic procedure, which helps to improve the smile and the overall appearance of an individual. The colour of the pigmented gingiva varies from light to dark brown or black. Consequently, wound healing within the oral cavity is an extremely complex mechanism where numerous characters may intervene, such as cell and/or tissue interrelations, growth factors and salivary components. The periodontium represents an inimitable histological system, because of the individual connection between the epithelium and connective tissue forming the dento-gingival junction. At the level of the superficial periodontium, the free gingival mucosa repairs through regeneration based on epithelial restoration, while the dento-gingival junction supports only the development of granulation tissue with subsequent cicatrization.

Epidermal growth factor (EGF) is a low molecular weight polypeptide which was first purified from the mice submandibular gland, but since then has been found in many human tissues including submandibular gland, parotid gland.8 EGF belongs to a family of growth factors that regulate cell proliferation, migration and differentiation through binding to receptor kinases on target cells. EGF is proved to act as a mitogen and also as a differentiation factor for many cell types. It can also be found in urine, saliva, milk, and plasma.9

EGF plays a role in a variety of biological actions, including promotion of epidermal development, wound healing, eruption of the incisors, activation of various transport systems and changes in cellular metabolism, in addition to mitogenesis, stimulation of pituitary secretion of adrenocorticotropic hormone (ACTH), growth hormone (GH), inhibition of gastric and thyroid hormone secretion. Moreover, most evidence indicates that it is an important hormone in the male reproductive system.10

Literature has shown an enhanced wound healing after application of epidermal growth factor. Recombinant human epidermal growth factor (REGEN-D 60) is supplied as a topical gel which is thought to have an important function in epidermal growth, differentiation and in reducing healing time drastically over the natural course leaving minimal scars with quality healing. Recently, a study showed enhanced healing of chronic diabetic foot ulcers (DFU) following application of REGEN-D TM 150 by significantly reducing the duration of healing in addition to providing excellent quality of wound healing and reepithelization.11

To the best of our knowledge, there are no available data showing the effect of REGEN-D 60 gel on the rate of healing of gingival epithelium. Therefore, the present study was undertaken to analyse the effect of REGEN-D 60 gel on the healing of epithelium following gingival depigmentation.

MATERIALS AND METHODS

The triple blind randomized controlled trial was carried out between February 2015 to August 2015 in accordance with the Helsinki declaration of 1975, as revised in 2000. The patients were selected from the outpatient section of the Department of Periodontology, Rajarajeshwari Dental College and Hospital, Bangalore, India. Each patient signed a written informed consent prior to his/her participation in the study after obtaining approval from the Institutional Ethical Committee. The trial was registered at Clinical Trial Registration of India (CTRI) bearing number REF:2016/03/011026. A total of 40 sites in 10 patients (6 males and 4 females, 18 to 32 years old) with uniformly dense bands of pronounced bilateral melanin pigmentation

ABSTRACT

Aim: To assess the effect of REGEN-D 60 Gel on the rate of healing of gingival epithelium.

Materials and methods: A total of 40 sites in 10 patients (6 males and 4 females, 18 to 32 years old) with uniformly dense bands of pronounced bilateral melanin pigmentation on the facial aspect of the maxilla and mandible were selected. Sites extending from distal of the right canine to the midline and distal of the left canine to the midline underwent depigmentation. In each patient 4 quadrants were randomly allocated to one of the four treatment groups. Group I with patients who underwent depigmentation with diode laser along with application of REGEN-D 60 gel, group II with patients who underwent depigmentation with diode laser alone, group III with patients who underwent depigmentation with tetrafluoroethane along with REGEN-D 60 gel and group IV with patients who underwent depigmentation with tetrafluoroethane alone. REGEN-D 60 gel was applied randomly to treated sites following depigmentation to assess the healing. Gingival index (GI) (Silness and Loe, 1964) was recorded at baseline and 1 month after the procedure. Healing Index (Landrey, Turnbull and Howley, 1988) was assessed at day 1, day 7, day 14 and at 1month respectively.

Results: Intergroup comparison of healing index scores at various time intervals showed a statistically significant difference (p˂0.0001). The change in the healing index scores between various groups from day 1 to day 14 was statistically significant (p˂0.05)

Conclusion: REGEN-D 60 gel may have a beneficial effect on the rate of healing of gingival epithelium.

KEYWORDS:

Epidermal growth factor, Gingival epithelium, Gingival index, Healing index.
on the facial aspect of the maxilla and mandible were selected. Sites extending from distal of the right canine to the midline of the left canine to the midline underwent depigmentation. Study groups were divided into four which included group 1 with patients who underwent depigmentation with diode laser along with application of REGEN-D 60 gel, group II with patients who underwent depigmentation with diode laser alone, group III with patients who underwent depigmentation with tetrafluoroethane along with REGEN-D gel and group IV with patients who underwent depigmentation with tetrafluoroethane alone.

