

## Repair of Mucosal Defects With Atelocollagen and Its Indications

Koji Yoshiga, Takeshi Ichikawa, Yoshiaki Kayada, Shinobu Miyauchi, Masanori Tsumura,  
Kazuhiro Sakurai, Masanori Kunishi and Kazuaki Takada

(Received for publication, September 30, 1995)

### Summary

In this study, we assessed its indications and usefulness in treating tissue defects in the oral area using artificial skin composed of atelocollagen in fiber form processed by briefly heat-crosslinking collagen (Terudermis®) in 13 patients with oral mucosal defects. Good tissue repair was achieved in all of patients in which this material was indicated.

The course of healing was faster than when conventional tissue covering materials are used, suggesting that new surgical procedures may be developed using Terudermis®. No increases in the IgA and IgG antibodies were monitored.

No complications were noted and antigenicity was low. Thus, Terdamis R appears to be a useful biomaterial for a variety of applications in the oral area.

### Introduction

Artificial organs and artificial biocompatible materials have been studied extensively in recent years. The frequency with which artificial biocompatible materials are used to treat skin defects caused by trauma, burns, etc., is increasing, and there is a desire for medical materials with higher affinity.

Until now collagen non-woven fabric, and chitin non-woven fabric, and Biobrane, etc., have been available to temporarily cover skin defects as a means of preventing loss of exudate fluid and preventing bacterial infection, and

poly-L-leucine sponge, etc., is also available to cover and protect wound surfaces for a limited period of time.

In contrast, materials are also available which are gradually replaced by autologous tissue. A great deal of research has been conducted on wound covering materials using collagen for this purpose, and such materials are now available for clinical use.

However, since these covering materials inhibit the breakdown of collagen in the body, collagen's high affinity for cells and tissue has been reduced by subjecting it to the proper degree of crosslinking.

As a means of solving these problems, briefly heat-crosslinked fiber atelocollagen compounds have been developed as opposed to collagen derived from the body.

Mucosal defects often occur in the oral region as a result of trauma or surgery, and up until now such defects have been treated with temporary covering material such as pig skin, fibrin membrane, sheep membrane, chitin membrane, etc., or with autologous skin or mucous membrane grafts. In this study, we used a bilayer material produced by attaching heat-crosslinked atelocollagen and heat-denatured atelocollagen to a silicone membrane as the artificial oral mucosa, and assessed its usefulness and indications.

### Material and Methods

#### 1. subjects

The subjects of this study were 13 patients with defects of the oral mucosa. There were 7 men and 5 women who ranged in age from 1 to 74 years. Eleven patients were treated for defects in the mucosa overlying the palate and alveolar sockets and two were treated for defects in mobile mucosa.

The indications were palatine mucosa and periosteal defects following palatoplasty for cleft palate in 4 patients defects after resection of tumors of the buccal mucosa in 2 patients, maxillary erythroplakia, oronasal fistula following

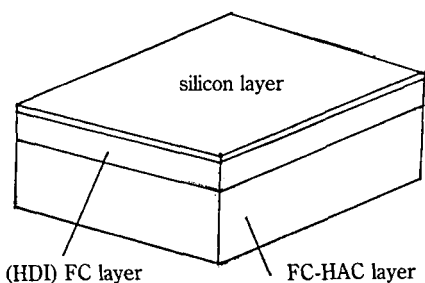
---

Hiroshima University, School of Dentistry, Department of Oral and Maxillofacial Surgery I (Chairman; Kazuaki Takada)

Correspondence of Dr. Koji Yoshiga, Hiroshima University, School of Dentistry, Department of Oral and Maxillofacial Surgery I, Kasumi 1 chome, Minami-Ku, Hiroshima City 734, Japan.

**Table 1** the cases underwent the treatment of artificial mucousmembrane

No	Name	Age	Sex	Diagnosis	Location	Tissue Defect
1.	A.F.	71	m	amyloidosis	palate	mucousmembrane
2.	T.Y.	1.5	m	Cleft Palate	palate	mucoperiostium
3.	Y.Y.	1.0	m	Cleft Palate	palate	mucoperiostium
4.	K.M.	1.10	m	Cleft Palate	palate	mucoperiostium
5.	K.C.	1.0	f	Cleft Palate	palate	mucoperiostium
6.	A.Y.	12	m	Oronasal Fistula	palate	mucoperiostium
7.	N.N.	37	m	Palatal Tumor	palate	mucoperiostium
8.	M.S.	70	f	Erythroplakia	palate	mucousmembrane
9.	M.S.	70	f	Erythroplakia	alveole palate	mucoperiostium
10.	M.S.	70	f	Erythroplakia	alveole palate	mucoperiostium
11.	M.H.	74	m	Carcinoma	buccal	mucousmembrane
12.	S.T.	63	m	Leukoplakia	buccal	mucousmembrane
13.	T.Y.	20	f	Oroantral Fistula	Alveole	mucousmembrane alveolar bone sinus membrane

**Fig 1** The shema of double layers of atelocollagen

surgery for a cleft lip and palate, and defects after resection for palatine tumor, palatine amyloidosis and oroantral fistula after tooth extraction in one patient each.

