Clinical Evaluation of atelocollagen sponge(TRE-641) in tooth extraction wounds.
School of Medicine, Yokohama City University Department of Oral and Maxillofacial Surgery

[Introduction]

In dental/oral surgery field, tooth extraction surgery is the most common surgery, but healing of the resultant tooth extraction wound takes a complex process associated with regeneration of soft tissue or bones.

Generally in the healing process of the tooth extraction wound of healthy people\(^1\), blood clot is formed at first, which is retained to be matrix. Blood vessels and fibroblast are generated and grow within the blood clot to form granulation tissue. Then with the growth of fibroblast in this granulation tissue, osteoblast appears and formation of a new bone trabecula is advanced. Internal remodeling is performed by repeated absorption by osteoclast as well as proliferation of the new bone by osteoblast, leading to compact bone in 3-4 months. Closure of the tooth extraction wound by the gingival epithelium is done by introversion of the free gingiva and proliferation of the epithelium from the free margin of gingiva, and closure is completed almost in 2-3 weeks. Like this, in many cases of tooth extraction of healthy people, the whole tooth extraction wound is filled with the new bone trabecula in about 1 months and healed by natural healing strength without any special treatment.

While in patients with blood disease, patients treated by drug therapy such as coagulant and steroid drug, patients with liver disease, patients with easily hemorrhagic or infectious disease such as diabetes mellitus, patient with difficulty in bite hemostasis after tooth extraction due to physical or mental disorder (referred to as "morbid people" in this trial) do not always take satisfactory healing process\(^2-5\), and the dentist sometimes encounters patients with postoperative spontaneous pain, haphalgesia, bleeding, inflammation, infection, healing failure (in particular, dry socket), and sometimes antrooral fistula (maxillary sinus perforation, hereinafter referred to as "perforation"), and has a hard time in treatment. However, currently there are no effective medical materials that are usable for these patients.

Thus, we have devised the use of atelo-collagen material\(^1\) which has good clinical results for mucosal defect wound in the buccal area\(^6-11\), and suggesting effective for bone defect wound\(^12\) in order to provide brief treatment for such patients, and examined its usefulness by applying this to various kinds of tooth extraction wounds.
### Test Materials and Methods

#### [Implementation facilities]

<table>
<thead>
<tr>
<th>Institution</th>
<th>Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faculty of Medicine, Asahikawa Medical College</td>
<td>Department of Oral and Maxillofacial Surgery</td>
</tr>
<tr>
<td>School of Dentistry, Niigata University</td>
<td>The Second Department of Oral and Maxillofacial Surgery</td>
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<tr>
<td>Faculty of Dentistry, Tokyo Medical and Dental University</td>
<td>The Second Department of Oral Surgery</td>
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<tr>
<td>School of Dental Medicine, Tsurumi University</td>
<td>The First Department of Oral and Maxillofacial Surgery</td>
</tr>
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<td>School of Medicine, Yokohama City University</td>
<td>Department of Oral and Maxillofacial Surgery</td>
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<tr>
<td>School of Medicine, Nagoya University</td>
<td>Department of Oral Surgery</td>
</tr>
<tr>
<td>School of Dentistry, Aichi-Gakuin University</td>
<td>The Second Department of Oral and Maxillofacial Surgery</td>
</tr>
</tbody>
</table>

#### [Subject patients]

Subject patients were selected among the following patient groups and a consent for this clinical trial was obtained: [1] tooth extraction of patient affected by disease, [2] normal tooth extraction, complicated exodontia, and impacted tooth extraction of healthy people, [3] tooth extraction alveolus re-curettage /postoperative hemorrhage, [4] maxillary sinus stoma (perforation) by tooth extraction. In this, patient affected by disease is defined as various kinds of patients including patients with blood disease, patients receiving anticoagulant therapy like warfarin/hepalin or patients administered with steroid, patients with hepatic failure, diabetes mellitus, or hypertension, and patients after radiation exposure, handicapped persons who are not cooperative for bite hemostasis, tending to be hemorrhagic and infectious and having a high possibility of incomplete healing for tooth extraction alveolus. With re-curettage of tooth extraction alveolus /postoperative hemorrhage, they are evaluated comprehensively because both are secondary use method after tooth extraction. With regard to maxillary sinus stoma (perforation), maxillary
sinus stoma and maxillary sinus perforation were comprehensively evaluated.

[Test material]

Test material used was bullet atelo-collagen sponge (clinical test number: TRE-641), and the following 2 types were selected depending on situation.

Type 1: Single layer structure with collagen sponge alone (Fig.1).
Type 2: Double layer structure of collagen sponge attached by silicone membrane.

The size used was S size (8 mm in outer diameter, 25 mm in length), M size (15 mm in outer diameter, 20 mm in length), and L size (22 mm in outer diameter, 30 mm in length).

For patients affected by disease, microfibrous atelo-collagen (2.5×5 cm) which is available on the market, absorbable hemostat, was used as control.

Fig.1 Appearance of TRE-641 From left S, M, and L size (all are product no.1)

[Use method]

The size fitted well to the size of tooth extraction wound was selected and after removal of blood on surface of bleeding wound, material was filled into tooth extraction wound, holding with dressing forceps. Sufficient adhesion was provided by pushing gently with absorbent gauze. Suture of gingiva or floor splint for hemostasis were used depending on patients.
[Evaluation method]

With degree of pain, degree of bleeding, epithelization completion period or fistula closure period, degree of depression, degree of inflammation, presence or absence of infection and operability were clinically evaluated.

1. Observation period

In principle, wound surface and symptom were observed immediately after tooth extraction, at 1 day, 3 days, 1 week after tooth extraction, and then every week until 4 weeks after. If within 4 weeks epithelization is completed or maxillary sinus stoma (perforation) is closed, test was to be finished at the time. However, if at 4 weeks after, epithelization is not completed or maxillary sinus stoma (perforation) is not closed, observation was to be continued at 5 or 6 weeks after.

2. Observation item

The following items were evaluated and recorded at observation days. Observation days for each observation item were shown in Table 1.

