J Oral Maxillofac Surg
70:2221-2228, 2012

Clinical Application of a Hyperdry Amniotic Membrane on Surgical Defects of the Oral Mucosa

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Purpose: The aim of this study was to evaluate the usefulness of a hyperdry amniotic membrane (AM), a novel preservable human amnion, as a wound-dressing material for surgical defects of the oral mucosa.

Materials and Methods: A hyperdry AM was used in the treatment of 10 patients who had developed secondary defects in the tongue and buccal mucosa after the surgical removal of cancerous or precancerous lesions. The effectiveness of the hyperdry AM was assessed by scoring its operability during the surgical procedure and by the hemostatic status, pain relief, feeding situation, epithelialization, and scar contracture in the postoperative period. Its usefulness was evaluated by considering its effectiveness and safety based on the absence of wound infection and graft rejection.

Results: The membrane was found to be easy to handle as an oral-dressing material. It adhered well to the bare connective and muscular tissues. One lingual case showed slight postoperative bleeding, which astriction then stopped. No remarkable adverse effects were observed in the process of wound healing. The average score of the patients was 11.2 points (10 to 13 points) in the present evaluation, with 14 being the highest possible score.

Conclusions: This study showed the clinical usefulness of the hyperdry AM as an intraoral wound-dressing material. Although the number of cases was small, the results suggested that the hyperdry AM is biologically acceptable to oral wounds and could be a suitable clinical alternative for the repair of the oral mucosa.

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Raw wounds in the oral cavity are prone not only to infection but also to contraction by scarring and often need a proper dressing to prevent these complications. Autografts using free mucosal and split-skin grafts, which seem biologically ideal, have been used to cover raw wounds in the oral cavity.[1-4] Those grafts, however, require a separate surgical procedure at donor sites and often cause morbidity associated with delayed healing of the donor site. Donor site limitations in mucosal grafts are disadvantages of autografts, as are the low mobility, mismatching of color, and hair growth in skin grafts.

Through the ages, biological materials have been used to cover surgical defects that could not be closed primarily. From lyophilized porcine skin to fibrin and chitin membranes, different materials have been investigated in an attempt to develop ideal covers for oral wounds.[5-8] Each material has its advantages and

Received from the Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan.

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0278-2391/12/7009-0$36.00/0
doi:10.1016/j.joms.2011.09.033

disadvantages in biocompatibility and availability. Of these materials, bovine-derived collagen products have been used under various clinical situations in oral areas.[8-10] However, unlike allografts, the donors for which are selected by a series of screening tests for pathogenic microorganisms, those xenogenous materials require more severe processing during production to remove or inactivate unknown or unnoticed zoonotic pathogens. Such processing may decrease not only the pathogenicity and immunogenicity but also the desirable biological activities of the original materials.

The amnion is the innermost layer of the placenta. It is composed of 3 layers: an epithelial monolayer, a thick basement membrane, and underlying stroma.[11] The amnion has been considered a suitable tissue for allografts, based on its low immunogenicity.[12,13] It also possesses anti-inflammatory, wound-protecting, and scar-reducing properties. Preserved amnions have been used for decades in various clinical fields, including ophthalmology and wound care.[14-19] For preservable amnions, cryopreservation of amniotic membranes is the most common preservation method and it is used widely to treat various wounds. However, there have been some problems in the storage and sterilization of the material in this method. To resolve these problems, the authors developed a hyperdry amniotic membrane (AM), which is processed using far-infrared rays and microwaves and then sterilized by γ-ray irradiation (Fig 1).[20] This membrane can be stored at room temperature for a long period. The hyperdry AM has been applied clinically in the fields of ophthalmology, otology, and neurosurgery and has been a useful substrate to treat corneal perforation, bony surfaces of the mastoid cavities, and dural defects, respectively.[21-23]

The present study evaluated the usefulness of the hyperdry AM for covering surgical defects in the oral environment.

