·Clinical Research·

Clinical and impression cytology findings of amniotic membrane and oral mucosal membrane transplantation for the management of socket contracture

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Abstract

• AIM: To investigate and compare the cytopathological and clinical effects of amniotic membrane transplantation (AMT) and oral mucosal membrane transplantation (OMMT) in socket contraction.

• METHODS: Twelve patients who could not be fitted with ocular prosthesis due to socket contracture were included in this study. Seven patients underwent AMT and 5 patients underwent OMMT. Thirteen patients who had healthy sockets were included as control group. Depth of inferior fornix, degree of inflammation, extent of the socket contracture and socket volume were measured in the preoperative period and at sixth and twelfth weeks postoperatively. Impression cytology of conjunctival fornices and tear transforming growth factor beta-1 (TGFβ1) levels were determined.

• RESULTS: In the AMT group, socket volume and lower fornix depth values were significantly higher (P=0.030 and P=0.004 respectively) and inflammation levels and impression cytology stages (P=0.037 and P=0.022 respectively) were significantly lower in postoperative period compared to preoperative period. In the OMMT group, no statistical differences were found in terms of clinical parameters, inflammation levels and impression cytology stages of preoperative versus postoperative values. Preoperative tear TGFβ1 levels were higher in AMT and OMMT groups compared to the control group (25.5 ng/mL, 26.3 ng/mL and 21.7 ng/mL respectively). Decreased tear TGFβ1 levels were observed in both the AMT and OMMT groups postoperatively (median decrease value=2.1 ng/mL and 2.7 ng/mL respectively).

• CONCLUSION: AMT is associated with postoperative improvement in inferior fornix depth, socket volume, inflammation and impression cytology levels and may be a more proper alternative method than OMMT in the management of socket contracture.

• **KEYWORDS:** socket reconstruction; amniotic membrane; oral mucosal membrane

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INTRODUCTION

Socket contracture is characterized by loss of fornices with scar formation and the presence of granulation tissue [1-3]. Histopathologically it is distinguished by inflammatory cell infiltration, conjunctival epithelial metaplasia, decrease of goblet cell density and increased keratinization [3,4]. Socket contracture may occur secondary to fibrosis, implant migration, chronic inflammation or infection [1-4]. Various surgical methods are used to address socket contracture; these include amniotic membrane transplantation (AMT), oral mucosal membrane transplantation (OMMT), the use of hard palate mucosal grafts and dermal fat grafts [4]. Socket reconstruction aims to create deep fornices and large conjunctival surface. In this surgery, providing adequate lid function and preventing the formation of fornix contraction or scar tissue is very important [4]. The most common reason for socket contracture in early postoperative period is the inadequate initial surgical dissection. Other causes include: excessive conjunctival loss due to thermal and chemical burns as well as radiation exposure. On the long run, chronic infection and inflammation are the most common etiologic factors for socket contracture[4].

AMT has been used with success in fornix reconstruction, symblepharon surgery, symptomatic conjunctivochalasis, primary and recurrent pterygium, entropion surgery, strabismus reoperations to prevent postoperative adhesions and for the treatment of ocular chemical and thermal burns, corneal and scleral ulcers, and bullous keratopathy [5-11]. AMT is thought to promote epithelialization, and reduce inflammation, vascularization and scarring[9,11].

Impression cytology as a safe, simple and noninvasive technique, plays an important role in diagnosis of ocular surface diseases and can be an alternative method to repeated biopsies[12-14].

TGFβ is a profibrotic cytokine and elevated levels of TGFβ may play an important role in collagen and extracellular matrix (ECM) deposition, wound healing and scar formation[15-18].

This study aimed to investigate and compare the cytopathological and clinical effects of AMT and OMMT use in socket contraction. Based on the role of fibrotic damage in pathogenesis of socket contraction, we also researched tear transforming growth factor beta-1 (TGFβ1) levels in contracted socket reconstructed by AMT and OMMT.