The depth of the wound defect was subperiosteal in 9 cases, above the muscle layer in 2 cases, supraperiosteal in 1 case, and below the mucosa of the maxillary antrum in 1 case.

## 2. Test material and surgical procedure

Fiber atelocollagen (FC) was obtained using bovine skin collagen as the source material. Enzyme-solubilized atelocollagen from which the highly antigenic telopeptide had been removed was used as the raw material, dissolved in a 2 mM aqueous hydrochloric acid, neutralized with 37°C phosphate buffer, and FC was obtained. Heat-denatured atelocollagen (HAC) was obtained by heating an

atelocollagen hydrochloric acid solution at 60°C for 30 min. The FC solution was lyophilized, made into sponge form, and dehydrated, heat — crosslinked with 110°C in a vacuum for 24 hr, and crosslinked fiber atelocollagen was obtained, which was bilayer material attached to silicon (Dawcorning Co.). there are three size of material, 10×10 mm, 5×5 mm, 5.0×2.5 mm and the thickness is uniform, 6 mm. The articles was packed in an aseptic condition.

**Surgical procedure:** The artificial mucousmembrane was chosen according to the size of mucousmembrane defects after surgery. The material was trimmed in the shape of tissue defect, adapted to the wound and sutured to the intact mucousmembrane. In the case of cleft lip and palate, two application method were induced, that in the case with or without palatal plate after surgery. In the case of a large tissue defect in the thickness, a bilayer material without the silicon layer was packed to a wound excessively. A case of oroantral fistula after tooth extraction was treated without mucosal flap, that after enough hemostasis in bottom of a wound adjacent to maxillary sinus a bilayer atelocollagen was sustained with absorptive thread and a normal bilayer material was inserted into the residual wound area and sutured to the gingival margin with silk. No protection was provided on top of the artificial mucous membrane.

### 3. The following factors were evaluated in each patient:

- 1) Detachment or peeling of the silicone membrane
- 2) Hemostasis or bleeding
- 3) Postoperative analgesic effect (rated on a 4-point scale: extremely good, good, fair, poor)
- 4) state of epithelialization (rated on a 4-point scale: extremely good, good, fair, poor)
- 5) contraction of the wound area (none, slight, moderate, severe)
- 6) postoperative infection

### 4. serum titer of antibody to bovine skin

Changes in titers of antibody to bovine skin were measured as described below when artificial mucous membrane was repeatedly applied to the same patient.

Study of human antibody response to bovine collagen, main component of TERUDERMIS, by ELISA (enzyme-linked immunosorbent assay)

#### Schedule of blood Samples

1993	3	4	First-grafted TERUDERMIS
1993	7	25	Second-grafted TERUDERMIS
1993	8	10	Blood drawn at 16 days after second-grafting (sample 1)
1993	8	26	Blood drawn at 32 days after second-grafting (Sample 2)
1993	9	30	Blood drawn at 67 days after second-grafting (Sample 3)
1993	10	17	Blood drawn at 12 days after second-grafting (Sample 4)

#### Preparation of serum samples

Blood samples were collected in tubes free of heparin and then were allowed to clot at room temperature for 2 hours.

The serum was separated by centrifugation, added  $\text{NaN}_3$  to make a final concentration 0.1% and stored at 4°C until using.

Blood samples were taken from a patient followed the schedule in above.

#### ELISA (enzyme-linked immunosorbent assay)

The technique for ELISA followed the basic protocol outlined below modified from Voller et al (1979)<sup>1</sup> and Frank et al (1991)<sup>2</sup>

#### Reagents:

#### Antigens:

Bovine collagens were purified from KOKEN crude atelocollagen

Type I collagen (purity 95%)

$\alpha 1$  (I)

$\alpha 2$  (II)

Type III collagen (purity 86%)

Porcine collagens were purified from NITTA Cellmatrix I-C

Type I collagen (purity 96%)

Type III collagen (purity 67%)

Human collagens were purified from Hoechst pepsin-solubilized collagen

Type I collagen (purity 100%)

Type III collagen (purity 77%)

Rat collagens were prepared from rat dermis

Type I collagen (purity 90%)

Type III collagen (purity 80%)

The purities were measured by SDS-polyacrylamide 5% gel electrophoresis.

