

Use of amnion as a graft material in vestibuloplasty: A preliminary report

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Objective. The aim of this study was to evaluate the clinical use of amnion as a biodegradable graft material for vestibuloplasty.

Study design. Seven subjects who had been referred for preprosthetic surgery underwent mandibular vestibuloplasty using Clark's technique and amnion as graft material. Fresh amniotic membrane was placed in the area and an acrylic splint was used with soft liner and 0.4-mm wires to cover the surgical site. The area was reexamined after 1 week, 2 weeks, 4 weeks, 3 months, and 6 months.

Results. A white necrotic soft tissue layer could be seen with underlying hyperemic tissue and an average reduction of 1 to 3 mm in the depth of the labial vestibule after a week. By the end of the second week, the necrotic layer had disappeared, leaving slightly hyperemic mucosal tissue under. By the third week, the graft area could be noticed but the amnion had completely degenerated and disappeared. After 4 weeks, the subjects could be referred for their prosthodontic treatment. The reduction in the depth of the buccal vestibule ranged from 17% to 40% after 6 months' follow-up.

Conclusion. Amnion might be used as a potential graft material for vestibuloplasty.
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Various materials have been used to cover the area of denuded periosteum after mandibular vestibuloplasty, such as mucosal and skin grafts. However, palatal grafts can provide only a limited amount of transplantable mucosa. Furthermore, they are associated with patient discomfort at the donor site. Skin grafts also have disadvantages including a lack of denture adhesion, the presence of hair, contraction of the graft, and an extensive graft donor wound in skin.¹

Fetal membranes are biodegradable materials that have been used as graft material since their first introduction in 1910.² Amniotic membrane is the innermost layer of the fetal membranes. It has a stromal matrix, a thick collagen layer, and an overlying basement membrane with a single layer of epithelium.³ The amnion has been used successfully as a wound dressing for burns and as a dressing to promote healing of chronic

ulcers of the leg.⁴⁻⁶ It has also been used in surgical reconstruction of artificial vagina, for repairing omphaloceles, and to prevent tissue adhesion in surgeries of the abdomen, head, or pelvis.^{2,7-10} The amnion has the following advantages: it promotes secondary epithelialization and angiogenesis,⁵ it is antibacterial,¹¹ and it causes no immune reactions.¹² It is also inexpensive and readily available in large amounts.

The use of amnion in vestibuloplasty has only been once reported by Güler et al,¹³ who concentrated on blood flow to the graft. The aim of this study was to evaluate the clinical use of amnion as a biodegradable graft material for mandibular vestibuloplasty.

SUBJECTS AND METHODS

Seven subjects consisting of 4 women and 3 men (mean age = 63 years) with no known systemic disease who had been referred from the Department of Prosthodontics for mandibular vestibuloplasty took part in this study. The institutional review board of our institution approved this study. Informed consent was obtained before an oral and maxillofacial surgeon began the operation.

Fresh amniotic membrane was provided from healthy seronegative mothers who underwent caesarian section. Small clean sections (6 × 10 cm²) of placenta were first rinsed with sterile saline and then kept in 400 mL of saline containing 1 000 000 IU penicillin at 4°C up to 24 hours.

All subjects received bilateral inferior alveolar nerve block injections of 2% lidocaine plus 1/100 000 epinephrine. Clark's technique¹⁴ was used for vestibuloplasty in the approximate area between the second

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Table I. Changes in the depth of the labial vestibule (mm).

Subject	Before surgery	After surgery	1 week	2 weeks	4 weeks	3 months	6 months
1	0	6	5	5	*	*	4
2	6	12	12	*	*	*	10
3	3	13	12	*	*	*	*
4	0	13	13	*	*	*	*
5	0	10	7	7	*	*	6
6	0	10	10	9	*	7	*
7	2	10	8	6	*	*	*

*Subject did not return for examination.

premolars, up to 10 mm below the alveolar crest. Then, an impression of the subject's alveolus was taken, using resin splints and soft liner to prevent formation of dead space.

Fresh amniotic membrane was placed in the area and sutured, with the mesenchymal side lying on the periosteum (Fig 1). The splint was covered with a topical gel of 3% tetracycline and placed on the alveolus before stabilization with 2 pieces of 0.4-mm wire. The splint was removed after a week.

The area was reexamined after 1 week, 2 weeks, 4 weeks, 3 months, and 6 months, and items such as pain, edema, hematoma formation, infection, and paresthesia were determined. The soft liner was replaced at regular intervals. The depth of mandibular labial vestibule was determined by measuring the height of the labial border of the splint with a special gauge of 0.01-mm accuracy by an oral-maxillofacial surgeon. In 2 subjects, an elliptical histological sample was taken from the marginal graft area with their informed consent at 1-week, 2-week, and 3-month intervals. Subsequent to fixation with formalin, routine hematoxylin and eosin staining was carried out, and 4-micron sections were cut.