SUBJECTS AND METHODS

Subjects The study was undertaken at a university based hospital and was designed as a prospective longitudinal clinical study. Twelve patients who could not be fitted with ocular prosthesis due to socket contracture were included in this study. Seven of these patients underwent AMT and 5 of them underwent OMMT. The surgery groups were formed randomly. Thirteen patients who had healthy sockets and who could retain ocular prosthesis were included as control group to compare the preoperative socket characteristics of the surgery groups. This study was carried out with the Institutional Review Board/Ethics Committee approval. Informed consent was obtained from each patient. The research adhered to the tenets of the Declaration of Helsinki.

For all subjects, the lower fornix depth, degree of conjunctival inflammation, stage of socket contracture as well as socket volume were determined. To measure the depth of the lower fornix; sterile, soft, bendable rulers were used. Socket volume was evaluated in the supine position and measurements were taken by a 5 mL syringe containing saline solution. With the eyelids fully opened, saline solution was dripped into the socket bed and the value at which overflow began was noted.

Socket contracture staging was described by Krishna in 1980[6]. A narrow lower fornix was defined as stage 1, loss of upper and lower fornices was defined as stage 2, loss of all fornices was defined as stage 3, loss of all fornices and palpebral narrowing was defined as stage 4, presence of contraction secondary to previous reconstructions was defined as stage 5[6].

Conjunctival vascularization was graded as 0-3 according to the degree of conjunctival inflammation. No vascularization was defined as stage 0, presence of mild and superficial vascularization was defined as stage 1, medium and deep vascularization was stage 2 and the presence of intense and deep vascularization was defined as stage 3[12].

Tears were taken from the lower temporal fornix of the patients using capillary tubes. Tear TGFβ1 levels were determined quantitatively by enzyme linked immuno sorbent assay (ELISA) method with Biosource Multispecies Kit (Biosource International, NJ, USA). The technique is described in detail in a previous study[19].

Impression cytology technique Impression cytology samples were taken from the lower fornix of the study group patients. Impression cytology was carried out using a 25 mm diameter and pore width of 0.22 mm Millipore (cellulose acetate) filters [13-15]. The Millipore filter paper was placed and firmly pressed on the area of lower fornix bed for a period of two minutes following a topical anesthetic drop. Samples were not air dried and were stored in 20 mL containers containing 95% alcohol. Periodic acid-Schiff (PAS) or Papanicolaou (PAP) stains were used for cytological evaluation. The stained samples were evaluated by light microscopy according to Nelson staging including levels described as 0-3[13,14]. The stages were described as below:

Stage 0: Small round epithelial cells, eosinophilic PAS (+) cytoplasm, large basophilic core, the nucleus/cytoplasm (N/S)=1/2, abundant goblet cells.

Stage 1: Large polygonal epithelial cells, eosinophilic PAS (+) cytoplasm, small core, the N/S=1/3, few goblet cells.

Stage 2: A large, multi-nucleated epithelial cells were polygonal, slightly PAS (+) cytoplasm, small core, n/s=1/4-5, few goblet cells.

Stage 3: Large polygonal epithelial cells, basophilic cytoplasm, small pyknotic core, the N/S=1/6, no goblet cells.

Methods Patients underwent socket reconstruction with either AMT or OMMT surgical techniques. Briefly, in the AMT procedure, human amniotic membrane was obtained under a sterile condition from seronegative donors for HIV, HCV and HBV after an elective caesarean section and prepared as reported [9]. Eyelids were put on traction with 2-0 silk sutures, following which a horizontal incision through the inferior fornix was made. Subepithelial dissection was carried out as necessary. Hemostasis was achieved by micro-etching and subconjunctival scar and granulation tissue were removed. The cryopreserved amniotic membrane 2.5×2-cm^2 with its epithelial side up was sutured in the incision area with interrupted 8-0 vicryl sutures. The conformer was placed in the socket bed. Temporary tarsorrhaphy was performed to protect the conformer.

