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Physical characterization of a new biomaterial for wound management

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The aim of this work was to evaluate some physical characteristics of a new biosynthetic membrane (Bioprocess®) used as temporary skin substitute for wound management. The tested characteristics, important for the clinical use of the membrane, were: permeability to some gases (O_2, CO_2 and N_2) and water vapour, and absorption of fluids simulating a physiological-like environment. The tests were completed by scanning electron microscope (SEM) observations in order to verify the surface morphology before and after the tests. The results confirmed the good *in vivo* performance of this material.

1. Introduction

A great variety of substances and procedures have been used for the protection of lesions characterized by skin loss, but only recently, due to a better understanding of wound healing physiology, substantial improvements have been made. The materials currently available to cover lesions involving extensive skin loss can be divided into three categories: traditional absorbent or impregnated dressings, synthetic dressings such as semipermeable films, foam dressings, hydrogels, hydrocolloids and xerogels and biological dressings created wholly or in part from human or animal tissue. Many of the properties of these materials overlap, but none of them meet all the criteria for an ideal dressing, that should be easily sterilized, non-allergenic, free of contaminants, able to absorb wound debris and excessive exudate and to protect the lesion from dehydration, temperature changes and bacterial contamination. It should also be easy to handle and to apply, comfortable when in place and cost-effective.

This work considers a new biosynthetic material for wound healing called Bioprocess®. Bioprocess® has been employed in the treatment of burns, abrasions, skin grafts and ulcers with positive clinical results [1–6]. Once moistened with normal saline solution, it adheres firmly to the damaged area by its exudate and may be left in place for weeks. The application of the film promptly relieves pain since it protects nerve endings. The strong adhesion to the wound prevents bacterial contamination and leaves the wound practically sealed. Hydro-electrolytic losses are reduced, with shortening of the scar forming time. Moreover, the transparency of the film enables constant inspection of the lesion, without the need of removing the dressing for periodical clinical controls.

As many properties of Bioprocess®, defined in preliminary testing, were to be confirmed through systematic investigations, we have undertaken a series of tests aimed at characterizing the new material from a physical point of view.

2. Materials and methods

Bioprocess® (Farmitalia Carlo-Erba) is a novel microfibrillar cellulose film biosynthesized by bacteria of the genus *Acetobacter Xylinum*. These bacteria, seeded in a culture medium supplied with carbohydrate nutrients associated with a nitrogen source, produce a cellulosic capsular 'zoogloea' which surrounds the microorganisms. This biomass is formed by the interweaving of bacterial chains surrounded by their cellulosic capsules. Elimination of water from the biomass produces pure cellulose films with a certain permeability due to the microscopic spaces existing among fibres. The films have a translucent appearance and a thickness ranging from 0.005 to 0.020 mm. The thickness of the films depends on various factors, such as the dosage of the nutrients, temperature, time, the saturation point of the colony and the kind of culture medium used.

In practice, Bioprocess® is obtained by inoculating a suitable culture medium with Acetobacters, incubated under strictly controlled conditions for 48 h. Crude films are then collected, washed, dried, packed and sterilized [7].

One of the most important factors for wound healing is the maintenance of a moist environment at the surface of the lesion to help cell regeneration.

To test the permeability of Bioprocess® to water vapour, the ASTM-E 96-90 standard was adopted, that evaluates water vapour transmission through membranes with the water method [8]. Three aluminium circular plates (diameter = 6.2 cm; height = 2.7 cm) capable of containing 40 cm^3 of water were employed. Each plate was filled with distilled water, covered with a sample of Bioprocess® membrane

(code n. 247; thickness = 0.019 mm), weighed (analytical balance Precisa 120 A, Italy), and put in a thermostatic oven at a constant temperature of 32 °C and 49% relative humidity. A suitable amount of desiccant material was placed in the oven in order to maintain relative humidity values constant and guarantee favourable conditions for the water vapour transmission. Two or more weighings of the plates were carried out daily for 6 days, in order to obtain the profile of weight changes as a function of time. The graphs of water vapour transmission rate make it possible to calculate the mean value of the membrane's permeability to water vapour.

In order to evaluate the ability of the membrane to exchange gases with the environment, its permeability to oxygen, carbon dioxide and nitrogen was tested using the system described as ASTM-D 1434-75 standard [9], modified by Zanderighi [10]. The system is composed essentially of three parts: a permeation unit, a gas cromatograph and a sampling cell. The permeation unit consists of two semicells with the testing membrane in between. This test was performed using three Bioprocess® samples (code n. 251) of the same size (area = 50 cm^2; thickness = 0.010 mm). Every test was carried out at 35 °C and with a difference of gas partial pressure between the two cells of 76 cmHg. The detected value of volumetric flow permitted to calculate gas permeability.

