Preclinical evaluation of skin substitutes

A. Nangia* and C. T. Hung*

*Department of Pharmacy, University of Otago, Dunedin, New Zealand and *Zenith Technology Co. Ltd, Dunedin, New Zealand.

The important requirements of a skin substitute such as water vapour permeability, adherence to the excised wound surface, oxygen permeability, mechanical properties, impermeability to micro-organisms and exudate soaking capacity have been highlighted. Two commercial synthetic skin substitutes, Bioclusive and Geliperm, have been used to establish the preclinical assessment procedures for skin substitutes. Two in vitro techniques, the 'Water Cup' and the 'Inverted Cup,' and two in vivo methods involving a 'Ventilated Hygrometer Chamber' system and an Evaporimeter have been employed to assess and compare the water vapour permeability of the skin substitutes under controlled conditions. An Evaporimeter, which is very simple to operate, provides more accurate results. A simple test has been designed to evaluate the early adherence of the skin substitutes to the excised wound surface of rats. The pulling force and the peeling force required to remove the membrane from the wound surface have been measured and these forces have been found to depend upon the composition of the membrane. An oxygen permeability cell has been fabricated which measures the dissolved oxygen permeability of the skin substitutes. The detection of oxygen is based on the electrocatalytic reduction of oxygen at the surface of a noble metal. The tensile properties of the skin substitutes have been measured by an International Standard procedure and both the skin prostheses are associated with some drawbacks. An in vitro method of testing the microbial permeability of the skin substitutes has been designed which simulates an oozing colonized wound that a skin substitute faces in cases of septicaemia. Both the test materials are impermeable to both bacteria and fungi and must provide an effective barrier. The effectiveness of the skin substitutes to absorb wound exudate from the wound surface has been evaluated by soaking the pieces of the membranes in water, plasma and serum and observing their weight gain. The soaking capacity depends upon the composition and nature of the material. The procedures developed have been employed to evaluate a hydrogel-type synthetic skin substitute recently formulated in our laboratory.

Introduction

Protection of an open wound by skin substitutes other than the patient's own skin has become popular in the past two decades (Park, 1978; Demling, 1985). Considerable efforts have been made in the design, development and evaluation of various types of skin substitutes involving both synthetic and natural materials (Brown and Barot, 1986). Successful application of these skin substitutes to the management of burns demands that they fulfill various requirements (Tavis et al., 1978; Bartlett, 1981; Pruitt and Levine, 1984). Therefore, during the development of skin substitutes, they are subjected to various preclinical assessment procedures (Queen et al., 1986a). Most of the conventional testing procedures involve in vitro evaluation of skin substitutes (Park et al., 1978; Moserova and Houskova, 1979; Andrews and Kamyab, 1986; Queen et al., 1986b; 1987a,b; Jones and Hudson, 1988). Although these testing procedures provide some indication of their effectiveness, they do not simulate the actual conditions at the burn surface. In particular, these in vitro testing procedures are not suitable for evaluating some of the synthetic skin substitutes which behave differently at the burn surface (Thomas and Hay, 1987; Jonkman et al., 1988). It is therefore important that appropriate testing procedures are made available to evaluate skin substitutes. These are not only important from the point of view of designing an effective skin substitute but in providing screening processes to reduce the number of expensive clinical trials. Appropriate evaluation techniques will save time, expense and energy spent in the development of skin substitutes.

In designing a skin substitute, it is important to know the demands which it should fulfill. An effective skin substitute should be able to control excessive evaporative water loss (EWL) from the wound surface, should have uniform adherence to the wound surface, should allow gaseous exchange and should be elastic in nature. In addition, it should be impermeable to micro-organisms, soak up wound exudate, be non-toxic and non-antigenic, easy to apply and remove, easy to sterilize. Able to deliver antimicrobial agents, have a long shelf-life and be inexpensive (Tavis et al., 1978; Yannas and Burke, 1980; Chvapil, 1982).

Some of the above factors are interrelated and independent. In this investigation, only the important physical parameters such as the ability to control water loss, adherence to the wound surface, gaseous permeability, mechanical properties, impermeability to micro-organisms and exudate soaking capacity have been monitored. Two commercially available skin substitutes, Bioclusive and Geliperm dry are used to establish and validate the assessment procedures. Bioclusive, an adhesive-backed polyurethane membrane, is an occlusive skin substitute, whilst Geliperm dry is a hydrogel-type skin substitute. The procedures established are then employed for the evaluation of a hydrogel-type skin substitute (HSS) recently developed in our laboratory.
Experimental

Materials
Gift samples of Geliperm and Bioclusive were obtained from Geistlich Sons Ltd (Chester, UK) and Johnson & Johnson Products Inc (New Brunswick, NJ, USA), respectively. Human plasma was isolated from human plasma using thrombin solution (Thrombostat, Parke-Davis, NJ, USA). Driente, the desiccant, was obtained from W. A. Hammond Driente Co. (Xenia, Ohio, USA).

