Original Article - Comparative Studies

Comparison of Vestibular Depth Relapse and Wound Healing After Reconstructive Preprosthetic Surgery Using Cryopreserved Amniotic Membrane and Acellular Dermal Matrix - A Comparative Study

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Abstract

Introduction: The significance of membranes as wound dressing in oral surgeries has been reported by previous studies. The aim of the present split-mouth randomized clinical study was to assess and compare the wound dressing properties of acellular dermal matrix (ADM) and cryopreserved human amniotic membrane (AM) after reconstructive preprosthetic oral surgery. **Materials and Methods:** Twenty-eight patients with complete mandibular edentulism and resorbed alveolar bone were included. After taking mandibular impression, a clear acrylic splint with increased labial flange height was created. In each participant, labial vestibular depth was elevated using the Clark's technique. Subsequently, half of the exposed periosteum was covered with ADM while the other half was covered with cryopreserved human AM. Vestibule depth and relapse in the two sides were measured immediately after vestibuloplasty and at the end of the 1st week, 2nd week, 1st month, and 3rd months with graduations of 0.1 mm. Furthermore, after 3 and 7 days, samples were collected from graft material, and the macrophage population was analyzed by flow cytometry. **Results:** There was no significant difference in the relapse of vestibule depth between the two grafts at different time intervals. However, the frequency of wound-infiltrating macrophages ($CD68^+$ cells) was significantly higher in areas covered by ADM after 3 and 7 days. **Discussion:** ADM is as effective as cryopreserved AM in terms of maintaining the postoperative vestibular depth. On the other hand, our results suggested that the onset of healing phase in ADM-covered areas occurs faster compared to the periosteum covered with cryopreserved human AM. This clinical trial showed significantly faster postoperative healing onset when ADM was used than when cryopreserved human AM was applied on the periosteum.

Keywords: Acellular dermal matrix, $CD68^+$ cell, cryopreserved human amniotic membrane, vestibular depth, vestibuloplasty

Introduction

The height of lower anterior ridge plays a crucial role in stability, convenience, and function of the complete mandibular removable prostheses.[1] In order to obtain a satisfactory denture-bearing area, lower anterior ridges with insufficient quality and quantity can undergo different types of procedures such as implant placement and vestibuloplasty.[2,3] It has been shown that dental implants can not only provide appropriate stability and convenience but can relatively prevent alveolar bone resorption as well.[4-7] However, in addition to prohibitive cost and difficulties in providing the necessary hygiene, implant

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placement is contraindicated in patients with epilepsy, diabetes, endocarditis, osteoradionecrosis, cardiac transplant, myocardial infarction, immunosuppressive disorders, active treatment of malignancies, and drug abuse. Therefore, vestibuloplasty can be employed as a promising surrogate to overcome these contraindications.[4] Through this surgical procedure, the soft-tissue attachments are retracted to improve the residual bone height and to provide a nondisplaceable denture-bearing base.[8] Following this procedure, the exposure of periosteum can lead to common adverse consequences such as infection, relative relapse of initial attachments, patient discomfort, scar formation, and poor healing.[8-10] Previous studies suggested that covering the raw periosteal surfaces can prevent these complications. Among various covering materials, split-thickness skin grafts and human amniotic membrane (AM) are considered as the favorable covering materials in oral and maxillofacial surgical procedures.[11-14] However, there is no well-documented evidence that indicates which of one can serve as the candidate covering material in the oral cavity.

Applications of fresh and preserved forms of the human AM in clinical studies suggest that it is a descent membrane for wound covering, thanks to its low immunogenicity, low cost, availability, epithelialization-stimulating potential, and anti-inflammatory properties.[11-13,15-20] Although the acellular dermal matrix (ADM) was firstly developed for covering the full-thickness burn lesions, the histologic and clinical results from oral and craniofacial studies suggested that its unique characteristics including easy handling, keratinization inducing nature, appropriate root coverage, and scar-relieving potential make it a promising substitute for other covering materials in the dental and oral surgeries.[21-26]

It has been previously shown that both ADM and AM as wound-dressing membranes contribute to improve wound healing after surgical procedures in the oral cavity.[11,23,24,27-29] However, there was no study comparing the efficacy and wound-covering properties of these two membranes. The current split-mouth study aims to implement a reliable comparison between these two membranes on both macroscopic and molecular scales. For this purpose, the relapse of vestibular depth and the influx of macrophages to the periosteum were investigated and compared in the presence of cryopreserved AM and ADM.

