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Biocompatibility study for PVP wound dressing obtained in different conditions

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Abstract

Hydrogels composed of PVP, PEG and agar, produced by simultaneous crosslinking and package sterilization by ionizing radiation, are used mainly as wound dressing. In this study, membranes prepared in different conditions were tested for their properties including in vitro biocompatibility. The results showed that the mechanical properties were in an acceptable range of values and that the membranes can be considered as non toxic and non hemolytic to the cells.

Keywords: Biocompatibility of PVP membranes; Cytotoxicity test; Hemolysis

1. Introduction

Hydrogels to be used as wound burn dressings were invented by Rosiak et al. (1989) and they have many interesting properties: immediate pain control; easy replacement; transparency to allow healing follow up; absorb and prevent loss of body fluids; barrier against bacteria; good adhesion; good handling; oxygen permeability; control of drug dosage and so on.

PVP wound dressing were prepared by radiation crosslinking and simultaneous sterilization of poly(vinyl-1-pyrrolidone) [PVP], polyethyleneglycol(PEG) and agar. The technology had to be adapted to the needs of the physicians, climate and raw materials. Key points of these technology have been studied: hydration/dehydration and mechanical properties were studied as a function of PVP molecular weight, PEG, agar concentration and irradiation dose, as well the polymeric structure of membrane (Lugão et al., 1998). Even though the starting materials were very pure, analytical or medical grade, the final product has to be biologically evaluated.

The aim of this work was to study in vitro biocompatibility of PVP membranes prepared in different conditions, determining the hemolysis and cytotoxicity of the membranes.

2. Experimental

2.1. Preparation of hydrogel membrane

2.1.1. Materials

PVP with molecular weight of 1.2×10^6 (K-90) and 2.8×10^6 (K-120), from GAF Co.; PEG with molecular weight of 400, from Oxiteno and technical grade agar supplied by Oxoid. The hydrogel mixture solutions prepared according to the formulations listed in Table 1 were poured into molds, properly packed and sub-

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Table 1
Mechanical properties and swelling of the hydrogel membranes

	Composition/Irradiation				Mechanical properties		
Sample number	PVP (%)	PEG (%)	Agar (%)	Radiation dose (kGy)	Peak load (kPa)	Extension at break (%)	Sorption of water (%)
1	8 (K-90)	1.5	1.0	EB/20	22.8	160.2	176
2	8 (K-90)	1.5	1.0	EB/25	23.1	130.3	162
3	8 (K-90)	1.5	1.0	EB/50	16.1	56.7	129
4	8 (K-90)	3.0	1.0	EB/25	17.0	155.5	198
5	16 (K-90)	1.5	1.0	EB/25	27.9	120.0	209
6	6 (K-120)	1.5	1.0	EB/25	17.2	134.6	156
7	10 (K-120)	1.5	1.0	EB/25	27.1	132.8	181

mitted to irradiation in a Dynamitron electron accelerator with 1.5 MeV energy, dose rate of 11.3 kGy/s.

2.2. Hydrogel properties measurement

The measurements of mechanical properties were carried out using an Instron machine at a strain rate of 10 mm/s. Swelling was determined by the membranes mass variation after immersion in water for 120 h at room temperature. The gel fraction was obtained in Soxhlet extractor with water. The dehydration rate was determined by thermogravimetric curves in a Shimadzu TGA-50 thermobalance at 37°C, dynamic atmosphere of synthetic air (50 mL/min) (Lugão et al., 1998).

2.3. Cytotoxicity assay

The cytotoxicity test was carried out with dilution of the extracts of hydrogel membranes numbered 2, 4 and 7, in contact with Chinese Hamster Ovary(CHO) cells culture, from ATCC. Phenol solution (0.02%) and high density polyethylene (HDPE) extracts were used as positive and negative controls, respectively. The extracts were prepared with about 1.4 cm^2 superficial area of each type of membrane and HDPE per mL of RPMI-FCS (RPMI 1640 culture medium supplemented with 10% calf fetal serum and antibiotics), (Nakamura et al., 1989; ISO 10 993-5 1992). For preparation of cell culture dishes 2 mL of 1×10^2 CHO cells/mL suspension were seeded to each 60 mm diameter assay culture dish and incubated for about 5 h at 37°C in a humidified 5% CO_2 air incubator, for cell adhesion. The medium then was removed and replaced with 5 mL of fresh RPMI-FCS as control, undiluted and serial diluted extract of test materials, in triplicate. After 7 days incubation, the colonies were fixed with 10% formalin in 0.9% saline and stained with Giemsa. The amount of visible colonies on each dish was counted and compared with the result from CHO control dish.

2.4. Hemolytic activity

The hydrogel samples were tested by direct and indirect contact methods, according to ISO 10 993-4 (1992).

