

# Melanocyte Response Following Depigmentation by Cryosurgery and Mucosal Excision: A Comparative Clinical and Histopathological Study

## Abstract

**Aims:** The main aim of this study was to evaluate the melanocyte response following depigmentation by cryosurgery and mucosal excision at both clinical and histopathological levels during 6 months. **Subjects and Methods:** A total of 15 individuals, aged 25–45 years with hyperpigmentation on the facial aspect of the gingiva present in the esthetic zone were included. A split-mouth approach was used, wherein one side received depigmentation by a scalpel and the other side received cryotherapy, with the choice of the therapy and side chosen by the spin of the coin. For determining the melanocyte assay, a small amount of gingival tissue that was excised was studied under a special staining technique, i.e., Masson–Fontana method for melanin, a histochemistry procedure which determines the activity of melanin both quantitatively and qualitatively. Clinical and histological observations for the intensity of pigmentation were recorded at baseline and 6 months after surgery. **Statistical Analysis Used:** Paired and unpaired *t*-test. **Results:** The mean value of the Dummert Oral Pigmentation Index (DOPI) was  $2.44 \pm 0.51$  at baseline; following depigmentation with scalpel technique, the mean DOPI was  $1.05 \pm 0.23$  at 6 months; and there was a statistically significant difference in the mean DOPI when compared with that of the baseline ( $P = 0.001$ ). Following depigmentation with cryosurgery technique, the mean DOPI was  $0.33 \pm 0.48$  at 6 months which also varied statistically significantly with that of the baseline ( $P = 0.001$ ). The mean value of melanin histopathological count (MHC) was  $95.53 \pm 9.03$  at baseline; following depigmentation with the scalpel technique, the mean DOPI was  $89.49 \pm 7.56$  at 6 months, which was not statistically significant ( $P = 0.795$ ). Following depigmentation with the cryosurgery technique, the mean MHC was  $75.38 \pm 5.63$  at 6 months, which was not statistically significant ( $P = 0.285$ ). **Conclusions:** The mean histologic count of the melanocytes, which accounts for the re-pigmentation process has shown low recurrence values in cryosurgery over the scalpel technique even though the difference was not statistically significant.

**Keywords:** Cryosurgery, gingival depigmentation, melanocytes, re-pigmentation

## Introduction

The science of beauty, that is, esthetics, curtails to every particular detail that makes either an animate or an inanimate object appealing to the eye. On the other hand, advances in esthetic/cosmetic dentistry allow a dental practitioner in achieving an environment that promotes optimal dental esthetics.<sup>[1]</sup> Appearance of the face relies on a multitude of entities encompassing oral and extraoral factors. Gingiva being a vital intraoral tissue plays a major role in the appearance of the face, among which pigmentation is one of the key criteria. Among the various features depicting the appearance and health of the gingiva such as size, shape, consistency, and contour, the

color of the gingiva plays a vital role in the overall appearance of the face.

Pigmentation, which is the underlying feature that plays an important role in the depiction of the color of the gingiva, often occurs as a melanin pigmentation which is a result of an abnormal deposition of melanin due to which the gums appear black to brownish black.

This gingival hyperpigmentation, which occurs due to excessive production of melanin, is usually physiological and is a genetic trait in some population irrespective of age and gender. This degree of pigmentation varies from individual to individual depending on the melanoblastic activity.<sup>[2]</sup>

Although melanin pigmentation is completely benign and does not pose a

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medical problem, patients' complaint of black gums is common due to their unacceptable esthetics which demands cosmetic therapy.

In recent times, periodontal esthetics has been considered to play a major role in the overall esthetic program, and it has become an integral part of esthetic dentistry wherein gingival depigmentation has become an integral part of periodontal plastic procedures.<sup>[3]</sup>

In an attempt to attain a gingiva that is free of pigmentation, various techniques have been in vogue for gingival depigmentation which include gingivectomy, mucosal excision by scalpel, abrasion techniques, free gingival grafts, chemical methods using escharotic agents, electrosurgery, cryosurgery, and more lately lasers.<sup>[4]</sup>

Although the abovementioned procedures have their own advantages and limitations, the selection of a technique for gingival depigmentation is based on the clinical experience, patient's affordability, and individual preferences.

