Evaluation of the Efficacy of the Acellular Dermal Matrix Allograft with Partial Thickness Flap in the Elimination of Gingival Melanin Pigmentation. A Comparative Clinical Study with 12 Months of Follow-Up

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ABSTRACT

Acellular dermal matrix (ADM) has been used as a substitute for gingival allografts. The aim of this study was to evaluate the effectiveness of ADM with partial thickness flap in the elimination of gingival melanic pigmentations. Fifteen patients presenting bilateral gingival melanin pigmentation were selected for this study. Each side of the pigmentation was randomly assigned to be part of a group. The sites of the experimental group had a partial thickness flap raised, excised, followed by adaptation and suture of the ADM. On the opposite side (ie, sites from the control group), the oral epithelium was removed with a diamond bur. The healing process was evaluated at 1 and 2 weeks, and 1, 3, 6, 9, and 12 months postoperatively. Images of the patient's follow-up were digitalized, and occurrence and quantification of clinical repigmentation were evaluated. After 12 months, minimal repigmentation (mean 3.14 ± 7.45%) was noted in 8 of 15 sites from the ADM group, while significant repigmentation (mean 55.84 ± 27.25%) was seen in 15 of 15 abrasion sites. According to our results, it can be concluded that ADM may be successfully used in the elimination or greater reduction of gingival melanic pigmentations, and is more efficient than epithelium abrasion after 12 months ($p < 0.0001$).

CLINICAL SIGNIFICANCE

Gingival pigmentation is a common finding in several ethnic groups. Although physiologic melanin pigmentation is not a medical problem, cosmetic demands may turn it into a problem of social acceptance in different cultures. In this way, different treatment modalities have been used with the aim of removing pigmentations for esthetic reasons. In the present study, the use of ADM with partial thickness flap resulted in more effective elimination of gingival melanin pigmentation, as repigmentation occurred later and was much weaker when compared to the outcomes of epithelial abrasion.

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INTRODUCTION

Gingival pigmentation is a common finding in several ethnic groups. Although physiologic melanin pigmentation is not a medical problem, cosmetic demands may turn it into a problem of social acceptance in different cultures. Reports can be found of patients who were submitted to self-induced gingival tattoo in order to achieve darker gingival tissues,[1,2] and also the opposite, that is, patients willing to eliminate gingival pigmentations.[3,4]

Melanin pigmentation is frequently caused by melanin deposition by active melanocytes located mainly in the basal layer of the oral epithelium. Pigmentations can be removed for esthetic reasons and different treatment modalities have been used with this aim, including epithelial abrasion,[5,6] autologous gingival grafts,[7] cryosurgery,[3,8] laser therapy,[4,9–11], gingivectomy,[12] and, more recently, acellular dermal matrix (ADM) allograft.[13]

Predictability of the esthetical result varies considerably regarding different techniques and follow-up duration. According to Farnoosh[5] and Permutter and Tal,[6] removal of the epithelium with a diamond bur was successful up to 2 years after surgery, after which repigmentation occurred. Using autologous gingival grafts, Tamizi and Taheri[7] observed that the recidive occurred more than 4 years after baseline. Despite the good long-term outcomes, this technique presents some disadvantages such as an additional surgical site, limited amount of donor tissues, and unesthetic final clinical aspect resulting from color and texture differences between the buccal and palatal tissues.[14]

Tal and colleagues,[8] after an evaluation of cryosurgeries, found that repigmentation was not observed until more than 20 months after surgery. In spite of the effectiveness of this technique, there seem to exist a lack of precision related to tissue removal. Furthermore, the cost of the required equipment is high. The use of laser has also been studied for the same purpose, and repigmentation seems to take place after 12 to 24 months.[9–11]. Similar to cryosurgeries, laser therapy requires specific equipment, and must be used carefully, especially in delicate areas such as gingival margins, as thermal damage may result in alterations in the gingiva, bone, periosteum, and enamel.[9]