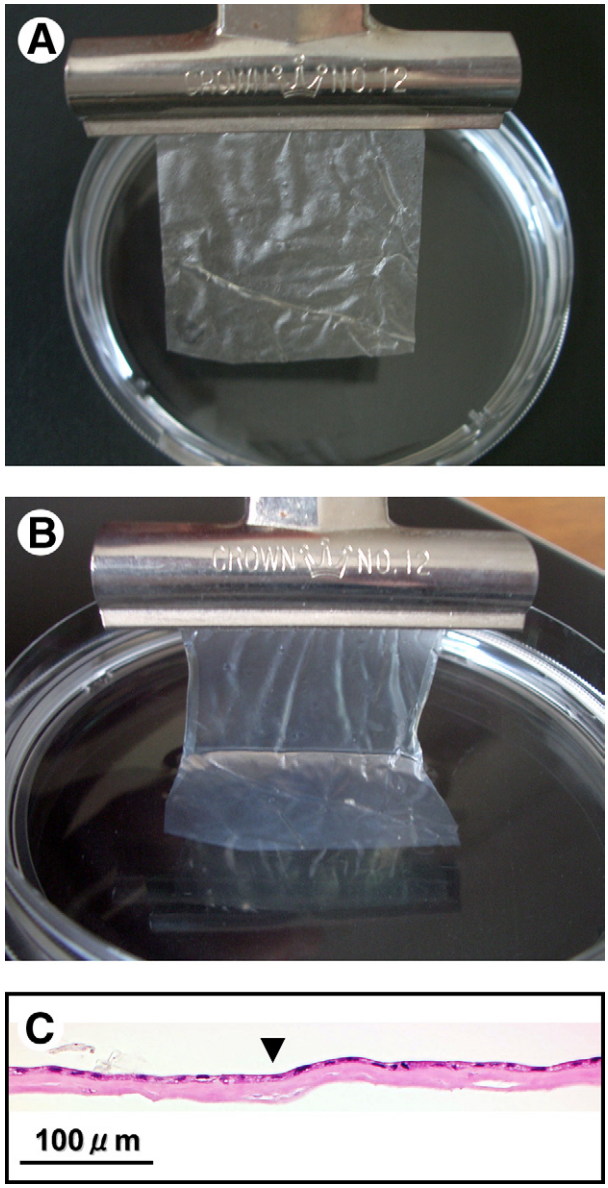

FIGURE 1. Macroscopic appearance of hyperdry amniotic membrane in the *A*, dry and *B*, hydrated states and *C*, histologic findings of the hydrated hyperdry amniotic membrane, including an epithelial monolayer (*arrowhead*) of the amnion.

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Materials and Methods

The hyperdry AM was prepared as follows. Fresh human amnions were obtained with the consent of donors who were seronegative for syphilis, human immunodeficiency virus, human T-cell lymphotrophic virus type 1, and hepatitis B and C viruses and who were scheduled to undergo cesarean section at Toyama University Hospital. The collected amnions were washed with sterile phosphate buffered saline without removing the epithelial cell layers. They were then dried under consecutive far-infrared rays and microwaves at temperatures lower than 60°C using a hyperdrying device (Sakura, Nagano, Japan). Thereafter, the amniotic membranes were cut into adequate sizes and were vacuum-packaged. For sterilization, the packages were irradiated with γ-rays (25 kGy).

This study was performed with the approval of the ethics committee of Toyama University according to the guidelines of the Declaration of Helsinki. All patients were informed of the risks and alternative treatments and underwent grafting of the hyperdry AM at Toyama University Hospital from June 2008 through April 2011.