Enzyme — conjugated secondary antibody:

Affinity purified goat anti-human IgG, and IgA specific antibodies were conjugated with horseradish peroxidase, Zymed Laboratories, Inc.

#### Process:

1 mg/ml solution of antigens in 3 mM HCl were diluted 1:100 with 0.1 M carbonate buffer, pH 9.6, immediately before use. The diluted antigen solution were then coated to E.I.A. 96 wells plates (Linbro/Titertek, Flow Laboratories, Inc.) These were accomplished by adding 100  $\mu$ l of the freshly diluted antigen solutions to each well of the plates and incubating at 4°C overnight. The whole added antigens were adsorption onto the well surface after this incubation. The coated plates were washed three times with T-PBS, added 300  $\mu$ l of 1% BSA per well and incubated at room temperature for 1 hour for the purpose of blocking sites non already coated with, thus decreasing any subsequent nonspecific binding of serum proteins. A part of sera were diluted 1:9 with 1% BSA and 100  $\mu$ l of the diluted serum per well was added to the plates removed the blocking BSA. The plates were incubated at room temperature for 2 hours and then washed three times with T-PBS to any unbound antibody. The wells were next exposed to 100  $\mu$ l of an enzyme-conjugated secondary antibody diluted 1:1000 with 0.1% BSA free of  $\text{NaN}_3$  incubated at room temperature for 2 hours and washed three times with T-PBS.

Substrate for the conjugated enzyme, 200  $\mu$ l of a 0.4 mg/ml solution of o-phenylenediamine in 0.1 M citric acid-sodium phosphate (pH 5.0) with 50  $\mu$ l of 5%

hydrogen peroxide per 25 ml of *o*-phenylenediamine solution as catalyst, was incubated in the plates at room temperature for 5–10 minutes until solution in wells were changed to yellow. The color reaction was quenched by adding 50  $\mu$  l of 10 NH<sub>2</sub>SO<sub>4</sub> to each well. Absorbances were read at 492 nm with a Labsystems iEMS reader.

## Results

Artificial mucous membrane was used in 13 patients. The diseases were treated with this material were as follows: mucoperiosteal defect wounds after Palatoplasty in 4 cases, residual fistulous opening in cleft lip and palate and mucoperiosteal defect wound after cancellous bone grafting in 1 case, a suprapariosteal mucosal defect after excision of palatal amyloidosis in 1 case, mucoperiosteal

defect after resection of a benign palatal tumor, and mucoperiosteal resection was performed in three stages to treat extensive erythroplakia involving the alveole, the palate, the gingivobuccal sulcus, and it was used to cover the defects. It was also used to treat mobile mucous membrane in the form of a defect above the buccal mucous membrane muscle layer, and artificial mucous membrane was used for closure of an oroantral fistula associated with an extensive bone defect. (this operation differed from conventional surgery in that no surrounding mucosal flap was used).

The minimum, maximum and mean area of the artificial mucous membrane used in this study was 1.5×1.5 cm, 7.5×2.0 cm, and 7.33 cm<sup>2</sup> respectively. Three artificial oral mucosa operations were performed in patient No. 8.

**Table 2** artificial mucousmembrane size and recipient tissue

No	Name	Age	Sex	Diagnosis	Terudermis Size(cm)	Recieipient Tissue
1.	A.F.	71	m	amyloidosis	3.5×2.5	periostium
2.	T.Y.	1.5	m	Cleft Palate	3.0×0.5	palatal bone
3.	Y.Y.	1.0	m	Cleft Palate	3.0×1.0	∕
4.	K.M.	1.10	m	Cleft Palate	3.0×2.0	∕
5.	K.C.	1.0	f	Cleft Palate	3.0×1.0	∕
6.	A.Y.	12	m	Oronasal Fistula	2.5×1.1	∕
7.	N.N.	37	m	Palatal Tumor	3.0×2.5	palatal bone
8.	M.S.	70	f	Erythroplakia	3.0×3.0	periostium
9.	M.S.	70	f	Erythroplakia	7.5×2.0	palatal bone
10.	M.S.	70	f	Erythroplakia	5.0×2.5	∕
11.	M.H.	74	m	Carcinoma	4.0×3.0	muscle
12.	S.T.	63	m	Leukoplakia	4.0×3.0	muscle
13.	T.Y.	20	f	Oroantral Fistula	1.5×1.5	alveolar bone