More recently, the use of ADM has been successfully reported as a substitute for autologous gingival grafts in order to increase attached gingiva, promote root coverage in gingival recessions,[15–17] increase gingival thickness in edentulous areas,[18,19] act as a barrier in guided bone regeneration,[20] and in association with immediate implants.[21] Despite some histological similarities between autologous gingival graft and ADM,[22] the latter presents some advantages as it avoids a second surgical approach to obtain donor tissues, provides unlimited amount of graft material, and produces predictable and satisfactory esthetic results.[15,16] These characteristics were underlined in a case report from Novaes Jr. and colleagues,[13] who first described the use of ADM to eliminate melanin pigmentation. The authors compared two surgical protocols in removing a bilateral melanin gingival pigmentation: on one side ADM was placed, while on the other the epithelium was removed with a diamond bur. In the ADM site, there were no signs of repigmentation up to 24 months after surgery, while in the abrasion side recidive was observed 6 months after surgery.

Thus, the aim of the present study was to further investigate the efficacy of ADM with partial thickness flap in the elimination of gingival melanin pigmentation compared with oral epithelium abrasion, through the quantification of repigmented areas after 12 months of follow-up.

MATERIALS AND METHODS

The protocol of the present study was approved by the Institutions Human Research Committee. Fifteen subjects signed a consent form after receiving an explanation concerning the research project and

being presented with the following eligibility criteria: (1) complaint of bilateral dark gingiva and the wish to remove it, (2) nonsmoking habit, and (3) negative report for the presence of systemic diseases. The patients who matched these criteria were women of African-American heritage, aged 18 to 45 years.

The pigmented areas extended bilaterally from the upper or lower central incisors to the canines or first bicuspids, reaching 15 to 20 mm in width and 5 to 8 mm in height. Both sides were photographed and randomly assigned, by a coin toss, to be part of either the ADM group or the control group. Surgical procedures were performed by the same operator at the same session (Figures 1 and 2).

In the ADM sites, after local anesthesia, two vertical incisions were performed mesially and distally to the surgical sites using a #15 scalpel blade. In sequence, an intracrevicu-

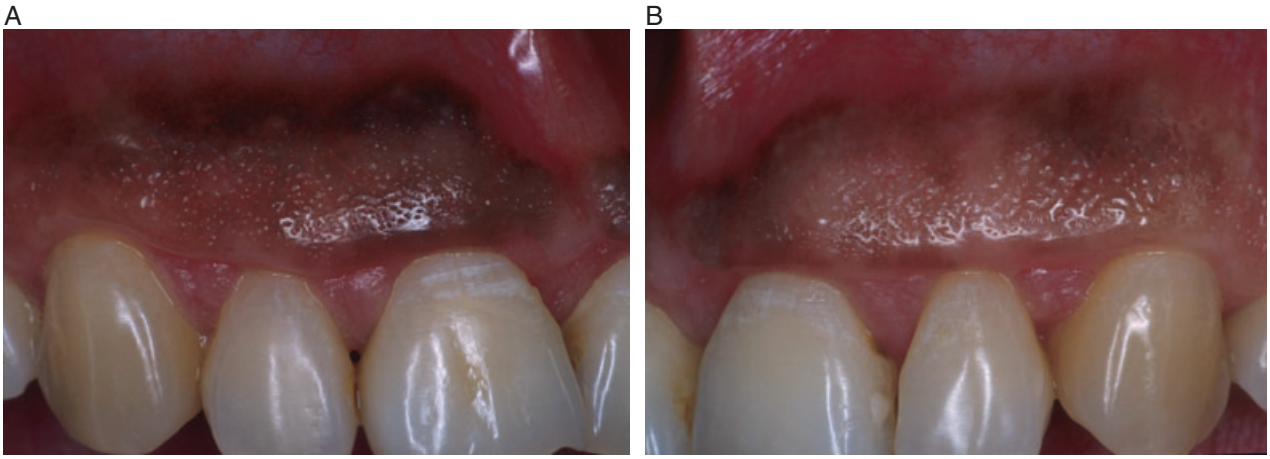

Figure 1. Preoperative view of the (A) control and (B) ADM site.