Ten patients with oral cancer or precancerous lesions in the tongue or buccal mucosa were included in this study (Table 1). After excision of the oral lesions, a hyperdry AM was used to cover the second-

Table 1. CHARACTERISTICS AND OUTCOMES OF CASES TREATED WITH THE HYPERDRY AMNIOTIC MEMBRANE

Case	Age (yr)	Gender	Site	Size (mm)	Ope	Hem	Pain	Feed	Epi	Scar	Safety	Total	Usefulness
1	54	F	tongue	27 × 18	2	2	1	2	1	1	2	11	very
2	54	M	tongue	40 × 28	2	0	1	2	2	2	2	11	very
3	79	F	tongue	38 × 25	2	2	2	1	2	2	2	13	very
4	66	F	tongue	55 × 34	2	2	1	1	1	1	2	10	useful
5	70	M	tongue	30 × 23	2	2	1	2	2	1	2	12	very
6	78	F	buccal	34 × 24	2	1	2	1	1	1	2	10	useful
7	76	M	buccal	25 × 22	2	1	1	2	1	2	2	11	very
8	89	F	buccal	60 × 35	2	1	2	2	0	1	2	10	useful
9	74	F	buccal	40 × 30	2	2	2	1	1	1	2	11	very
10	72	M	buccal	40 × 40	2	2	2	2	1	2	2	13	very

Abbreviations: 0, poor; 1, fair; 2, good; Epi, epithelialization; F, female; Feed, feeding situation; Hem, hemostatic status; M, male; Ope, operability; Pain, pain relief; Scar, scar contracture.

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ary mucosal defects that were too large to close primarily. Wounds with bone exposure were excluded from this study. Before use, a membrane was cut into a suitable shape that was a little larger than the actual wound. It was then placed directly on the wound and stabilized using a pressure dressing of antibiotic ointment gauze (Fig 2). The pressure was removed approximately 1 week after the operation. Wound sites and sizes are listed in Table 1.

The effectiveness and usefulness of the hyperdry AM were evaluated by scoring the following parameters in the intraoperative and postoperative periods: operability, hemostatic status, pain relief, feeding situation, epithelialization, scar contracture, and safety. The scoring pattern for the hyperdry AM was determined by referring to the criteria described by Bessho et al[9] and Rastogi et al.[10] The result of each parameter was judged by 2 doctors, each with more than 10 years of clinical experience, as good (2 points), fair (1 point), or poor (0 point). The criteria for the judgment in this study are presented in Table 2.

The operability of the hyperdry AM was evaluated based on the operators' impressions during the operation of the handling properties in cutting and shaping and of the membrane's adherence to the wound surface. Hemostatic status was assessed postoperatively on the next day and after removal of the pressure dressing. An absence of bleeding was considered good, insignificant bleeding such as oozing was considered fair, and bleeding that required hemostatic intervention was considered poor. Pain relief soon after the removal of the pressure dressing during the disinfection procedure of the wound was examined as haphalgesia using a moistened benzalkonium chloride pledget. Pain relief was categorized based on the patients' own words as good (none to mild), fair (slight to moderate), or poor (severe). The patients' diets were changed from smooth to normal according to the time oral feeding commenced and the recovery time. It was categorized as good (oral feeding throughout the postoperative course, recovering to a normal diet within 2 weeks after surgery), fair (oral feeding throughout the postoperative course, recovering to a normal diet within 4 weeks), or poor (tube diet required, or recovering to a normal diet later than 4 weeks). Epithelialization was noted at 1 month after the operation and rated as good (entire wound), fair (nearly the entire wound), or poor (inadequate). Scar contracture of the wound was assessed at 2 months after surgery and categorized as good (none or little), fair (<50%), or poor (>50%). This was evaluated by comparing the degree of mouth opening and tongue movement before and after the operation.

The effectiveness of the hyperdry AM was judged by the total scores of these 6 parameters. A score of 10 to 12 was considered very effective, 7 to 9 effective, and 0 to 6 ineffective.

The safety of membrane use was assessed simply as good (2 points) or poor (0 point), depending on the absence or presence of an allergenic reaction and wound infection after grafting. When no signs of the reaction and infection were observed, it was judged good. Otherwise, it was considered poor even if intervention was not required.

The usefulness of the material was judged by summing the effectiveness and safety scores; 11 to 14 points was considered very useful, 7 to 10 points useful, and 0 to 6 points useless.

