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고분자 인공피부의 합성과 성질(Ⅰ)
가교젤라틴과 폴리우레탄으로 된 복합막의 평가

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Synthesis and Properties of Polymeric Skin Substitute (Ⅰ)
Evaluation of Bilayer Membrane Composed of Crosslinked Gelatin and Polyurethane

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요 약 : 표피성분으로는 의료용폴리우레탄을, 진피성분으로는 다공성의 가교화 젤라틴—폴리비닐알코올을 사용하여 이중막을 제조하였다. 인공피부로서 이용가능성을 검토하기 위하여 건조상태와 습윤상태에서의 인장강도 및 신장율, 팽윤도, 생체분해율, 수증기 및 산소투과성을 측정하였다. 가교제의 농도가 증가함에 따라 팽윤도는 감소하였으며, 인장강도는 건조상태에서는 500kg/cm² 에서 720kg/cm² 까지, 습윤상태에서는 150kg/cm² 에서 260kg/cm² 까지 증가하였다. 신장율은 건조상태에서는 7%에서 50%까지, 습윤상태에서는 10%에서 65%까지 변화하였다. 생체분해율은 초기에는 매우 빠르게 증가하였으나 4~6일 이후에는 평형상태로 거의 변화하지 않았다. 폴리우레탄막의 건조시간에 따라 수증기투과율은 6~1.8(mg/cm²/hr), 산소투과계수는 10^{-7}~10^{-8}(cm³(STP)cm/cm²·sec·cmHg)으로 변화하였다. 이상의 실험결과로 부터 본 연구에서 측정한 이중막의 물성값들은 인공피부를 설계하는 데 있어서 목표값들을 대부분 만족하였다.

Abstract : The bilayer membrane which comprises a upperlayer of a medical grade polyurethane and a underlayer of a porous corsslinked network of gelatin and poly(vinyl alcohol) was prepared. Possibilities of the membrane for the usuage as skin substitute were estimated by measuring tensile strength and elongation under dry or wet condition and biodegradation ratio, and also studied by permeabilities of water vapor and oxygen. The degree of swelling was decreased with increasing the concentration of cross-linking agent. And the tensile strength was increased with increasing the concentration of cross-linking agent, from 500kg/cm² to 720kg/cm² at dry condition and from 150kg/cm² to 260kg/cm² at wet condition. The value of elongation was varied within the limits from 7% to 50% at dry condition and from 10% to 65% at wet condition. The rate of biodegradation was very fast at the initial state but it became slow down after 4~6 days and accessed to the equilibrium state. Water vapor transmission rate and permeability coeffcient of O_2 varied with the change of evaporating time of polyurethane membrane and their values were controlled 6~1.8(mg/cm²/hr) and [10^{-7}~10^{-8}cm³(STP)cm/cm²·sec·

cmHg), respectively. These values of the bilayer membrane measured in this study were satisfied with those of ideal skin substitute.

INTRODUCTION

Numerous skin replacements for burn wound healing have been investigated. These have generally consisted of biological coverings from the patient, other human or animal donors. (autograft, homograft and xenograft, respectively).[1] Among these, autograft is the most ideal, but it has the limitation of grafting when the amount of skin loss is excessively extensive. Homograft or xenograft which is frequently used to dress burn wound seems to be an ideal prosthetic method, however, its rejection symptoms, such as its antigenicity and bacterial contamination, force to seek for synthetic skin substitutes.[2] The ideal skin substitute should be adherent, hygroscopic, elastic, durable, non-antigenic, and non-toxic, and it must have proper water vapor transport and permeability of oxygen and protect against bacterial invasion, therefore promote remedial value.[3]

Destruction of the body skin by burn injury brings about increase in fluid loss. And individuals who have suffered extensive skin loss get into dehydration state and also lose the equilibrium of body temperature. By contrast to the above, if the moisture flux through the skin substitute is excessively restrained, water accumulates at the interface between woundbed and impermeable graft. And this makes inflammation, which increase the procrastination of covalescence, and consequently proper water vapor transmission rate and hygroscopic are the most important properties in designing a skin substitute.