Instruments
A constant temperature and humidity cabinet (Corhythm Scientific Co., New Zealand) was used to determine EWL by the ‘Water Cup’ and the ‘Inverted Cup’ techniques. A Rotameter (Platun, CA Platun Ltd, Basingstoke, UK) was used to control the flow of compressed air. A Reese Drum Dermatome (Allen and Hanburys Ltd, UK) was used to excise the dorsal skin of rats. An Evaporimeter Model EP 1 (Servomed AB Stockholm, Vallingby, Sweden) was used to determine in vivo evaporative water loss from the excised wound of rats.

Water cup assembly to study in vitro EWL
Aluminium cups (60 mm i.d. x 45 mm deep) were used for this study. The synthetic skin substitute sample of approximately 65 mm diameter was placed on the aluminium mesh and fixed onto the cup. A neoprene rubber gasket was used to provide a water-tight seal to the assembly.

Ventilated hygrometer chamber system to study in vivo EWL
The instrument used in this study was similar to that described by Wang et al. (1980). The apparatus consisted of a flowmeter, a manometer and a hemispherical open-bottomed glass hygrometer chamber with outer diameter of 2.6 cm and thickness of 0.3 cm (cross-sectional area equal to 3.14 cm²) and a U-tube containing approximately 20 g of desiccant. Dry compressed air, with an inlet pressure of 20 mmHg and a flow rate of 1 l/min, was used as a carrier gas. The absorption of moisture by the U-tube from the atmosphere was checked by a guard tube filled with the desiccant.

Oxygen permeability cell
A commercially available Clark type oxygen permeability cell (Rank Brothers, Cambridge, UK) was modified to study the oxygen permeability of skin substitutes. Figure 1 shows the construction of the modified oxygen permeability cell. The cell consists of two units (A and B) joined together with a locking ring. The lower unit (B) consists of an oxygen sensor mounted on the body made from perspex. The electrodes are composed of a platinum disc cathode of diameter 1.6 mm and a large annular silver anode, insulated from each other by epoxy resin. The space between and around the electrode was filled with a 0.5 M potassium chloride solution. The upper unit consists of a cylindrical tube enclosed in a thermostated jacket. A firmly mounted Teflon film was placed between the electrodes and the skin substitute.

Instruments for testing mechanical properties
A TSK Tensile Testing Machine (J. J. Instrument, CR 450, UK) was used to determine the mechanical properties of the membranes. The thickness of the membrane strips was measured with a spring-loaded micrometer (R & B Cloth Thickness Tester, James H. Heal & Co. Ltd, Halifax, UK) which had a sensitivity of 0.01 mm.

Glass diffusion cell for micro-organism permeability
Figure 2 shows the glass diffusion cell used to study the micro-organism permeability of skin substitutes. The glass cell consists of a receptor and donor compartment held together by a spring clamp. Two sampling ports, one on each side of the cell, were used to introduce nutrient broth into the cell and to withdraw the samples.

Methodology
In vitro EWL measurements with the water cup assembly
An in vitro testing procedure recommended by the American Society for the Testing of Materials (ASTM-E96-81, 1981) was used to evaluate the skin substitutes. Circular pieces of Geliperm and Bioclusive membranes were cut with a sharp scalpel using a template. After putting 50 ml of distilled water in the aluminium cup, the membrane was mounted. Care was taken not to crease or rupture the membrane during clamping. The water cup assembly, covered with the membrane, was placed in the humidity control cabinet at 35 ± 1°C and relative humidity of 35 ± 2 per cent. EWL was determined by weighing the cup assembly after 24 h on an analytical balance. With the ‘Inverted Cup’ technique, the assembly was kept in the inverted position.

In vivo EWL measurements using the ventilated hygrometer chamber
The effectiveness of the skin substitutes in controlling evaporative water loss from the wound surface was evaluated using an excised rat model as previously described (Nangia and Hung, 1989).

Figure 1. Cross sectional view of the oxygen permeability cell.