Whatever is the procedure advocated, re-pigmentation is likely to occur, which is mainly because of the activity of melanocytes, as early as 6–24 months.

This period of re-pigmentation varies depending on the various methods employed for depigmentation. While on the one hand studies have shown recurrence of pigmentation as early as 6–12 months in scalpel technique, procedures such as cryosurgery and laser have shown a bit prolonged duration extending over a period of 24 months.

The scalpel technique is one of the first and foremost popular techniques to be employed for the advocacy of depigmentation. The procedure advocates the surgical removal of gingival epithelium along with a layer of the underlying connective tissue, thereby allowing the denuded connective tissue to heal by secondary intention, thereby resulting in an epithelium that is devoid of melanin pigmentation.<sup>[5]</sup>

On the other hand, the application of cryosurgery appears to be a simple, time-efficient, cost-effective, and minimally invasive alternative procedure for gingival melanin depigmentation wherein the esthetic outcome may be attained and maintained and also has the advantage of being used safely wherein surgery is contraindicated, thereby offering a practical technique to improve patient esthetics.

Even though studies in literature do exist comparing the various techniques in their efficacy of attaining de-pigmentation,<sup>[4,6]</sup> not many studies in literature have shown the response of melanocytes following depigmentation by cryosurgery and mucosal excision.<sup>[6]</sup> Hence, the present study was intended to evaluate the melanocyte response following depigmentation by cryosurgery and mucosal excision at both clinical and histopathological levels.

## Subjects and Methods

### Patient selection

Convenience sampling technique was employed to select the study population. The study participants were selected from patients reported to the Department of Periodontics, Vishnu Dental College, who intend to undergo cosmetic therapy for hyperpigmented gums [Figures 1 and 2]. Fifteen volunteer patients of either sex were selected, and after administering routine oral prophylaxis and plaque control instructions and ensuring disease-free gingiva in the patients selected, the two treatment modalities were carried out contralaterally in the anterior region of the mouth. All patients were informed about the procedure and had agreed to keep the scheduled recall appointments for data collection. Ethical approval from the institutional ethical committee was obtained. Informed letter of consent was obtained from the volunteered patients. The choice of the particular procedure in the right or left side was decided by the spin of the coin.

### Inclusion criteria

1. Patients who were aware of the nature of physiological hyperpigmentation of their gums and who requested for improvement of their gingival appearance
2. Patients with the presence of moderate-to-severe physiological melanin pigmentation of the gingiva in the anterior region [Figures 1 and 2].

### Exclusion criteria

(1) Patients with systemic diseases that can interfere with healing and medically compromised conditions, (2) pregnant patients, (3) patients with a history of postsurgical keloids, (4) patients who smoke, (5) patients with acute gingival and periodontal diseases, (6) patients with pathologically pigmented lesions of gingiva, and (7) patients showing extremely thin gingiva and gingival recession.

### Surgical procedure

#### Scalpel method

After administration of local anesthetic, a Bard-Parker® (B-P) handle with a No. 11 blade was used to remove the pigmented layer. Pressure was applied with a sterile gauze soaked in local anesthetic agent to control hemorrhage during the procedure. The entire pigmented epithelium along with a thin layer of connective tissue was removed with the scalpel. The exposed surface was irrigated with saline, and the surgical area was covered with a periodontal dressing [Figures 3 and 4].