RESULTS

Following the removal of the splint a week after the operation, a white necrotic soft tissue layer could be seen with underlying hyperemic tissue. An average reduction of 1 to 3 mm in the depth of the labial vestibule could be noted (Table I). By the end of the second week, the necrotic layer had disappeared, leaving slightly hyperemic mucosal tissue.

Three of our subjects did not return after the second week. A soft tissue layer similar to attached mucosa with the appropriate consistency was noticed 3 weeks after the procedure. At this time, the graft area could be noticed but the amnion had completely degenerated and disappeared. After 4 weeks, the subjects were referred for their prosthodontic treatment (Fig 2). By the third month, the graft area could not be distinguished from

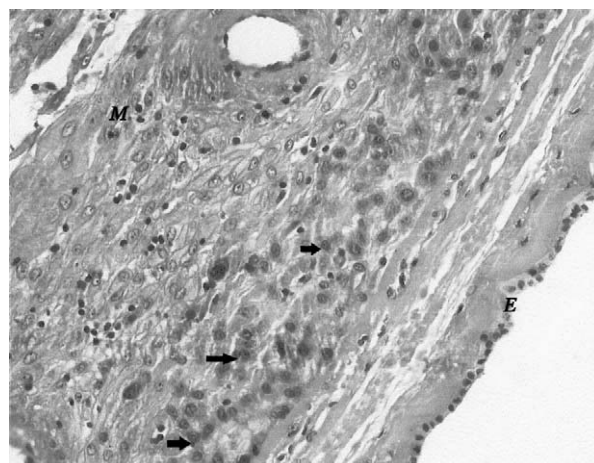


Fig 1. Amniotic membrane with its Ectodermal (E) and Mesenchymal (M) layers. The arrows indicate the undifferentiated mesenchymal cells; H & E stained, $\times 200$.

normal mucosa and an appropriate amount of attached mucosa had formed. The reduction in the depth of the buccal vestibule ranged from 17% to 40% after 6 months' follow-up. No complications such as infection, a burning sensation, or graft rejection were noted.

Histological examination of the 1-week sample showed viable and necrotic remains of the amniotic tissue attached to the underlying cells (Fig 3). Epithelium had started to migrate over the graft area from the margins and the underlying connective tissue showed evidence of granulation tissue formation. In the 2-week sample, the amniotic membrane had remained only in small sections in a few areas (Fig 4). Acanthosis and rete peg formation could be observed in the epithelial layer that was forming. After 3 months, epithelial tissue of appropriate thickness had completely covered the area with slight acanthosis (Fig 5). The underlying connective tissue consisted of fibrous components with a few inflammatory cells, indicating normal healing similar to oral tissues.

DISCUSSION

Amniotic membrane has unique properties including antiadhesive effects, bacteriostatic properties, wound protection, pain reduction, and epithelialization effects. Another characteristic of amniotic membrane is the lack of immunogenicity.¹⁵ Amniotic membrane has been used as a surgical material for several decades.¹⁶

There are only few reports in the literature on reconstruction of oral tissues using amnion. Lawson in 1985¹⁷ studied the use of amniotic membrane along with pectoralis major muscle for oral cavity reconstruction. He concluded that placement of amnion over the deep aspect of the muscle that is exposed in the oral cavity resulted in