Results

The hyperdry AM is a semitransparent thin membrane. In the dry state, it possesses morphologic stability and therefore is easy to shape with scissors. Upon hydration, however, it thickens and becomes flabby, and its transparency increases. It is known that the histologic structures of hydrated hyperdry AM are similar to those of fresh amnions (Fig 1).

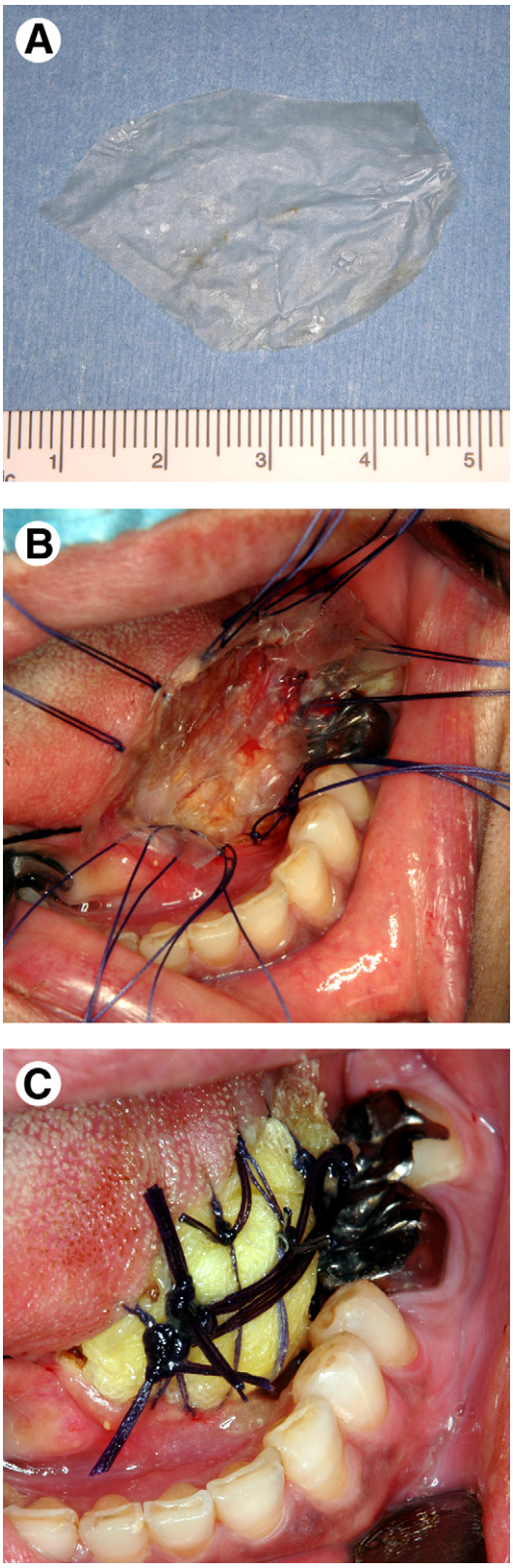

FIGURE 2. Photographs showing the placement procedure of the membrane. The hyperdry amniotic membrane was *A*, shaped with scissors, *B*, placed on the wound, and *C*, then stabilized using a pressure dressing.

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Table 2. CRITERIA FOR JUDGMENT OF THE HYPERDRY AMNIOTIC MEMBRANE

Score	Definition
Operability	
Good	easy to use
Fair	acceptable
Poor	impractical
Hemostatic status	
Good	no bleeding
Fair	slight bleeding, no hemostasis required
Poor	bleeding that required hemostasis
Pain relief	
Good	none to mild
Fair	slight to moderate
Poor	severe
Feeding situation	
Good	oral feeding, normal diet within 2 wk
Fair	oral feeding, normal diet within 4 wk
Poor	combination of tube diet
Epithelialization	
Good	entire wound
Fair	nearly entire wound
Poor	inadequate
Scar contracture	
Good	none or little (<25%)
Fair	slight (25-50%)
Poor	serious (>50%)
Effectiveness	
Very effective	score 10-12
Effective	score 7-9
Ineffective	score 0-6
Safety	
Good	no adverse effect
Poor	any adverse effect
Usefulness	
Very useful	11-14 points, no adverse effects
Useful	7-10 points, no adverse effect
Useless	0-6 points

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The mean follow-up period after the use of the hyperdry AM was 20.9 months (range, 3 to 36 mo). This study included 10 patients 54 to 89 years old. Of these patients, 5 had lesions on the lateral border of the tongue and 5 on the buccal mucosa. The wounds ranged from 25 to 60 mm in diameter (Table 1).