The skin substitute is usually employed for short-term (1 to 3 weeks)and long-term (1 to 4 months) wound treatments. The skin substitute for short-term treatment has optimal water vapor transmission rate and good adherence to prevent bacterial infection. And the skin substitute for long-term treatment has not only all of the properties of short-term skin substitute but also capacity of being readily degraded by extracellular enzymes or by simple hydrolytic scission into non-toxic and nonantigenic materials which improve remedial value.[2,6,12]

Various types of polymeric materials called synthetic skin substitute or wound dressing, such as foam, film, velure, sponge and laminate have been produced and used to cover donor site or early second-degree burns made use of silicon rubber, Nylon 6, polypeptide, fibrin and collagen. But clinical practice showed that there is still a great need for new, more perfect skin substitute.[2,3,13]

In this study, we have produced a bilayer membrane which comprises a upperlayer of a medical grade polyurethane and a underlayer of a gelatin obtained from collagen by hydrolysis.[4] This biosynthetic skin substitute was produced in order to improve treatment by performing prevention of excessive fluid loss and making formation of epidermis and granulation tissue. The underlayer adhered closely to woundbed was freeze dried to have macroporous structure which facilitate proper tissue ingrowth.[5] This layer is finally hydrolized and replaced with newly synthesized connective tissue. The upperlayer compensates the deficient flexibility of underlayer, protects the woundbed against bacterial invasion and ensures a proper water vapor transport through the bilayer membrane. It is finally stripped off from the rehealed wound.[7]

Possibilities of the membrane for the usuage as skin substitute were estimated by measuring tensile strength under dry or wet condition, and also studied by permeabilities of water vapor and oxygen.

EXPERIMENT

Materials

Pharmaceutical gelatin (Won Hee Trade Co.)

was used by drying under reduced pressure (gel strength : 280−320(6 $\frac{2}{3}$%), viscosity : 45-55mps (60℃, 6$\frac{2}{3}$%), pH : 4.5~5.5).

Poly(vinyl alcohol)(MW : 200,000, Taiwan, Polyol Co.) was used by drying under reduced pressure.

Medical grade polyurethane (Estane 5714 Fl, Goodrich Co.) was used for the preparation of the upperlayer. Glutaraldehyde (25% aqueous solution)(Japan, Kokusan Co.) was used as crosslinking agent. Pepsin, chymotrypsin, trypsin and protease(Sigma Co.) were used as enzymes.

Preparation of the Underlayer

Preparation of Crosslinked Gelatin Membrane

Aqueous gelatin solution(10wt%)was cast onto the PMMA plate and air dried at room temperature. The PMMA plate covered with gelatin membrane was dipped into ethanol solution to separate gelatin membrane from the PMMA plate, and the membrane was precipitated with glutaraldehyde solution in order to have crosslinked network for 24 hrs. Crosslinked membrane was washed by water at 40℃ to remove unreacted gelatin and glutaraldehyde.

The crosslinking solutions whose concentrations were 0.2, 0.4 and 0.6wt% were used and their corresponding samples were named as G−1, G−2 and G−3, respectively.

Preparation of Crosslinked Gelatin−Poly(vinyl alcohol) Membrane

Aqueous gelatin−poly(vinyl alcohol) solutions containing 3wt% of gelatin and 6wt% of poly(vinyl alcohol)were prepared. Hydrochloric acid, as an acid catalyst, was added gradually to this polymer solution up to pH 1. Then, glutaraldehyde was added to this solution, and the solution was stirred efficiently. And it was cast onto the PMMA plate and gradually crosslinked as membrane state at room temperature for 48 hrs. Glutaraldehyde solutions whose concentrations were 0.2, 0.4 and 0.6wt% were used. And their corresponding samples were named as PG-1, PG-2 and PG-3, respectively.