1) Effectiveness evaluation

[1] Degree of pain

Evaluation was done by 3 grades: “no pain (10 scores)”, “bearable pain (5 scores)”, and “marked pain (0 score)”.  

[2] Degree of bleeding

Evaluation was done by 3 grades: “no bleeding (10 scores)”, “bleeding to degree of infiltration from the surrounding (5 scores)”, and “marked bleeding (0 score)”.  

[3] Epithelization completion period (except for maxillary sinus stoma (perforation))

Evaluation was done by 7 grades: “within 8 days after filling (30 scores)”, “9-12 days after filling (25 scores)”, “13-16 days after filling (20 scores)”, “17-20 days after filling (15 scores)”, “21-24 days after filling (10 scores)”, “25-28 days after filling (5 scores)”, and “no epithelization at 28 days after filling (0 or 3 scores)”.  

[4] Fistula closure period (in case of maxillary sinus stoma (perforation))

Evaluation was done by 7 grades: “within 8 days after filling (30 scores)”, “9-12 days after filling (25 scores)”, “13-16 days after filling (20 scores)”, “17-20 days after filling (15 scores)”, “21-24 days after filling (10 scores)”, “25-28 days after filling (5 scores)”, and “no fistula closure at 28 days after filling (0 or 3 scores)”.

[5] Degree of depression

Evaluation was done by 3 grades: “no depression (10 scores)”, “bearable depression (5 scores)”, and “marked depression (0 score)”.  

[6] Degree of inflammation

Evaluation was done by 3 grades: “no inflammation (10 scores)”, “bearable inflammation (5 scores)”, and “marked inflammation (0 score)”.  

[7] Presence or absence of infection

Evaluation was done by 2 grades: “no infection (10 scores)”, and “infection (0 score)”.  

[8] Operability

Evaluation was done by 2 grades: “no operability (10 scores)”, and “operability (0 score)”.  

filling (15 scores), “21-24 days after filling (10 scores), “25-28 days after filling (5 scores), and “no closure at 28 days after filling (0 or 3 scores).”

[5] Degree of depression
Evaluation was done by 3 grades: “extremely few depression, and aberrance of pieces of foodstuff was prevented (10 scores),” “a few depression, and aberrance of pieces of foodstuff was rarely observed (5 scores),” and “marked depression, and aberrance of pieces of foodstuff was observed (0 scores).”

[6] Degree of inflammation
Evaluation was done by 3 grades: “no flare, swelling, and haphalgesia (5 scores),” “mild flare, swelling, and haphalgesia (3 scores),” and “marked flare, swelling, and haphalgesia (0 score).”

[7] Absence or presence of infection
Evaluation was done by 3 grades: “no infection (5 scores),” “infection was observed, however, maintained by other treatment (3 scores),” and “infection was observed, and this product was removed (0 score).” With microbiological examination, culture examination was done to examine absence or presence of infection or identification of strain when necessary.

[8] Operability
Evaluation was done by 3 grades: “extremely simple (10 scores),” “slightly simple (5 scores),” and “complicated (0 score).”

2) Safety evaluation
[1] Clinical laboratory test value
The following items were, in principle, examined before test, and at 1 week of examination. Evaluation was done by 3 grades: “no abnormal variation,” “abnormal variation (no causal relationship with test material),” and “abnormal variation (causal relationship with test material)” by variation of laboratory test value before and after test referring to “Judgment Criteria of Adverse Reaction/Abnormal Clinical Laboratory Test Value” by Judgment Criteria Committee for Japanese Chemical Therapy Adverse Reaction.
General blood test: red blood cell count, white blood cell count, hematocrit value, platelet count

Blood biochemical test: total protein, A/G ratio, BUN, creatinine, GOT, GPT

[2] Absence or presence of adverse reaction

Evaluation was done by 4 grades depending on degree of adverse reaction: “no adverse reaction”, “adverse reaction was observed, however, special treatment was needed”, “adverse reaction was observed, and use of test material was kept by other treatment”, and “adverse reaction was observed, and use of test material was suspended”. And if adverse reaction occurs, the symptom, date of onset, severity, and the cause were observed and examined.

Table 1 Observation variables and day

<table>
<thead>
<tr>
<th>Observation variables</th>
<th>Immediate after</th>
<th>After 1 day</th>
<th>After 3 days</th>
<th>After 1 week</th>
<th>After 2 weeks</th>
<th>After 3 weeks</th>
<th>After 4 weeks</th>
<th>After 5 weeks</th>
<th>After 6 weeks</th>
</tr>
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<tbody>
<tr>
<td>An extent of pain</td>
<td>-</td>
<td>O</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>An extent of bleeding</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fistula closure period</td>
<td>-</td>
<td>Evaluation when it is necessary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelization complete period</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>An extent of depression</td>
<td>-</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>An extent of inflammation</td>
<td>-</td>
<td>O</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Presence or absence of infection</td>
<td>-</td>
<td>O</td>
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<td>O</td>
<td>O</td>
<td>O</td>
<td>O</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Operability</td>
<td>O</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

[Usefulness judgment method]

Mean score of each observation item through observation period was calculated and effectiveness of test material was judged in accordance with the total value (Table 2). And safety was judged based on variation of clinical laboratory test and absence or presence of adverse reaction (Table 3). Furthermore, usefulness was judged based on combination of effectiveness and safety (Table 4) and finally shown by 4 grades:
“extremely useful”, “useful”, “slightly useful”, and “not useful”.