Figure 2. Glass diffusion cell used to study micro-organisms' permeability of skin substitutes. A, Cotton plug; B, sampling ports; C, skin substitute to be tested; D, wire mesh. A spring-loaded clamp was used to hold the receptor (1) and donor (2) compartments together.
In vivo EWL measurements using the Evaporimeter

An Evaporimeter is an electronic device which is able to measure the water vapour gradient in the layer adjacent to the wound surface which is proportional to the exchange of water vapour from that surface (Nilsson, 1977). Therefore, an Evaporimeter was used to determine the occlusiveness of the skin substitutes. An area of 2 x 2 cm on the dorsal surface of Sprague-Dawley rats was excised with a Reese Drum Dermatome as explained previously (Nangia and Hung, 1989). After injury, the blood was absorbed by a gauze pad and the excised area was covered with a 3 x 3 cm square piece of membrane. Adhesive tape was used to tape the four sides of the membrane to the intact skin. Plastic collars were placed around the neck of the rats to prevent them from removing the membranes. All the rats were housed individually to prevent them from interfering with each other’s wound covering. Water loss measurements (g/m²/24 h), at 24 h after injury, were made by placing the Evaporimeter probe above the surface of the membrane until a constant reading was observed. All measurements were performed at a room temperature of 20 ± 2°C and a relative humidity of 35 ± 5 per cent.

Adherence of skin substitutes to the wound surface

The methods developed by Skornik et al. (1968), Gulddalian et al. (1973) and Oluwasanmi and Chvapil (1976) were modified in this investigation to evaluate the Phase I adherence and the peeling force of synthetic skin substitutes. The apparatus used for studying adherence of skin substitutes to the wound surface is shown in Figure 3. The apparatus consisted of a rubber stopper with a bottom diameter of 2.5 cm, suspended on one side of a balance and then counterbalanced. Sprague-Dawley rats with a split-thickness excised wound were used in this investigation. A circular piece of the skin substitute of diameter 2.5 cm was applied onto the excised wound of an anaesthetized rat. A spring-loaded micrometer was used to measure the thickness of the hydrated membrane. As the hydrated membrane distorted the micrometer anvil force was uniformly distributed by sandwiching the membrane between two glass coverslips. The thickness of the membrane was calculated by subtracting the thickness of the coverslip from the combined thickness. The membrane was then placed on top of the cell body, next to the Teflon film and secured with the cylindrical tube. The membrane was then covered with a thin layer of distilled water to prevent desiccation. The electrodes were operated at 0.6 V. The top unit of the cell was flushed with oxygen-free nitrogen with a flow rate of 20 cm³/min until the meter read zero. Compressed air with a flow rate of 20 cm³/min was then allowed to enter the cell and the current-voltage curve for oxygen reduction was recorded on chart paper. Before entering into the cell, the inlet gases were allowed to attain a temperature of 20°C by immersing the inlet metallic tubings in a controlled-temperature water-bath. All the permeability measurements were carried out at 20 ± 2°C.

The detection of oxygen in the oxygen permeability cell is based on the electrocatalytic reduction of oxygen on the surface of a noble metal. When an electrode of a noble metal, such as platinum, is polarized at 0.6 V with respect to a suitable reference electrode placed in a suitable electrolyte (KCl), the dissolved oxygen is reduced at the surface of the electrode. By separating the cathode, anode and potassium chloride solution from the test membrane by a fine Teflon membrane permeable to oxygen but not to the ions, the current produced is directly proportional to the oxygen permeated through the test membrane.

\[
O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \quad (1)
\]

The transient current observed with the amount of oxygen passed through the skin substitute membrane after a given time interval is given by the following equation (Aiba et al., 1968):

\[
i_t = NFA_PD \frac{P_0}{b} \left( \sum_{n=1}^{\infty} (-1)^n e^{-n^2D_{m/v}/b} \right) \quad (2)
\]

where \(i_t = \) steady-state current, \(\mu A\)

\(P_D = \) dissolved oxygen permeability coefficient, cm³ (STP) cm/sec cm Hg

\(F = \) Faraday’s constant, 96 490 coulombs per gram-equivalent

\(A = \) area of cathode, 0.0198 cm²

\(N = \) number of electrons involved per molar unit of reaction, 4

\(P_{O_2} = \) partial oxygen pressure of oxygen in the dry air, 15.92 cm Hg

\(b = \) thickness of the membrane, cm

\[
i_\infty = NFA_PD \frac{P_{O_2}}{b} \quad (3)
\]
The permeability coefficient PD can be calculated using the following equation (4):

$$P_D = \frac{i x b}{N F A P_0}$$

where: 
- $i$ is the current density, 
- $b$ is the thickness of the membrane, 
- $FA$ is the flux, 
- $P_0$ is the permeability coefficient of the membrane.