In the *direct contact method* 0.2 mL of whole rabbit blood was added to 10 mL of (i) 0.9% NaCl solution (SC) containing 2 g of each membrane; (ii) SC for negative control; and (iii) distilled water as positive control. Then the contents of the tubes were gently mixed and placed in a water bath at 37°C/1 h. After incubation time the absorbance of the supernatant of each tube was determined at 545 nm in a Spectrophotometer Ultrospec III, Pharmacia LKB and the percentage of hemolysis was calculated. A mean hemolysis value from two test samples of 5% or less was considered acceptable.

In the *indirect contact method* were used 5 mL of isotonic aqueous extract from hydrogel membranes with 0.25 mL of 10% suspension of human erythrocytes. To prepare the isotonic aqueous extracts, 20 g of each membrane (1.5 cm^2 pieces) was allowed for 72 h at 37°C in 100 mL of sterilized bidistilled water and after 0.9 g NaCl was added. The negative control was 0.9% NaCl solution and 100% hemolysis was obtained in bidestilled water. After incubation at 37°C/24 h the absorbance of the supernatant was read at 540 nm and the percentage of hemolysis calculated. The test material has no hemolytic action if the average percent of hemolysis is not higher than 1.

3. Results and discussion

The membrane swelling and mechanical properties were shown in Table 1. Sorption of water, tensile strength and elongation decreased with increasing dose

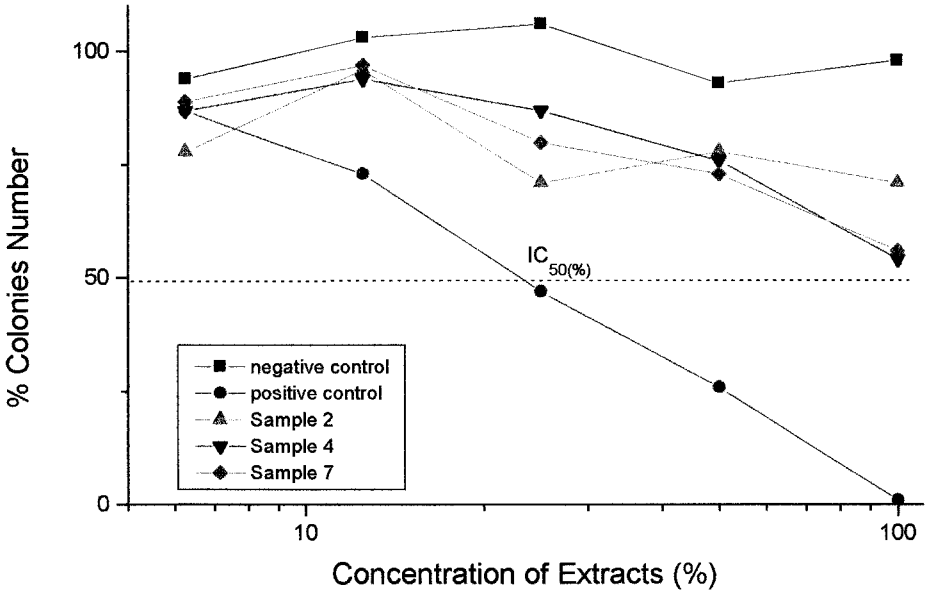

Fig. 1. Colony suppression curves of the hydrogel membranes.

of radiation, but the values were in an acceptable range. The effect of increasing PVP concentration from 8.0 to 16.0% was shown with the tensile strength raised values, which could be attributed to the cross-linking formation caused by the closer proximity of PVP molecules in higher concentration.

In the cytotoxicity assay the relative percentage of visible colonies number at different concentration of membrane extracts was calculated and plotted in the graphic presented in Fig. 1. The concentration of extract necessary to kill half of the cell population is known as cytotoxic index, expressed as $IC_{50(\%)}$. The negative control should not present toxic effect as observed with HDPE($IC_{50(\%)} > 100$) and the positive control should present cytotoxic effect, as phenol solution which kills 50% of cells at the concentration of 56% ($IC_{50(\%)} = 56$), as shown in Fig. 1. None of the hydrogel membranes were considered cytotoxic, showing $IC_{50(\%)}$ greater than 100.

The hemolysis testing of biomedical materials has been used to measure hemocompatibility. Under the conditions of this study, blood in contact with tested PVP membranes would not be considered hemolytic, showing mean hemolysis value $\leqslant 1.9\%$ in the direct contact assay and $\leqslant 1\%$ in the indirect contact assay (Table 2).

The in vitro study of biocompatibility carried out with the hydrogel membranes prepared in different conditions, showed no evidence of cell toxicity and they were considered no hemolytic.

Table 2
Hemolytic activity of the PVP hydrogel membranes under test

Sample number	Mean of % hemolysis	
	Direct contact	Indirect contact
1	0.2	0.0
2	0.5	0.0
3	0.8	1.0
4	1.9	0.0
5	0.7	0.0
6	0.9	0.4
7	0.2	0.0

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