### Table 2  Effectiveness evaluation

<table>
<thead>
<tr>
<th>Total score</th>
<th>Effectiveness</th>
</tr>
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<tbody>
<tr>
<td>66-80</td>
<td>Extremely effective</td>
</tr>
<tr>
<td>46-65</td>
<td>Effective</td>
</tr>
<tr>
<td>25-45</td>
<td>Slightly effective</td>
</tr>
<tr>
<td>0-24</td>
<td>Not effective</td>
</tr>
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</table>

### Table 3  Safety evaluation

<table>
<thead>
<tr>
<th>Laboratory test values</th>
<th>Presence or absence of adverse reaction</th>
<th>Safety</th>
</tr>
</thead>
<tbody>
<tr>
<td>No abnormal variation</td>
<td>No adverse reaction.</td>
<td>No problems in safety</td>
</tr>
<tr>
<td></td>
<td>No adverse reaction.</td>
<td>Some problems in safety</td>
</tr>
<tr>
<td></td>
<td>Adverse reaction was observed, but treatment was necessary.</td>
<td>Problems in safety, but discontinuation was not necessary.</td>
</tr>
<tr>
<td>Abnormal variation (no causal relationship)</td>
<td>Adverse reaction was observed, but use was continued by other treatment.</td>
<td>Problems in safety, and use of test materials was discontinued.</td>
</tr>
<tr>
<td></td>
<td>No adverse reaction.</td>
<td>No problems in safety</td>
</tr>
<tr>
<td></td>
<td>No adverse reaction.</td>
<td>Some problems in safety</td>
</tr>
<tr>
<td></td>
<td>Adverse reaction was observed, but treatment was necessary.</td>
<td>Problems in safety, but discontinuation was not necessary.</td>
</tr>
<tr>
<td>Abnormal variation (causal relationship was observed)</td>
<td>Adverse reaction was observed, but use was continued by other treatment.</td>
<td>Problems in safety, and use of test materials was discontinued.</td>
</tr>
</tbody>
</table>
### Table 4  Usefulness evaluation

<table>
<thead>
<tr>
<th>Effectiveness</th>
<th>Safety</th>
<th>Usefulness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No problems in safety</td>
<td>Extremely useful</td>
</tr>
<tr>
<td></td>
<td>Some problems in safety</td>
<td>Useful</td>
</tr>
<tr>
<td>Extremely effective</td>
<td>Problems in safety, but discontinuation was not necessary.</td>
<td>Slightly useful</td>
</tr>
<tr>
<td></td>
<td>Problems in safety, and use of test materials was discontinued.</td>
<td>Not useful</td>
</tr>
<tr>
<td>Effective</td>
<td>No problems in safety</td>
<td>Useful</td>
</tr>
<tr>
<td></td>
<td>Some problems in safety</td>
<td>Useful</td>
</tr>
<tr>
<td></td>
<td>Problems in safety, but discontinuation was not necessary.</td>
<td>Slightly useful</td>
</tr>
<tr>
<td></td>
<td>Problems in safety, and use of test materials was discontinued.</td>
<td>Not useful</td>
</tr>
<tr>
<td>Slightly effective</td>
<td>No problems in safety</td>
<td>Slightly useful</td>
</tr>
<tr>
<td></td>
<td>Some problems in safety</td>
<td>Slightly useful</td>
</tr>
<tr>
<td></td>
<td>Problems in safety, but discontinuation was not necessary.</td>
<td>Slightly useful</td>
</tr>
<tr>
<td></td>
<td>Problems in safety, and use of test materials was discontinued.</td>
<td>Not useful</td>
</tr>
<tr>
<td>Not effective</td>
<td>Regardless of safety</td>
<td>Not useful</td>
</tr>
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### Test Implementation Cases

The number of patients subjected for this test was 169. Out of them, 2 were rejected case in both effectiveness and safety evaluations, 1 was rejected case in effectiveness evaluation, and 1 was rejected case in safety evaluation. Therefore, effectiveness evaluation subject was 166 patients, and safety evaluation subject 166 patients, finally usefulness was judged with regard to 165 patients.

Two patients of rejected case in both effectiveness and safety evaluations included 1 patient who disobedied subject case and 1 patient who did not visit our clinic since 1st day after filling, thus, follow-up observation was impossible. One patient who was rejected case in effectiveness evaluation did not visit our clinic since 2 weeks after filling, thus, follow-up observation was impossible. One patient who was rejected case in safety evaluation experienced dropout of test material on the filling day. Even if there was more or less a gap regarding follow-up observation period, if the physician in charge judged appropriate, the patient was selected as subject.
[Distribution of by sex/age]

The number of subject patients by sex was 92 males and 77 females. Distribution by age was as follows: 10-19 years, 14 patients (8.3 %); 20-29 years, 32 patients (18.9 %); 30-39 years, 12 patients (7.1 %); 40-49 years, 21 patients (12.4 %); 50-59 years, 31 patients (18.3 %); 60-69 years, 42 patients (24.9 %); 70-79 years, 14 patients (8.3 %); 80-89 years, 2 patients (1.2 %); and 90 years or greater, 1 patient (0.6 %), with an average of 47.3 years.

[Distribution by application]

The number of subject patients by application is shown in Table 5, that is, with patients affected by disease, the number of patients receiving medical therapy were 40 patients (23.7 %), the largest in number, followed by blood disease 33 patients (19.5 %), hepatic failure 14 patients (8.3 %), handicapped 8 (4.7 %), dialysis patient 3 (1.8 %), and other patients affected by disease 21 patients (12.4 %). Normal tooth extraction, complicated exodontia, and impacted tooth extraction of healthy people were 28 patients (16.6 %). Tooth extraction alveolus re-curettage/postoperative hemorrhage were 6 patients (3.6 %), and maxillary sinus stoma (perforation) 16 patients (9.5 %).

<table>
<thead>
<tr>
<th>Indication</th>
<th>TRE-641</th>
<th>Control</th>
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<tbody>
<tr>
<td>Tooth extraction of morbid people</td>
<td>66</td>
<td>53</td>
</tr>
<tr>
<td>Tooth extraction of healthy people</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Recurettage/postoperative bleeding of</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>the tooth extraction cavity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antrooral fistula (perforation)</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>53</td>
</tr>
</tbody>
</table>

Test Results and Outcome

[Results of effectiveness evaluation]

Results of effectiveness evaluation for all patients applied for TRE-641 and
control is shown in Fig. 2. The ratio of effective or greater (effectiveness rate) was 93% in the whole TRE-641. By application, it was 93% for patients affected by disease, 96% for normal tooth extraction, complicated exodontia, and impacted tooth extraction of healthy people, 83% for tooth extraction alveolus re-curettage/postoperative hemorrhage, and 86% for maxillary sinus stoma (perforation). On the other hand, effectiveness rate of microfibrous atelo-collagen which was used as control was 81%.