Materials and Methods

In this comparative study, twenty-eight patients consisting of 15 females and 13 males (mean age = 58 years), referred from the Department of Prosthodontics, were included between August 2017 and March 2018. The inclusion criteria were a minimum bone height of 2 cm and no previous alveolar bone augmentation procedure in the mandible. Patients with medical contraindications, those who smoke or consume alcohol, and those who are on medications were excluded to avoid their interaction with wound healing. Approval for the study was obtained from the Iranian Registry of Clinical Trials (IRCT 2017082835160N1).

Informed consent, approved by the research ethics board, was signed by each patient prior to the surgical procedure. The inclination of the anterior wall of the mandibular symphysis and the alveolar bone height were determined using a lateral cephalogram and a panoramic radiograph, respectively [Figure 1].

In the first step, preoperative mandibular impression was taken, and a study cast was made for each patient for fabricating clear acrylic splints. The labial vestibular depth was increased 1 cm by scrapping the cast. Next, considering the inclination of the anterior wall of the body of the mandible, the splint was fabricated enough to cover the scrapped area, finished, and polished.

Two hours prior to the surgery, all patients received a preoperative dose of 2 g amoxicillin orally. After receiving local anaesthesia of lidocaine 2% with epinephrine 1/100,000, a labial-based mucosal supraperiosteal flap was released from the underlying periosteum through a horizontal incision of the mucogingival border, extending from the right to the left premolar area. The flap was then apically positioned and sutured with 4-0 Vicryl to the periosteum at the depth of newly created labial vestibule, which was 10 mm apical to the initial attachment. In each patient, the right and left exposed periostea were covered with cryopreserved human AM group (NEOX 100 Wound Allograft, AMNIOX) and ADM group (AlloDerm, BioHorizons), randomly. The splint, lined with the soft liner to establish minimal dead space, was fixed with two 7-mm bone screws [Figure 2]. Oral regimen of antibiotics of 0.5 g TID was continued for 7 days.

Preoperative and postoperative assessments were performed by a blinded examiner. After removing the splint, vestibular depth and infection of the areas were examined on days 7, 14, 28, and 90. The labial vestibular depth measurement was performed during follow-up sessions, at three points, with graduations of 0.1 mm: at the proximal and distal end of the grafts as well as the center. The average height of these three points was reported as the final vestibular depth in each area.

To evaluate the infiltration of macrophages into the wound site, tissue samples, harvested from the graft margins at days 3 and 7, were analyzed by flow cytometry. The collected

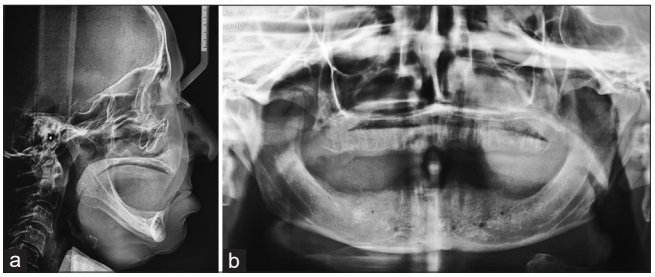

Figure 1: Preoperative radiographs: (a) Lateral cephalometric radiograph demonstrating the inclination of the anterior wall of the mandible symphysis. (b) Panoramic radiograph demonstrating adequate bone height for vestibuloplasty