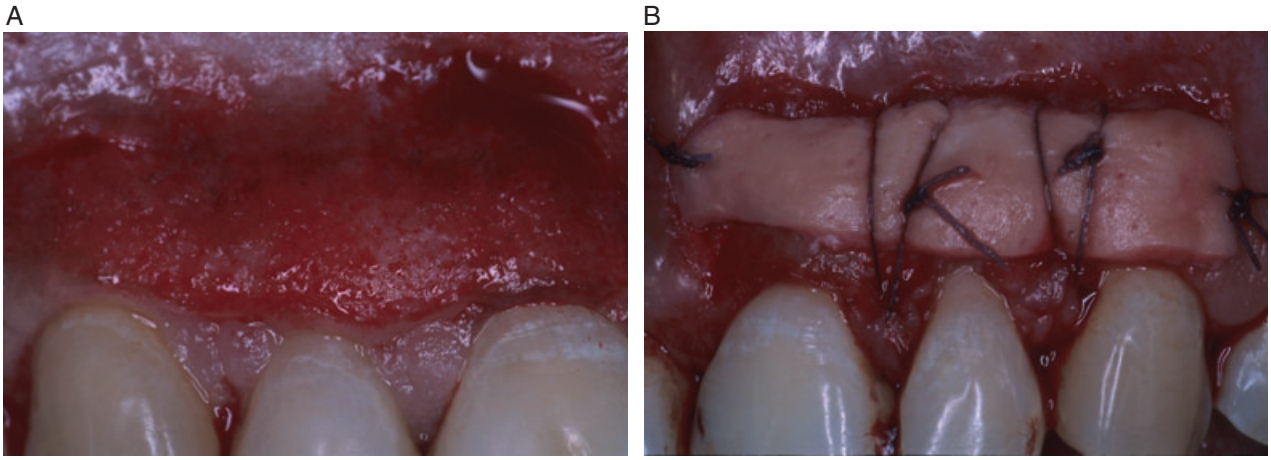

Figure 2. Clinical aspect after surgical procedure in the (A) control and (B) ADM site.

lar incision was made and a partial thickness flap was raised beyond the mucogingival junction. Then, a horizontal incision was made in the apical margin of the pigmentation in order to completely remove it. Sterile saline-soaked gauze was pressed against the recipient bed to control bleeding. ADM (Alloderm, LifeCelll Corporation, Woodlands, TX, USA) was hydrated according to the manufacturer's instructions, trimmed to fit the recipient bed, placed with the connective matrix facing the periosteum, and fixed with lateral and interproximal bioabsorbable poliglactin 910 5.0 sutures (Figure 2B). Grafts were tested for stability after suturing by moving the patient's lips. The area was then compressed with a saline-soaked gauze for 3 to 5 minutes in order to adapt the graft to the recipient bed.

In the control group, under local anesthesia, the oral epithelium was removed by abrasion with a diamond bur and abundant saline irrigation. This procedure continued until no clinical signs of pigmentation could be observed and a bleeding surface of connective tissue was achieved (Figure 2A).

Periodontal dressing (Coe-Pak, GC America Inc., Alsip, IL, USA) was applied over the two areas and was replaced after 7 and 14 days. After surgery, the patients were instructed to use an analgesic during the first 3 days and a 0.12% chlorhexidine digluconate rinse, three times daily, for 14 days. The healing process was clinically evaluated and the patients were photographed after 1 (Figure 3, A and B) and 2 weeks, and after 1, 3, 6, 9, and 12 months (Figure 4, A and B). During this period, photographs from both sides were taken. The baseline, 6-, and 12-month photographs were digitalized and evaluated for occurrence and quantification of repigmentation.

A blind analysis of the digitalized pictures was performed by the same examiner. Identification of repigmentation was dichotomous, representing the presence or absence of detectable clinical repigmentation. The area of recidive was quantified with a computer image analysis program (Image Tool, UTHSCSA, San Antonio, TX., USA). Each image was evaluated three times, and a mean value was obtained and transformed into percentage, considering the initial pigmented area as 100%. For statistical analysis, the Mann-Whitney test was used to determine if the two groups had