**Table 3** Results of treatment with artificial mucousmembrane

No	Name	Diagnosis	Fixation	Detachment	Hemostasis	Analgesic effect	Contraction	Infection
1.	A.F.	Amyloidosis	suture	—	good	extrem. good	—	—
2.	T.Y.	Cleft Palate	splint	—	∕	good	—	—
3.	Y.Y.	Cleft Palate	suture	—	∕	good	—	—
4.	K.M.	Cleft Palate	∕	—	∕	extrem. good	—	—
5.	K.C.	Cleft Palate	∕	—	∕	fair	—	—
6.	A.Y.	Oronasal Fistula	splint	—	∕	good	—	—
7.	N.N.	Palatal Tumor	suture	partial+(3rd day)	∕	fair	—	—
8.	M.S.	Erythroplakia	∕	—	∕	good	—	—
9.	M.S.	Erythroplakia	∕	—	∕	extrem. good	—	—
10.	M.S.	Erythroplakia	∕	—	∕	extrem. good	slight	—
11.	M.H.	Carcinoma	∕	partial+(1st day)	∕	good	slight	—
12.	S.T.	Leukoplakia	∕	partial+(5th day)	∕	extrem. good	slight	—
13.	T.Y.	Oroantral Fistula	∕	—	∕	extrem. good	—	—

The second operation was performed 4 months and 21 days after the first, and the third operation was performed 6 months and 21 days later. The underlying tissue was palatine bony tissue in 8 cases, periosteal tissue in 2 cases, muscle tissue in 2 cases, and alveolar bone in 1 case. The artificial mucousmembrane was fixed at its periphery with silk sutures, but a palatal splint was used in 1 case after palatoplasty and postoperatively in the patient who underwent iliac spongy bone fragment graft to close an oronasal fistula.

Postoperative hemostasis was good in all of the patients. There was early postoperative partial detachment of the silicone from the wound in 3 of the patients (23%). Detachment occurred in patient no. 7, who had a palatal tumor, and in patient No. 11 and 12, after resection

of a tumor of the buccal mucosa. The partial detachment in these patients was observed on postoperative days 1, 3, and 5, respectively.

Of the 13 patients, postoperative wound pain was extremely mild in 6 (43%), fairly mild in 2 (15%) and mild in 5 (38%), with none of the patients experiencing severe pain.

Slight postoperative contraction of the wound area was noted in 3/13 of the patients (23%).

The artificial mucousmembrane had been used to treat mobile buccal mucosa in two of them, while in the third patient it was used to cover an area from the alveole to the gingivobuccal sulcus and the buccal mucosa. The wound healed well in all of the patients, and infiltration of healthy granular tissue into the collagen was observed through the silocone membrane in the early postoperative period.