Fig. 2 Evaluation results of effectiveness by application. A numerical value in the graph indicates the number of patients.

- Not effective
- Slightly effective
- Effective
- Extremely effective

1. Whole TRE-641
2. Morbid people (TRE-641)
3. Morbid people (control)
4. Tooth extraction of healthy people
5. Recurettage/postoperative bleeding of the tooth extraction cavity
6. Antrooral fistula (perforation)

Evaluation results by observation item of the whole TRE-641 is shown in Figs 3-9. With degree of pain, the ratio of not feeling pain at 1 day after was 62%, and 3 days after 87% (Fig. 3). With degree of bleeding, ratio of no bleeding immediately after was 39%, and at 1 day 89% (Fig. 4). With epithelization completion period, the patient who showed epithelization at 13-16 days was 36%, the maximum in frequency. With fistula
closure period, 29% were within 8 days, while there were 5 patients (3.6%) who did not have closure within 28 days (Fig. 5). With degree of depression, ratio of combination of slight depression observed with marked depression observed was 22% at 2 weeks after and 31% at 3 weeks after (Fig. 6). With degree of inflammation, at 1 day after, flare, swelling, haphalgesia were observed in 65%, at 1 week after, it decreased to 16% (Fig. 7). With absence or presence of infection, at 3 days after, 1 patient was infected, at 4 weeks after, 2 infected. One of 2 was recovered (Fig. 8). With operability, extremely simple was in 83%, and complicated in 1 patient (1%) who used type 2 (Fig. 9).

Evaluation results by observation item by application are shown in Fig. 10-38, the outline is described below.

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**Fig. 3**  Whole TRE-641 (An extent of pain).

① After 1 day  ② After 3 days

A numerical value in the graph indicates the number of patients

- □ Unbearable pain
- □ Bearable pain
- □ No pain

**Fig. 4**  Whole TRE-641 (An extent of bleeding).

A numerical value in the graph indicates the number of patients.

- □ Marked bleeding
- □ Blood stained from around
- □ No bleeding

① Immediately after  ② After 1 day  ③ After 3 days  ④ After 1 week
Fig. 5  Whole TRE-641 (epithelization complete period or fistulous opening closure period).

- Within 8 days
- 9-12 days
- 13-16 days
- 17-20 days
- 21-24 days
- 25-28 days
- Epithelization not completed or fistulous opening not closed in 28 days.

Fig. 6  Whole TRE-641 (An extent of depression).

A numerical value in the graph indicates the number of patients.

- Marked depression and aberrance of food debris was frequently observed.
- Some depressions and aberrance of food debris was rarely observed.
- Extremely less depression and aberrance of food debris was prevented.
Fig. 7  Whole TRE-641 (An extent of inflammation).
A numerical value in the graph indicates the number of patients.

Marked flare/swelling/haphalgesia
Slight flare/swelling/haphalgesia
No flare/swelling/haphalgesia

Fig. 8  Whole TRE-641 (Presence or absence of infection).
A numerical value in the graph indicates the number of patients.

Infection was observed and this product was removed.
Infection was observed and use was continued by other treatment.
Infection was not observed.

Fig. 9  Whole TRE-641 (Operability)

Extremely easy  Slightly easy  Complicated
Fig. 10 Morbid people (An extent of pain).

A numerical value in the graph indicates the number of patients.

☑️ Unbearable pain ☒ Bearable pain ☐ No pain

Fig. 11 Morbid people (An extent of bleeding).

A numerical value in the graph indicates the number of patients.

☑️ Marked bleeding ☒ Blood stained from around ☐ No Bleeding

Fig. 12 Morbid people (Epithelization complete period or fistulous opening closure period).

☐ Within 8 days ☑ 9-12 days ☒ 13-16 days ☐ 17-20 days ☒ 21-24 days ☐ 25-28 days

☑️ Epithelization not completed or fistulous opening not closed in 28 days.
Fig. 13  Morbid people (An extent of depression).

A numerical value in the graph indicates the number of patients.

Marked depression and aberrance of food debris was frequently observed.

Some depressions and aberrance of food debris was rarely observed.

Extremely less depression and aberrance of food debris was prevented.

Fig. 14  Morbid people (An extent of inflammation).

A numerical value in the graph indicates the number of patients.

Marked flare/swelling/haphalgesia

Slight flare/swelling/haphalgesia

No flare/swelling/haphalgesia
Fig. 15  Morbid people (Presence or absence of infection).
A numerical value in the graph indicates the number of patients.

- ☒ Infection was observed and this product was removed.
- ☒ Infection was observed and use was continued by other treatment.
- ☐ Infection was not observed.

Fig. 16  Morbid people (Operability)

- ☒ Extremely easy
- ☒ Slightly easy
- ☐ Complicated

Fig. 17  Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (An extent of pain).
A numerical value in the graph indicates the number of patients.

- ☒ Unbearable pain
- ☒ Bearable pain
- ☐ No pain
Fig. 18  Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (An extent of bleeding).

A numerical value in the graph indicates the number of patients.

☑️ Marked bleeding  
☑️ Blood stained from around  
☐ No Bleeding

Fig. 19  Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (Epithelization complete period).

☐ Within 8 days  ☐ 9-12 days  ☑ 13-16 days  ☐ 17-20 days  ☑ 21-24 days  ☑ 25-28 days  
☒ Epithelization not completed or fistulous opening not closed in 28 days.
Fig. 20 Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (An extent of depression).

A numerical value in the graph indicates the number of patients.

- Marked depression and aberrance of food debris was frequently observed.
- Some depressions and aberrance of food debris was rarely observed.
- Extremely less depression and aberrance of food debris was prevented.

Fig. 21 Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (An extent of inflammation).

A numerical value in the graph indicates the number of patients.

