

## Comparison of the effect of exposed (semi-covered) technique of ADMA (Acellular Dermal Matrix Allograft) in increasing the width of attached gingiva

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### ABSTRACT

**Background & objectives:** The presence of adequate zone of attached gingiva is considered critical for the maintenance of marginal tissue health and for the prevention of continuous loss of connective tissue attachment. Free grafts have been commonly used due to their predictable results, however, some disadvantages such as patient's discomfort and colour difference were reported.

Acellular Dermal Matrix (ADM) Allografts may be utilized as an alternative for autogenous grafts, but increasing the width of Keratinized tissue following the use of these grafts may not be as predictable as Free Gingival Grafts (FGG) due to high shrinkage rate (3,7).

**Material and methods:** In this split mouth study, six patients with bilateral inadequate keratinized tissue were selected. In case groups, after securing the Sure-Derm, the alveolar mucosa was displaced coronally to cover half of the graft. Patients were followed and measurements were done once after one month and again after 4 months. Results were analysed by Wilcoxon Signed Rank Test.

**Results:** No statistically significant differences existed between control and case groups at baseline. Amount of the attached gingiva in the exposed groups reached from  $0.53 \pm 0.8$ mm preoperative, to  $4.06 \pm 1.16$ mm in one month. In semi coverage groups, amount of the attached gingiva reached from  $0.6 \pm 1.12$ mm preoperative to  $3.4 \pm 0.82$ mm in one month. In the fourth month post surgery, the amount of attached gingiva reached to  $2.7 \pm 0.98$ mm in the exposed groups, and  $2.7 \pm 0.91$ mm in the semi coverage ( $p < 0.9$ ). In the fourth month post surgery, both techniques proved capable of increasing the amount of AG, with no statistically significant differences.

**Conclusions:** The present study indicated that both techniques can increase the amount of attached gingiva and the relapses in both were the same and equal to 74%.

**KEYWORDS:** Mucogingival surgery, attached gingiva, Keratinized gingiva, Acellular Dermal Matrix Allograft (ADMA), Sure-Derm

### INTRODUCTION

Adequate width of attached gingiva is required for resistance against functional stresses and providing periodontal health [1,2,3,4]. Attached gingiva augmentation is classically recommended to control the microbial plaque accumulation and prevention of more recession [3,5], and attachment loss [3]. Free Gingival Graft (FGG) has been extensively used in periodontal surgeries since 1963 (6). For the first time in 1996, Shulman used Acellular Dermal Matrix Allograft (ADMA) in augmentation of attached gingiva [2, 3]. FGG has defects such as having two surgical sites, limitation in donor graft site [5, 7], remaining lesion in palatal region for secondary healing and colour difference [3, 5, 6,7]. ADMA has many advantages which cover FGG defects. However, ADMA itself has defects such as high graft shrinkage and long term healing [3-10]. Resistance to functional stresses and microbial resistance as well as gingival health will be at risk [1, 2,3] and there will be increase in attached gingiva loss [3, 7] unless the short width of attached gingiva is treated.

The high percentage of shrinkage of ADMA was demonstrated by some researches [3 -10] as well as its advantages in comparing with FGG [1,5,6,7,9], but there is not any data about different surgical methods for using ADMA and reduction its high shrinkage. Therefore, this study was performed with the aim of comparing the effect of using two different methods of ADMA i.e. exposed and semi-covered by alveolar mucosa on increasing width of attached gingiva in patients with inadequate width of gingiva who referred to Tehran Islamic Azad University during 2010-2011.

### METHOD AND MATERIALS

In this split mouth clinical trial, patients were selected among those who had bilateral loss of attached gingiva (less than 2 millimetres) in canine, premolar, and first molar regions. Those with systemic and

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autoimmune diseases, pregnancy, and removable dentures were excluded. In a meeting, the study was explained for the patients, and a written consent was taken. There were 6 patients who underwent split mouth treatment. One side of mouth was considered as case and the other as control. Then the first phase of periodontal treatments including scaling, smoothing and polishing root surface with hand instruments and ultrasonic tools was performed. Periodontal parameters were evaluated after two weeks. Periodontal parameters include Attached Gingiva (AG), keratinized Gingiva (KG), Gingival Index (GI), Gingival Recession (GR), Probing Depth (PD) measured by a periodontist using Williams probe.