In OMMT, eyelids were put on traction using 2-0 silk sutures. Similar to the AMT technique, a horizontal incision in the inferior fornix was made and the incision was expanded through the deeper subconjunctival tissues. After achieving hemostasis, scar tissue and granulation tissue were removed. The lower lip was everted with 2-0 silk traction sutures and the mucosal epithelium was exposed and put on stretch. Following incision of the lower lip, an oral mucosal membrane was removed. After removing adipose tissue from the membrane, the 2.5×2-cm^2 graft was transplanted to socket

Table 1 Age, gender and socket contracture levels of subjects included in the study

Parameters	AMT group (*n*=7)	OMMT group (*n*=5)	Control group (*n*=13)	*P*
Age $\bar{x}$±SD (range)	44±12.1 (14-74)	60±14.2 (42-74)	35±13.3 (22-63)	0.241
Gender (F/M)	2/5	3/2	7/6	0.217
Socket contracture stage $\bar{x}$±SD	2.8±2.0	2.2±2.8	0.0	0.001

F:Female; M: Male; AMT: Amniotic membrane transplantation; OMMT: Oral mucosal membrane transplantation; SD: Standard deviation.

Table 2 Within and between groups comparisons of clinical findings and impression cytology stages of the study subjects at the time of enrollment into the study and postoperatively

Parameters	AMT group (*n*=7)				OMMT group (*n*=5)				[1]*P*		
	Preop.	Postop 6th week	Postop. 12th week	*P*	Preop.	Postop. 6th week	Postop. 12th week	*P*	Preop.	Postop. 6th week	Postop. 12th week
Lower fornix depth (mm)	3 (1-6)	4 (3-7)	5 (3-9)	0.004	3 (1-5)	4 (3-5)	4 (3-6)	0.211	[1]0.001	[1]0.001	[1]0.002
Socket volume (mL)	0.5 (0-1)	0.7 (0-2)	1 (0-2)	0.030	0.5 (0-2)	1 (1-2)	1 (1-2)	0.058	[1]0.001	[1]0.001	[1]0.001
Inflammation level	1 (1-2)	0 (0-1)	1 (0-1)	0.037	1 (1-2)	1 (0-1)	1 (0-1)	0.097	[1]0.001	[1]0.038	[1]0.326
Impression cytology stage	3 (1-3)	1 (0-3)	0 (0-3)	0.022	3 (0-3)	2 (0-3)	0 (0-3)	0.148	[1]0.001	[1]0.007	[1]0.270

AMT: Amniotic membrane transplantation; OMMT: Oral mucosal membrane transplantation; [1]The statistical difference between study groups according to the preoperative, postoperative 6th and 12th week values.

bed and sutured with interrupted 8-0 vicryl sutures. The primary donor site was closed with 2-0 silk sutures. Temporary tarsorrhaphy was performed to protect the conformer that was placed in the socket bed.

Different surgery procedures and suture materials can be used in socket reconstruction that were reported in such studies [5,7-9,20]. We often prefer absorbable vicryl sutures for AMT and OMMT for socket reconstruction as described above.

Statistical Analysis Data analysis was performed by using SPSS for Windows (version 11.5). Friedman test was used for changes over time among groups. Kruskal-Wallis test was used for the group comparisons at study time points. Descriptive statistics were provided for tear TGFβ1 values because the presence of dry eye precluded the acquisition of tear samples from a significant proportion of study subjects. Scoring was performed to evaluate the stages of impression cytology, socket contracture and inflammation. Median values (instead of mean values) were provided when the number of observations were insufficient. According to the results of statistical analysis, $P<0.05$ was regarded as significant.

RESULTS

Twenty-five patients were included in the study. Surgical intervention group consisted of 7 patients in the AMT group and 5 patients in the OMMT group. Control group consisted of 13 subjects who had healthy sockets. The mean age of all subjects was 44.1±17.7y (range 14-74y). The mean ages of study subjects were shown in Table 1. The stage of socket contracture ranged between 1-5 in AMT group and 1-4 in OMMT group. All patients in the control group had stage 0 socket contracture. There was a significant difference in the mean preoperative socket contracture stages between the study groups and control group ($P=0.001$) (Table 1).