- Marked flare/swelling/haphalgesia
- Slight flare/swelling/haphalgesia
- No flare/swelling/haphalgesia
Fig. 22  Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (Presence or absence of infection).

A numerical value in the graph indicates the number of patients.

- Infection was observed and this product was removed.
- Infection was observed and use was continued by other treatment.
- Infection was not observed.

Fig. 23  Ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people (operability)

- Extremely easy
- Slightly easy
- Complicated
Fig. 24  Recurettage/postoperative bleeding of the tooth extraction cavity
(an extent of pain).

A numerical value in the graph indicates the number of patients.

- ☒ Unbearable pain  ☑ Bearable pain  ☐ No pain

Fig. 25  Recurettage/postoperative bleeding of the tooth extraction cavity
(An extent of bleeding). A numerical value in the graph indicates the number of patients.

- ☒ Marked bleeding  ■ Blood stained from around  ☐ No bleeding

Fig. 26  Recurettage/postoperative bleeding of the tooth extraction cavity
(epithelization complete period).

- ☐ Within 8 days  ☐ 9-12 days  ☐ 13-16 days  ☐ 17-20 days  ☒ 21-24 days  ☐ 25-28 days

- ☒ Epithelization not completed or fistulous opening not closed in 28 days.
Fig. 27  Recurettage/postoperative bleeding of the tooth extraction cavity (An extent of depression).

A numerical value in the graph indicates the number of patients.

- Marked depression and aberrance of food debris was frequently observed.
- Some depressions and aberrance of food debris was rarely observed.
- Extremely less depression and aberrance of food debris was prevented.

Fig. 28  Recurettage/postoperative bleeding of the tooth extraction cavity (An extent of inflammation).

A numerical value in the graph indicates the number of patients.

- Marked flare/swelling/haphalgesia
- Slight flare/swelling/haphalgesia
- No flare/swelling/haphalgesia
Fig. 29  Recurettage/postoperative bleeding of the tooth extraction cavity (Presence or absence of infection).

A numerical value in the graph indicates the number of patients.

- Infection was observed and this product was removed.
- Infection was observed and use was continued by other treatment.
- Infection was not observed.

Fig. 30  Recurettage/postoperative bleeding of the tooth extraction cavity (Operability)

- Extremely easy
- Slightly easy
- Complicated

Fig. 31  Antrooral fistula (perforation) (An extent of pain)

A numerical value in the graph indicates the number of patients.

- Unbearable pain
- Bearable pain
- No pain
Fig. 32 Antrooral fistula (perforation) (An extent of bleeding)

A numerical value in the graph indicates the number of patients.

- Marked bleeding
- Blood stained from around
- No bleeding

Fig. 33 Antrooral fistula (perforation)

(Epithelization complete period).

- Within 8 days
- 9-12 days
- 13-16 days
- 17-20 days
- 21-24 days
- 25-28 days

Epithelization not completed or fistulous opening not closed in 28 days.

Fig. 34 Antrooral fistula (perforation) (An extent of depression).

A numerical value in the graph indicates the number of patients.

- Marked depression and aberrance of food debris was frequently observed.
- Some depressions and aberrance of food debris was rarely observed.
- Extremely less depression and aberrance of food debris was prevented.
Fig. 35  Antrooral fistula (perforation) (An extent of inflammation)

A numerical value in the graph indicates the number of patients.

- Marked flare/swelling/haphalgesia
- Slight flare/swelling/haphalgesia
- No flare/swelling/haphalgesia

Fig. 36  Antrooral fistula (perforation) (Presence or absence of infection)

A numerical value in the graph indicates the number of patients.

- Infection was observed and this product was removed.
- Infection was observed and use was continued by other treatment.
- Infection was not observed.

Fig. 37  Antrooral fistula (perforation) (Operability)

- Extremely easy
- Slightly easy
- Complicated
Fig. 38 Results of usefulness evaluation by indication

A numerical value in the graph indicates the number of patients.

- ■ No useful
- ■■ Slightly useful
- ■■■ Useful
- ■■■■ Extremely useful

1. Tooth extraction of patients affected by disease

Test was performed by selecting microfibrous atelo-collagen sheet available on the market as a control.

With degree of pain, ratio of feeling of no pain at 1 day after was 63 % for TRE-641 and 64 % for control (Fig. 10), suggesting no difference. With degree of bleeding, ratio of no bleeding immediately after was 39 % for TRE-641 and 35 % for control (Fig. 11), showing TRE-641 had slightly higher value. With epithelization completion period, patients who had epithelization within 16 days was 71 % for TRE-641 and 62 % for control. The ratio of no epithelization at 28 days was 2 % for TRE-641 while 6 % for control (Fig. 12). The patient in whom test material was dropped at the filling day or the patient who was infected at 9 days after filling and underwent re-curettage were excluded from total, because judgment for epithelization completion period is impossible. With degree of depression, ratio of combination of a little depression with marked depression at 3 weeks was 20 % for TRE-641, while 62 % for control (Fig. 13), suggesting fewer depressions in TRE-641. With degree of inflammation, in TRE-641 at 1 day after, marked flare, swelling, haphalgesia were observed in 4 patients (6 %) (Fig. 14), all of which are patients received severe invasion into surrounding tissues due to tooth extraction. With absence or presence of infection, in TRE-641 at 3 days after, mild infection was observed in 1 patient (2 %), and in control at 3 weeks after, mild infection was observed in 1 (7 %) (Fig. 15), however, they recovered by treatment like cleaning without problem. With operability, patients who evaluated as extremely simple were 82 % for TRE-641, while 31 % for control, showing overwhelmingly high % for TRE-641 (Fig. 16).
Among patients with blood disease, in case of tooth extraction of patients with anaplastic anemia, good hemostasis was obtained by filling TRE-641. In patients who have physically and mentally disorder and in whom, hemostasis by bite absorbent gauze is impossible, sufficient hemostasis was obtained by filling TRE-641 into the tooth extraction wound. Some of patients administered warfarin underwent tooth extraction without suspension of administration by judgment of physician, considering general state of patients. And good healing was obtained without apprehensive sustaining of bleeding or healing disorder.