The hyperdry AM showed good operability in all cases. It was not only easy to cut and shape but also adhered well to the irrigated wound surface. By virtue of the transparency and good adherence of the hyperdry AM, the wound tissues could be clearly seen through the membrane (Figs 2B, 3A, 4A).

Hemostasis was generally good, and no obvious bleeding was observed in the buccal mucosa cases. Among the tongue cases, 1 showed bleeding after removal of the pressure dressing (case 2). The bleeding was slight and could be stopped by astriction using an absorbable local hemostat.

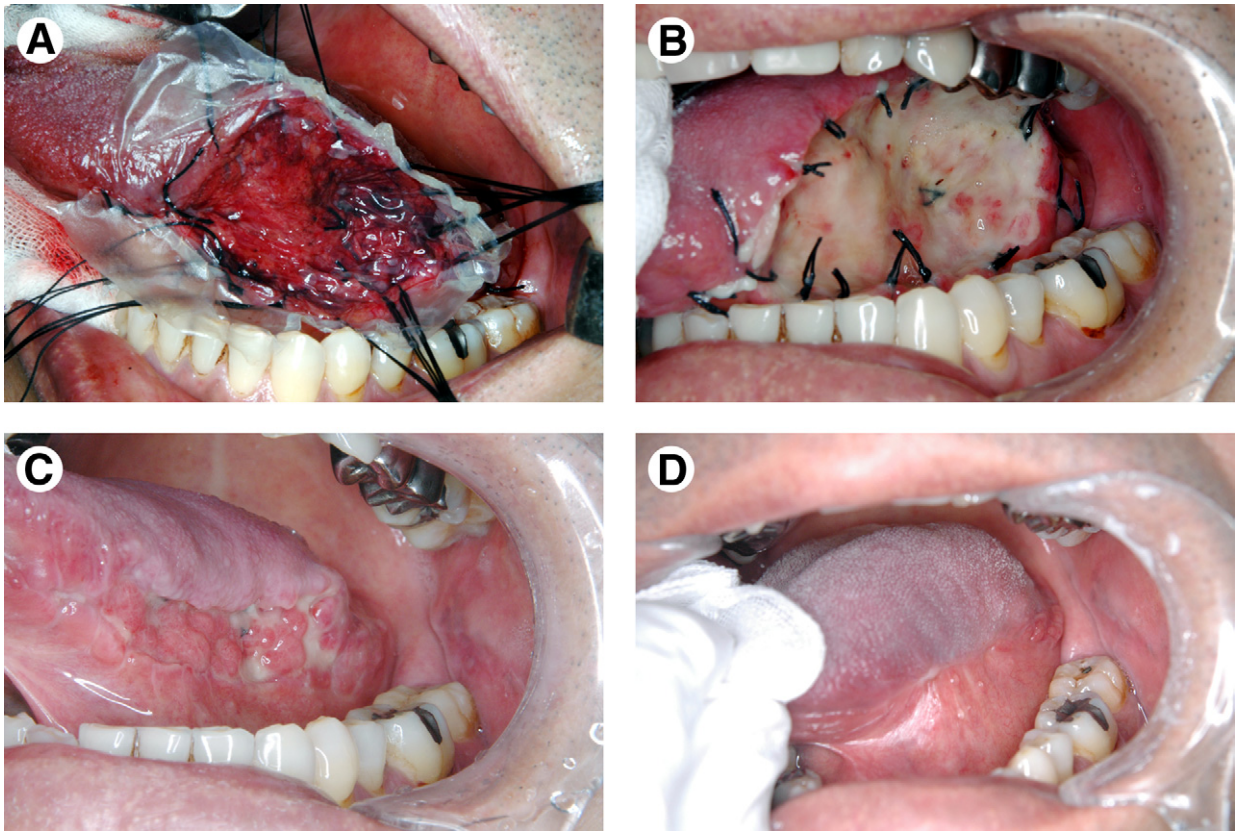

FIGURE 3. Photographs of a patient with a tongue lesion (case 2). *A,* Intraoperative findings of the hyperdry amniotic membrane placed on the wound. Intraoral findings at *B,* 6 days (just after removal of the pressure dressing), *C,* 12 days, and *D,* 32 days after surgery. *D,* Postoperative view showing wound repair with minimal contraction.

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Pain relief was good in 5 cases and fair in 5 cases. When the pressure dressing was removed at about 1 week after the operation, the wound surface appeared smooth and glossy. Although it was difficult to observe the membrane at this time, haphalgesia was absent or slight in all cases. In feeding situations, all patients were able to ingest orally and did not need another alimentation such as tube feeding.

Epithelialization was good in 3 cases, fair in 6 cases, and poor in 1 case. For scarring contracture, 4 cases were good and 6 were fair. Epithelialization was poor in 1 buccal case with the largest wound among the cases (case 8). It took about 6 weeks until the entire wound was epithelialized, whereas the scar contracture was slight in this patient.

Based on the results of these parameters, the hyperdry AM was very effective in 3 patients, effective in 7, and ineffective in none. None of the patients showed any allergenic reaction or wound infection locally and in hematologic examinations or complained of any notable symptoms such as dysesthesia. Therefore, of the 10 patients examined in the study, the hyperdry AM was very useful in 7 and useful in 3 (Table 1). The average score of the patients was 11.2 points (range, 10 to 13 points) of a possible 14 points.

The intraoral appearance of representative tongue and buccal mucosa cases is shown (Figs 3, 4).

Discussion

Raw wounds of the oral mucosa, like any other wound, generally heal by granulation and after epithelialization. It is known that the incidence of infection and the degree of contraction caused by scarring are decreased when wounds are covered by biological materials rather than uncovered or covered by nonbiological materials.[24-27] Thus, biological coverings that remain stable for an adequate time may be useful for wound healing. Unlike other organs such as the skin, grafts to oral wounds have some special problems. First, the oral environment is always wet by salivary secretion and food ingestion. Second, there are constant movements of the cheek and tongue by articulation, mastication, and deglutition. These factors may interfere with the adherence and retention of the graft materials, resulting in the failure of epi-