The period of prosthesis use between groups was not significantly different ($P=0.17$) and ranged from 6mo to 50y in all groups. In the AMT group, evisceration surgery had been performed in 1 case due to glaucoma, in 2 cases due to trauma, in 2 cases due to chemical burns and in 2 cases due to choroidal melanoma. In OMMT group evisceration surgery had been performed in 2 cases due to trauma, in 1 case due to endophthalmitis, in 1 case due to basal cell carcinoma and in 1 case due to mucoepidermoid carcinoma. The primary indications for globe removal in the control group were trauma for 8 patients, choroidal melanoma for 4 patients, and phthisis bulbi for 1 patient. In the AMT group, 3 patients had previous history of socket surgery (OMMT in all patients). Three patients in OMMT group had received radiotherapy to the orbit. A history of continuous prosthesis dislocation had been identified for one patient in each surgery groups. No prosthesis dislocation had been identified in control group.

In the AMT group, postoperative values of lower fornix depths were significantly higher as compared to their respective preoperative values ($P=0.004$) (Table 2). The socket volumes demonstrated a significant increase during follow-up ($P=0.030$). In OMMT group, neither the lower fornix depths ($P=0.211$), nor the socket volume ($P=0.058$) parameters were statistically different between the preoperative versus postoperative time points (Table 2).

In the AMT group, the stage of inflammation and impression cytology levels during the postoperative follow-up were significantly lower than their preoperative levels ($P=0.037$, $P=0.022$). In the OMMT group, the level of inflammation and impression cytology stages did not reveal any significant changes during the follow-up period.

In both study groups, preoperative and postoperative 6th week median impression cytology levels ($P=0.001$, $P=0.038$) and inflammation stages ($P=0.001$, $P=0.007$) were significantly higher. However, at the postoperative 12th week, the median

impression cytology levels (P =0.270) and the level of inflammation (P =0.326) were not significantly different between groups (Table 2). Impression cytology stages did not reveal significant changes in 2 patients in AMT group and 1 patient in OMMT group after the surgical intervention.

Preoperative tear TGFβ1 levels in AMT, OMMT and the control group were 25.5 ng/mL, 26.3 ng/mL and 21.7 ng/mL respectively. In surgical intervention groups, preoperative tear TGFβ1 levels were higher compared to the postoperative 6th week and 12th week median tear TGFβ1 levels. Tear TGFβ1 levels could not be measured in 1 patient in AMT group and 2 patients in OMMT group who had severely dry sockets. However, a decrease was shown in postoperative tear TGFβ1 values as compared to preoperative values in each group. The median decrease in the tear TGFβ1 level was 2.15 ng/mL the in AMT group and 2.7 ng/mL in the OMMT group during the postoperative 6 weeks and 12 weeks. Median values (instead of mean values) were provided when the number of observations was insufficient. That is why we could not give P value for TGFβ1 levels.

Bleeding due to the process, granuloma formation, graft rejection and infectious complications did not develop in our cases. In the OMMT group 1 patient complained of oral discomfort. At the conclusion of this study, 1 patient in both intervention groups could not be fitted with ocular prosthesis because of persistent socket contracture despite the application of amniotic membrane or oral mucosal membrane. Nevertheless, success rates of prosthesis use were 86% in AMT group and 80% in OMMT group (P =0.7).

DISCUSSION

The successful revision of the socket depends on the removal of scarred and contracted conjunctival tissue. For optimum surgical outcomes, fornix depth and conjunctival surface area should be augmented with the use of appropriately sized grafts. The choice of grafts is determined generally by the surgeon's preference and include, buccal membranes, nasal mucosa grafts, followed less frequently by nasal septum or soft palate grafts[5-8].

Currently, oral mucosal membranes are the most commonly used grafts for socket reconstruction. The advantages of these grafts are; easily accessibility of grafts in sufficient size even for repeated procedures and a high stability [21]. However, the use of buccal mucosa requires the availability of donor tissue, necessitates a long operation time and may result in a discomfort in the donor tissue site. In addition, oral mucosa may be associated with a color mismatch of the graft[5,7,8].