#### Cryosurgical procedure

The cryosurgical apparatus used was Cryo Super Deluxe model no. 004B manufactured by BascoCryos Company, Chennai, Tamilnadu, India. this works on the Joule–



Figure 1: Baseline intraoral photographs showing gingival pigmentation (cryotherapy)



Figure 2: Baseline intraoral photographs showing gingival pigmentation (Scalpel method)



Figure 3: Depigmentation by mucosal excision



Figure 4: Periodontal dressing placed

Thomson principle using nitrous oxide gas at a temperature of  $-70^{\circ}\text{C}$  to  $-80^{\circ}\text{C}$  at the probe tip. Cryosurgery was performed with the probe tip placed on the surgical area, and the tissues were allowed to freeze until a visible icicle was formed. The entire surgical area was frozen in concentric circles following which a repeated cycle of application was done for another 10 s to ensure gingiva completely free of pigmentation [Figure 5].

#### *Melanocyte assay*

For determining the melanocyte assay, a small amount of gingival tissue was excised using the B-P handle with a No. 11 blade, especially at the distal surface of the first premolar after determining the site for scalpel procedure. The blade was positioned toward the interdental gingiva similar to that of an external bevel distal to the first premolar. Utmost care was ensured that a small triangular portion of the tissue was taken following the interdental gingival architecture. The shape of the interdental gingiva was maintained, and the contour of the gingiva was unaltered. The excised area was covered with a periodontal dressing for due comfort of the patient.

After 6 months of postoperative period, a small amount of gingival tissue was excised using the B-P handle with

a No. 11 blade from the same area where it was excised priorly at the scalpel-performed site; along with this, a small amount of gingival tissue was excised from the cryosurgery-performed area for intergroup comparison of the melanocyte response.

The excised gingival tissue was studied under a special staining technique, i.e., Masson-Fontana method for melanin, a histochemistry procedure which determines the activity of melanin both quantitatively and qualitatively.

After subsequent procedures, the patients were prescribed ibuprofen 400 mg three times daily for 3–5 days to prevent postoperative pain. The patients were recalled after 1 week and were monitored monthly for 6 months [Figures 6 and 7]. All the cases were examined by the same operator to ensure that there was no examiner variability.

#### **Results**

A total of fifteen patients, aged between 25 and 45 years, who reported to the Department of Periodontics, Vishnu Dental College, with physiological gingival melanin pigmentation were recruited for the study. The results obtained were statistically analyzed by paired and unpaired *t*-test.



Figure 5: Depigmentation using cryotherapy



Figure 6: One-week re-evaluation after depigmentation



Figure 7: Six-month re-evaluation

The mean value of the Dummett Oral Pigmentation Index (DOPI) was  $2.44 \pm 0.51$  at baseline.

- Following depigmentation with the scalpel technique, the mean DOPI was  $1.05 \pm 0.23$  at 6 months
- There was a statistically significant difference in the mean DOPI when compared with that of the baseline ( $P = 0.001$ )
- Following depigmentation with the cryosurgery technique, the mean DOPI was  $0.33 \pm 0.48$  at 6 months

- This also varied significantly with the baseline ( $P = 0.001$ )
- When assessed after 6 months, the difference between the depigmentation values over the two techniques showed higher values of recurrence of pigmentation in scalpel when compared to that of cryosurgery [Table 1].

The mean value of melanin histopathological count (MHC) was  $95.53 \pm 9.03$  at baseline.

- Following depigmentation with scalpel technique, the mean DOPI was  $89.49 \pm 7.56$  at 6 months
- However, the difference was not statistically significant in mean MHC when compared with baseline ( $P = 0.795$ )
- Following depigmentation with the cryosurgery technique, the mean MHC was  $75.38 \pm 5.63$  at 6 months
- However, the difference in MHC was not statistically significant with  $P = 0.285$  [Table 2]
- When compared with baseline, the DOPI showed the mean value of recurrence of pigmentation as  $1.05 \pm 0.23$  for scalpel and  $0.33 \pm 0.48$  for cryosurgery. This difference was statistically significant ( $P = 0.001$ )
- The recurrence of pigmentation when assessed with DOPI showed a recurrence percentage of 43.04% in the scalpel technique and 13.53% in the cryosurgery technique [Table 3].

The recurrence of pigmentation when assessed with regard to the mean histological count showed a recurrence of 93.67% in the scalpel technique and 78.90% in the cryosurgery technique. This elicits a superiority of cryosurgery over the scalpel technique.