GI index was evaluated using the Loe & Silness criteria [0,1,2,3].

Probing depth was measured using a Williams probe (figure 1). The distance from CEJ to gingival margin was measured using Williams probe in order to define Gingival recession (GR) (figure 2). Then Keratinized Gingiva (KG) was measured as the distance from gingival margin to mucogingival line. The last step was Attached Gingiva (AG) evaluation which consists of the distance from sulcus base to mucogingival line.

2% lidocaine and 1/100000 epinephrine were locally injected at the beginning of the surgery. Then a horizontal incision was made in the mucogingival line using a #15 scalpel. And a partial thickness flap was done on periosteum in apical position. The connective tissue fibres were completely eliminated from the underlying tissue to prevent graft mobility after the procedure (figure 3).

As the factory has recommended, rehydration of Sure-Derm was performed by putting Sure-Derm in normal saline solution for 10 minutes and removing the sheath over it using a forceps (figure 4).

The dermal surface of Sure-Derm was put on the defect. In order to recognize the dermal surface, both sides of Sure-Derm were moistened by the patient's blood. In this way, the dermal surface clearly remains red after washing with normal saline (figure 5). In the control group, Sure-Derm was put on underlying tissue and was fixed to the underlying periosteum and adjacent tissues using reverse cut 50 vicryl sutures. After fixing Sure-Derm in the case group, alveolar mucosa is coronally repositioned to cover half of the graft (figure 6); then the site was covered by periodontal pack.

500 mg Amoxicillin, TDS for one week, was used in order to prevent infection, and 400 mg Ibuprofen was administered for pain alleviation. Washing with 12% chlorhexidine was initiated from the day after surgery, and was continued until patients could mechanically control the microbial plaque using toothbrush and dental floss. Periodontal pack and sutures were removed after 10 days.

Patients were followed for one and four months after surgery. AG, KG, GR, GI, and PD measurements were repeated during follow-ups, and the results were recorded and analysed (figure 7-12).

Freedman test has been used in each case and control group in order to compare the rate of each index before treatment, one and four months after treatment. Also, Wilcoxon Signed Rank Test has been used in order to compare the rate of indices in each time in case and control groups.

## RESULTS

The study was performed on both sides and 30 tooth regions including 15 control (semi-covered) and case (covered) teeth of 6 patients. Patients were 4 women and 2 men with age of  $36.6 \pm 8.8$ .

Table 1 indicates the width of attached gingiva in baseline and follow-up steps for each surgical method. The table shows that the width of attached gingiva at the beginning, had no significant difference. The rate of one month change was  $2.6 \pm 1.4$  in the semi-exposed group and  $3.3 \pm 1.35$  in the exposed group which is not statistically significant ( $p < 0.2$ ). Its changes were the same in the fourth month ( $p < 0.5$ ). However, there were statistically significant changes of each method within each group in one and four month periods ( $p < 0.000$ ).

Table 2 indicates that the width of keratinized gingiva had no significant difference at the baseline, after one month, and after the fourth month of the study between groups. The rate of one month changes was  $2.9 \pm 1.5$  in the semi-exposed group and  $2.8 \pm 1.3$  in the exposed group which was not statistically significant ( $p < 0.8$ ). The same changes were seen in the fourth month ( $p < 0.8$ ). However, changes in each method within each group after one and four months were statistically significant ( $p < 0.000$ ).

Table 3 indicates that the amount of probing depth at the start, the first month and the fourth month of follow-up within and between the two groups were not significantly different.

Table 4 indicates that the amount of graft shrinkage from the first to the fourth month is  $1.05 \pm 0.8$  in the semi-covered group, and  $0.83 \pm 0.72$  mm in the exposed group. The difference between groups is statistically significant ( $p < 0.05$ ).