The values of $N$, $F$, $A$, $P_0$, and $b$ are known. The $P_D$ for the test membrane can be calculated if $i x b$ is determined.

Integrating equation (2) from $t = 0$ to a specific time $t'$ at which steady state is attained:

$$\int_0^{t'} dG = GFAPD \int_0^{t'} \left( t - \frac{b^2}{6Dm} \right)$$

where $G$ is the amount of electric charge (coulombs), $F$ and $A$ are the diffusion coefficient of the membrane (cm$^2$/s), $t'$ and $b$ are known. The permeability coefficient of the Teflon membrane and the skin substitute can be calculated using this equation (9).

The relationship between the combined permeability coefficient of the two membranes, i.e. the Teflon plus the skin substitute, is determined by the above method.

The exudate uptake capacity is the amount of exudate taken up by the skin substitute expressed in mg/day. The results were compared with other investigators using the water cup technique at 35°C and 35 per cent RH.

Results

In vitro EWL measurements using the water cup assembly

In order to compare our results with other investigators, the EWL for the skin substitutes are expressed in g/m²/24 h. Table 1 shows the values of EWL (g/m²/24 h) obtained with Geliperm and Bioclusive skin substitutes using the ‘Water Cup’ and the ‘Inverted Cup’ techniques. EWL from the free water surface (uncovered cup) in the ‘Water Cup’ technique at 35°C and 35 per cent RH was found to be 4521 ± 57 g/m²/24 h which is approximately half of that observed (10 419 ± 689 g/m²/24 h) by other workers (Queen et al., 1987a) under similar conditions. In general, the EWL using the ‘Inverted Cup’ technique was observed to be higher than using the ‘Water Cup’ method. Using the ‘Inverted Cup’ method, Geliperm showed EWL values more than eight times those of the ‘Water Cup’ method, whilst the increase was only 1.5 times in the case of Bioclusive. Similar results have also been obtained by other investigators with Op-Site, an adhesive-backed polyurethane film synthetic skin substitute (Queen et al., 1987a). In the case of the ‘Water Cup’ technique, the average EWL through the Geliperm and Bioclusive were 1872 ± 66 g/m²/24 h and 270 ± 41 g/m²/24 h. However, in the case of the ‘Inverted Cup’ technique, higher EWL of 14 080 ± 1213 g/m²/24 h were observed for Geliperm and Bioclusive respectively, which are in good agreement with those (Geliperm 11 275 ± 598 g/m²/24 h and Bioclusive 382 ± 26 g/m²/24 h) reported by Queen et al. (1986a, 1987a). These in vitro results indicate that Geliperm provides virtually no protection in controlling the water loss from the wound surface and Bioclusive checks water loss to an appreciable extent.
In vivo EWL measurements using the ventilated hygrometer chamber

The average EWL through the intact skin of rat was 1238 ± 350 g/m²/24 h (at 35°C body temperature) while an EWL of 13 392 ± 273 g/m²/24 h, which is approximately ten times that of the intact skin, was observed for the freshly excised wound. Geliperm with an average EWL of 11 392 ± 53 g/m²/24 h had little effect in reducing the rate of water evaporation. Bioclusive reduced water loss of the excised wound to 153 ± 775 g/m²/24 h, which is slightly more than that for the intact skin.

In vivo EWL measurements using the Evaporimeter

The intact skin of the rat was found to have an EWL of 180 ± 6 g/m²/24 h, similar to that of 204 ± 12 g/m²/24 h observed from intact human skin using the Evaporimeter. The average EWL for Geliperm was 2902 ± 314 g/m²/24 h, which affirms that it has no effect in controlling excessive water loss from the uncovered excised wound (3004 ± 183 g/m²/24 h). Bioclusive with an EWL of 528 ± 23 g/m²/24 h provides maximum occlusion.

Adherence of the skin substitutes to the excised wound surface

The pulling force and peeling force required to remove the Geliperm and Bioclusive from the wound surface are presented in Table II. The results indicate that Geliperm has a lower adherence than Bioclusive on the freshly excised wound. The pulling force required to remove thin Bioclusive film from the wet wound surface was found to be three times higher than that of Geliperm. Geliperm after hydration in water was evaluated for its adherence. Once the end of the Bioclusive was detached, the whole strip peels off easily. In the case of Geliperm, peeling was very smooth.