samples were washed in phosphate-buffered saline (PBS), containing antibiotics (penicillin and streptomycin). After measuring the sample's wet mass, they were cut into 1–3 mm pieces. The pieces were added to PBS on ice and washed 3 times. These were digested with 1.2 IU of Dispase (Roche, Indianapolis) for 1 h at 37°C. Minced tissues were then incubated with trypsin-ethylenediaminetetraacetic acid 0.05% solution for 10 min at room temperature. The cell suspension was sieved through a fine mesh to eliminate clumps and segments. This was followed by centrifugation of dissociated cells at 400 g for 10 min at 2°C and resuspension of the cell pellet in fluorescence activated cell sorting (FACS) buffer. Next, to count the living cells, 10 µl of the sample was mixed with trypan blue solution and counted using a hemocytometer. The results gave an estimate of 2.8–3 × 10^6 cells/gram of tissue. Then, 1 × 10^6 of the obtained cells were washed with staining buffer for 5 min. Fc receptors were blocked, and cells were incubated with phycoerythrin-conjugated anti-CD68 monoclonal antibody (Y1/82A; mouse IgG2b) or appropriate isotype control antibody for 20 min. Once washed with the staining buffer, flow cytometric assay was conducted and all data were analyzed using FlowJo software (Flowjo LLC, Ashland, OR, USA).

All results were expressed as mean ± standard deviation. Statistical analysis was performed by GraphPad Prism v6.07 software (GraphPad Software Inc., San Diego, CA, USA). First, the Shapiro–Wilk test was used to evaluate the normality of data sets acquired from each experiment. Comparisons between the two groups were made by Student's *t*-test, and $P < 0.05$ was considered to be statistically significant.

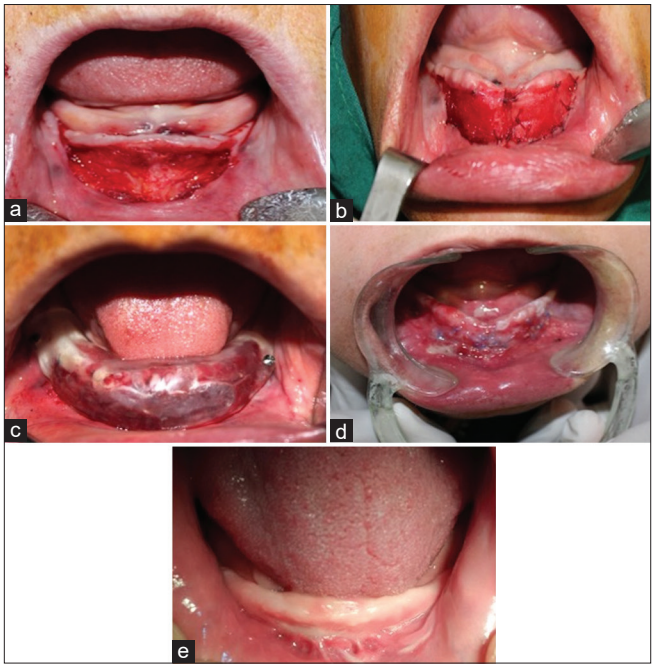

Figure 2: Vestibuloplasty: (a) Apically positioned supraperiosteal flap. (b) Suturing of amniotic membrane and acellular dermal matrix to denuded periosteum. (c) Fixing the lined splint by bone screws. (d) Surgical site 2 weeks postoperatively. (e) Surgical site 3 months postoperatively

Results

After 3 months, the tissues resulting from both membranes were clinically nonkeratinized and fixed to the underlying bone. There were no complications such as burning sensation, clinical evidence of infection, mental nerve paresthesia, or graft rejection on either side in all participants.

The Shapiro–Wilk test showed that the distribution of data collected from the vestibule depth and relapse in both types of the grafts was normal ($P > 0.05$). The results indicated that the reduction rate and vestibular depth loss were remarkably higher in the AM group comparing to that of the ADM group at different time intervals [Table 1]. Moreover, a similar trend of reduction in vestibular depth was observed on both sides [Figure 3]. These data suggested that during the follow-up period, ADM can maintain the vestibule depth more efficiently, resulting in less morbidity at the donor site.