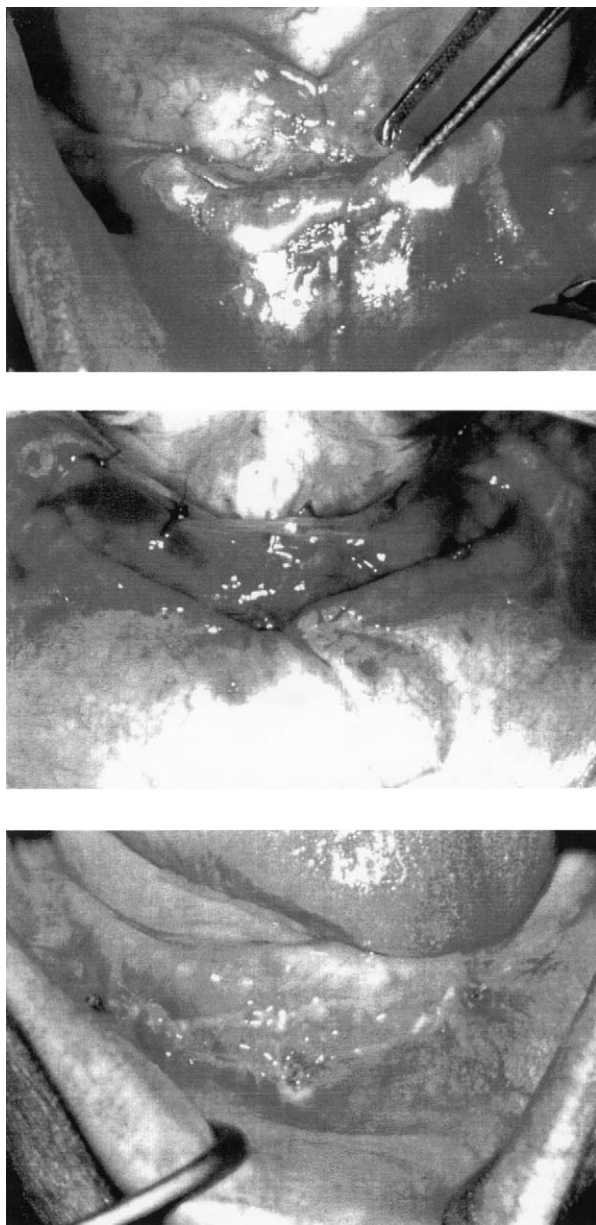


Fig 2. The alveolar ridge: 1. before surgery, 2. after placement of the graft, and 3. after the surgical procedure.

a more rapid development of mucosa. When muscle was used without amniotic membrane, the healing process usually took twice as long. Also, when amnion was not used, one saw a significant amount of wound contracture. Lawson suggested that amnion enhanced re-epithelialization of the oral cavity and reduced the contracture effects in moderate-sized defects.

Several characteristics explain why the amniotic membrane can be useful to promote epithelial healing. For example, the epithelium produces various growth factors and the basement membrane facilitates migration

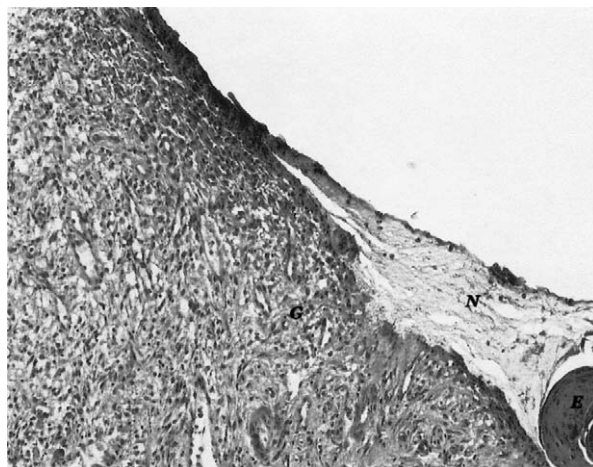


Fig 3. The graft margin 1 week after the procedure. Necrotic remainder of the graft (N) can be viewed on top of inflamed granulation tissue (G) with signs of epithelial migration (E); H & E stained, $\times 100$.

of epithelial cells, reinforces adhesion of basal epithelial cells, and may promote epithelial differentiation.⁵ The probable mechanism of amnion's antiadhesive characteristic is contact with healthy tissue that induces an arrest in tissue proliferation. In addition, the amniotic membrane transplant may also function as an anatomical barrier to fibrous tissue proliferation. Another unique characteristic of amniotic membrane is its lack of immunogenicity; the tissue does not express the usual major histocompatibility antigens such as HLA-A, B, or DR.^{15,18,19} As a result, amniotic membrane does not induce immunological rejection after its transplantation. The membrane, especially the epithelium, also produces various growth factors including basic fibroblast growth factor, hepatocyte growth factor, and transforming growth factor β .²⁰

Güler et al¹³ in 1997 concluded that grafts of amnion might be better than other grafts in mandibular vestibuloplasty because of early healing. In their study of blood flow to the amniotic grafts with 2 different surgical techniques, there was a rapid increase in blood flow during the first 10 days, whereas palatal grafts have shown a reduction in blood flow during the same period.²¹ They stated that the angiogenic function of the amnion occurred within the first 10 to 15 days and the blood flow returned to normal by 30 days after surgery.