Permeability to dissolved oxygen

In this investigation, only Geliperm after hydration in water was evaluated for its PD. Bioclusive due to its self-sticking nature was very difficult to apply into the cell and was not investigated in this study. Teflon film (17.5 μm), and polyethylene film (72 μm) used in this study were found to be highly permeable to oxygen with a PD of 5.14 x 10⁻⁹ cm³ STP/cm² sec cmHg and 0.21 x 10⁻⁹ cm³ STP/cm² sec cmHg respectively, which are in good agreement with those reported in the literature (Aiba et al., 1968). The validity of equations (7)-(9) to determine the PD of the test skin substitute was checked by measuring the PD of the Teflon combined with polyethylene. Results obtained are shown in Table III. The PD of the composite membrane of Teflon and polyethylene was found to be similar to the calculated value. Therefore, these equations were used in the determination of PD of the synthetic skin substitute. The PD of the fully hydrated Geliperm was found to be 3.48 ± 0.42 x 10⁻⁹ cm³ STP/cm² sec cmHg. The results obtained with Geliperm cannot be compared as no study has been carried out previously to determine its dissolved oxygen permeability. Nonetheless, this study indicates that Geliperm when hydrated at the wound surface, is highly permeable to dissolved oxygen.

Mechanical properties of skin substitutes

The stress-strain curves observed for Geliperm and Bioclusive are shown in Figure 4. The elongation at break of Bioclusive (657 ± 21 per cent) was approximately three times higher than that of Geliperm (234 ± 12 per cent). In contrast, Geliperm had a lower initial modulus

<table>
<thead>
<tr>
<th>Skin substitute</th>
<th>'Water cup' method</th>
<th>'Inverted cup' method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncovered control</td>
<td>4521 ± 57</td>
<td>14080 ± 1213</td>
</tr>
<tr>
<td>Geliperm</td>
<td>1872 ± 66</td>
<td>428 ± 80</td>
</tr>
</tbody>
</table>

All measurements were made at 35°C and 35 per cent relative humidity. Mean of four determinations. Results shown are mean ± s.d.

Table II. Pulling and peeling forces required to remove the Geliperm and Bioclusive from the excised skin of rats*

<table>
<thead>
<tr>
<th>Pulling force (g/cm²)</th>
<th>Peeling force (g/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geliperm</td>
<td>5.23 ± 1.06</td>
</tr>
<tr>
<td>Bioclusive</td>
<td>15.08 ± 3.22</td>
</tr>
</tbody>
</table>

Results shown are mean ± s.d. *Measurements were made 1 h after application of skin substitutes to the excised wound surfaces.

Table III. Oxygen permeability coefficients of polymeric membranes

<table>
<thead>
<tr>
<th>Material type</th>
<th>Thickness (μm)</th>
<th>PD x 10⁻⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teflon</td>
<td>17.5</td>
<td>5.138</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>72.0</td>
<td>0.211</td>
</tr>
<tr>
<td>Teflon + Polyethylene</td>
<td>89.5</td>
<td>0.259**</td>
</tr>
<tr>
<td>Geliperm</td>
<td>970</td>
<td>3.482</td>
</tr>
</tbody>
</table>

*at ambient temperature (20°C) **calculated value *experimental value

<table>
<thead>
<tr>
<th>Physical characteristics</th>
<th>Experimental value (Mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EWL (g/m²/24h)</td>
<td>2264 ± 186**</td>
</tr>
<tr>
<td>Pulling force (g/cm²)</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td>Peeling force (g/cm²)</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>PD (cm² STP/cm sec cm Hg 10⁻¹⁰)</td>
<td>73.6 ± 7.8</td>
</tr>
<tr>
<td>Elongation at break (%)</td>
<td>473 ± 42</td>
</tr>
<tr>
<td>Tensile strength at break (MPa)</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>Initial modulus (MPa)</td>
<td>0.85 ± 0.24</td>
</tr>
<tr>
<td>Water uptake capacity (%)</td>
<td>716 ± 2R</td>
</tr>
</tbody>
</table>

*Measured using Evaporimeter **Mean of six determinations *Mean of four determinations
Figure 4. Stress–strain curves for skin substitutes Geliperm (A) and Bioclusive (B).

Figure 5. Uptake capacity (%) of Geliperm at different time intervals. Water (■); serum (□); plasma (▲).