Degree of Swelling

The weight of completely dried samples was measured directly. And these samples were dipped into the test tube filled with water whose temperature was maintained at 36℃ in incubator. Degree of swelling, Qw(%), of these samples was calculated by the equation, $Qw(\%) = (x_2 - x_1)/x_1 \times 100$, where x_1 is the weight of dried sample and x_2 is the weight of swollen sample. This measurement had been repeated until the weight of these samples was in fully swollen state.

Tensile Strength and Elongation under Dry and Wet State

Tensile strengths under dry and wet condition at 36℃ were measured by Instron (Toyo Baldwin UTM−4−100) at a cross−head speed of 5mm/min and chart speed of 20mm/min. And 5kgf load cell was used.

Biodegradation Ratio

Biodegradation ratio was greatly influenced by enzymes released from lysosome. In this experiment, we selected Aiba's method which made use of protein hydrolysis enzymes.[3,8] In order to examine the biodegradability of underlayer, protein hydrolysis enzymes were used by dissolving to buffer solution which made enzymes have maximum activity. The enzyme solutions used in this experiment are pepsin solution (2,940 unit/mg, pH2.0, 1mg/ml, 36℃), protease solution (10 unit/mg, pH7.6, 4mg/ml, 36℃), α-chymotrypsin solution (51 unit/mg, pH7.6, 4mg/ml, 36℃) and trypsin solution (1,000 unit/mg, pH8.1, 1mg/ml, 36℃), respectively.

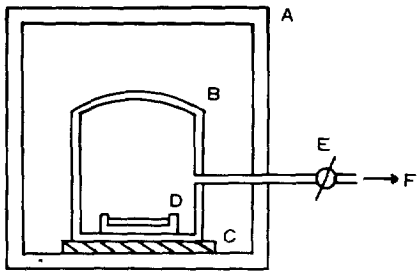
After being dipped into these solutions, samples were washed and dried completely. And then their weight loss was measured.

Freeze Dry[9]

The freeze drying apparatus constructed in our laboratory is schematically shown in Fig.1. Sufficiently swollen samples were hastily freezed by liquid nitrogen and placed onto sample holder. And vacuum was maintained with an oil diffusion pump which consistently gave a chamber pressure of 10^{-3}mmHg. And the interior of refrigerator was constant temperature level, near −40℃

Preparation of Bilayer[10]

Polyurethane was cast from the solution (10wt%) onto the surface of freeze dried underlayer. After



A : Refrigerator
B : Freeze drying chamber
C : Insulating block
D : Sample holder (with sample)
E : Vacuum cock
F : Outlet to vacuum pump

Fig. 1. Schematic diagram of apparatus used to freeze dry.

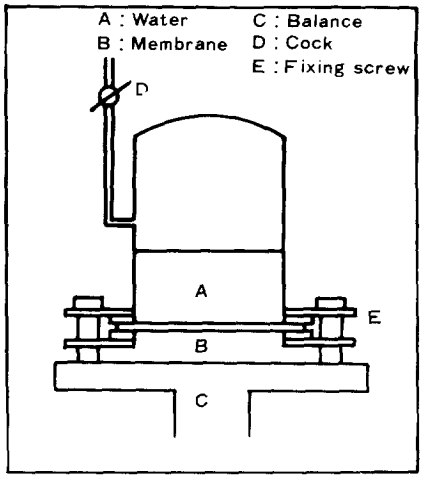


Fig. 2. Schematic diagram of water vapor transmission apparatus.

being dried under reduced pressure at constant time interval, each 10 min, cast polyurethane was solvent-exchanged by water to control the porosity.

Water Vapor Transmission Rate[3,10]

Schematic diagram of water vapor transmission apparatus is shown in Fig.2. The cell was made of PMMA and placed in incubator at constant temperature level, 36℃. The weight of the cell filled with water was measured. The amount of water vapor loss through the membrane was determined by measuring the weight decrease of the cell after 24 hrs.