Only motivated patients who were conscious about their esthetics were enrolled for the study. Smokers, patients with diabetes and other debilitating systemic diseases or conditions, pregnant or lactating females, patients with clinically diagnosed periodontitis, pathologic factors causing gingival pigmentation were excluded from the study. In each patient 4 quadrants were randomly allocated to one of the four treatment groups as depicted in consort flowchart (Fig-1). REGEN-D 60 gel was applied randomly to treated sites following depigmentation to assess the healing. Gingival index (GI) (Silness and Loe, 1964) was recorded at baseline and 1 month after the procedure. Healing Index (Landrey, Turnbull and Howley, 1988)21 was assessed at day 1, day 7, day 14 and 1 month respectively.  

CLINICAL PROCEDURES
Laser technique
The diode laser (SIRO Laser Xtend, Sirona Dental Systems, Germany, 970 nm) was set at 3 W and ablation was performed in a contact, continuous wave mode following infiltration anesthesia. The laser beam was guided in a 'brushstroke' pattern from the mucogingival junction towards the free gingival margin including the interdental papilla until the entire area was free of pigmentation.

Cryosurgery by tetrafluoroethane
Tetrafluoroethane (TFE) (DUPONT Fluorochemicals, USA) was applied after the pigmented area was isolated, air dried and anaesthetised. TFE was sprayed on a cotton swab and immediately rolled gently over the pigmented area maintaining a freezing zone for about 30-40 seconds.

Application of REGEN-D 60 gel
REGEN-D 60 gel (Bharath Biotech International Limited, India) was applied over the treated depigmentation sites using a cotton swab in a swiping motion.

STATISTICAL ANALYSIS
Data analysis was performed using the patient as the experimental unit. The statistical analysis was done using SPSS version 15.0 statistical analysis software. The gingival index scores and healing index scores were statistically analysed by Kruskal Wallis ANOVA. The intragroup comparison was done using Wilcoxon’s signed rank test to evaluate the difference between gingival index scores at baseline and one month and healing index scores at various time intervals. The intergroup comparison was done using the Mann-Whitney U-test to determine if a difference exists between the 4 groups.

RESULTS
Forty sites were evaluated in the study of which 20 sites underwent depigmentation followed by application of REGEN-D 60 gel. Throughout the study no local allergic reaction, pain, swelling, or any side effects were observed.

The difference in the mean gingival index in between the groups at baseline and 1 month was statistically significant (p=0.0001) (Table 1). Pair wise comparison of gingival index at baseline and at 1 month revealed statistically significant difference in the mean gingival index (p<0.05) as shown in Table 1. Reduction in the mean gingival index scores from baseline to 1 month in group III and group IV was statistically significant (p<0.05) (Table 1).

Inter group comparison of healing index scores at various time intervals showed a statistically significant difference (p=0.0001) (Table 1). Pair wise comparison in between the groups also revealed a statistically significant result as shown in Table 2. The change in the healing index scores between various groups from day 1 to day 14 was statistically significant (p=0.05) (Table 2).

Group III showed the greatest reduction in gingival index scores when compared to all the groups (Fig 2) while Group I showed the best improvement in healing when compared to other groups (Fig 3).

Figure 1:- Consort flow chart

**Table 1:** Comparison of four groups (I, II, III, IV) with respect to gingival index scores at baseline and 1 month by Kruskal Wallis ANOVA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean</th>
<th>S.D</th>
<th>Mean Diff</th>
<th>SD Diff</th>
<th>% of change</th>
<th>Z-value</th>
<th>p-value</th>
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<tr>
<td>Group I Baseline</td>
<td>0.77</td>
<td>0.25</td>
<td>0.18</td>
<td>0.29</td>
<td>23.18</td>
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<td>1 month</td>
<td>0.59</td>
<td>0.22</td>
<td>0.18</td>
<td>0.29</td>
<td>19.90</td>
<td>1.36</td>
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<td>Group II Baseline</td>
<td>0.39</td>
<td>0.21</td>
<td>0.10</td>
<td>0.18</td>
<td>9.82</td>
<td>1.04</td>
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<td>1 month</td>
<td>0.49</td>
<td>0.21</td>
<td>0.15</td>
<td>0.21</td>
<td>29.68</td>
<td>2.49</td>
<td>0.0125</td>
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<tr>
<td>Group III Baseline</td>
<td>0.57</td>
<td>0.18</td>
<td>0.08</td>
<td>0.14</td>
<td>25.44</td>
<td>2.49</td>
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<td>1 month</td>
<td>0.42</td>
<td>0.14</td>
<td>0.15</td>
<td>0.15</td>
<td>25.44</td>
<td>2.49</td>
<td>0.0125</td>
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*Note:* *p<0.05,* **p<0.0001