Although application of occlusive dressings onto the damaged area may restore the fluid balance (May, 1984), it facilitates fluid accumulation at the wound surface and may increase the risk of wound infection (Mertz and Eaglstein, 1984; Katz et al., 1986; Barnett et al., 1986), although some data argue against this conclusion (May, 1984). It certainly increases patient discomfort (Ehleben et al., 1985). Therefore, one of the clinical requirements of a successful skin substitute is that it must have an optimum water vapour permeability, which can provide a satisfactory moisture balance at the repairing wound site.

The in vitro measurements were carried out at 35°C because it has been observed (Lamke et al., 1977) that the average temperature of the wound surface is 35°C. A relative humidity of 35 per cent was maintained inside the chamber to provide an adequate water vapour driving force. The humidity is also similar to that maintained in some burn units (Liljedahl et al., 1979). The large difference in EWL between the ‘Water Cup’ and ‘Inverted Cup’ techniques is attributed to the relative humidity gradient developed at the air/membrane interface within the cup (Queen et al., 1987a). In the ‘Water Cup’ method, the space between the membrane and the water within the cup may not reach 100 per cent RH. Such an effect will reduce the EWL of the test membrane. The magnitude of this humidity gradient is dependent upon the water permeability and nature of the membrane. The difference in EWL, observed by the Water Cup technique, between this study and that observed by other workers (Queen et al., 1987a), under similar conditions, may be due to the difference in geometry of the cup used. The aluminium cups (i.d. of 60 mm and 45 mm deep) used in this study will provide greater boundary layer resistance than the cup (i.d. of 70 mm and 25 mm deep) used by Queen et al. (1987a). In the case of the ‘Inverted Cup’ technique, where boundary layer resistance is negligible, similar results have been recorded. From this study, it is apparent that the ‘Inverted Cup’ technique is preferred to the ‘Water Cup’ method as it is less dependent on the geometry of the cup assembly and reproducible results are attainable with different skin substitutes.
In the case of the 'Ventilated Hygrometer Chamber' system, the average EWL through the intact skin of rats was $1238 \pm 350 \, \text{g/m}^2/24 \, \text{h}$ (at $35^\circ\text{C}$ body temperature), which is approximately twice the EWL ($624 \pm 168 \, \text{g/m}^2/24 \, \text{h}$ at $30^\circ\text{C}$) observed by other investigators (Wang et al., 1980). This difference in EWL is probably due to the higher flow rate ($2 \, \text{l/min}$) and lower temperature ($30^\circ\text{C}$) used by Wang et al. (1980). The freshly excised skin wound with a ten-fold increase in EWL compared with the intact skin is in good agreement with that reported by other workers (Jelekko, 1967). These in vivo results with Geliperm and Bioclusive, obtained by using the Ventilated Hygrometer Chamber system and the Evaporimeter, show a trend similar to that observed in vitro. From the results it is obvious that there is no correlation between the in vitro and the in vivo experiments. Indeed, different investigational techniques will produce different EWL of the same skin substitute (Aiba et al., 1985).

The investigation by Lamke et al. (1977) using the Evaporimeter technique determined the EWL from burns of different types, granulating wounds and donor sites. Granulating wounds were found to have an EWL around 20 times ($5138 \pm 201 \, \text{g/m}^2/24 \, \text{h}$) that of the intact skin ($204 \pm 12 \, \text{g/m}^2/24 \, \text{h}$). Relative water vapour permeabilities of different grafts and synthetic skin substitutes applied to various wound surfaces have also been observed. Based on the results of the above study, various investigators (Wong, 1980; Queen et al., 1987a), irrespective of the technique employed, have advocated an EWL in the range of 2000–2500 g/m²/24 h for an ideal skin substitute. The reason for such a recommendation is that an EWL of 2000–2500 g/m²/24 h is approximately half that of the granulating wound and will provide an adequate moisture balance at the wound surface thus preventing wound desiccation. In view of the fact that different EWL will be obtained using different evaluation methods, the value of 2000–2500 g/m²/24 h will be inappropriate as the standard EWL for an ideal skin substitute. Instead, an effective burn cover should have an EWL sufficient to keep the wound surface just moist to obtain the benefits of accelerated healing (Winter, 1972) but without pooling of fluid between the wound and the covering because of the risk of infection. Such conditions can be best obtained by using a material with both high absorption and water vapour transmission properties (Quinn, 1987). As there are large variations in the EWL results using the in vitro techniques and due to the complex design and operation of the ventilated hygrometer system, the use of the Evaporimeter is preferred over other techniques. It is simple to operate and immediate results can be obtained from excised wounds with or without skin substitutes.