The water absorption test was performed using seven membranes (code n. 251; area = 25 cm^2, thickness = 0.010 mm) dipped in a thermostat (37 °C) filled with distilled water buffered with phosphate (pH 7.4). The samples were kept in the bath for 24 h, 5, 10, 15, 20, 25 and 30 days, respectively. After removal from the bath, water excess was eliminated with blotting paper and the samples were weighed. They were subsequently put in a desiccator with partial vacuum and weighed again after 1 h in order to estimate the amount of water lost during this period of time. Each sample was observed under a scanning electron microscope (SEM XL 40, Philips, the Netherlands) to detect the morphological changes produced by immersion in fluid. A mechanical test was also undertaken in order to check tensile strength of the samples by means of a tensile strength tester (Metrocom Universal, Italy).

3. Results

The water vapour transmission (WVT) of Bioprocess® was calculated according to the following formula:

$$WVT = G/t \cdot A = (G/t)/A \text{ in g h}^{-1}\text{ m}^{-2}$$

where: G = weight change of the samples (g)
t = duration of the test (h)
G/t = slope of the line (g/h)
A = area of the test membrane (m^2)

Weight changes actually measured are shown in Fig. 1. The test was repeated three times and the mean value of G/t was chosen. Applying the formula, the value of WVT was found to be equal to 74.8 $g\,h^{-1}\,m^{-2}$.

This value allows to calculate the permeance of the membrane, since

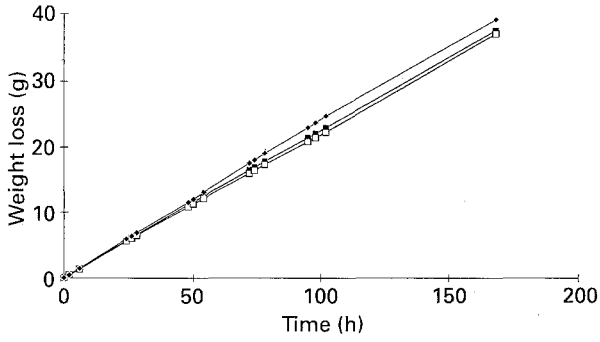

Figure 1 Graph of weight changes relative to the water vapour transmission test.

$$\text{permeance} = WVT/S(R_1 - R_2)$$

where: S = saturation pressure of vapour at test temperature, equal in our case to 46.66×10^2 Pa (at 32 °C)

R_1 = relative humidity inside the plate, as fraction
R_2 = relative humidity in the thermostatic oven, as fraction.

Developing this formula, the value of permeance was found to correspond to $3.1 \times 10^{-2}\,g\,Pa^{-1}\,h^{-1}\,m^{-2}$. This value is greater than that of the human skin ($P = 4 \times 10^{-3}\,g\,Pa^{-1}\,h^{-1}\,m^{-2}$) [11].

Since permeance, multiplied by the thickness of the membrane, gives its permeability:

$$\text{permeability} = \text{permeance} \times \text{thickness}$$

it was found that the value of permeability is $5.9 \times 10^{-7}\,g\,Pa^{-1}\,h^{-1}\,m^{-1}$. This value was compared with values reported in the literature for similar products, such as OpSite® (Smith and Nephew Ltd.), Omiderm® (Omikron Scientific Ltd.), Biobrane® (Hall, Woodroof Inc.) and Tecoflex EG-80A® (Thermedics Inc.). These are polymeric film dressings that are gas and water permeable, except OpSite®, which is considered an occlusive material [12–14]. Bioprocess® had a WVT value greater than OpSite® but lower than the others. As for thickness, Bioprocess® membrane is thinner than other polymeric materials, but similar to OpSite®, from which it differs in the lack of adhesive coating that makes it easier to apply.

The test of oxygen permeability of the membrane was carried out using the quoted system that allows one to measure some indirect parameters evolved in the formula of permeability:

$$\text{permeability} = \frac{m\,V_c\,L\,273}{1000\,V_i\,A\,\Delta p\,(273 + t)}$$

where m = angular coefficient of the line of the oxygen flow/time, in µl/s,
V_c = gas volume of the circuit of the system (cc)
L = thickness of the membrane (cm)
V_i = volume of the sampling cell in the system [10], (cc)
A = area of the membrane (cm^2)
Δp = difference of gas partial pressure at the two sides of the membrane in the cell (cmHg)

t = temperature at which gas calibration is made (25 °C)

Values of oxygen flow, assessed at various times, are reported in Fig. 2. The angular coefficient calculated from the regression line estimated from the first experimental points (points relative to the stationariety of the system) shown in the graph, was found to be equal to 1.38×10^{-2} µl s^{-1}.