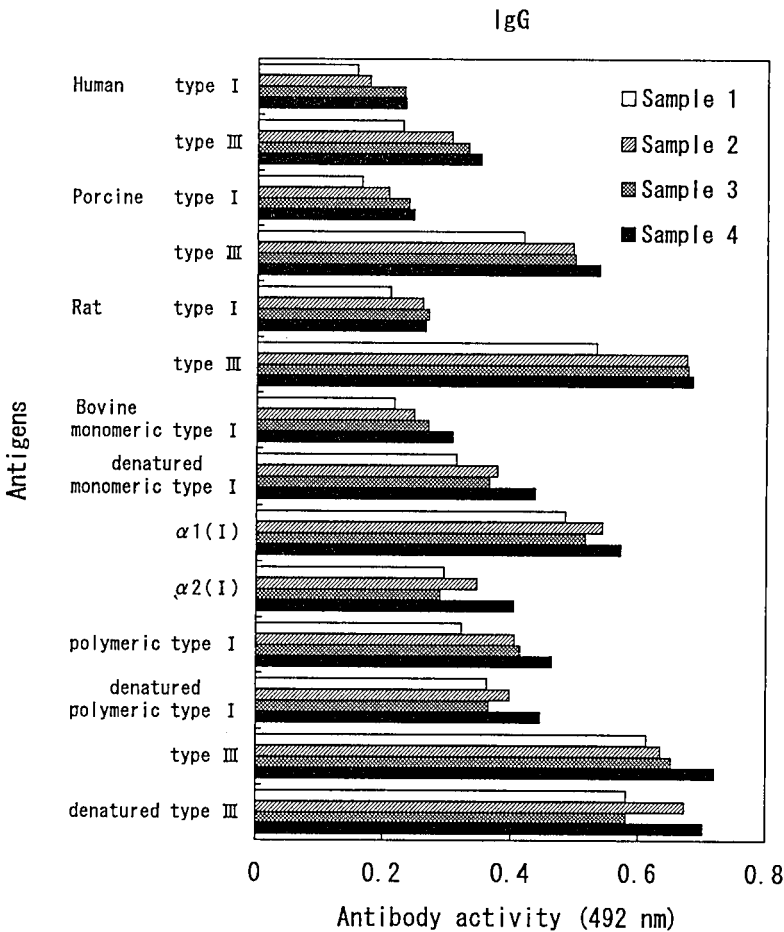
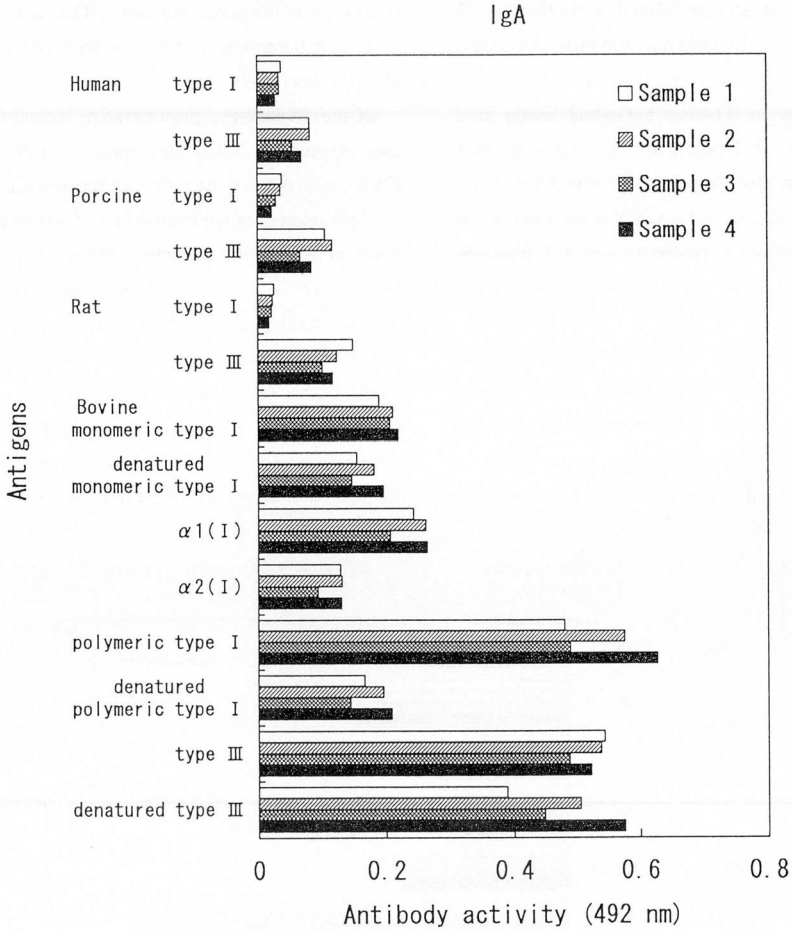


Fig 2 sample 1;16 days after surgery  
 sample 2;32 days after surgery  
 sample 3;67 days after surgery  
 sample 4;84 days after surgery



**Fig 3** sample 1;16 days after surgery  
 sample 2;32 days after surgery  
 sample 3;67 days after surgery  
 sample 4;84 days after surgery

Routine measures were taken to prevent postoperative infection, and no infections were observed. There were no systemic or local complications.

Human antibody response to bovine collagen:

Fig 2. Fig 3 showed the results of serum antibody. In this study, human serum samples prepared for measurement was diluted 10 times. IgA antibody activity of this patient was not different from those of porcine and rat. There was not significant differences among 4 samples. IgG antibody activity was also not different from controls.

Case 1: A 71-year-old man with amyloidosis of the palate.

The patient presented with complaints of bleeding from the palate. At the time of initial examination, a slightly swollen, red mucosal lesion of the palate was note (Fig. 4).