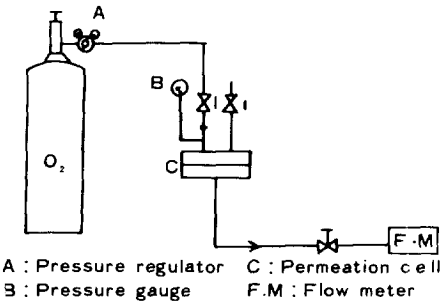


Fig. 3. Schematic diagram of gas permeation apparatus.

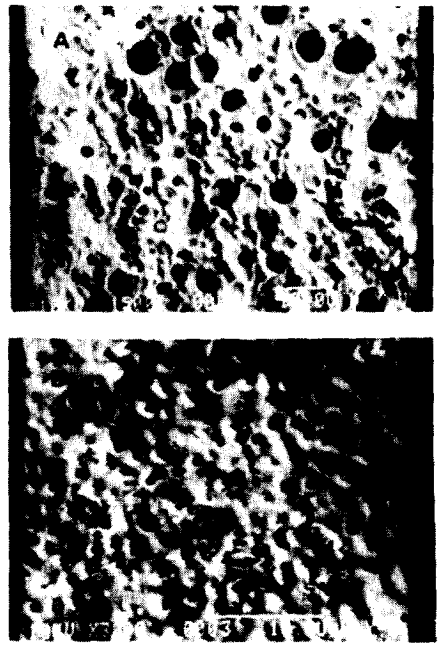


Fig. 4. Scanning electron micrograph of bilayer membrane. A) surface of underlayer, B) surface of upperlayer

Permeability of Oxygen[11]

Apparatus of gas permeation measurement is shown in Fig.3. Samples were placed in the inner part of the permeation cell. The amount of oxygen permeated through the cell was detected by soap film flow meter. Fig.4 is a typical micrograph of skin substitute prepared in this study. The upperlayer has microporous structure and prevents bacterial infection. The macroporous

structure of underlayer enables a strong attachment and proper tissue ingrowth.

RESULT AND DISCUSSION

Degree of Swelling

The degree of swelling of underlayer is shown in Fig.5. In the case of crosslinked gelatin membrane, the degree of swelling was decreased with increasing the concentration of crosslinking agent, in the order of G-1, G-2 and G-3, respectively. And in the case of crosslinked poly(vinyl alcohol)-gelatin membrane, the degree of swelling was decreased in the order of PG-1, PG-2 and PG-3, respectively. The degree of swelling was increased very fast at initial state but increased slowly at later state and reached at equilibrium state after 24 hrs. All of the samples were swollen sufficiently and regarded as not making fluid pocket which led to inflammation.

Tensile Strength

The tensile strength and elongation under dry and wet condition of these samples are shown in Table 1 and Table 2. The tensile strength of the crosslinked gelatin membrane was increased with increasing the concentration of crosslinking agent, in the order of G-1, G-2, and G-3, respectively, but the elongation was decreased. And the tensile strength of the crosslinked poly(vinyl alcohol)-gelatin membrane was increased with increasing the concentration of crosslinking agent. In the case of elongation, the value of PG-2 is best among them. As compared with dry condition, the tensile strength of all the samples showed a trend of decrease. Nevertheless all of the swollen samples have the tensile strength over 100kg/cm^2. And the value of the elongation of PG-2 is 63%. Therefore, the samples could be considered as stable and durable skin substitute when they were soaked by fluid.

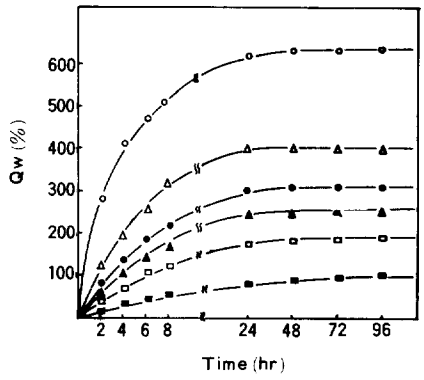


Fig. 5. Degree of swelling vs. swelling time of crosslinked gelatin membranes and crosslinked PVA−gelatin membranes at 36℃. ○, G−1 ; △, G−2 ; ●, PG−1 ; ▲, PG−2 ; □, G−3 ; ■, PG−3