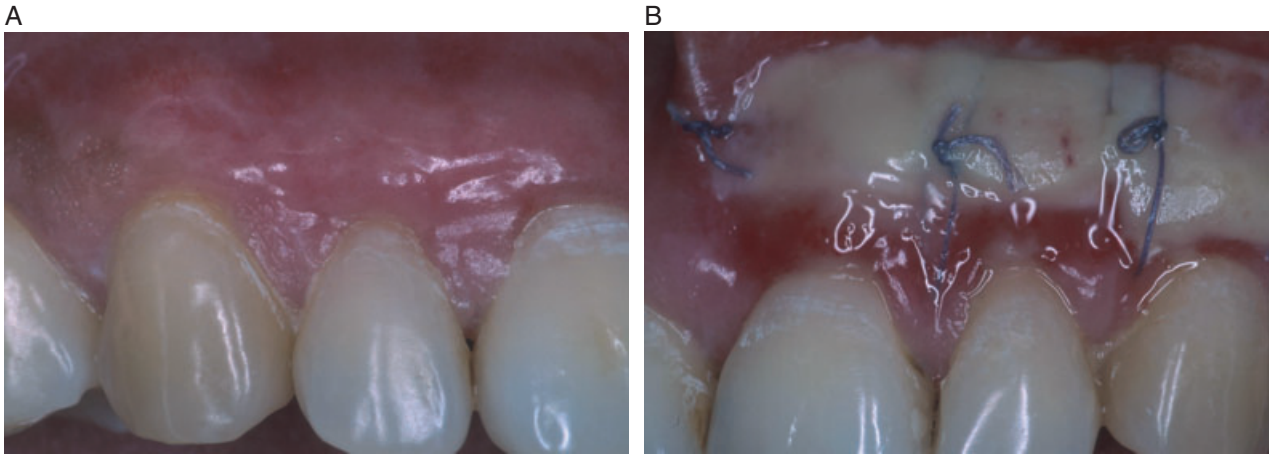

Figure 3. One-week clinical view of the (A) control site with evidence of epithelium formation and of the (B) ADM site with the graft in position.

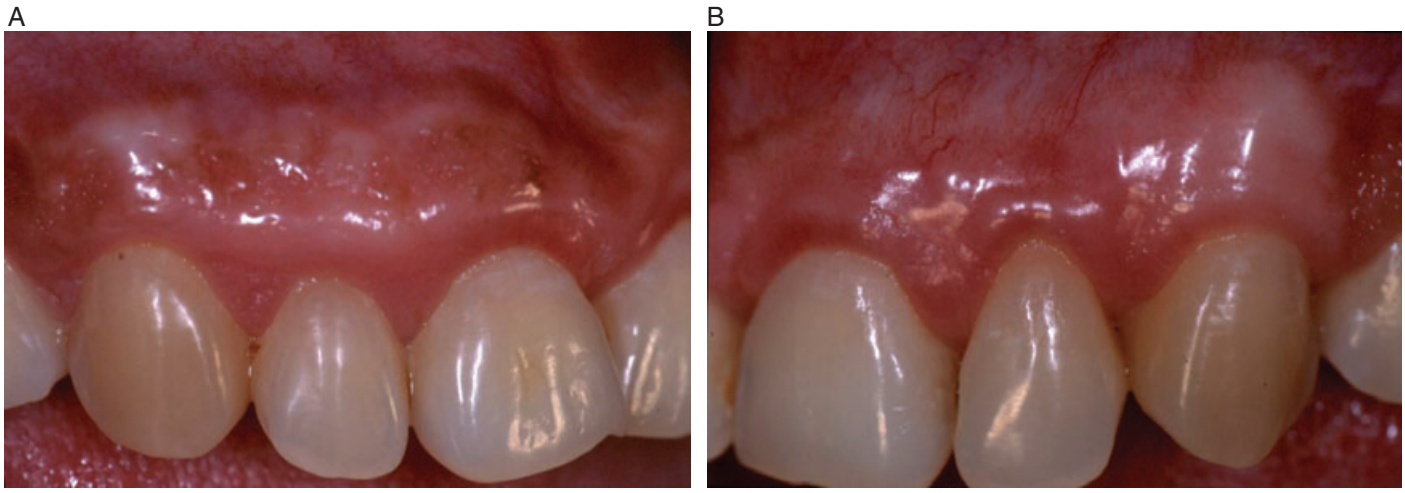

Figure 4. Twelve-month clinical view of the (A) control site with evidence of repigmentation and of the (B) ADM site with no signs of repigmentation.