**Table 2:** Comparison of baseline and 1 month with respect to gingival index scores in four groups (I, II, III, IV) by Wilcoxon matched pairs
Table 3: Comparison of four groups (I, II, III, IV) with respect to healing index scores at day1, 7, 14 and month by Kruskal Wallis ANOVA test

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<th>Group</th>
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*p<0.05, **p<0.0001

Table 4: Comparison of four groups (I, II, III, IV) with respect to healing index scores at changes from day1 to 7, 14 and month by Kruskal Wallis ANOVA

<table>
<thead>
<tr>
<th>Group</th>
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DISCUSSION

To the best of our knowledge, this is the first trial to demonstrate a significant and positive effect of hEGF on the rate of healing of gingival epithelium.

The gingival tissue is continuously subjected to mechanical and bacterial aggressions. The saliva, epithelial surface and the initial stages of the inflammatory response provide resistance to these actions. The degree of keratinisation and cell turnover rate are key considerations for the protective function of the epithelium. The oral epithelium undergoes continuous renewal and its thickness is maintained by a balance between new cell formation in the basal and spinous layers and the shedding of old cells at the surface.

In our study we have performed gingival depigmentation with diode laser and followed by application of REGEN-D 60 gel at randomly selected sites. No adverse effects were experienced by the patient throughout the study. The drug administered was in a gel base and was supplied as a 7.5g tube consisting of 60μg of recombinant human epidermal growth factor allowing for even application on the treated sites using a sterile cotton swab.

REGEN-D 60 epidermal growth factor is a new generation therapy with a novel factor used for first and second degree burns, skin grafts and bed sores. It was reported that the application of various epidermal growth factor (EGF) formulations onto experimentally induced wounds enhances epithelialization with the concurrent accumulation of granulation tissue. In particular, the topical application of EGF has been shown to accelerate the healing rate of open wounds.

A double-blind randomized pilot study was previously conducted by Falanga et al. with use of human recombinant epidermal growth factor (h-EGF) to treat 44 patients with venous ulceration of the lower extremities. There were no untoward side effects related to the application of h-EGF. However, the authors concluded that it was safe but failed to significantly enhance reepithelialization of venous ulcers.

A previous animal study revealed that topical application of epidermal growth factor accelerates wound healing by myofibroblast proliferation and collagen synthesis in rats. A randomized, double-blind human clinical trial was conducted using skin-graft-donor sites to determine whether epidermal growth factor would accelerate the rate of epidermal regeneration. The authors concluded that epidermal growth factor accelerates the rate of healing of partial-thickness skin wounds.

Complete healing was observed in all the groups with the highest healing index score being observed in group I. This could be attributed to the healing properties of the REGEN-D 60 gel coupled with the biostimulatory properties of diode laser.

It has been reported that repeated treatment with EGF increases the epithelial cell proliferation in a dose dependent manner and accelerates the wound healing process, whereas a single EGF treatment has not demonstrated a noticeable effect on the wound-healing rate. However in contrast to the previous study, present study demonstrated single application of REGEN-D 60 gel which showed a significant gain in the healing index scores in various groups at 14th day. Significant results were not achieved at one month postoperatively. Repeat application of EGF during study period might have improved
the gain in healing index at 1 month. As previously mentioned, this being the first study to assess the healing capacity of REGEN-D 60 gel on oral wounds; the authors decided to perform only a single application of the gel to evaluate its response on the oral mucosa.

Greater reduction in gingival index scores was noted in group 1 and 3 where the REGEN-D 60 gel was used. This further reinforces the evidence available in literature regarding its potential in accelerating wound healing. In the present study we did not encounter any dropout as only patients who were esthetically conscious were recruited.

In conclusion, the topical application of rhEGF gel can induce rapid wound healing in gingival epithelium by accelerating epithelial cell proliferation. Our study demonstrated that topical application of REGEN-D 60 gel provided faster and predictable healing following gingival depigmentation either with laser or tetrefluoroethane. However more scientific research needs to be carried out for better understanding of its exact role in wound healing.

Conflict of interest
Authors Dr. Shivaprasad BM, Dr. Vinaya Kumar R, Dr. Sruthi K Nair, Dr Nayan Jyoti Deka, Dr. Rekha BM states that there are no conflicts of interest.

REFERENCES