Biodegradation Ratio

The result of biodegradation by trypsin, pepsin, α-chymotrypsin and protease are shown in Fig.6, Fig.7, Fig.8, and Fig.9, respectively. From these results, we knew that gelatin-poly (vinyl alcohol) membranes were degraded partially by the protein hydrolysis enzymes but still maintained a function of membrane. Thus we recognized that the degraded part of these mem-

Table 1. Tensile Strength and Elongation of Dry Crosslinked Gelatin Membranes and Crosslinked PVA−Gelatin Membranes at 36℃

Sample	Tensile strength (kg/cm^2)	Elongation (%)
G−1	501	11
G−2	694	8
G−3	721	7
PG−1	620	20
PG−2	644	49
PG−3	678	12

Table 2. Tensile Strength and Elongation of Swollen Crosslinked Gelatin Membranes and Crosslinked PVA−Gelatin Membranes at 36℃

Sample	Tensile strength (kg/cm^2)	Elongation (%)
G−1	150	22
G−2	220	12
G−3	267	10
PG−1	212	32
PG−2	210	63
PG−3	229	28

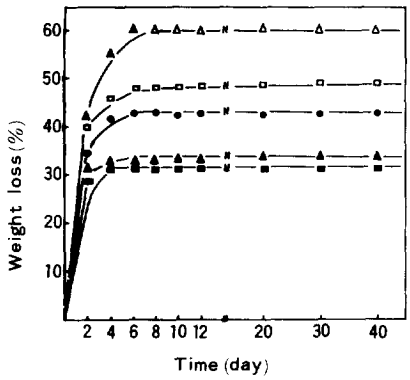


Fig. 6. Biodegradation of crosslinked gelatin membranes and crosslinked PVA−gelatin membranes by trypsin at 36℃.
△, G−2 ; □, G−3 ; ●, PG−1 ;
▲, PG−2 ; ■, PG−3.

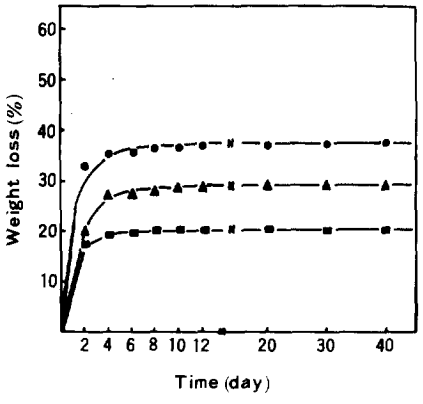


Fig. 7. Biodegradation of crosslinked gelatin membranes and crosslinked PVA−gelatin membranes by pepsin at 36℃.
●, PG−1 ; ▲, PG−2 ; ■, PG−3

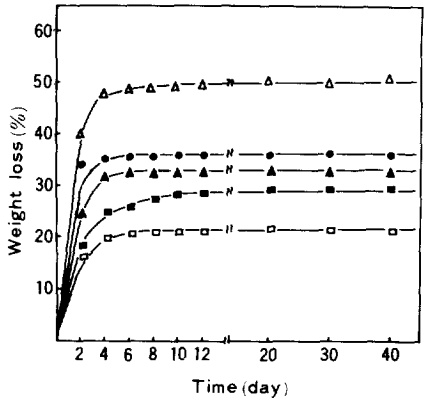


Fig. 8. Biodegradation of crosslinked gelatin membranes and crosslinked PVA−gelatin membranes by α-chymotrypsin at 36℃.
△, G−2 ; ●, PG−1 ; ▲, PG−2 ;
■, PG−3 ; □, G−3