Representative cases are presented hereinafter.

Case 66 (50 years old, female) was a patient with anaplastic anemia, and preoperative number of platelet was as low as 23,000/mm³. After tooth extraction of 8, early hemostasis was obtained by filling TRE-641 into the tooth extraction wound, in which liquid thrombin was immersed, and at 8 days after surgery, epithelization was obtained (Fig. 39 a-c).

Case 106 (15 years old, male) was a patient with autism, and what is called, non-cooperative for tooth extraction. Under general anesthetic, after tooth extraction of 8, TRE-641 was filled into tooth extraction wound. Sufficient hemostasis and good healing course were obtained (Fig. 40 a-c).

Case 129 (56 years old, male) was a patient after undergoing displacement of aortic valve, and without suspension of warfarin, after tooth extraction of 3, TRE-641 was filled into tooth extraction wound. Immediately after filling, hemostasis effect was excellent and at 22 days after operation epithelization was obtained (Fig. 41 a-d).

2. Normal tooth extraction, complicated exodontia, and impacted tooth extraction of healthy people

With degree of pain, there was 1 patient (7 %) who complained of unbearable pain at 3 days after (Fig. 17), while the remaining 11 patients (79 %) had no pain. With degree of bleeding, no marked bleeding was observed immediately after filling (Fig. 18). Epithelization completion period was within 16 days in 83 % (Fig. 19). With degree of depression, degree of depression showed a tendency to increase with time and at 3 weeks
after, slight depression was observed in 60 % (Fig. 20). With degree of inflammation, at 1 day after, mild inflammation was observed in 78 %, and at 1 week after, disappearance of inflammation was observed in 81 % (Fig. 21). Infection was not observed (Fig. 22). Operability was evaluated as extremely simple in 89 % and complicated in 0 % (Fig. 23).

Representative cases are presented hereinafter.

Case 81 (27 years old, female) was a patient with half impacted tooth. Gingival incision and ablation were performed, and after corona separation removal, TRE-641 was filled into tooth extraction wound. The diameter of aperture produced by tooth extraction was 10 mm, showing a large defect, however, at 2 weeks postoperatively, excellent healing with little depression was obtained (Fig. 42 a-d).

3. Tooth extraction alveolus re-curettage/postoperative hemorrhage

With the number of days after tooth extraction, in case of tooth extraction alveolus re-curettage, at 2 days after, tooth extraction was in 2 patients and at 7 days in 1 patient, and in case of postoperative hemorrhage, at 0 day after, tooth extraction was in 1 patient, at 5 days in 1 patient, and after re-curettage, at 1 day in 1 patient. With degree of pain, at 1 day after, 3 patients (60 %) had bearable pain, while others did not have pain (Fig. 24). With degree of bleeding, immediately after, no bleeding was found in 2 patients (33 %) and bleeding to degree of infiltration, in 4 patients (67 %), in any of which, bleeding was not observed at 1 day after (Fig. 25). Epithelization completion period was at 6, 22, and 28 days after filling, respectively in tooth extraction alveolus re-curettage. Patients who needed 28 days infected after tooth extraction, which may affect delay of epithelization. Similarly, in postoperative hemorrhage, epithelization was completed at 6, 11, and 32 days after filling, respectively. Patients who needed 32 days infected after tooth extraction, and underwent re-curettage, which may have provided large invasion on epithelization (Fig. 26). With degree of depression, extremely small depression at 1 week after was found in 3 patients (60 %), at 2 weeks after, in 2 patients (50 %) (Fig. 27). With degree of inflammation, no inflammation was observed except for mild inflammation at 1 day after, in 3 patients (60 %), and at 3 days after, in 1 patient (33 %) (Fig. 28). There was no infection observed (Fig. 29). With operability, it was evaluated
as extremely simple in all except for slightly simple in 2 patients (Fig. 30).

Representative cases are presented hereinafter.

Case 33 (50 years old, male) was a patient who had marked pain and flare and mild pus discharge since 3 days after tooth extraction of \( 6 \). Because of this, re-curettage was performed at 1 week after tooth extraction, TRE-641 was filled and gingival suture was done. At 3 days after re-curettage, pain and flare disappeared, since then no problem occurred, and at 3 weeks after, re-curettage epithelization was completed (Fig. 43 a-d).

4. Maxillary sinus stoma (perforation)

With degree of pain, at 3 days after, there were no pain in all (Fig. 31). With degree of bleeding, bleeding of infiltration degree was observed immediately after in 71 % (Fig. 32), which may be caused by frequent bleeding due to curettage of surrounding granulation. With stroma closure period, patients who showed closure within 8 days were 29 %, 9-12 days 7 %, 13-16 days 14 %, 21-24 days 14 %, and patients who did not show closure at 28 days was 36 % (Fig. 33). One of patients who did not show closure at 28 days was a patient who was doubted as having infection at 32 days after filling and underwent curettage of the wound. With degree of depression, since a week after, depression was observed in about 38 % (Fig. 34). With degree of inflammation, at 1 day after, flare, swelling, and haphalgesia were observed in 64 %, and at 1 week after, it decreased to 23 % (Fig. 35). Infection was observed at 4 weeks after, in 2 patients (Fig. 36). One developed mild infection, thus, no special treatment was given except for cleaning, and the other 1 patient underwent curettage of the wound. With operability, extremely simple in 86 % and complicated in 0 % (Fig. 37).

Representative cases are presented hereinafter.

Case 61 (27 years old, male) was a patient with old maxillary sinus stoma which happened to develop after tooth extraction of \( 6 \), and spontaneous closure was not expected even at 207 days. After the membrane around the stroma was curetted, and bleeding was performed, TRE-641 was filled. At 1 week after, in the center of collagen filled, a small perforation was produced, then gradually the perforation decreased and at 8
weeks after filling, it completely closed (Fig. 44 a-d).