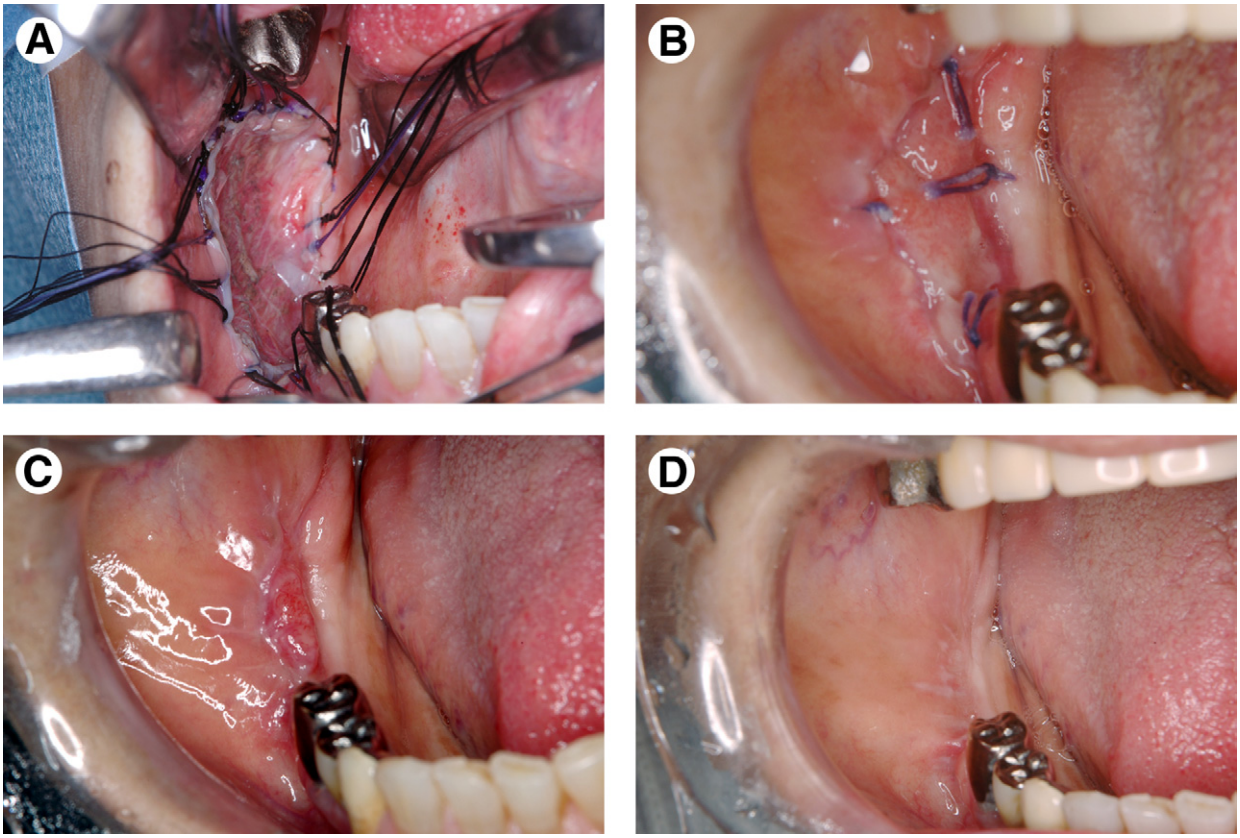

FIGURE 4. Photographs of a patient with a buccal mucosa lesion (case 6). *A,* Intraoperative findings of the hyperdry amniotic membrane placed on the wound. Intraoral findings at *B,* 14 days, *C,* 24 days, and *D,* 52 days after surgery. *B,* Some sutures were left for indications of the wound border. *D,* Postoperative view showing wound repair with minimal contraction.

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thelialization and in the formation of scar tissues. Impaired healing of large mucosal defects can be a source of discontent to patients because oral functions are highly sensitive to contraction caused by scarring.[9,28]

The usefulness of the hyperdry AM was examined for the healing of secondary defects of the lingual and buccal mucosa by assessing operability, hemostatic status, pain relief, feeding situation, epithelialization, scar contracture, and safety. In the present method, the wound is protected not only by the membrane but also by a pressure dressing for the first week after surgery. To understand the protective effect of the hyperdry AM itself, the degree of pain was examined after the removal of the dressing gauze. The results indicated that pain relief was good or fair in all patients, and that all were capable of oral ingestion throughout the postoperative course. The bleeding observed in a tongue case was slight and stopped easily by astriction, but this result was judged as poor because a hemostatic procedure was necessary. Because it occurred after the pressure dressing had been removed and after the patient had eaten, the masticatory force and direct stimulation by ingested food might have been involved in this adverse event. Epithelialization was judged poor in 1 buccal case; this patient was the oldest and had the largest wound among the patients. It is noteworthy that, although this patient needed much more than 1 month to complete the epithelialization of the relatively large buccal wound, the degree of scar contracture was judged as fair. Operability, pain relief, feeding situation, scar contracture, and safety were good or fair in all patients. The number of cases was small in this study, but the present results showed that the hyperdry AM is a useful graft material in the oral cavity, without any abnormal reactions.