Applying the formula to actual values, the value of oxygen permeability was found to be equal to 1.2×10^{-9} cm s^{-1}.

The test of permeability to CO_2 and N_2 gave the same result, indicating that Bioprocess® membrane is equally permeable to these gases.

The profile of the test of water absorption is given in Fig. 3. As shown in the graph, there is an initial quick, though small (0.5%) absorption of water in the first hours of immersion, after which no more water is absorbed until the end of the test, when some further absorption (less than 1%) occurs. This means that the membrane dehydrates quickly and preserves its integrity for 25–30 days, without any evidence of degradation or reabsorption. The matrix of the surface is partially disrupted, but not yet reabsorbed. This evidence is confirmed by mechanical resistance values. In fact the maximum tensile strength at break ranges from 111 N for the original membrane to 77 N for the 30-day-immersion dried sample. The behaviour of tensile strength is correlated with that of water absorption, as its decrease is maximum during the first 5 days of immersion and remains constant thereafter.

Microscopic examination of Bioprocess® membrane shows a homogeneous surface crossed by foldings due to its thickness. At higher magnification a great number of fibrils disposed along various directions are visible. Among many fibrils there are cross linkings that probably explain the relatively high mechanical strength of this material (Fig. 4a, b, c). At higher magnification the number of the cross linking points appears decreased and the surface shows a large number of 'closed pores' having a diameter ranging from 0.1 to 0.4 µm (Fig. 5a, b, c). This size is compatible with the 'bacterial barrier' concept, as shown experimentally by Audrito [15] for the following micro-organisms: Salmonella abony, Escherichia coli, Pseudomonas diminuta and Staphylococcus aureus.

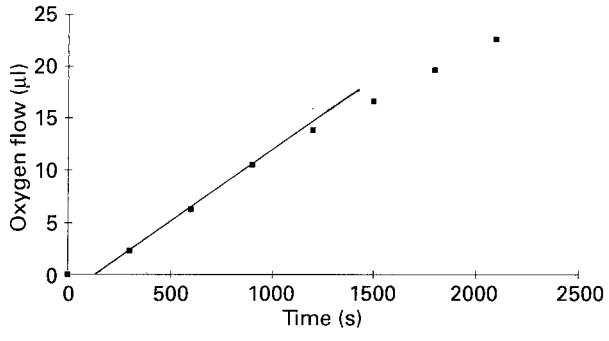

Figure 2 Graph of oxygen flow through the membrane as a function of time.

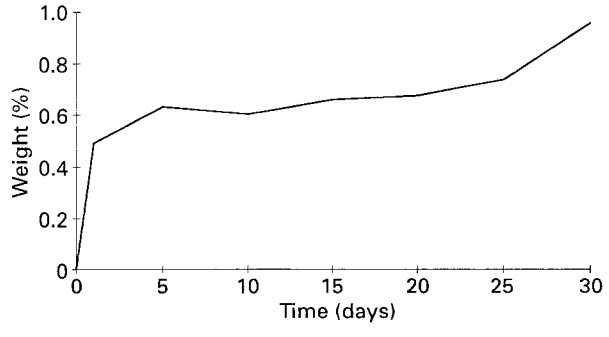

Figure 3 Graph of water absorption of the membrane as a function of time.