Table 5 indicates the amount of alloderm shrinkage according to the graft width in the first and fourth month for each surgical method. The rate of graft width was the same and equalled 10 in all surgeries. In the statistical analysis, the rate of shrinkage in the first month was  $61\% \pm 8\%$  in the exposed group and  $67\% \pm 4.5\%$  in the semi-covered group which this difference is not statistically significant ( $p > 0.05$ ). The rate of shrinkage in the fourth group is also  $74\% \pm 7.5\%$  in the exposed group and  $74\% \pm 6\%$  in the semi-covered group. This difference is not statistically significant ( $p > 0.05$ ).

Table 1) width of attached gingiva according to follow-up times and their changes in each surgical method (mm)

| Follow-up time       | Primary              | At first month        | At fourth month      | Changes              |         |                     |         |
|----------------------|----------------------|-----------------------|----------------------|----------------------|---------|---------------------|---------|
|                      |                      |                       |                      | After one month      |         | After four months   |         |
|                      |                      |                       |                      | Rate                 | Test    | Rate                | Test    |
| Semi-exposed<br>N=15 | 0.6+ <sub>1.12</sub> | 3.4+ <sub>0.82</sub>  | 2.7+ <sub>0.91</sub> | 2.6+ <sub>1.4</sub>  | P<0.000 | 1.8+ <sub>1.1</sub> | P<0.000 |
| Exposed<br>N=15      | 0.53+ <sub>0.8</sub> | 4.06+ <sub>1.16</sub> | 2.7+ <sub>0.98</sub> | 3.3+ <sub>1.35</sub> | P<0.000 | 1.5+ <sub>1.4</sub> | P<0.000 |
| Test result          | P<0.6                | P<0.2                 | P<0.9                | P<0.2                | P<0.2   | P<0.5               | P<0.5   |

Table 2) width of keratinized gingiva according to follow-up times and their changes in each surgical method (unit: millimetre).

| Follow-up time       | Primary              | At first month       | At fourth month      | Changes             |         |                      |         |
|----------------------|----------------------|----------------------|----------------------|---------------------|---------|----------------------|---------|
|                      |                      |                      |                      | After one month     |         | After four months    |         |
|                      |                      |                      |                      | Rate                | Test    | Rate                 | Test    |
| Semi-exposed<br>N=15 | 1.4+ <sub>1.05</sub> | 4.5+ <sub>0.74</sub> | 3.7+ <sub>0.9</sub>  | 2.9+ <sub>1.5</sub> | P<0.000 | 2.1+ <sub>1.3</sub>  | P<0.000 |
| Exposed<br>N=15      | 2.07+ <sub>0.7</sub> | 5.1+ <sub>1.1</sub>  | 3.7+ <sub>0.97</sub> | 2.8+ <sub>1.3</sub> | P<0.000 | 1.5+ <sub>1.15</sub> | P<0.000 |
| Test result          | P<0.3                | P<0.2                | P<0.9                | P<0.8               | P<0.8   | P<0.8                | P<0.8   |

Table 3) probing depth according to follow-up times and their changes in each surgical method (unit: millimetre)

| Follow-up time       | Primary               | At first month        | At fourth month      | Changes               |       |                      |       |
|----------------------|-----------------------|-----------------------|----------------------|-----------------------|-------|----------------------|-------|
|                      |                       |                       |                      | After one month       |       | After four months    |       |
|                      |                       |                       |                      | Rate                  | Test  | Rate                 | Test  |
| Semi-exposed<br>N=15 | 1.13+ <sub>0.35</sub> | 1.26+ <sub>0.46</sub> | 1+ <sub>0</sub>      | 0.13+ <sub>0.5</sub>  | P<0.5 | 0.13+ <sub>0.3</sub> | P<0.5 |
| Exposed<br>N=15      | 1.5+ <sub>0.67</sub>  | 1.13+ <sub>0.35</sub> | 1.2+ <sub>0.41</sub> | 0.19+ <sub>0.66</sub> | P<0.3 | 0.13+ <sub>0.8</sub> | P<0.2 |
| Test result          | P<0.2                 | P<0.5                 | P<0.4                | P<0.8                 | P<0.8 | P<0.9                | P<0.9 |

