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Irradiated PVAl membrane swelled with chitosan solution as dermal equivalent

A.C.D. Rodas [a,*], T. Ohnuki [a], M.B. Mathor [a], A.B. Lugao [b]

[a] *Instituto de Pesquisas Energéticas e Nucleares – CTRD, Av. Professor Lineu Prestes, 2242, Cidade Universitária, CEP 05508-900 São Paulo – SP, Brazil*
[b] *Instituto de Pesquisas Energéticas e Nucleares – MQA, Av. Professor Lineu Prestes, 2242, Cidade Universitária, CEP 05508-900 São Paulo – SP, Brazil*

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Abstract

Synthetic membranes as dermal equivalent can be applied at in vitro studies for developing new transdermal drugs or cosmetics. These membranes could be composed to mimic the dermis and seed cultivated keratinocytes as epidermal layer on it. The endothelial cells ingrowth to promote neovascularization and fibroblasts ingrowth to promote the substitution of this scaffold by natural components of the dermis. As, they can mimic the scaffold function of dermis; the membranes with biological interaction could be used for in vivo studies as dermal equivalent. For this application, poly(vinyl alcohol) (PVAl) membranes crosslinked by gamma radiation were swelled with chitosan solution. PVAl do not interact with the organism when implanted and is intended to mimic the mechanical characteristics of the dermal scaffold. The chitosan as a biocompatible biosynthetic polysaccharide were incorporated into PVAl membranes to improve the organism response. Degradation of chitosan by the organism occurs preferably by hydrolysis or enzymatic action, for example, by lysozyme. For this purpose the swelling kinetic of PVAl membranes with chitosan solution were performed and it was verified their degradation in vitro. The results showed that the swelling equilibrium of the PVAl membranes with chitosan membranes was reached in 120 h with average swelling of 1730%. After swelling, PVAl and chitosan/PVAl membranes were dried and immersed in phosphate buffer solution pH 5.7 and pH 7.4, with and without lysozyme, as those pH values are the specific physiologic pH for external skin and the general physiological pH for the organism, respectively. It was verified that the pure PVAl membrane did not showed change in their mass during 14 days. PVAl membranes swelled with chitosan solution showed mass decrease from 1 to 14 days inside these solutions. The highest mass decrease was verified at pH 5.7 in phosphate buffer solution without lysozyme. The smallest mass decrease was verified at pH 7.4 in phosphate buffer solution without lysozyme. In general, PVAl membranes swelled with chitosan solution showed a clear mass decrease at pH 5.7.

* Corresponding author.
E-mail address: arodas@ipen.br (A.C.D. Rodas).

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1. Introduction

The skin is considered the largest organ of the body and plays an environmental barrier that protects the body of fluid loss and chemical attack [1]. Three distinct layers compose the skin: the epidermis, the dermis and the hypodermis (subcutaneous layer) [1].

The dermal layer could be mimic by development of synthetic membranes [2]. These membranes could be composed to mimic the mechanical characteristics of the dermis and cultivated keratinocytes could be seed to grow and to promote an epidermal layer on it. The endothelial cells ingrowths to promote neovascularization and fibroblasts ingrowths promote the substitution of this scaffold by natural components of the dermis [2,3]. For this application, poly(vinyl alcohol) (PVAl) membranes crosslinked by gamma radiation were swelled with chitosan solution. The PVAl does not interact with the organism when implanted and is intended to mimic the mechanical characteristics of the dermal scaffold by variation of preparation [4]. The chitosan as a biocompatible biosynthetic polysaccharide was incorporated into PVAl membranes to improve the organism response as vessels invasion into the scaffold [5]. The chitosan degradation by the organism occurs preferably by hydrolysis or enzymatic action, for example, by lysozyme [6–8]. For this purpose the swelling kinetic of PVAl membranes with chitosan solution were performed and it was verified their degradation in vitro.

2. Methodology

PVAl (72 K – Química Especializada Erich Ltda) solution was prepared at 10% (w/v) in water, which was poured on Petri dishes, sealed and irradiated at gamma source of ^{60}Co (Gamma Cell 200 – Atomic Energy of Canadá Limited – Ottawa, 14 cm of diameter and 20 cm of height) until 10 kGy at dose rate of 4.43 kGy/h. Membranes were freeze dried for 24 h. Dried membranes were immersed in chitosan solution of 2% (w/v) in acetic acid 0.6 mol/l and the increase of the weight were followed until constant weight at 22 °C. The swelling degree were calculated using the expression:

$$S\% = \frac{w_t - w_i}{w_i} \times 100,$$

where S% is the swelling degree in percentage, w_i is the initial weight in grams and w_t is the weight at each time in grams.

The chitosan used to perform the test was purchased from Polymar–Ceará and was characterized as average molecular weight average 330 Da and deacetylation degree 54%.

The potential degradation of the chitosan inside the membrane was verified. Two pH value were choosed, 5.7 which is the physiological pH of the skin, and 7.4, which is the physiological body's pH. The experiment was performed with lysozyme (abundant enzyme in immunological cells) added to the buffer solution.