Under general anesthesia, the area of amyloidosis above the periosteum, including the normal palatal mucosa, was



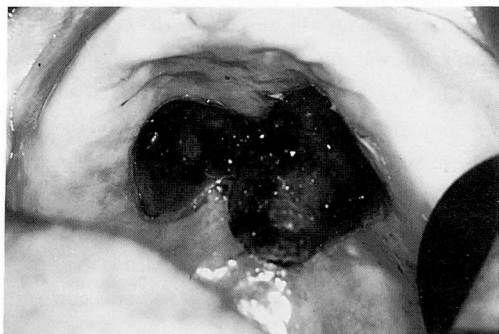
**Fig 4** Case 1 (No 1), anterior part of the hard palate was light swelling with redness.

resected (Fig. 5). The silicone layer was sutured to the palatal mucosa, and the area where the tissue defect was deep was filled with heat-denatured atelocollagen alone (Fig 6). Early palatal mucosa healing was observed 4 weeks postoperatively. (Fig 7)

Case 2: A 68-year-old woman with erythroplakia of the alveolar socket and palatal area.

The patient presented with a chief complaint of ill-fitting

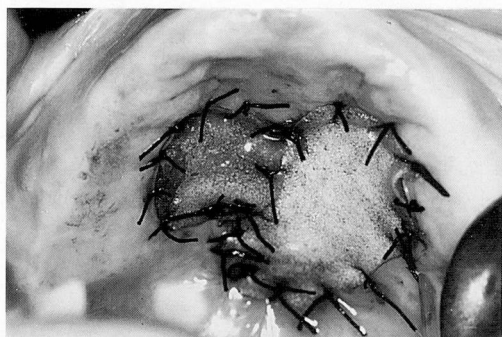
dentures. During the initial examination, a thickened red patch was observed extending from the alveole and palate to a portion of the buccal mucosa. The lesion was diagnosed histologically as epithelial dysplasia, and partial resection was performed. During the first operation, the left side of the palate and alveole were resected and artificial mucous membrane was applied to the defect. The second resection was performed 4 months after the



**Fig 5** The lesion of amiloidosis was excised over the periostium layer.



**Fig 8** Case 2 (No 8), Erythroplakia of the upper jaw gingiva and hard palate



**Fig 6** The wound was covered with artificial mucous membrane (Terudermis<sup>R</sup>)



**Fig 9** The upper jaw gingiva was widely resected



**Fig 7** The anterior hard palate was almost epithelialized 4 weeks after surgery



**Fig 10** Artificial mucous membrane was applied and the margin of silicon was sutured



first operation when the red patch was observed to be spreading into the healed mucosa, primarily the alveole (Fig 8, 9.). The artificial mucous membrane used which measured 7.5×1.5 cm, was sutured at the margins.

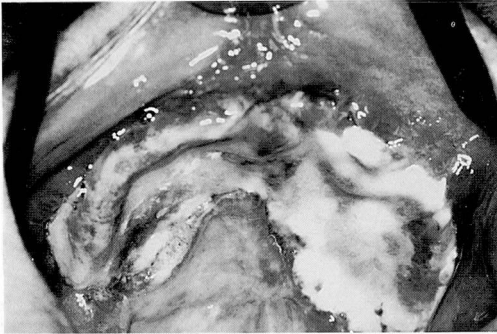


Fig 11 The oral view 2 weeks after treatment



Fig 12 The oral view 2 month after treatment. The upper jaw was covered with normal mucosa

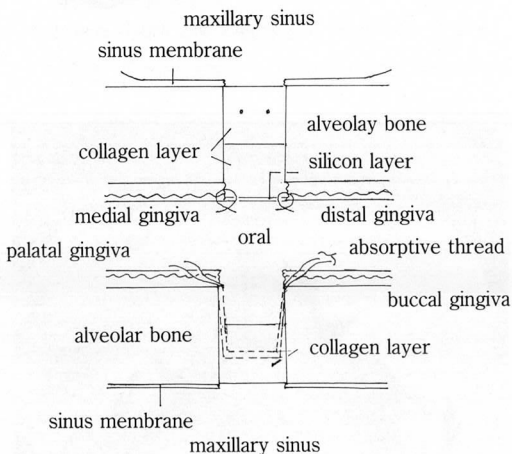


Fig 13 The shema of new operation method for oroantral fistula

Two weeks postoperatively, there was no exposed bone and epithelialization had progressed (Fig. 10, 11). 3 months postoperatively, the alveole and palate was epithelized. (fig 12)

Case 3: A 23-year-old woman an oroantral fistula. The patient underwent extraction of a first upper molar tooth, and postoperatively a communication was found between oral cavity and maxillary sinus. At the time of the initial examination, there was a bony defect of approximately 15 mm in the apical area of the socket, and closure with a mucosal flap was considered necessary. As shown in (Fig. 13), after good hemostasis was achieved atelocollagen from which the silicone had been removed was fixed to the maxillary sinus side with absorbable sutures, and atelocollagen with the silicone membrane was sutured to the gingival margin on top of it (Fig. 14).