TABLE 1. MEAN, SD, AND MEDIAN FOR MELANIN PIGMENTATION AREAS (IN PIXELS^2) AT BASELINE, FOR BOTH GROUPS.

	Control Group	Test Group
Mean ± SD	53744.71 ± 48197.62	59441.16 ± 56986.11
Median	30448.67	26825.33

No significant difference was detected between test and control groups (Mann-Whitney test, $N = 15$, $p = 0.19$).

similar-sized melanin pigmentations preoperatively, and the Wilcoxon test evaluated the differences between the two treatments 12 months after the surgery. For all statistical analysis, $p < 0.05$ was selected.

RESULTS

Both groups had statistically (Mann-Whitney test, $p = 0.19$) similar-sized melanin pigmentations at baseline (Table 1). Surgical procedures were well tolerated by all patients. During clinical follow-up, differences were observed between the groups. The ADM sites remained covered by a white pseudomembrane during the first and/or second weeks, after which it changed gradually to an intense red surface with inflammatory characteristics that lasted approximately 3 weeks. On the other hand, in control sites, epithelium formation could be clinically detected within a 2-week period.

After 6 months, repigmentation occurred in only one site of the ADM group (mean repigmentation area of 0.50 ± 1.74%), and in 10 sites of the control group (mean repigmentation area of 32.58 ± 29.84%).

Within all periods, repigmentation in the ADM sites was less intense than in the control group (Figure 5, A and B). After 12 months, 8 sites out of 15 from the ADM group presented repigmentation with a mean 3.14 ± 7.45% of the total original pigmentation, ranging from 0.00% to 29.21%, while all sites from the control group presented recidive (mean 55.84 ± 27.25% of the total original pigmentation, ranging from 13.57% to 99.38%); these results are shown in Table 2 and Figure 6. Statistical analysis of mean repigmentation values demonstrated a significant

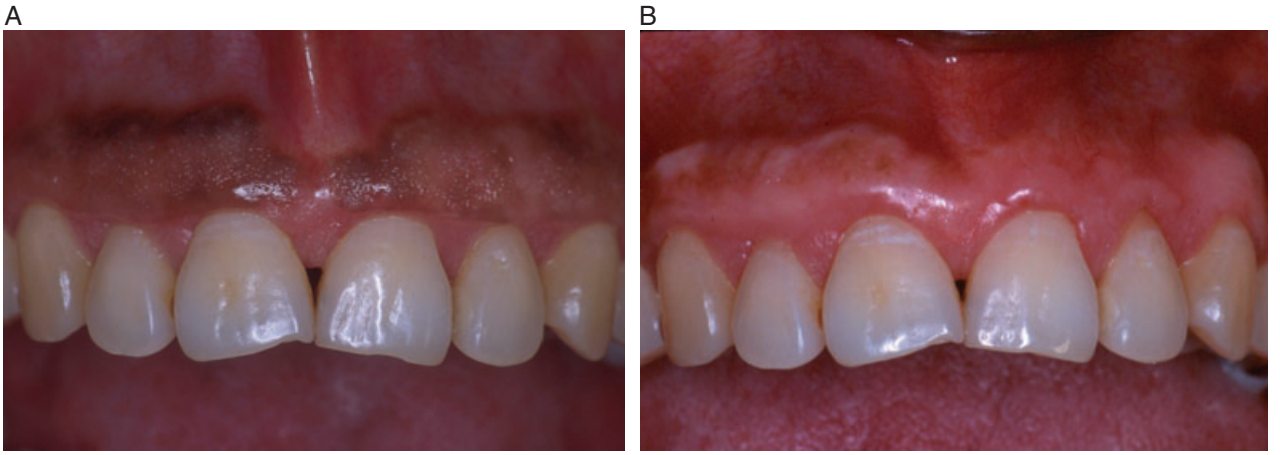

Figure 5. Bilateral clinical aspect at (A) baseline and at (B) 12 months after the surgical procedure.