The phosphate buffer solution was prepared at pH 5.7 and at pH 7.4. The lysozyme (Sigma-L6876) was added to part of buffer solution reaching the concentration of 4 mg/ml. Membranes swelled with chitosan solution were dried, cut, weighted and placed in a vessel with 5 ml of different buffer solution and incubated at 37 °C. For each time and buffer 6 samples were examined.

The membranes were dried and weighted again. The loss of weight were determined by using the equation

$$WL\% = 100 - \left(\frac{w_t - w_f}{w_f} \times 100\right),$$

where WL% is the weight loss in percentage, w_i is the initial dry weight and w_f is the final dry weight.

3. Results and discussion

Fig. 1 shows the swelling of PVAl membranes with chitosan solution. The swelling equilibrium of the PVAl membranes with chitosan solution was reached in 120 h with average swelling of 1730%.

The membranes obtained by gamma ionizing radiation crosslinking showed adequate characteristic as dermal equivalent. The methodology applied to incorporate chitosan inside the membranes was efficient, where the chitosan solution could be observed homogeneously into the membrane.

Fig. 2 shows the decrease of weight of the PVAl membranes swelled with chitosan solution at different experimental conditions. It was verified that the pure PVAl membrane did not showed change in their weight during 14 days. PVAl membranes swelled with chitosan solution showed mass decrease from 1 to 14 days inside these solutions. The highest mass decrease was verified at pH 5.7 in phosphate buffer solution without lysozyme. The smallest mass decrease was verified at pH 7.4 in phosphate buffer solution without lysozyme. In general, PVAl membranes swelled with chitosan solution showed a clear mass decrease at pH 5.7.

A well-designed dermal equivalent with the purpose to be implanted, should be present degradable by the body fluids. To analyze these characteristic in vitro buffers could be used as

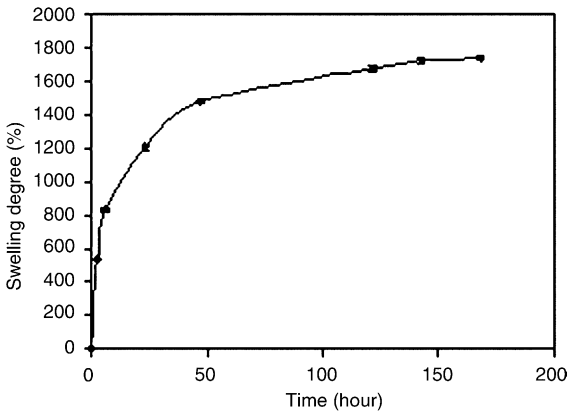

Fig. 1. Swelling degree of PVAl membranes with chitosan solution 2% (w/v) in acetic acid 0.6 mol/l at 22 °C.

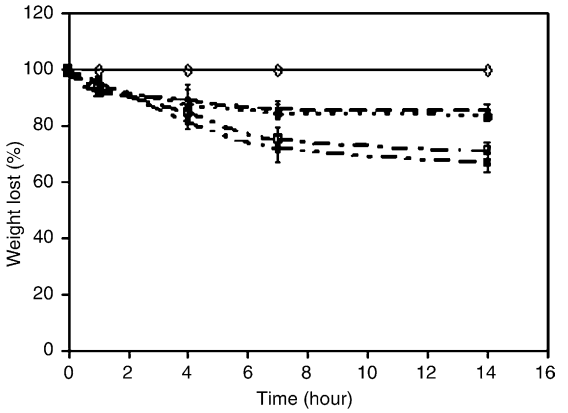

Fig. 2. Evaluation of the weight loss of the PVAl membrane swelled with chitosan solution. (◇) PVAl without chitosan immersed in phosphate buffer solution pH 5.7 and 7.4 with and without lysozyme; (□) PVAl swelled until equilibrium with chitosan solution and immersed in phosphate buffer solution pH 5.7 with lysozyme; (■) PVAl swelled until equilibrium with chitosan solution and immersed in phosphate buffer solution pH 5.7 without lysozyme; (△) PVAl swelled until equilibrium with chitosan solution and immersed in phosphate buffer solution pH 7.4 with lysozyme; (▲) PVAl swelled until equilibrium with chitosan solution and immersed in phosphate buffer solution pH 7.4 without lysozyme.

body fluids, and the pH could be chosen for the equivalent to the specific site of the body.

While the lysozyme is an abundant enzyme in inflammatory response and breaks polyssacharides in the specific link β-(1 → 4) [1], the same of the chitosan units, it was added in the buffer solution [6,8].

The test applied to verify the degradation of the membranes could not detect the action of the lysozyme added to the solution. The loss weight could be related to the capacity of the membrane release the chitosan. It is more intense at pH 5.7 because its chemical dissolution properties [7,9,10].

Present results indicate that PVAl membranes swelled with chitosan solution can be used as dermal equivalent. Biological interactions of these membranes will be studied in the future.

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