Two weeks postoperatively, epithelialization was occurring from the periphery, and wound healed without the use of a mucosal flap (fig 15).

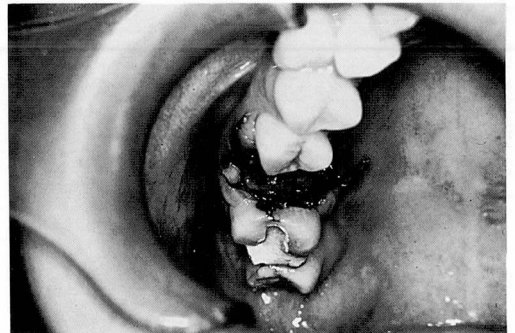


Fig 14 Case 3 (No 13, The socket of tooth extraction was closed with artificial mucosmembrane



Fig 15 The fistula was closed gradually 2 weeks after treatment



## Discussion

In recent years, there has been a great deal of research and development concerning of artificial organs and artificial biomaterials. The need for artificial biomaterials to treat skin defects resulting from trauma, burns, etc., is increasing because of the problem of infection and there has been an increasing demand for medical materials with greater affinity.

The reason for using them to treat skin defects is principally to prevent exudative fluid loss and to prevent bacterial infection, and this involves temporarily covering the wound. The materials available to date for this purpose include chitin non-woven fabric, pig skin, fibrin membrane, poly-L-leucine sponge, etc.<sup>3,4</sup>. These materials have also been employed as covering materials for mucosal defects. On the other hand, Yannas, Burke et al.<sup>5</sup> reported a new form of artificial skin with a bilayer structure composed of an inner layer consisting of substance produced by lyophilizing and crosslinking bovine skin-derived collagen with chondroitin 6-sulfate, a glycosaminoglycan (CAG), as the inner layer for contact with the wound surface, and an outer layer of silicone, as a stage I membrane<sup>6,7</sup>.

Since the highly antigenic telopeptide was removed from the artificial mucous membrane (Terudermis<sup>®</sup>) used in the present study by treating atelocollagen collected from bovine dermis with pepsin, its antigenicity was lower than that of the insoluble collagen used by Yannas et al.<sup>8-9</sup> The physical strength of fiber atelocollagen is also superior, and it is resistant to degradation by enzymes such as collagenase. However, it has poorer cell penetration. Heat-denatured atelocollagen, on the other hand, has superior cell chemotactic ability, is highly soluble, has lower physical strength, and involves the problem of persistence in the body. However, taking advantage of both these properties by mixing them in a ratio of 9 to 1 yields a new artificial material in which persistence in the body is increased and cell penetration is maintained.

The artificial mucous membrane is a product of brief heat dehydration and crosslinking of collagen, and this feature appears to be responsible for its superior bioaffinity compared with chemical crosslinking with glutaraldehyde, as performed by Yannas et al.<sup>10,11</sup>

In the present study, we evaluated Terudermis<sup>®</sup> as an artificial mucous membrane in the treatment of mucoperiosteal defects associated with oral mucosal defects, and

alveolar bone defects in 13 patients. We assessed its usefulness as an artificial mucous membrane in terms of: 1) the safety of the silicone membrane, 2) the postoperative hemostatic effect, 3) analgesic efficacy, 4) epithelialization, 5) wound contraction, and 6) wound infection.

Assessment of the stability of the silicone membrane showed that when smaller sizes were used they became slightly more difficult to suture.

It is important to conform the artificial membrane mucous to the wound and to fix it by suturing. In two of the cases of early silicone membrane detachment, the site was the buccal mucosa, i.e., mobile mucosa, and there were never any problems with mucosa attached to the palate, etc. Nevertheless, there were cases in which breaks occurred at the margins when suturing, and it seemed that reinforcing the silicone membrane margin would both facilitate suturing and at the same time reduce the occurrence of early partial detachment.

Good postoperative hemostasis was achieved in every patient. This is believed to be attributable to collagen's own coagulation-promoting and platelet-aggregating actions. Although it was possible to adequately promote healing by suturing alone, depending on the wound, it would seem better to increase the adherence with palatal splints, etc. Our impression was that, since the silicone margin is weak when fixation is performed by the tie-over technique, there would be less detachment if the suturing were mixed without using penetrating sutures on the silicone whenever the tie-over technique is used.

There was extremely little postoperative pain in 46% of the patients, regardless of whether or not a splint was used, and the analgesic effect was good or better in 99% of the patients. Since there was little postoperative pain and Terudermis<sup>®</sup> had good hemostatic efficacy, broader indications, not just for inpatient surgery, but for outpatient surgery as well are expected in the future.