Among the various wound dressing materials that have been used in the oral area, autogenous skin grafts[3,4] and xenogenous collagen products[8-10] seem to be predominant. Autogenous grafts are immunologically ideal graft materials. However, the quantity available for grafting is limited and a certain degree of donor site morbidity is inevitable. Collagen-based biomaterials, especially bovine xenogenous collagen sheets, have been used widely in oral surgery.[8-10] The structures of type I collagen are well conserved in animals. The homology of amino acid sequences be-

tween humans and cows is high (98% in the α_1 chain and 93% in the α_2 chain).[29] Collagen materials have also been proved to possess biocompatibility, good conformability to the mucosa, and a hemostatic effect.[30-32] In contrast, collagen membranes undergo slow collagenolysis by an inflammatory reaction. This lysis is compounded by the oral environment, which is characterized by moisture and constant movement. The weakening of collagen can be controlled by crosslinking, although it may change the original properties of the collagen.

The hyperdry AM is processed consecutively by far-infrared rays and microwaves and then sterilized by γ-ray irradiation.[20] During the drying process, the temperature inside the hyperdrying device does not exceed 35°C. Therefore, only the most superficial surface of the AM is heated to 60°C. Although the data are unpublished, the authors previously confirmed that the structures of collagen in the stroma are not destroyed by hyperdrying. The hyperdry AM possesses several advantages as a product. It can be cut easily to the desired size and shape with scissors just before application. It can be preserved in a dry state at room temperature without a time limitation. Moreover, it returns to a layered structure similar to that of fresh amnion when it absorbs water, suggesting that the membrane may have sufficient strength. A dried amnion produced by a freeze-drying method has been recently reported to be clinically useful in the ophthalmologic field.[33,34] Those lyophilized amnions were, like the hyperdry AM, sterilized by γ-ray irradiation and allowed long storage at room temperature. Upon hydration, however, the freeze-dried amnions did not recover their thickness, and their histologic appearance was no longer similar to that of a fresh AM.[21]

The hyperdry AM is histologically composed of an epithelial monolayer, a thick basement membrane, and a collagen-rich stromal layer.[11,21] As described above, collagen conforms to wounds and has a hemostatic effect on the wounds. In this study, therefore, this 2-sided hyperdry AM was placed on surgical defects of the oral mucosa so the stromal layer faced the wound surface. This membrane adhered well along the irregular surfaces of the wounds. It is still unclear how and how long the membrane remains on the wound surface in the oral environment. Upon removal of the pressure dressing, however, the wound surfaces were very smooth, with little or no haphalgesia in any of the patients. These findings suggest that the membrane may be adherent to and present on the wounds even after the pressure is removed and the wound is exposed to the oral environment. The adherence of the hyperdry AM is thought to be a result of a fibrin-collagen interaction, because a fibrin-like whitish substance appeared beneath the smooth wound surfaces (Fig 3B). The stability of the hyperdry AM in the oral environment remains to be elucidated. This membrane keeps its strength and morphology at least 1 month in vitro when soaked in sterilized physiologic saline solution at room temperature. Further investigation is needed to evaluate whether the membrane is robust enough to resist the masticatory and salivary effects for a sufficient time and is biodegradable for subsequent repair and maturation of the mucosal tissues.

The amnion, which is a source of the hyperdry AM, has been reported to possess properties such as the suppression of inflammation and neovascularization, the inhibition of scarring, and the promotion of re-epithelialization.[35] Preliminary studies have shown that some of these properties might remain in the hyperdry AM. From an allogenous perspective, the hyperdry AM does not fulfill all the requirements of an ideal autograft. It is therefore advocated as a dressing biomaterial in the oral cavity when a patient is not indicated for autogenous skin grafting but needs more biological activities than other materials, such as xenogenous collagen products.

In conclusion, cases of secondary defects of the tongue and buccal mucosa were successfully treated using a new type of dried AM called the hyperdry AM. The results showed that the membrane was useful for all patients examined in this study. This method may become an alternative treatment to manage surgical wounds in the oral cavity, not only in the tongue and buccal mucosa but also in other regions, such as the vestibule, palatal mucosa, and floor of the mouth.

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