Novel Xyloglucan Sheet for the Treatment of Deep Wounds: Preparation, Physicochemical Characteristics, and in Vivo Healing Effects

Kaoru Hirose, Masanaho Sasatsu, Tatsunori Toraiishi, and Hiraku Onishi

INTRODUCTION

The number of elderly individuals with pressure ulcers is expected to increase in Japan. Therefore, it is important to develop appropriate methods to treat such deep wounds.1) Recently, moist healing, including occlusive dressing therapy, is considered the most acceptable. This method can promote wound healing by sealing wounds and keeping wound healing factors.2–5) Also, patient-friendly wound dressings are required for the enhancement of QOL.6–8)

Ointments and creams are formulations are frequently used because they can be treated and adjusted in the wound moist environment.9,10) However, the exchange of these medicines is sometimes accompanied by difficulty in removal and induction of pain.11) A spray type of medicine containing basic colloid (3M Health Care, MN, U.S.A.) and Viewgel® (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) are used as commercial products.

Animals Male Wistar rats (7 weeks old, 200–210 g) were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan), and used in animal studies within one week. Rats were fed using the breeding diet MF (Oriental Yeast, Tokyo, Japan) with water ad libitum at 23 ± 1°C and a relative humidity of 60 ± 5%. The animal experiment protocol was approved by the Committee on Animal Research of Hoshi University (Tokyo, Japan). Animal experiments were executed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University.

Preparation of Xyl Sheets Xyl was dissolved in water with a magnetic stirrer for 24 h, leading to a polymer solution of 3, 4.5, and 6% (w/v). Xyl solution (4 g) mixed with Suc (1, 2, or 4 g) was poured into a square plastic box (4 × 4 × 2 cm (in depth)), and the box was placed horizontally on a hot plate warmed at 37°C. The time at which the fluidity of the mixture was lost was evaluated as the sheet formation time.

In the present study, their preparation, physicochemical characteristics and in vivo efficacy were investigated.

MATERIALS AND METHODS

Materials Xyl was supplied by DSP Gokyo Food & Chemical Co., Ltd. (Osaka, Japan). Sucrose (Suc) was provided by Kozakai Pharmaceutical Co., Ltd. (Tokyo, Japan). All other chemicals were of reagent grade. Tegaderm™ hydrocolloid (3M Health Care, MN, U.S.A.) and Viewgel® (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) were used as commercial products.

Animals Male Wistar rats (7 weeks old, 200–210 g) were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Tokyo, Japan), and used in animal studies within one week. Rats were fed using the breeding diet MF (Oriental Yeast, Tokyo, Japan) with water ad libitum at 23 ± 1°C and a relative humidity of 60 ± 5%. The animal experiment protocol was approved by the Committee on Animal Research of Hoshi University (Tokyo, Japan). Animal experiments were executed in compliance with the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University.

Preparation of Xyl Sheets Xyl was dissolved in water with a magnetic stirrer for 24 h, leading to a polymer solution of 3, 4.5, and 6% (w/v). Xyl solution (4 g) mixed with Suc (1, 2, or 4 g) was poured into a square plastic box (4 × 4 × 2 cm (in depth)), and the box was placed horizontally on a hot plate warmed at 37°C. The time at which the fluidity of the mixture was lost was evaluated as the sheet formation time.
Measurement of Physical Characteristics Tensile strength and extensibility were measured using the rheometer FUDOH RHEO METER RTC-2005 D (Rheotech Co., Ltd., Tokyo, Japan). The sheet was cut into 3×4 cm, and the upper side (1.5 cm) and bottom side (1.5 cm) were fixed using clamps. The sheet was pulled at 6 cm/min. Strength immediately before the sheet broke was measured as tensile strength. Young’s modulus was calculated using the following equation.