With regard to the recurrence of pigmentation, clinically, cryosurgery is far better than scalpel which has shown statistically significant values in our study, whereas for the mean histological count, though low recurrence rate was recorded in cryosurgery, the effect of cryosurgery over scalpel was not statistically significant.

## Discussion

The physiological pigmentation of the oral mucosa, which is a common finding in the oral cavity, varies depending on the quantity and depth or location of the pigment. The mechanism behind this pigmentation is the production of melanin by melanocytes in the basal cell layer of the epithelium and sometimes in the upper portion within the lamina propria and the transfer of the melanin to adjacent keratinocytes via membrane-bound organelles called melanosomes. Variations in the activity of melanocytes in the basal cell layer of the oral epithelium is the reason behind the ascribable variations in the physiological pigmentation of the oral mucosa, resulting in a color range from light brown to almost black.

This physiological pigmentation of the oral mucosa is common in Black persons, with the frequency being higher in dark-skinned Whites (Caucasians) than in light-skinned

**Table 1: Comparison of mean Dummett Oral Pigmentation Index pre- and postoperatively in the two groups using paired t-test**

	Mean±SD	T	P
DOPI			
Preoperative	2.4444±0.51131	11.747	0.001
Postoperative scalpel	1.0556±0.23570		
DOPI			
Preoperative	2.4444±0.51131	15.364	0.001
Postoperative cryosurgery	0.3333±0.48507		

DOPI: Dummett Oral Pigmentation Index; SD: Standard deviation

**Table 2: Comparison of mean melanin histopathological count pre- and postoperatively in the two groups using paired t-test**

	Mean±SD	T	P
MHC			
Preoperative	95.5394±90.38463	0.265	0.794
Postoperative scalpel	89.4911±75.68726		
MHC			
Preoperative	95.5394±90.38463	1.105	0.285
Postoperative cryosurgery	75.3833±56.38162		

MHC: Melanin Histopathological count; SD: Standard deviation

**Table 3: Comparison of mean postoperative Dummett Oral Pigmentation Index and melanin histopathological count between scalpel and cryosurgery groups using unpaired t-test**

	Mean±SD	T	P
DOPI			
Postoperative scalpel	1.0556±0.23570	5.682	0.001
Postoperative cryosurgery	0.3333±0.48507		
MHC			
Postoperative scalpel	89.4911±75.68726	0.634	0.530
Postoperative cryosurgery	75.3833±56.38162		

DOPI: Dummett Oral Pigmentation Index; MHC: Melanin histopathological count; SD: Standard deviation

Whites, and the determination of the physiological oral pigmentation by genetic factors is strongly evident in the literature.

Although the reason behind this variation of physiological pigmentation in different people is unknown, the microscopic examination of the pigmented oral mucosa has evidenced increased melanin in the basal layer.

Even though melanin pigmentation may involve any part of the oral mucosa, pigmentation may be patchy or uniform, and the attached gingiva is the most common structure to be evidenced with pigmentation, with an unusual involvement into the mucogingival junction and free gingiva with an occasional involvement of the alveolar mucosa. Furthermore, an extensive pigmentation in the anterior than in the posterior part of the oral cavity with the buccal gingiva being more intensely pigmented than the lingual gingiva

is apparent. Males and females are equally affected, with a greater incidence of bilaterally symmetrical involvement.

Even though melanin hyperpigmentation may possess a defensive role,<sup>[7]</sup> an unattractive soft tissue owing to a pigmented gingiva can have a negative impact on the overall esthetic appearance.<sup>[8]</sup>

Because of the pandemic of this oral melanin pigmentation and the gingiva being the most frequently pigmented intraoral site, pigmented gingiva may cause esthetic problems and embarrassment, especially in patients with a gummy smile. As the esthetic needs of the patient are increasing day by day, gingival depigmentation has become an integral part of periodontal plastic surgery.