Flow cytometry analyses confirmed the presence of $CD68^+$ in wound regions at different time intervals [Figure 4]. The Shapiro–Wilk test indicated that the distribution of all data obtained from flow cytometry analysis was normal ($P > 0.05$). The results were expressed as mean ± standard error of mean of absolute number of cells per gram of wet tissue. As it is shown in Figure 5, the frequency of wound-infiltrating macrophages ($CD68^+$ cells) per gram of collected tissue was significantly higher in the ADM group after 3 and 7 days ($P < 0.05$), suggesting that the wound healing procedure was initiated earlier in the presence of ADM.

Discussion

In the current study, the healing process in wounds covered by ADM and cryopreserved human AM as periosteum-covering membranes was investigated and compared. Since this study

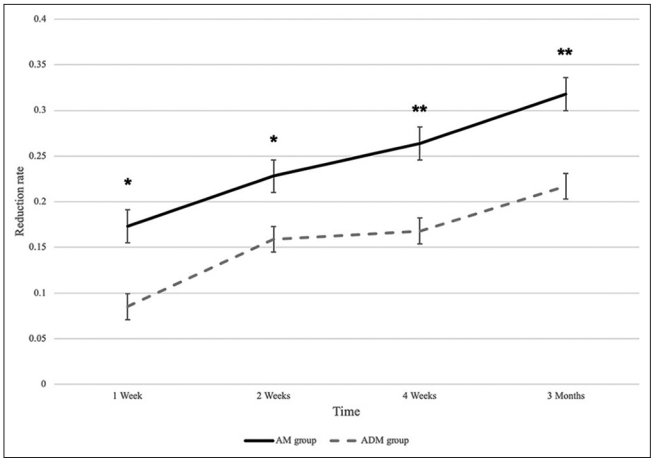

Figure 3: The trend of reduction rate in vestibule depth among AM and ADM groups. Assessing the relapse of vestibule depth showed that the reduction rate was significantly higher in amniotic membrane-covered regions than ADM group at four different points of follow-up within 3 months. The trend of reduction rate was similar in two groups. * and ** indicate $P < 0.05$ and $P < 0.01$, respectively.

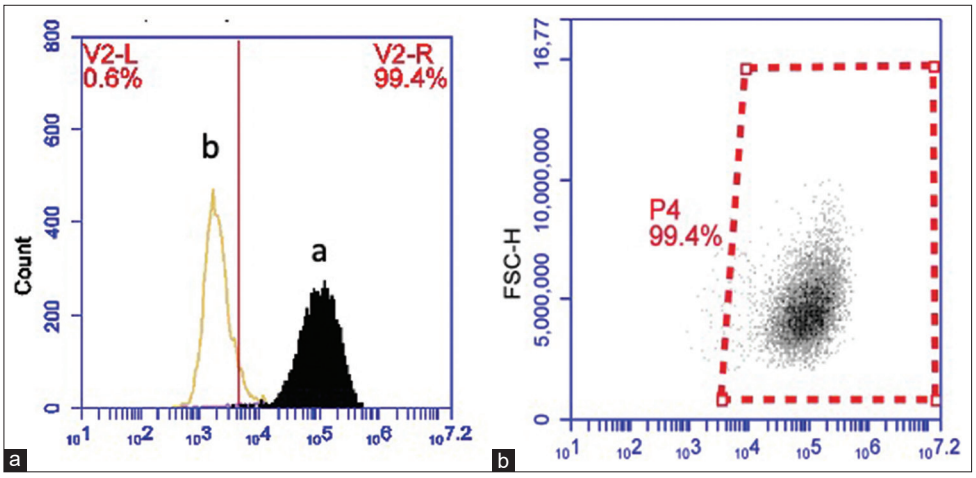

Figure 4: Cell surface marker expression profile of extracted cells. Flow cytometric analysis demonstrating that obtained cells can be categorized into negative (a) and positive (b) for CD68. Only representative examples are shown here