Young’s modulus = tensile strength / extensibility

Adhesion characteristics to the plastic plate were investigated by referring to the 12th edition of the Japanese Pharmacopoeia and Frandol tape interview form. The sheet (4×4 cm) was fixed to the bottom plate of the rheometer. A disk-shaped bakelite plate (2 cm) fixed to the upper plate of the rheometer was attached to the above sheet with a force of 10 N for 1 min. The lower plate was then pulled down at 2 cm/min. The time at which the sheet separated from the bakelite plate was defined as the adhesion time, and strength immediately before separation was obtained as adhesion strength. The adhesion time was not markedly prolonged warming (24 h) in all formulations. Physical characteristics of Xyl sheets were examined at several concentrations of Xyl and Suc (Table 1). The thickness of the sheet was slightly larger when the concentrations of Suc were higher. At 6 and 24 h, tensile strength was greater when the concentration of Suc was lower. The gelation time became shorter as the concentration of Suc increased. Tensile strength was higher at 24 h than at 6 h in every formulation, suggesting the sheet strength should be greater with the decrease in water content (see next section). Extensibility decreased with time in every formulation, which was due to the loss of water with prolonged warming (24 h). Young’s modulus increased after prolonged warming (24 h) in all formulations.

Efficacy Studies in Rat Wound Models The deep wound model was created according to the method reported by Machida et al. Rats were anesthetized by an intraperitoneal (i.p.) injection of pentobarbital at 25 mg/kg (4 mL/kg in saline), and their back hair was shaved. A 1.0-cm circular flap of skin located 1.0 cm from the midline to the right was removed. A brass cylindrical tube with a circular basement (diameter: 1.0 cm) and wall thickness of 0.02 cm was charged dry ice-acetone mixture. This tube was placed on the wound, which was covered with a plastic film, for 3 min using tube weight only. After the frostbite had been produced, the wound was covered with 6 sheets of gauze and protected by surgical adhesive tape. Each injured rat was bred with one animal per cage.

The treatment was performed 24 h after the production of frostbite. A Xyl sheet (3.0% Xyl/33.3% (w/w) Suc) was used in the in vivo test. Previously reported Xyl/Suc hydrogel, made by mixing 3.0% Xyl solution (4.0 g) and Suc (4.0 g), was used as Xyl hydrogel. A CATHEREEP FS ROLL was used as a polyurethane film. After these preparations were applied to wound surfaces, they were covered with 3 sheets of gauze. Gauze alone (3 sheets) was used as the control. Finally, each was fixed using surgical adhesive tape. Each preparation was changed to new one once every day, when wound surface state and size were observed. Every time changing the preparation, the wound surface was washed with saline and wiped gently with a non-woven wiper. The wound area was determined as follows, and its ratio to the area immediately before treatment was calculated.

Wound area

\( = \text{length of the longest axis in the wound (cm)} \times \text{length of the axis vertical to the longest axis (cm)} \)

Statistical Analysis In the in vivo studies, each dressing was compared with the control (gauze alone) using ANOVA followed by the Dunnett’s post hoc test. The criterion for significant difference was set at \( p < 0.01 \).

RESULTS AND DISCUSSION

Formation of Xyl Sheets Xyl forms a gel under the coexistence of sugar of 40–65% (w/w) in the solution. Therefore, Xyl and Suc were mixed in water, and water was evaporated gradually on the hot plate at 37°C to form the wet sheet. In every formulation, sheets were formed within 4 h (Fig. 1). Therefore, Xyl sheets were produced by warming at 37°C for 6 h in all formulations.

Physical Characteristics of Xyl Sheets Physical characteristics of Xyl sheets were examined at several concentrations of Xyl and Suc (Table 1). The thickness of the sheet was slightly larger when the concentrations of Suc were higher. At 6 and 24 h, tensile strength was greater when the concentration of Suc was lower. The gelation time became shorter as the concentration of Suc increased. Tensile strength was higher at 24 h than at 6 h in every formulation, suggesting the sheet strength should be greater with the decrease in water content (see next section). Extensibility decreased with time in every formulation, which was due to the loss of water with prolonged warming (24 h). Young’s modulus increased after prolonged warming (24 h) in all formulations.