Although various de-pigmentation methods have been advocated to attain a pleasing and esthetic smile, re-pigmentation is an alarming issue which is of concern to both the patient and the dentist. While the literature states that the mechanism of re-pigmentation is unclear, one hypothesis suggests that the melanocytes from the adjacent pigmented tissues migrate to the treated area and cause re-pigmentation. Taking this aspect into consideration, our study aimed at evaluating the melanocyte response following depigmentation by scalpel and cryosurgery techniques. Our study included 15 patients who intended to undergo cosmetic therapy for hyperpigmented gums. After administering a routine oral prophylaxis and plaque control instructions and ensuring disease-free gingiva in the patients selected, the two treatment modalities, namely scalpel and cryosurgical techniques, were performed contralaterally in the anterior region of the oral cavity.

The mucosal excision method by scalpel was performed using a B-P blade No. 11 until the gingival surface appeared clinically free of pigmentation. On the other hand, gingival cryosurgery was performed with a gas expansion cryoprobe using liquid nitrous oxide by employing Joule–Thompson effect. Concentric circles of gingiva would be frozen for 10 s and thawed immediately under repeated cycles.

On assessment after 6 months, the difference between the depigmentation values over the two techniques showed higher values of recurrence of pigmentation in scalpel when compared to that of cryosurgery. These results are in accordance with that of Patil *et al.* who carried out a split-mouth comparative study between scalpel and cryosurgery techniques for performing gingival depigmentation.<sup>[6]</sup>

Similar results were also reported by Ahmed *et al.* wherein depigmentation was performed on 21 patients with no report of adverse effects and re-pigmentation for a period of 30 months.<sup>[9]</sup>

However, a delay in the recurrence of gingival pigmentation wherein cryosurgery is advocated was also observed in a case report after 20 months following freezing.<sup>[9]</sup>

In the present study, an attempt was made to evaluate the melanocytic response following cryosurgery and scalpel excision in gingival depigmentation. For determining the melanocyte assay, a small amount of gingival tissue was taken through biopsy from the distal papilla adjacent to the canine and first premolar.

The gingival tissue that was excised was studied under a special staining technique, that is, Masson–Fontana method for melanin which is a histochemistry procedure that would determine the activity of melanin both qualitatively and quantitatively. In our study, the mean histologic count of the melanocytes, which accounts for the re-pigmentation process, has shown low recurrence values in cryosurgery over the scalpel technique even though the difference was not statistically significant.

This can probably be attributed to the fact related to the consistency in reports showing delayed recurrence in cryosurgery, attributing to the larger time taken for the reactivation of melanocytes. In the literature, on the other hand, apart from the advocacy of cryosurgery for depigmentation, it is well established that the 5-year recurrence rate for primary basal cell carcinoma treated by cryosurgery is also as low as 7.5% as compared to the other treatment procedures.

The same also applies to other lesions such as leukoplakia wherein their results of cryosurgery seem to last longer. Some researchers also attributed this to extended T-cell activity.

It has been hypothesized that the melanocytes from the adjacent pigmented tissues migrate to the treated area and cause re-pigmentation.<sup>[10]</sup> It has also been hypothesized that the rate of melanin formation is higher in dark-complexioned people than fair-complexioned people.<sup>[11]</sup> The gingival pigmentation is more in the anteriors than posteriors and has been attributed to sunlight exposure.<sup>[12]</sup>

Probably, the results of our study can perhaps be ascribed to similar mechanism although the exact reason is still unclear.

This study being the first of its kind in literature to have evaluated the melanocytic response requires further clinical studies to authenticate its findings.

### Conclusion:

Gingiva and its display in a person accounts to one of the most important parameters that determines an esthetic smile and Gingival depigmentation is one of the commonly executed perioesthetic procedures. Although various methods of performing depigmentation do exist in the literature the predictability of one over the other needs to

be authenticated as the recurrence rate of the pigmentation is attributed to the underlying melanocytic response. Hence studies in future need to be more directed towards eliciting the underlying melanocytic responses as ours is the first of its kind attempt in doing so.

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### Conflicts of interest

There are no conflicts of interest.

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