Lai et al²² in 1995 used a single layer of fresh amnion for surgical treatment of oral submucous fibrosis. They observed no acute rejection of the graft, however there was a 62% decrease of interincisal distance in the range of 5 to 10 mm after 2 years of follow-up, compared with 50% and 38% decrease with split-thickness skin graft and pedicled buccal fat pad graft, respectively. The results showed that fresh amnion would not be effective

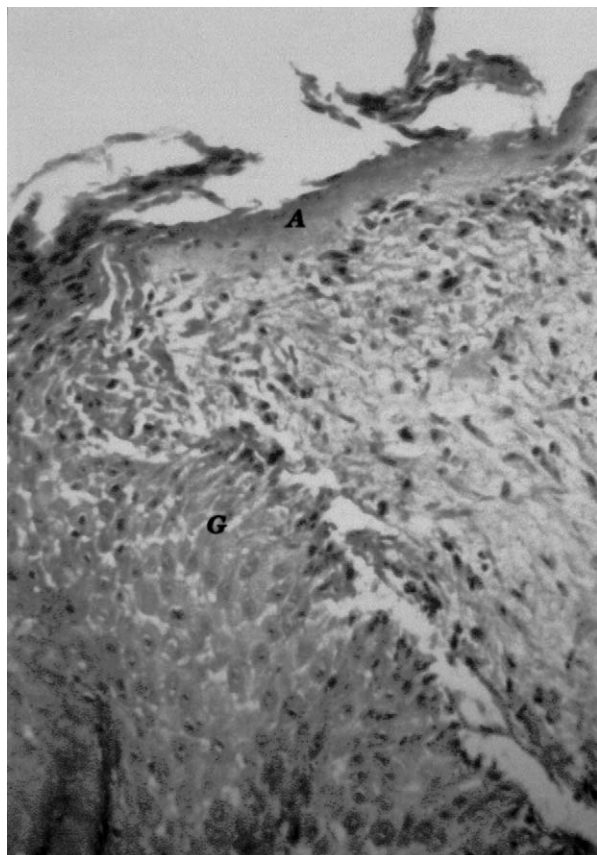


Fig 4. The graft margin 2 weeks after the procedure. A small segment of the amniotic membrane (A) that has not undergone necrosis is evident, with underlying inflamed granulation tissue (G); H & E stained, $\times 200$.

in a single layer over deep buccal clefts left after surgical treatment of submucous fibrosis.

Other materials have also been used for vestibuloplasty, such as a bilayer artificial dermis consisting of a collagen sponge with a silicone layer, which has been reported to produce similar results as with autogenous mucosa or skin grafts.²³ Raghoobar et al²⁴ in 1995 introduced the use of cultured mucosal grafts in vestibuloplasty, which were claimed to function as well as palatal mucosal grafts and eliminate donor site problems. Soft tissue profile changes²⁵ and residual ridge resorption²⁶ have been reported to be the same using either Edlan flap or mucosal and skin grafts. In another study, Gregory et al found no significant differences between fresh autogenous and freeze-dried allogenic skin after 6 months of follow-up.²⁷

Unfortunately, because of the small number of our subjects and the missing of 3 subjects after 2 weeks of follow-up, no statistical analysis was performed in this study. The reduction in the depth of the buccal vestibule ranged from 17% to 40% after 6 months follow-up; and,

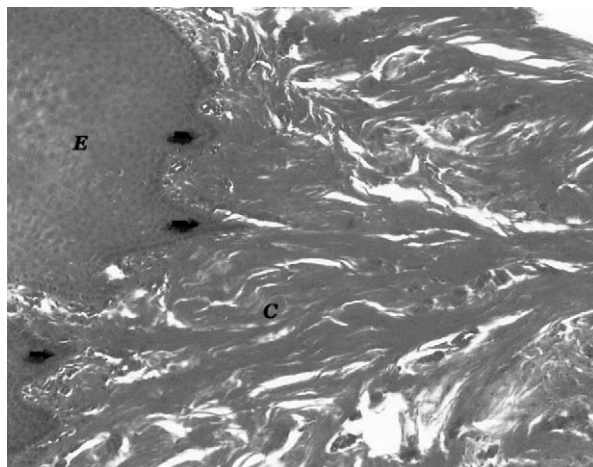


Fig 5. The area of graft 3 months after the procedure. Epithelial migration (E) with rete peg formation (arrows) can be seen adjacent to connective tissue (C) with slight inflammation; H & E stained, $\times 100$.

generally, the graft area could not be differentiated from nongrafted tissue after 3 months. No complications such as immunologic rejection and infection occurred in our subjects, and the prosthetic treatment could be started a month after the surgery. Histological assessment indicated normal healing in the presence of the amniotic membrane graft, similar to pattern of healing by secondary intention.

CONCLUSION

The results generally show that amniotic membrane might be a favorable graft material for vestibuloplasty, promoting healing and preventing relapse. However, further study with larger samples and longer follow-up is certainly recommended.

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