Case 124 (58 years old, female) is the patient with maxillary sinus perforation which developed on occasion of tooth extraction of 6. Immediately TRE-641 was filled into the tooth extraction wound, and follow-up observation revealed that maxillary sinus stroma did not develop, and at 15 days after filling, complete closure was obtained (Fig. 45 a-d).

[Safety evaluation results]
Abnormal variation of clinical laboratory test value associated with test material and adverse reaction were not observed in all the patients.

[Usefulness judgment results]
According to combination of effectiveness and safety, usefulness judgment results by application are shown in Fig. 38. Rate of "useful or grater" for TRE-641 (usefulness rate) was as follows: by application, tooth extraction of patients affected by disease was in 93 %, normal tooth extraction, complicated exodontia, and impacted tooth extraction of healthy people in 96 %, tooth extraction alveolus, re-curettage /postoperative hemorrhage in 83 %, maxillary sinus stoma (perforation) in 86 % and total in 93 %. Microfibrous atelo-collagen sheet which was used as control for patients affected by disease was in 82 %.

Fig. 39-a-c Case No. 106 (patient with anaplastic anemia)
Fig. 39-a Prior to filling
Fig. 39-b Immediately after filling of TRE-641
Fig. 39-c 1 day after filling

Fig. 40-a-c Case No. 106 (handicapped)
Fig. 40-a Prior to filling of TRE-641
Fig. 40-b Immediately after filling of TRE-641
Fig. 40-c At the time of completion of suture

Fig. 41-a-d Case No. 129 (patient administered warfarin)
Fig. 41-a Prior to filling of TRE-641
Fig. 41-b Immediately after filling of TRE-641
Fig. 41-c At 8 days after filling
Fig. 41-d At 22 days after filling (healing was completed)
Fig. 42-a-d Case No. 81 (impaction tooth extraction of healthy people)

Fig. 42-a Prior to filling of TRE-641

Fig. 42-b Immediately after filling of TRE-641

Fig. 42-c At 8 days after filling

Fig. 42-d At 14 days after filling (healing was completed)

Fig. 43-a-d Case No. 33 (recurettage of the tooth extraction cavity)

Fig. 43-a Prior to filling of TRE-641

Fig. 43-b Immediately after filling of TRE-641

Fig. 43-c At 6 days after filling

Fig. 43-d At 22 days after filling (healing was completed)
Fig. 44-a-d Case No. 61 (antrooral fistula)
Fig. 44-a Prior to filling of TRE-641
Fig. 44-b Immediately after filling of TRE-641
Fig. 44-c At 7 days after filling
Fig. 44-d At 52 days after filling (healing was completed)

Fig. 45-a-d Case No. 124 (maxillary sinus perforation)
Fig. 45-a Prior to filling of TRE-641
Fig. 45-b Immediately after filling of TRE-641
Fig. 45-c At 8 days after filling
Fig. 45-d At 15 days after filling (healing was completed)
Discussion

Bovine dermis derivative atelo-collagen sponge that we used this time is made by mixing fibrillar atelo-collagen and heat denatured atelo-collagen, and it has both biostability and bioaffinity. It is a tissue replacement type medical material, so when it is patched on skin/mucosal defect portion, it makes blood vessel/cell invade from the surrounding self tissue for a short period, and gradually replaced by being resolved and absorbed in associated with generation/maturation of self tissue for a long period \(^{14,15}\). This sheet composed of bilayer structure of atelo-collagen sponge and silicone membrane has been currently used as graft for dermal defect (Terudermis\(^{8}\)) with an aim of repairing severe skin/mucosal defect from palatoplasty in patients with palate cleft, thermal burn, trauma, and surgical wound \(^{6-11}\). We have processed this atelo-collagen sponge into the bullet shape which is easy to apply to the tooth extraction wound \(^{13,16,17}\) and filled in the various tooth extraction wounds, and evaluated an extent of pain or bleeding, epithelization complete period, fistulous opening closure period, an extent of depression or inflammation, presence or absence of infection, and operability, then examined its usefulness as protective material for the tooth extraction wound.

[With regard to tooth extraction of morbid people]

As with patients with blood disease, we filled the tooth extraction wound of patients with anaplastic anemia with TRE-641 which was immersed in liquid thrombin, and obtained localized hemostasis. When this was compared with hemostatic floor splint conventionally used for patients with blood disease, equivalent hemostatic effect was obtained, and this was considered to be extremely easy localized hemostasis. In patients with haemophilia, when dose amount of VII factor preparation or IX factor preparation was decreased from conventional amount, hemostasis was possible in early stage postoperatively \(^{18}\). This may largely contribute to avoiding the adverse reactions due to use of blood preparation, and provide very easy hemostasis for the surgeon.

There are various kinds of handicapped patients with disorder of motor function or understanding ability, abnormality in behavior, problems in medical aspects, and complications of these \(^{19}\), and if they do not cooperate on tooth extraction, the surgeon may have difficulties in behavioral management and after-treatment for them. Using TRE-641 for these patients may provide particularly marked hemostatic effect and granulation formation effect, and may reduce aberrance of food debris to keep oral cleaning.

The causal diseases that may be subjects of administration of anticoagulant tend to be comparatively severe circulatory diseases, and bleeding problems frequently occur in tooth extraction treatment. Because of this, usually tooth extraction is done after discontinuation of anticoagulant for
3-4 days before performing tooth extraction. However, in this trial, taking systemic condition of patients into consideration, TRE-641 was administered to patients who underwent tooth extraction without discontinuing anticoagulant. Retaining bleeding for which we had misgivings was not observed with good treatment course.

[With regard to ordinary tooth extraction/difficult tooth extraction/of healthy people]

We have used TRE-641 for tooth extraction of 28 patients with no causal disease and found no patients who developed persisting swelling/bleeding/ pain excluding very invasive tooth extraction such as difficult tooth extraction/impaction tooth extraction. There were no patients with symptoms such as dry socket/postoperative bleeding, and good healing was obtained. This may suggest that filling TRE-641 immediately after tooth extraction for comparatively invasive tooth extraction such as difficult tooth extraction/impaction tooth extraction may prevent dry socket/postoperative bleeding as postoperative accidental disease.