Adherence of the skin substitutes to the excised wound surface
Adherence has been considered to be one of the most important requirements for an ideal skin substitute (Skornik et al., 1968; Tavis et al., 1976; Noe, 1978; Enterline and Salisbury, 1980; Lawrence, 1982; Morykwas et al., 1987). Effective skin substitutes should adhere readily to dry and wet wound surfaces with sufficient strength to resist lifting and slipping. Adherence must be intimate and uniform because small areas of non-adherence will lead to the formation of fluid-filled pockets which are ideal for bacterial proliferation (Frank et al., 1984). A uniform adherence will not only reduce infection (Gerding et al., 1988) but can also reduce pain (Malone, 1987) and promote wound healing (Kornberg et al., 1972). Adherence of skin substitutes onto the wound bed usually involves two distinct phases (Tavis et al., 1976, 1977). The initial phase, Phase I, lasts for about 72 h and involves fibrin bonding between the material and the wound bed. During this phase, removal of the cover is relatively easy and will not induce further damage to the wound bed. The second phase, Phase II, involves growth of fibrovascular tissues into the contact material. Because of the strong interaction, removal of the cover from the wound bed will damage the newly formed tissues. Therefore skin substitutes which involve Phase II adherence should be left on the wound bed until healing is completed. However, not all materials which allow fibrin bonding (Phase I adherence) will promote fibrovascular tissue ingrowth (Phase II adherence). High Phase I adherence indicates good natural adhesion of the synthetic skin substitute on the wound surface. However, a strong peeling force implies that skin substitute changes will be difficult.

The pulling force required to remove thin Bioness film from the wet wound surface was found to be three times higher than that of the Geliperm. The strong adherence of the hydrophobic film is due to its adhesive coated surface and the effect of atmospheric pressure due to displacement of exudate between the skin substitute and wound surface. Peeling of one end of the Bioness with concomitant detachment of the whole strip was probably due to the release of the vacuum seal between the Biofibrous and the wet wound surface. Such an observation also suggests that adherence of Biofibrous on the wet wound surface is mainly due to the effect of atmospheric pressure and the adherence related to the adhesive contact layer is minor.

Permeability to dissolved oxygen
One of the critical factors limiting the rate of wound healing is the extent of oxygen supply at the wound site. A number of studies have shown that oxygen is beneficial to epidermal wound healing (Starr, 1932; Hinman and Maibach, 1963; Kan et al., 1964; Shulman and Krohn, 1967; Fischer, 1969; Winter, 1972; Silver, 1972; Pai and Hunt, 1972; Hurlte et al., 1975). Accelerated epithelialization under high oxygen tension, in the absence of exudate, is considered partly due to the enhanced ability of phagocytes to destroy invading micro-organisms and partly to the improved energy supply provided by the oxidative process as compared to anaerobic glycolytic metabolism under hypoxic conditions (Silver, 1985). The availability of oxygen is also important in collagen synthesis (Kao et al., 1963), increased fibroblast activity (Silver, 1985) and increases in tensile strength of the wound (Kivisaari and Ninikoski, 1975). Therefore, it is essential that an effective skin substitute should be permeable to oxygen.

The measurement of oxygen permeability of skin substitutes usually involves the use of a gas–gas vacuum technique (British Standard Method BS 2782, 1979). In this method, the test membrane is exposed to the permeant on one side and a vacuum on the other. The rate of gas transmission is determined by measuring the increase in pressure on the vacuum side with time (Queen et al., 1986a). This technique, however, does not simulate the actual situation where the underside of the skin substitute is in contact with the wound exudate, as hydrated membranes usually have a different oxygen permeability from that of the dehydrated ones. Additionally, only skin substitutes with extremely high mechanical strength can withstand such harsh testing conditions. In view of the above shortcomings of the gas–gas vacuum procedure, the
method used to determine the 'dissolved oxygen permeability' ($P_D$) rather than 'gaseous oxygen permeability' ($P_g$) has been used. An electrochemical technique (Connelly, 1957) is employed since it has been routinely used in measuring the dissolved oxygen permeability of contact lenses (Refojo et al., 1977; Refojo and Leong, 1979) and other polymeric materials (Hitchman et al., 1984). It has been observed that oxygen permeates through the water phase of the hydrated hydrogels with little interaction with the polymer network. A linear relationship between the logarithms of $P_D$ and the reciprocal hydration of the membrane has been observed (Yasuda et al., 1968; Refojo et al., 1979). Therefore, before testing for $P_D$, the pieces of Geliperm membrane were fully soaked in water for 24 h. During initial experiments with Teflon, it was observed that there was no statistically significant difference in $P_D$ at ambient temperature (20 ± 2°C) and 35°C. Therefore, subsequent experiments were conducted at 20°C.