TABLE 2. MEAN, MEDIAN, AND SD OF QUANTIFICATION OF CLINICAL REPIGMENTATION FOR THE ADM AND CONTROL GROUPS.

Patient	ADM Group	Control Group
1	0.6	23.9
2	3.4	13.6
3	1.2	34.5
4	5.4	63.9
5	0.0	71.8
6	0.0	31.9
7	0.0	38.1
8	5.3	99.4
9	0.8	68.1
10	0.0	69.2
11	0.0	81.4
12	29.2	87.4
13	0.0	73.7
14	1.3	65.5
15	0.0	15.4
Mean ± SD	3.14 ± 7.45*	55.84 ± 27.25*
Median	0.57	65.47

ADM = acellular dermal matrix.
*Significant difference was detected between groups ($p < 0.0001$, Wilcoxon test).
Values for each patient are expressed as percentage of repigmentation after 12 months.

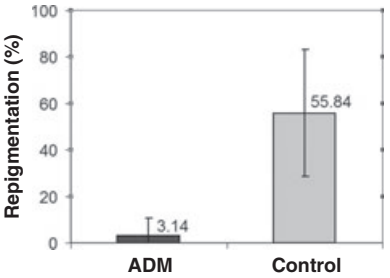

Figure 6. Histogram showing the percentage of repigmentation at the ADM and control groups. Means and SDs are represented in the graph.

difference between groups ($p < 0.0001$, Wilcoxon test).

In the 12-month recall visit, no complains about the esthetic outcome in the test side were noted. Although objective evaluations of patient satisfaction were not performed, the absence of patient dropouts and a favorable subjective response are potential indicators

toward the success of the therapy. Furthermore, 12 of the 15 patients in the control side asked to have the same surgical procedure that was made in the test side as, in their opinion, there was a better esthetical result with the ADM graft with partial thickness flap.

DISCUSSION

As the esthetic demand from the patients rises, reaching effective elimination of gingival melanin pigmentations can become a challenge. Thus, in the present study ADM was evaluated concerning its effectiveness in the elimination of gingival melanic pigmentation. Among the most common procedures that have been used for this purpose (ie, laser, cryosurgery, autologous gingival graft, epithelial abrasion, and ADM), ADM seems to present some advantages such as easy use, noncomplicated technique (does not require special equipment), unlimited amount of graft tissue, and satisfactory cosmetic results.[13]

The use of ADM to eliminate melanin pigmentations was first described by Novaes Jr. and colleagues[13] in a case report of a patient with bilateral gingival melanin pigmentation who received ADM on one side and epithelial abrasion on the other. The ADM became completely integrated to the recipient tissues, resulting in a very good cosmetic appearance. Clinical repigmentation did not occur up to 2 years after surgery on the site that received ADM, while it recurred after 6 months on the epithelium abrasion side.

To our knowledge there are no other reports regarding the use of ADM with partial thickness flap to eliminate gingival melanin pigmentation, nor the quantification of these pigmented areas—reasons why no direct comparisons with other studies can be made. The outcomes from the present study indicate that epithelial abrasion presents satisfactory, but short-term, esthetical results, after which repigmentation starts to occur in a gradual and progressive manner. In all sites where epithelial abrasion was performed, repigmentation occurred; on the average, more than 55% of the originally pigmented area presented recidive after 12 months of follow-up. On the other hand, eight sites among those that received ADM had repigmentation (mean of 3.14% of the originally pigmented area). Moreover, the intensity (darkness) of repigmentation in the ADM sites was significantly weaker compared with the sites from the control group.