Adhesion strength increased as the concentration of Suc decreased (Table 1). A lower concentration of Xyl slightly gave stronger adhesion. The adhesion time was not markedly affected by changes in the Suc concentration.

The adhesion properties of the 3% Xyl/33.3% Suc sheet were compared with those of Tegaderm® hydrocolloid and Viewgel® (Fig. 2). The adhesion strength of the Xyl sheet
was significantly greater than those of the commercial dressings. On the other hand, Tegaderm® hydrocolloid showed the longest adhesion time, which might be because Tegaderm® hydrocolloid has an adhesion layer. Shorter adhesion time is considered better in removal from wound surface. In addition, the high adhesion strength may be useful to fixation to the application site. The adhesion features of the Xyl sheet were similar to those of commercial formulations, suggesting that the sheet would be acceptable for handling.36,37)

### Table 1. Physical Parameters of Sheets Made of the Different Xyl and Suc Contents 6 and 24 h after Warming Xyl Solution at 37°C

<table>
<thead>
<tr>
<th>Xyl (%)</th>
<th>Suc (%)</th>
<th>6 h</th>
<th>24 h</th>
<th>6 h</th>
<th>24 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tensile strength (MPa)</td>
<td>Extensibility (%)</td>
<td>Young modulus (MPa)</td>
<td>Adhesion strength (MPa)</td>
<td>Adhesion time (s)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>1.36 ± 0.03</td>
<td>2.51 ± 0.12</td>
<td>300.57 ± 16.61</td>
<td>125.10 ± 3.65</td>
<td>0.45 ± 0.01</td>
<td>2.01 ± 0.04</td>
</tr>
<tr>
<td>33.3</td>
<td>0.29 ± 0.01</td>
<td>0.55 ± 0.12</td>
<td>140.67 ± 3.06</td>
<td>108.20 ± 7.53</td>
<td>0.21 ± 0.01</td>
<td>0.51 ± 0.08</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>50</td>
<td>0.06 ± 0.01</td>
<td>0.13 ± 0.02</td>
<td>218.07 ± 15.35</td>
<td>125.93 ± 6.56</td>
<td>0.03 ± 0.00</td>
<td>0.10 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>4.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.33 ± 0.04</td>
<td>3.01 ± 0.27</td>
<td>196.00 ± 50.78</td>
<td>88.07 ± 11.42</td>
<td>0.63 ± 0.13</td>
<td>3.44 ± 0.35</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>33.3</td>
<td>0.39 ± 0.10</td>
<td>1.03 ± 0.21</td>
<td>165.40 ± 0.30</td>
<td>136.57 ± 0.21</td>
<td>0.24 ± 0.06</td>
<td>0.75 ± 0.04</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.22 ± 0.10</td>
<td>3.01 ± 0.45</td>
<td>136.70 ± 27.01</td>
<td>75.78 ± 12.68</td>
<td>0.90 ± 0.01</td>
<td>4.06 ± 0.73</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>33.3</td>
<td>0.60 ± 0.13</td>
<td>1.49 ± 0.10</td>
<td>226.67 ± 41.63</td>
<td>165.50 ± 3.82</td>
<td>0.27 ± 0.02</td>
<td>0.90 ± 0.08</td>
<td>0.01 ± 0.00</td>
</tr>
<tr>
<td>50</td>
<td>0.06 ± 0.02</td>
<td>0.16 ± 0.02</td>
<td>195.00 ± 19.50</td>
<td>170.87 ± 34.78</td>
<td>0.03 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.00 ± 0.00</td>
</tr>
</tbody>
</table>

*a* The results are expressed as the mean ± S.D. (n = 3).