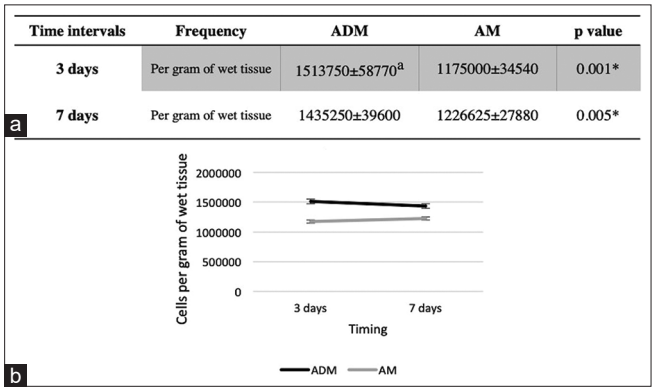

Figure 5: Macrophage infiltration assessment. (a) Number of macrophages at different sites and time intervals. (b) Representative data for CD68$^+$ cells based on the CD68$^+$ gate; the numbers indicate the absolute number of macrophages per gram of wet tissue. [a]Data are expressed as mean ± standard error of the mean for 28 patients/group. *indicates $P < 0.05$

Table 1: Reduction rate in vestibule depth at different time intervals

Time intervals	AM group	ADM group	*P*
1 week	0.173±0.021[a]	0.085±0.013	0.011*
2 weeks	0.228±0.016	0.159±0.017	0.032*
1 month	0.264±0.014	0.168±0.020	0.008**
3 months	0.318±0.022	0.217±0.017	0.007**

*P<0.05 and **P<0.01, respectively, [a]Data are expressed as mean±SEM of the reduction rate (n=28 patients/group). ADM: Acellular dermal matrix, AM: Amniotic membrane, SEM: Standard error of mean

was performed in a split-mouth randomized clinical fashion, it allowed a good comparison between these membranes regardless of the status of the participants.

There have been reports on covering the periosteum postoperatively in the oral cavity. Samandari *et al*. used fresh AM in vestibuloplasty. In this study, the clinical and histological assays revealed that fresh AM is an appropriate membrane for covering the denuded periosteum after vestibuloplasty, accelerating the healing procedure, and preventing the depth reduction in the buccal vestibule.[13] Similarly, fresh AM was used after mandibular vestibuloplasty in the studies of Kothari *et al.* along with Sharma *et al.* They reported that the use of AM results in less postoperative morbidity.[19,30] In a study conducted by Güler *et al.*, blood flow to the areas covered with lyophilized AM was measured after Clark's or Kazanjian's vestibuloplasty. Interestingly, it has been shown that it has promoted angiogenesis after 10–15 days.[31] Sikkerimath *et al.*, along the same lines, reported that the application of amnion as a graft material after Clark's vestibuloplasty maintained the buccal vestibular depth more effectively in comparison with areas not covered with amnion.[32]

Successful uses of ADM in gingival recession have been widely reported in the literature. They suggested that the usage of this covering material could provide appropriate keratinized tissue and complete root coverage.[28,33-35] In addition, in a split-mouth design study, Hashemi *et al.* found that the width of fixed tissue after vestibuloplasty was significantly lower in the ADM-covered area than that in the mucosal graft-covered areas. Nevertheless, there was no significant difference in the relapse of the depth of labial vestibule between the two groups.[29]

In accordance with previous reports, the results of the current study demonstrated that unlike the similar clinical results of both covering materials, the healing response at cellular level was initiated faster in ADM-covered periosteum. The porosity of the covering materials is one of the most crucial parameters that play a key role in cell migration and directing the tissue formation.[36-40] Furthermore, a scaffold with appropriate level of porosity can result in more efficient angiogenesis at the wound site.[13,37,38,41] The hypothesis that the pore size and microarchitecture within the ADM are more favorable for covering the periosteum was supported by the flow cytometry data. These observations indicated a higher level of infiltrating macrophages per mass of tissue, owing to the specific structure of ADM, providing a favorable anchorage for cell migration.

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Conflicts of interest

There are no conflicts of interest.

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