Table 4) Mean and standard deviation of the graft shrinkage from the first to the fourth month in exposed and semi-covered groups

| Shrinkage Group | Rate                  | Test Result |
|-----------------|-----------------------|-------------|
| Exposed         | 1.5+ <sub>0.8</sub>   | P<0.03      |
| Semi-covered    | 0.83+ <sub>0.72</sub> |             |

Table 5) mean and standard deviation of graft shrinkage according to follow-up times in each surgical method

| Shrinkage rate Group | First month         | Fourth month        | Test result |
|----------------------|---------------------|---------------------|-------------|
| Exposed              | %61+ <sub>8</sub>   | %74+ <sub>7.5</sub> | P<0.7       |
| Semi-covered         | %67+ <sub>4.5</sub> | %74+ <sub>6</sub>   |             |
| Test result          | p>0.05              | p>0.05              |             |

Figure 1.measurement of periodontal parameters



Figure 2. The distance from CEJ to gingival margin was measured using Williams probe in order to define Gingival recession (GR)



Figure 3. Preparation of underlying graft tissue

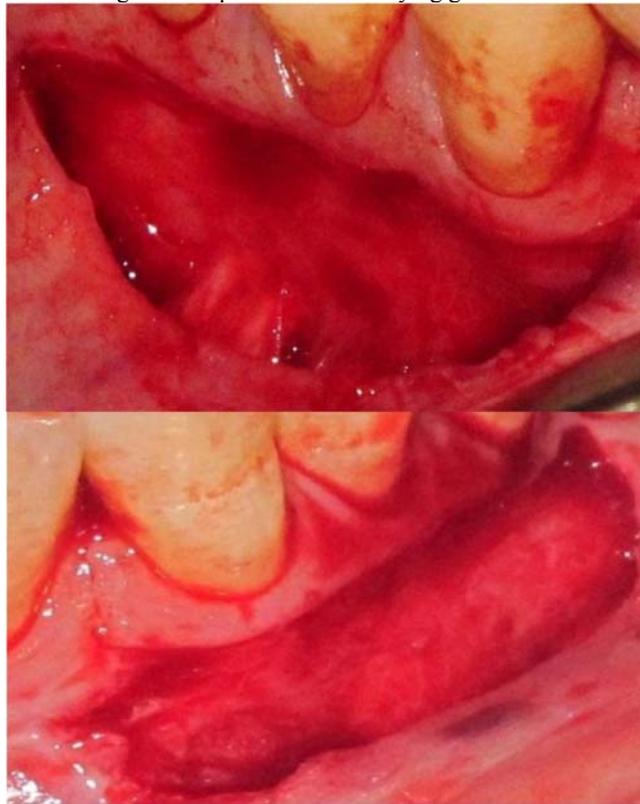


Figure 4. preparation of Sure-Derm



Figure 5. Method of recognizing two sides of Sure-Derm

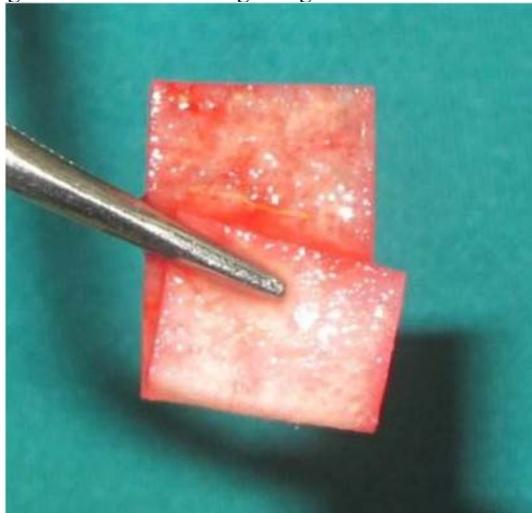


Figure 6. Exposed (right) and semi-covered (left) side at the end of surgery



Figure 7. Semi-covered side before surgery



Figure 8. Semi-covered side one month after surgery



Figure 9. Semi-covered side four months after surgery



Figure 10. Exposed side before surgery



Figure 11. Exposed side one month after surgery



Figure 12. Exposed side four months after surgery



## DISCUSSION

Results of this study demonstrated that using exposed and semi-covered methods of ADMA (Sure-Derm) leads to similar outcomes of increasing the width of keratinized gingiva. The rate of graft shrinkage in the first month was higher in semi-covered method in comparison with exposed method. At the end of four months, however, the rate of keratinized tissue in both methods was not significantly different, and the most graft shrinkage in exposed method took place in the third and fourth months after surgery.