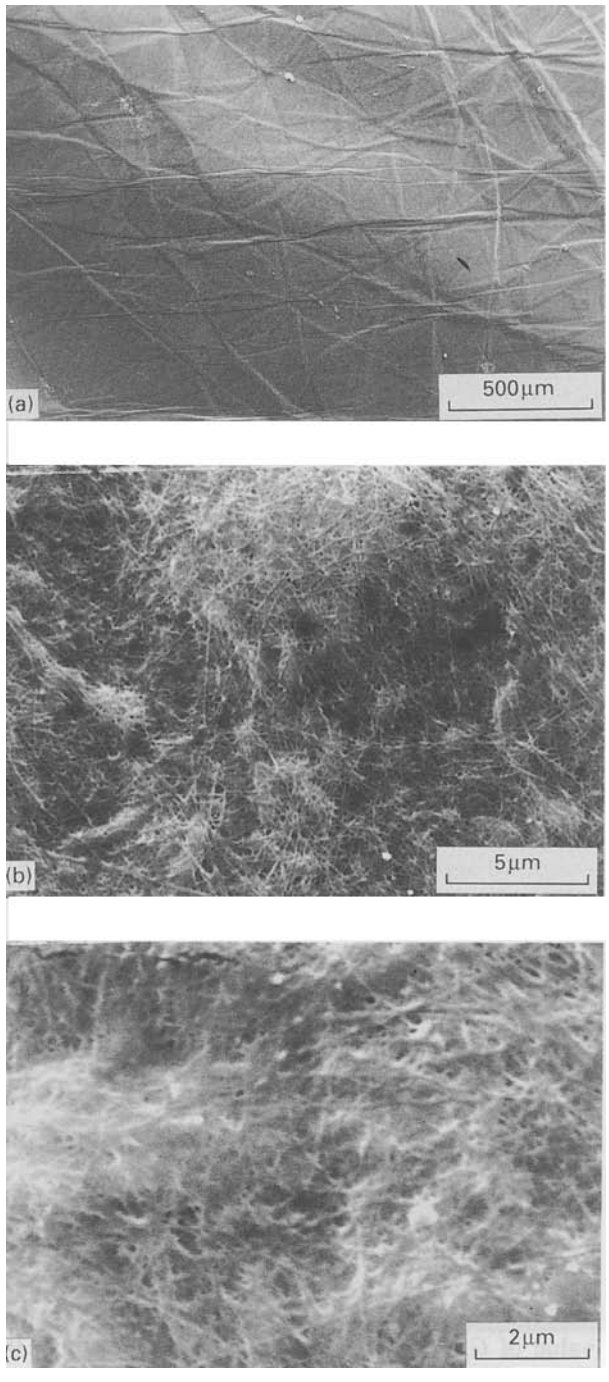

Figure 4 SEM microphotographs of Bioprocess® surface at three different magnifications.

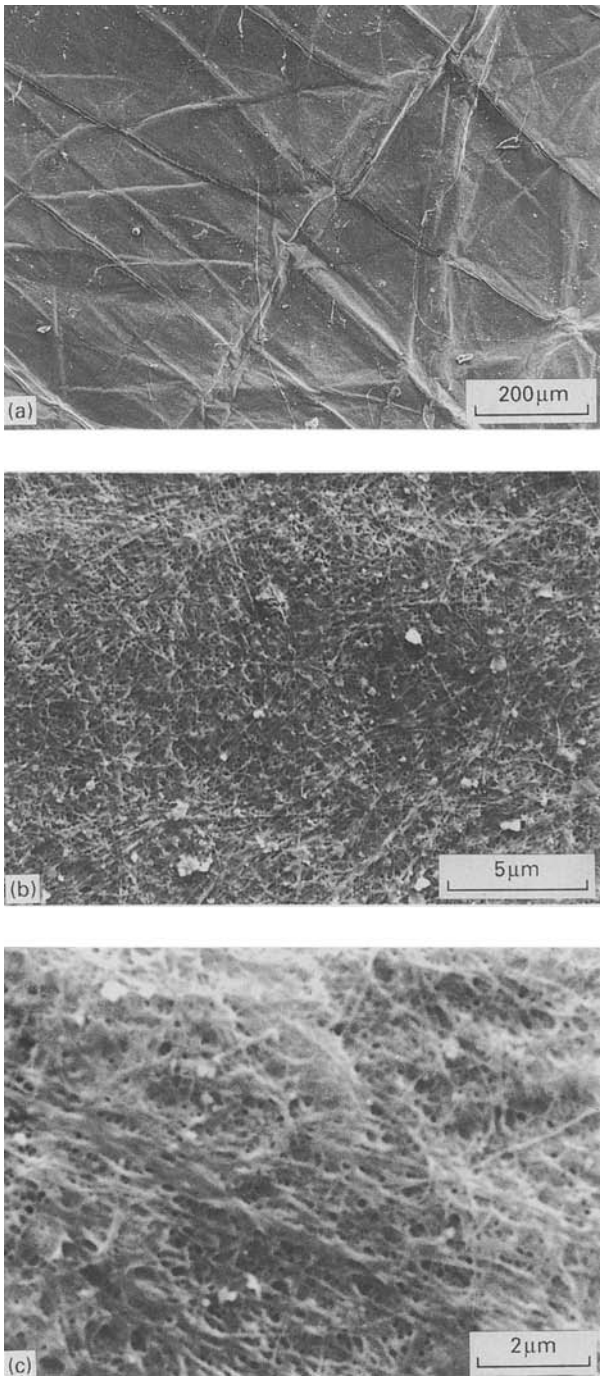

Figure 5 SEM microphotographs of Bioprocess® after 30 days immersion in water, at three different magnifications.

4. Conclusions

The results obtained for gas exchange and water vapour transmission support the claim that Bioprocess®, when applied to skin lesions, provides a suitable environment for healing, allowing the damaged tissue to breath and preventing access to bacteria. The negligible water absorption detected indicates that the wettability of the membrane is quite limited. This fact explains how the membrane, when applied to wounds, adheres to the substrate, without dehydrating it, and maintains a moist environment which favours the healing process. The good tensile strength of the membrane, also after prolonged water absorption, warrants the maintenance of these conditions, even in case of delayed removal.

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