There were three cases in which slight wound contraction was observed, and in two of them the defect was in mobile mucous membrane. Thus, it would seem necessary to both reinforce the margin of the silicone and make the collagen layer thicker when artificial mucous membranes are applied to defects in mobile mucosa.

No wound infection was observed in any of the patients. This is believed to be because of the protective effect of the silicone membrane and because of the fact that cell infiltration was induced by the collagen layer in the early postoperative period. Although there have been reports

of a delayed-type positive reaction, a bead-like reaction, petechial hemorrhages, etc., as side effects with conventional collagen for injection<sup>12)</sup>, the present material seemed to have low antigenicity, and since no changes in serum antibody titers were detected in the No 8 cases tested, it was concluded that its antigenicity is low. There did not appear to be any problems even when repeated surgery was performed using this material. However, there were also cases in which antibodies to bovine skin collagen were produced<sup>13)</sup>, and assessment in a larger patient population is necessary. A broader range of applications is also expected in the future, and as in case No. 13 reported in this paper, while a surrounding mucoperiosteal flap would generally have been used in the past to close an oroantral fistula caused by an extensive alveolar bone defect, the surgical procedure using this new artificial mucousmembrane suggested the possibility of developing new, completely different procedures with less local surgical invasiveness.

### Conclusion

Postoperative pain was mild and the hemostatic effect was adequate when Terudermis<sup>®</sup> which was developed as artificial skin, was used to treat various degrees of defects in the oral mucosa. Good penetration by granular tissue was observed in the atelocollagen layer in the early postoperative period, and good healing without sacrificing the surrounding tissue was confirmed. Reinforcement of the margin of the silicone layer, however, may be necessary to facilitate suture fixation during the surgical procedure. The results of this study suggest that use of Terudermis<sup>®</sup> may enable the development of new surgical procedures.

### Acknowledgments

We would like to express our gratitude to the Terumo Co., Ltd., for providing the Terudermis<sup>®</sup> used in this study.

### References

- 1) Voller, K.: The enzyme-linked immunoabsorbent assay (ELISA). Alexandria, Va.: Dynatech Laboratories, Inc., 10-56, 1979.
- 2) Frank, N.: Human antibody response following multiple injections of bovine collagen. *Plast Reconst. Surg.* 87, 1080-1088, 1991.
- 3) Elliot, R.A., Hoem, J.G.: Use of commercial porcine skin for wound dressing. *Plast. Reconst. surg.* 52, 401-405, 1973.
- 4) Chang, W.H., Gometz, N.H., and Edelstein, N.M.: Use of lyophilized pig skin for donor site cover. *Brit. J. Plast. Surg.*, 26, 147-49, 1973.
- 5) Burke, J.F., Yannas, I.V., and Quinby, W.C.: Successful use of a physiologically acceptable artificial skin in the treatment of extensive burn injury. *Ann. Surg.*, 194, 413-428, 1981.
- 6) Burke, J.F.: Observations on the development of an artificial skin: Meeting *J. Trauma*, 23, 543-551, 1983.
- 7) Dagalakis, N., Flink, J., and Stasikelis, P.: Design of an artificial skin III. control of pore structure. *J. Biomed. Mater. Res.*, 14, 511-528, 1980.
- 8) Yannas, I.V., Burke, J.F.: Design of an artificial skin. I. Basic design principles. *J. Biomed. Mater. Res.*, 14, 65-81, 1980.
- 9) Yannas, I.V., Burke, J.F., and Gorden, P.L.: Design of an artificial skin II. Control of chemical composition. *J. Biomed. Mater. Res.*, 14, 107-131, 1980.
- 10) Yannas, I.V., Burke, J.F. and Warpehosk, M.: Prompt, long-term functional replacement of skin. *Trans. Am. Soc. Artif. Intern. Organs.*, 27, 107-131, 1981.
- 11) Yannas, I.V., Burke, J.F., and Orgill, D.P.: Wound tissue can utilize a polymeric template to synthesized a functional extension of skin. *Science*, 215, 174-176, 1982.
- 12) Charriere, G., Bewjat, M., and Schmitzler, I.: Reactions to a bovine collagen implant: Clinical and immunologic study in 705 patients. *J. Am. Acad. Dermatol.*, 21, 1203-1208, 1989.
- 13) Frank, D.H., Vakassian, L., Fisher, J.C., and Ozkan, N.: Human antibody response following multiple injections bovine collagen. *Plast Reconst. Surg.*, 87, 1080-1088, 1991.