Using ADMA in order to increase the width of attached gingiva has been shown by many researchers. According to some studies, however, ADMA's impact has been considered to be less than FGG, and the rate of graft shrinkage is high [3-10]. Comparing FGG with ADMA (alloderm), Wei et al reported  $5.57 \pm 0.44$  mm and  $2.09 \pm 1.92$  mm increases in width of attached gingiva respectively. The shrinkage rate of ADMA was significantly higher than FGG ( $71\% \pm 10$  versus  $16\% \pm 12$ ). This was while the primary grafted tissue was  $8.81 \pm 0.46$  for ADMA versus  $6.7 \pm 0.89$  for FGG. According to histological studies, the tissue produced tissue by ADMA was similar to scar tissue (6).

Comparing FGG and ADMA (alloderm) in 2005, Ayoubian and Moghavvemi reported an increase in the width of attached gingiva from  $0.38 \pm 0.5$  mm before surgery to  $4.66 \pm 1.08$  mm after one month by using alloderm, while there was an increase from  $0.78 \pm 0.66$  mm before surgery to  $5.03 \pm 0.90$  mm after one month by using FGG. After three months, the rate of change in FGG and ADMA samples was  $4.5 \pm 0.82$  and  $3.56 \pm 1.09$  respectively. The shrinkage rate of ADMA after three months was  $49.11\% \pm 15.63$  while compared with  $10\% \pm 16.33$  for FGG [9].

Randall J. Harris stated that there is no statically significant difference between free gingival graft and graft of connective tissue and alloderm in increasing keratinized gingiva i.e. keratinized gingiva increased 4.1 mm by FGG, 6.3 mm by connective tissue, and 4.1 mm by alloderm (19).

In two case reports, RJ Harris put ADMA on periosteum and bone in order to increase the width of keratinized gingiva. ADMA behaved differently on these two tissues. It completely sloughed on the bone, and wound healing took place from similar region via denudation. On the periosteum, however, although the graft unified with underlying tissues, this method was considered to be a failure due to severe shrinkage [2, 13].

Yuknaet al.[23] reported shrinkage rates of 34.6% and 29.2% using Freezed Dried Skin Allograft (FDSA) and FGG respectively. Schoo and Coppes reported 63% shrinkage rate for Lyophilized Dura Matter Graft compared with 20% for FGG. However, Lyophilized Dura Matter Graft was more beautiful [24].

It is stated that in all dead grafts without any living cell such as FDSA, or Freezed Dura Matter is just a structure for fibroblasts, blood vessels, and epitheliums of adjacent tissues to rehabilitate. By persistent production of new connective tissue and decomposition of primary graft matrix and dead cells, the dead graft will be eventually completely replaced by host tissues [6]. High ADMA shrinkage rate is attributed to its nature since ADMA is also a kind of dead acellular graft again organized by adjacent tissues. Possible resources of granulation tissue formation followed by mucogingival surgery are: adjacent gingiva, periodontal ligament, remaining periosteum, bone marrow and alveolar mucosa space. In 1975, Karring et al demonstrated that only gingival connective tissue and PDL have the capacity of inducing keratinized epithelium [25].

Since ADMA (alloderm) works as a structure, it is invaded by some cells of adjacent cellular resources. During healing process, allodermunifies underlying tissue and undergoes remodelling. This is why the area grafted with alloderm indicates a transitional area between newly formed keratinized tissues and alveolar mucosa. Newly formed epithelium might be ortho-keratinized, para-keratinized, non-keratinized, or a combination of them based on the cellular resource taking part in alloderm reorganization [6, 17].