Amniotic membrane has been frequently used for the reconstruction of the ocular surface [9]. The amniotic membrane has a thick basement membrane and avascular stroma. It promotes the differentiation of the goblet cells and survival of conjunctival epithelium [9,10]. AMT accelerates epithelialization, reduces inflammation, scarring and vascularization [9,10,15]. Amniotic membrane is used in pterygium surgery, treatment of ocular burns, glaucoma surgery, limbal stem cell transplantation, and in the surgical management of symblephara [9,10,22]. It is also used to decrease adhesions after extraocular muscle surgery particularly during strabismus reoperations [11].The findings of our study support the use of amniotic membrane for the correction of socket contracture.

The use of AMT is relatively straight forward and is not associated with iatrogenic trauma to the oral mucosa, does not require nasal intubation and is completed in a shorter time interval.

Impression cytology has an important role in diagnosis of ocular surface diseases such as dry eye, limbal stem-cell deficiency, conjunctival melanosis and melanoma. It is a safe, simple and noninvasive technique. Impression cytology is usually applied to conjunctiva, cornea and the limbal region. Superficial cell layers of the conjunctiva and cornea can be easily collected. Thus this technique can be an alternative method to repeated biopsies [11-13]. In our study, the conjunctival impression cytology samples from subjects who underwent AMT exhibited findings indicative of lower stages of impression cytology in the postoperative period as compared to their preoperative values.

TGFβ is a central profibrotic cytokine and has an important role in collagen and extracellular matrix (ECM) deposition, wound healing and scar formation [15-18]. Amniotic membrane use has been shown to suppress TGFβ production [14-17]. In our study, a decrease was shown in the postoperative median tear TGFβ1 levels in both surgical groups, supporting the antifibrotic role of amniotic membrane. The decrease was higher in OMMT group than the AMT group during the follow-up. OMMT seems to be more effective to reduce the TGFβ1 levels in contrast to anti-fibrogenic properties of amniotic membrane.

Patients with socket contracture who become prosthesis intolerant can have social, economic, and psychological problems. Professional lives of patients have been affected with incompatible prosthesis due to failure of the socket. Psychosocial and aesthetic concerns due to the anophthalmic sockets of the patients can be evaluated elaborately and good cooperation should be provided among the oculoplastic surgeon, prosthetic technician and the patient.

Kumar *et al* [5] emphasized that AMT could be used as an alternative method to OMMT because of its less morbidity, faster recovery, better prosthetic fit, with no observed contractures and donor site complications.

The application of AMT for the management of socket contracture has been reported by Poonyathalang *et al* [7] . In their study, in which twenty patients were followed up for an average duration of 13.6mo, 80% of patients had successful outcomes and no complication such as infection or graft

rejection were reported[7].

Bajaj *et al*[8] applied AMT and OMMT for socket failure to twenty patients. The study evaluated patients for the depth of the lower fornix, socket volume and observed similar results in both of the groups. Cosmetically and functionally acceptable results were obtained in cases of mild to moderate levels of anophthalmic socket contraction with AMT [8]. In a previous study, the use of AMT in contracted sockets was associated with a more favorable outcome as compared to buccal mucosa and nasal mucosal use[10].

Repeated surgeries, radiotherapy applied to the orbit, chemical burns, advanced socket stage at initial presentation, dry socket, the presence of fibrosis are some of the negative factors that may affect surgical success. In our study, no complications related to the two procedures occurred in the surgical groups.

In conclusion, our findings suggest that AMT for socket reconstruction is a clinically significant and proper method based on clinical parameters and impression cytology findings. To the best of our knowledge, our study is the first in literature which compared the effects of AMT and OMMT use in socket contracture utilizing histopathological findings and ocular tear TGFβ level. To demonstrate the effectiveness of AMT, more patients and longer follow-up of prospective studies are needed. AMT could be used as an alternative method to OMMT in socket contracture.

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