**Mechanical properties of skin substitutes**

An effective skin substitute should have adequate toughness to withstand handling. The skin substitutes should also be elastic and flexible to allow conformation to uneven surfaces of the body (Queen et al., 1986a, 1987b). Skin substitutes with exceptional mechanical properties, i.e. high tensile strength, excellent elasticity and flexibility, are required for covering hand burns, facial burns and burns incurred around the joints. The membranes should not restrict the mobility of the injured areas as limited movements will retard recovery of the wound area and promote hypertrophic scarring. Bioclusive, due to its high elongation at break, can be stretched easily without exerting pressure on the underlying delicate tissues and thus will not affect the normal process of wound healing. In addition, the high tensile strength of this membrane will provide resistance to tearing and offer protection to the wound areas. Geliperm, due to its high initial modulus (stiffness), is not an ideal skin substitute for covering wound surfaces with sharp curvatures and joints. Similar observations have also been reported by other investigators (Queen et al., 1987a). In the case of Bioclusive, this drawback has been overcome by coating one side of the skin substitute with an adhesive material, which keeps it in close contact with the dry wound surface.

**Impermeability to micro-organisms**

Infection remains a major problem in burn care (McManus et al., 1981; Macmillan, 1982; Kagan et al., 1985). An effective skin substitute should prevent the passage of bacteria from the environment into the wound and vice versa, thus preventing cross-contamination (Lawrence, 1985). This is particularly important in a hospital environment where cross-infection can be a serious problem (Ayliffe and Lilly, 1985). The most common method employed to check bacterial impermeability of skin substitutes involves overlaying a piece of skin substitute over the surface of agar medium. A suspension of bacterial culture is applied to the surface of the skin substitute and the assembly incubated. Bacterial growth on the underlying media after incubation indicates that the skin substitute is permeable to bacteria (Bartzokas et al., 1983). However, negative results may be obtained because the inoculated micro-organisms fail to grow on the test membrane. To overcome this drawback, a similar technique with the skin substitute placed on an agar plate seeded with a bacterial culture (i.e. reverse of the above method) has been proposed for studying the bacterial impermeability of skin substitutes (May, 1984). The major drawback of these techniques is that false-positive results may be obtained through lateral migration of micro-organisms, especially air-borne bacteria like Pseudomonas aeruginosa (Thomas and Hay, 1987). While the latter technique yielded appropriate negative results in the case of OpSite, an occlusive polyurethane dressing, that technique might not be useful for all types of skin substitutes. The permeability cell used in this study eliminates the drawbacks of the above techniques. This technique also simulates an oozing colonized wound that a skin substitute faces in cases of septicaemia.

**Exudate uptake capacity**

Many species of pathogenic bacteria produce toxins and other metabolites that inhibit epithelial migration by digesting tissue proteins and polysaccharides present in the dermis (Lawrence, 1983). An ideal skin substitute should absorb exudate and other toxic materials from the wound surface. This will not only clean the wound but will increase patient comfort and promote wound healing.

Exudate soaking capacity is measured by soaking the skin substitute in the wound exudate and observing any weight gain (Kichkofen et al., 1986). It has been observed that the composition of exudate is similar to plasma but with reduced protein content (Nanto and Vilkanto, 1960; Heggers et al., 1980). Therefore, to study the effect of protein concentration on the soaking capacity of skin substitute, membranes were soaked in water, plasma and serum. Since no significant difference in the absorption capacity of water, serum and plasma was observed, water can be used as a medium for evaluating the exudate soaking capacity of the skin substitutes.

The assessment procedures established above were employed for the evaluation of a transparent semi-interpenetrating hydrogel-type skin substitute (HSS) recently formulated in our laboratory. The HSS is composed of a biocompatible polyaacrylamide gel, a polysaccharide and phospholipid. The physical properties obtained for this HSS are presented in Table IV. This table shows that the assessment procedures have assisted in the development of a skin substitute which possesses all the desirable properties. In further studies in rats, this wound covering was found to provide a faster rate of wound healing of the excised skin wound than Geliperm and Bioclusive (Nangia and Hung, 1990).

**Conclusions**

The present study demonstrates the importance of the preclinical assessment procedures in the evaluation of skin substitutes. The principal uses envisaged for such procedures are two fold. First, as a screening test to evaluate the performance of the existing skin substitutes and secondly as...
a quality control measure to control various physical characteristics of different batches of a finished product.

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References