Since ADMA (alloderm) is avascular, it is not able to anastomose with underlying tissue i.e. vessels should grow and spread in it. Therefore, new vessels formation would take a long time. Delay in new vessel formation of alloderm and the following deceleration of healing process might be one main reason for high alloderm shrinkage [9, 26].

Due to the high rate of ADMA shrinkage, It was decided in this study to cover the lower half of Sure-Derm with alveolar mucosa of underlying tissue which was separated from muscular attachments. In this way, new vessels would form in shorter time, healing would be faster, and the graft shrinkage would eventually decrease. Although semi-covered patients had less problems during wound healing and heading the process was more rapid, the graft shrinkage rate was not different. The width of attached gingiva reached from  $0.53 \pm 0.8$  before the surgery to  $2.7 \pm 0.89$  after four months in the exposed group, and from  $0.6 \pm 1.12$  before the surgery to  $2.7 \pm 0.91$  after four months in the semi-covered group. The amount of keratinized gingiva after four months was  $3.7 \pm 0.97$  and  $3.7 \pm 0.9$  in the exposed and semi-covered groups respectively. The graft shrinkage rate after one month was  $61\% \pm 8$  and  $67\% \pm 4.5$  in the exposed and semi-covered groups respectively, which eventually after four months reached  $74\% \pm 7.5$  and  $74\% \pm 6$  respectively.

This indicated that covering half of the graft does not prevent shrinkage, and there are other factors affecting shrinkage event, which most common of them is graft nature and its healing mechanism. However, vestibular depth is also an effective factor, and covering the lower half of the graft by alveolar mucosa might decrease vestibular depth and cause graft shrinkage. Nevertheless, it was tried to separate muscular connections from mucosa during surgery so that mucosa covered the graft without any attachment of fibres.

During a histological study of alloderm samples six months after healing, Wei et al reported that the resultant tissue of alloderm is mostly similar to scar tissue. It was variable in terms of keratinization so that keratinization decreases in apical direction, and it was mostly ortho-keratinized or para-keratinized toward gingiva, while non-keratinized toward alveolar mucosa [17].

Ayoubian and Mirsaedi indicated that adding PRGF growth factor to ADMA is not effective in increasing width of attached gingiva, so that AG rate was  $3.43 \pm 0.94$  and  $3.46 \pm 0.8$  in ADMA, and PRGF added to ADMA groups respectively three months after surgery; this underlines the graft nature and its way of scar healing [26].

Peacock states in a case report that shrinkage in ADMA was seen after the third week post-surgery [8], while according to Zingale, no change is seen in graft size and thickness even after 6 weeks [10].

In the present study, patients noted more bleeding in exposed site. There was no pain when using sedatives, and they only complained about postsurgical swelling. The exposed site indicated a longer healing course compared to semi-covered site. The tissue had more blood supply in semi-covered technique which might be one reason for more rapid healing.

Although occurring more shrinkage after healing, using alloderm leads to better results in terms of esthetic and graft consistency with adjacent tissues, and the patients' consent of postsurgical pain is higher by using ADMA. Furthermore, surgical methods limited to one surgical site are preferred. All mentioned studies agree upon such issue.

Therefore, using ADMA as a graft tissue for increasing keratinized gingiva should be limited to cosmetic areas in which FGG colour might not be accepted by patient. The same colour of ADMA in such situations would compensate its problem of high shrinkage rate. Moreover, patients who do not desire their palates to be used as donor site are proper candidates for using such material, and the surgical technique does not affect treatment outcome.

Considering the same results of producing keratinized gingiva in both exposed and semi-covered methods, using semi-covered method could be considered since patients faced less problems and there was a more rapid process of healing.

### **Conclusion**

According to results of this study, both exposed and semi-exposed methods cause similar increase in attached gingiva, and the results are clinically acceptable. The graft shrinkage rate is the same in both techniques and no one is preferred to the another one.

### **Suggestions and Recommendations**

Long-term studies are suggested in future to ensure conclusions. It is recommended to measure vestibular depth as one effective parameter.

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