[Recurettage/postoperative bleeding of the tooth extraction cavity]

In 3 patients who underwent recurettage of the tooth extraction cavity, early alleviation of pain was observed after filling TRE-641. The reason may be that early granulation formation and epithelization by TRE-641 covered the exposed bone surface, which may have led to alleviation of pain. Using TRE-641 for postoperative bleeding may provide sufficient hemostasis.

[With regard to antrooral fistula (perforation)]

For antrooral fistula for which natural closure is not expected, though a long period of time has passed since tooth extraction, we have filled TRE-641 after curettage/postoperative bleeding of mucosa around fistulous opening and obtained closure of the fistulous opening. Patients with maxillary sinus perforation caused by tooth extraction who underwent filling of TRE-641 had a complete closure in 2-3 weeks after filling except one patient. Some of these patients had comparatively large perforations caused by tooth extraction, but perforation was easily closed and as a result they had a course without accident of antrooral fistula. This may suggest that if perforation is developed in the maxillary sinus when the dentist carried out tooth extraction, filling TRE-641 as the first choice may have a possibility to prevent the occurrence of antrooral fistula, which may avoid unnecessary anxiety and a waste of time for both the dentist and patients. In 1 patient who did not achieve fistulous opening closure, marked pus discharge was observed at 4 weeks and infection was suspected, so TRE-641 was removed at discretion of the surgeon in charge. After that, the state of the
tooth extraction wound was good, and fistulous opening was reduced though it remained. Based on these results, in patients who show pus discharge for a long time of period, and cannot obtain resolution, application of TRE-641 may be difficult, so conventional maxillary sinus radical cure/plastic closure of oroantral opening may be applied.

[With regard to use method]

As use method of TRE-641, wiping of the tooth extraction wound and filling was sufficient to retain to the wound portion. But for patients with shallow tooth extraction wound, gingival suture of filling part or combination use of floor splint for hemostasis may provide easier fixation. TRE-641 is bullet shaped and there are 3 kinds of sizes in outer diameter and length available. It is important to use the size that adheres appropriately to the tooth extraction wound. If appropriate adhesion was obtained, it absorbs blood to be slightly swollen with humidity and more effective press hemostasis may be obtained.

[With regard to comparison with control]

Both TRE-641 and control are atelo-collagen materials, and though comparison by subjective judgment of the surgeon in charge, hemostatic effect of both may be by no means inferior as is shown in Fig 11. With operability, control was a sheet type and it required the operation of rounding or folding at the time of filling into the tooth extraction wound, whereas, TRE-641 was used as it is after being taken out from the package, so extremely easily and hygienically used. With aspect of granulation formation, as is shown in Fig. 13, TRE-641 was dissolved slowly, which provided less depression at the time of epithelization completion, whereas, control easily melt in the early stage immediately after filling, providing larger depression, which may provide scarce effect on granulation formation. When compared use feeling of TRE-641 to gelatin sponge preparation or oxycellulose preparation used with an aim of hemostasis, TRE-641 is easy to fill and has excellent adhesion to the tooth extraction wound. Experiment of filling to the tooth extraction wound of the canine showed less harmfulness compared to gelatin sponge preparation or oxycellulose preparation\textsuperscript{21, 22}, and considered to have higher tissue affinity to the wound as biomaterial.

[With regard to infection]

Three patients for whom TRE-641 was used developed infection, though similar administration method of antibiotic as the ordinary treatment. In 1 patient, pus discharge was observed from the wound, and it was removed by judgment of the surgeon in charge, and healing of tooth extraction after that was good. One patient developed infection at 3 days after surgery, and after
administration of antibiotics, the patient was recovered without problems by gargling. One patient was recovered without problems by cleaning the tooth extraction wound. There were no patients who developed infection. If infection is suspected, it will be necessary to take an appropriate measure such as cleaning or gargling in early stage.

[With regard to Safety]

In laboratory test pre- and post clinical trial, abnormal variations of test values that may be associated with test material were not observed in any patient. All through the period of this clinical trial, no patients showed symptoms suggesting adverse reaction due to TRE-641, indicating the material with higher safety.

Based on the above, for not only tooth extraction of healthy people but also that of morbid people accompanying with bleeding or infection, TRE-641 can be used safely if the control of the disease is sufficient. So, it was demonstrated that TRE-641 is the medical material excellent in postoperative hemostatic effect, alleviation of pain, and prevention of depression without delay of healing.

Conclusion

[1] Bovine dermis derivative atelo-collagen tooth extraction wound protective material TRE-641 was applied to various kinds of tooth extraction wounds, and its usefulness was examined comprehensively.

[2] Usefulness (more than useful) was 93% for the whole TRE-641. In morbid patients, clinical trial was performed with bovine tendon derivative fine fibrous atelo-collagen sheet as control, and it was 93% for TRE-641 and 82% for control. Usefulness was as high as 96% for ordinary tooth extraction/difficult tooth extraction/impaction tooth extraction of healthy people, 83% for recurettage/postoperative bleeding of the tooth extraction cavity, and 86 % for antrooral fistula (perforation).

[3] In blood disease patients, using TRE-641 for localized hemostasis provides sufficient localized hemostasis without using a floor splint for hemostasis depending on patients. This suggests a possibility to decrease the administration dose of substitution therapy which was conventionally required.
[4] For non cooperative patients who have physically and mentally disorders and for whom bite hemostasis is not possible, filling TRE-641 may provide sufficient hemostasis.

[5] For patients who have perforation at the maxillary sinus after tooth extraction, filling of TRE-641 as the first choice may prevent the subsequent accident of antrooral fistula. For the old antrooral fistula, filling after curettage/bleeding of the mucosa around the fistulous opening may have a possibility of closure.

[6] Even easily infectious patient such as diabetes mellitus, if control of disease is sufficient, it can be used without problems, by preforming normal infection prevention measure.

[7] Allergy reaction associated with the bovine dermis derivative was not observed in any patient, and there was no patients with problems regarding safety.