---

**Fig. 2.** Adhesion Strength and Adhesion Time of Xyl Sheet (3% Xyl/33.3% Suc), Tegaderm® Hydrocolloid and Viewgél®

The results are expressed as the mean ± S.D. (n = 3).

**Fig. 3.** Water Contents of Sheets Made of the Different Xyl and Suc Contents 6 and 24 h after Warming Xyl Solution at 37°C

The results are expressed as the mean ± S.D. (n = 3).
the water uptake by the formation of hydrogen bond networks with Suc. The commercially available dosage forms, Reflap® ointment (water-in-oil emulsion base) have similar water contents. Therefore, Xyl sheets may give wet condition adequately.

**Water Absorption by Xyl Sheets** Water absorption, caused because of the compensation for water loss by warming, progressed gradually. The water absorbed at 5 h was 0.5–0.7 mL at the conditions of 20% Suc and 33.3% Suc, but at a Suc concentration of 50%, the water of 0.3–0.5 mL was absorbed at 5 h. Xyl sheets of high Suc concentration might tend to show less water absorption. The water absorption profiles were compared among the 3% Xyl/33.3% Suc sheet, Tegaderm® hydrocolloid and Viewgel® (Fig. 4). The Xyl sheet showed a water absorption profile with a very slight deviation.

![Water Absorption Profiles of Different Types of Wound Dressings](image1)

**Fig. 4. Water Absorption Profiles of Different Types of Wound Dressings**

Data are Xyl sheet of 3% Xyl/33.3% Suc (●), Tegaderm® hydrocolloid (□), and Viewgel® (▲). The results are expressed as the mean ± S.D. (n = 3).

![Changes in Wound Surface Area after Application of Different Preparations in Rats with Deep Wound](image2)

**Fig. 5. Changes in Wound Surface Area after Application of Different Preparations in Rats with Deep Wound**

The results are expressed as the mean ± standard error (S.E.) (n = 4).

![Wound States during the Treatment Using Different Preparations](image3)

**Fig. 6. Wound States during the Treatment Using Different Preparations**

One interval of the scale = 1 mm.
The absorption rate was similar to that of Tegaderm™ hydrocolloid, whereas Viewgel® had a slower absorption rate. The Xyl sheet showed similar water absorptibility to the commercial dressings.

**Efficacy in the Severe Wound Model** Changes in wound areas are shown in Fig. 5. After 7d, the decrease was significantly greater with Xyl sheet than with gauze (p < 0.01). On day 15, the wound area almost completely disappeared. For the Xyl hydrogel, the wound area decreased faster at the later period. After 9d, the wound surface area with the Xyl hydrogel was significantly smaller than that with gauze (p < 0.01). Gauze and the polyurethane film removed exudates poorly, while the Xyl sheet and Xyl hydrogel effectively removed exudates, resulting in good healing effects. The Xyl sheet appeared to show higher absorption of exudate than the Xyl hydrogel, which would be related to water absorption characteristics of the Xyl sheet. This point might be the difference in effect between sheet and hydrogel.

Although the wound states were only observed visually, the Xyl sheet tended to suppress wound severity as shown in Fig. 6. In this efficacy studies, the Xyl sheet was softened obviously, which might be partly due to the absorption of the exudate. Suc was considered to accelerate the granulation of the wound surface from its biological function. From these results, the Xyl sheet was proposed to be superior for wound healing.

**CONCLUSION**

Xyl sheets could be produced using a simple casting method. The sheets had appropriate physical features for handling, and exhibited water absorption and retention similar to those of commercial wound dressings. The Xyl sheet exerted good healing effects in a rat severe wound model. The Xyl sheets could be produced using a simple casting method and PolyMem Silver®. An innovative bi-layered wound dressing made of silk and gelatin for accelerated wound healing. 

**REFERENCES**


39) Kowa Co., Ltd., Package insert: UPASTA KOWA OINTMENT·oint. 8g pack (2013).