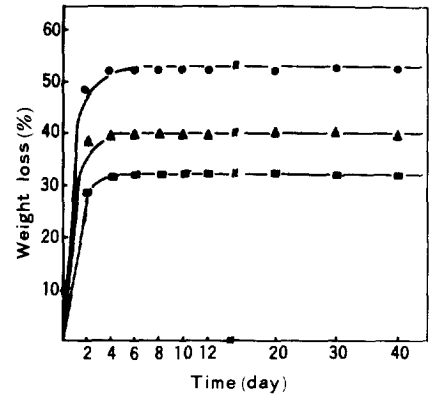


Fig. 9. Biodegradation of crosslinked gelatin membranes and crosslinked PVA−gelatin membranes by protease at 36℃.
●, PG−1 ; ▲, PG−2 ; ■, PG−3

branes improve healing effect and the rest part of these membranes keep their functions as real dermis when these membranes were really grafted onto burn woundbed.

Water Vapor Transmission and Oxygen Permeability

The relation between evaporating time and the water vapor transmission rate of polyurethane membrane is shown in Fig.10. Because the freeze dried underlayer was macroporous, the water vapor transmission rate of the skin substitute in this study was influenced primarily by the upperlayer of polyurethane membrane. Therefore, we carried out the experiment of the water vapor transmission rate against the evaporating time to control the water vapor transmission rate of polyurethane membrane. The water vapor transmission rate was decreased with increasing the evaporating time of polyurethane membrane.

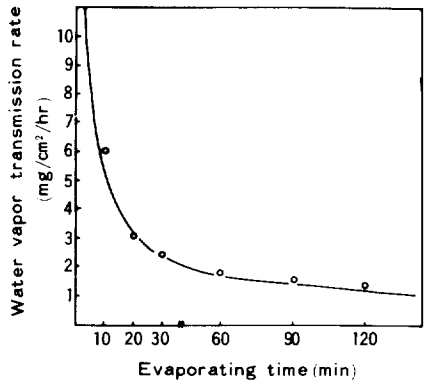


Fig. 10. Water vapor transmission rate of polyurethane membranes as a function of evaporating time.

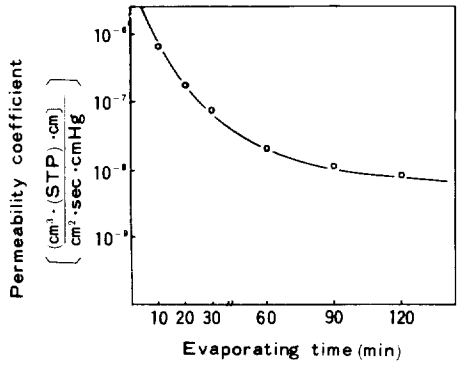


Fig. 11. Oxygen permeabilities of polyurethane membranes as a function of evaporating time.

The desirable value of the water vapor transmission rate was reported over 1.5mg/cm²/hr. Considering that the water vapor transmission rate of the normal skin is about 0.5mg/cm²/hr and that of open burn wound is about 13mg/cm²/hr, we would know that, in this study, the samples evaporated from 20 min to 1 hr under reduced pressure had an ideal water vapor transmission rate as a skin sbstitute.

The relation between the evaporating time and oxygen permeability of polyurethane membrane is shown in Fig.11. Oxygen permeability was also influenced chiefly by polyurethane membrane. And oxygen permeability was decreased with increasing the evaporating time of polyurethane membrane. We also knew that polyurethane membranes evaporated from 20 min to 1 hr under reduced pressure were satisfied with oxygen permeability of ideal skin substitute.

CONCLUSION

The degree of swelling was decreased with increasing the concentration of crossinking agent, but all of the samples satisfied with that of ideal skin substitute, over 100%. In the case of the elongation, samples under wet condition have better value than samples of under dry condition and their maximum value was not satisfied with that of ideal skin substitute. But elongation of bilayer membrane was improved from 20% to 25%, due to flexible upperlayer's supporting function. The rate of biodegradation was very fast at the initial state but it became slow down after 4~6 days. The water vapor transmission and oxygen permeability was influenced primarily by polyurethane membrane, and the samples evaporated from 20 min to 1 hr under reduced pressure had ideal value as a skin substitute. From these experimental results, most of the bilayer membrane were satisfied with those of ideal skin substitute.

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