Repigmentation is a common finding after different gingival surgical procedures to remove melanin pigmentation. In the present study more intense repigmentation was observed after epithelial abrasion and it occurred earlier compared with the results from sites that received ADM. This is in accordance with Novaes Jr. and colleagues,[13] who reported recidive 6 months after epithelial abrasion. Nevertheless, Farnoosh,[5] after 18 months of follow-up, observed repigmentation in only 2 patients, out of 20, who submitted to epithelial abrasion. These differences can be explained in part by the characteristics of our patients, who were all African-Americans. According to Barret & Scully,[23] African-Americans seem to have more melanin granules, which result in more intense melanin expression in their tissues. The melanocytes, which are the melanin-producing cells in the epithelium, are derived from neural crest cells.[24] The melanocytes are resident in the basal layer of the epithelium, and the melanin they produce is transferred to the basal epithelial cells. As the epithelium have projections and ridges into the connective tissue, the depth of epithelial removal can be another explanation for the poor results of the epithelial abrasion technique, because ridges at pigmented areas may harbor active melanocytes, and their persistence result in repigmentation after a short time. In the present study, epithelium was removed until a bloody surface of connective tissue without pigmentation was achieved. Farnoosh,[5] although using the same criteria, may have actually removed a thicker layer of

epithelium, as this is a very subjective clinical judgment, and this could have resulted in fewer remaining epithelial ridges in the connective tissue.

Regarding the test group, only a few, small and weakly repigmented areas were observed, and this occurred later than in the control group. During the preparation of the recipient tissues to receive the ADM, as the flap is dissected, only a few or maybe no epithelial ridges are likely to remain in the connective tissue. Thus, during the healing process, migration of inactive melanocytes from the surrounding, that is, nonpigmented areas, would hamper repigmentation.[25] The satisfactory esthetic result obtained with ADM is also attributed to the fact that, being an acellular collagen membrane, its healing occurs through the repopulation of cells and revascularization, which leads to the formation of tissues with similar characteristics, particularly in color and texture, to the surrounding tissues.[26]

Epithelial abrasion was chosen as the control technique to be compared with ADM with partial thickness flap for some specific reasons. First of all, the mere dissection and excision of gingival tissue without using a graft is not acceptable, as it causes excessive pain and discomfort. Healing occurs slowly by second intention and can even lead to bone resorption. Second, the use of an autogenous gingival graft was also discarded mainly because of the great amount of donor tissue needed to cover the area of pigmentation, which extended typically from the central incisor to the canine or first bicuspid. Moreover, the esthetic outcome of autogenous gingival grafts does not seem to be as favorable as that seen with ADM, as the formation of a pale and fibrotic tissue surrounded by a defined healing line is not uncommon.[27] Finally, the epithelial abrasion technique is simple and can be performed quickly, and it has been largely used in clinical practice.

According to the findings from the present study, the use of ADM with partial thickness flap resulted in a more effective elimination of gingival melanin pigmentation, as repigmentation occurred later and was much weaker when compared with the outcomes of epithelial abrasion. Despite the positive results obtained with the use of ADM allografts, longitudinal evaluations are still needed in order to verify long-term results. It could be concluded that ADM can be successfully used in the elimination or greater reduction of gingival melanic pigmentations over a 12-month observation period.

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COMMENTARY

EVALUATION OF THE EFFICACY OF THE ACELLULAR DERMAL MATRIX ALLOGRAFT WITH PARTIAL THICKNESS FLAP IN THE ELIMINATION OF GINGIVAL MELANIN PIGMENTATION: A COMPARATIVE CLINICAL STUDY WITH 12 MONTHS OF FOLLOW-UP

Edward P. Allen, DDS, PhD*

This randomized controlled clinical trial provides strong evidence that the use of an acellular dermal matrix is more effective in eliminating gingival pigmentation than de-epithelization without graft placement. The technique reported resulted in minimal repigmentation over the 12-month term of the study and indicates that it may be a useful method for esthetic management of gingival pigmentation.

Other reports cited by the author (references 5 and 6) indicate that epithelial removal is successful in eliminating gingival pigmentation for up to 2 years, after which repigmentation occurs. This study, with a term of only 12 months, does not allow comparison with the longer-term reports cited. Although this study reports positive findings and a clinically significant difference between groups, it does not provide information for the outcome beyond 12 months. It would be important to learn the outcome at 2 years after treatment.

Based on the results of this 12-month study, the use of acellular dermal matrix as a surface graft produced a better outcome in the treatment of gingival pigmentation than removal of epithelium by abrasion. While it might be assumed that the grafted sites were more comfortable than the denuded sites, postsurgical discomfort was not reported in this study.

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