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Blends of hyaluronic acid derivatives with ethylene-vinyl alcohol copolymers as potential biomaterials

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Films were prepared by solution casting from blends of hyaluronic acid derivatives and ethylene-vinyl alcohol copolymers. A chemico-physical and biological characterization was carried out on these "bioartificial materials" made of synthetic and biological polymers. The morphological and chemical properties of the films were investigated by scanning electron microscopy and differential scanning calorimetry. The transport properties of these films were tested in liquid systems to evaluate their possible use in dialysis and/or haemodialysis. The biocompatibility was investigated by a haemocompatibility test based on the contact activation of plasma prekallikrein. No particular interaction between the two components was observed. The results of the permeation tests were compared with those obtained using commercial products such as Cuprophane and poly(acrylonitrile) membranes. These tests indicate that the permeability of the blends decreases as the content of the synthetic polymer increases. The good haemocompatibility of these materials suggests their possible use as biomaterials.

1. Introduction

In the last few years great attention has been devoted to the preparation and the characterization of polymeric blends in order to obtain new materials with different properties from those of the two original polymeric components, and variable as a function of the composition.

Blends of natural and synthetic polymers are also the object of great interest in the biomaterial field for possible biomedical applications. Natural polymers (biopolymers) show good biocompatibility, but poor mechanical properties and often high production costs. On the other hand, synthetic polymers have, in general, much better physical–mechanical properties and low production costs, but their biocompatibility is often inadequate. Thus biopolymer/synthetic polymer blends were prepared to obtain new "bioartificial" [1] polymeric materials that could combine the good mechanical properties of the synthetic component with the biocompatibility of the natural polymer.

Bioartificial materials have been prepared using as biopolymers, hyaluronic acid (HA) and its derivatives (HYAFF), and as synthetic components, commercially available polymers such as ethylene-vinyl alcohol copolymers (tradename, Clarene®). These copolymers are already in use in the biomedical field owing to their hydrophilic properties and high biocompatibility. Since hyaluronic acid is hydrosoluble, the HA-based films redissolve when in contact with biological fluids. Therefore water-insoluble derivatives of HA, obtained by esterifying the carboxylic groups of HA with benzyl alcohol [2], have been used in this work.

This paper details the preparation and chemico-physical characterization of benzyl hyaluronic acid esters (HYAFF)/Clarene blends.

The transport properties of HYAFF/Clarene films obtained by casting were also studied to evaluate their possible use in haemodialysis. Haemocompatibility tests were carried out on both the pure polymeric components and the bioartificial materials.

2. Experimental procedures

2.1. Materials

Benzyl hyaluronic acid esters with degree of esterification of 75% (HYAFF11p75) and 100% (HYAFF11) were supplied by FAB S.p.A, Italy.

Clarene® with different ethylene content:

- Clarene® L6 (low ethylene content: 29 mol %)
- Clarene® P10 (moderate ethylene content: 36 mol %)
- Clarene® R20 (high ethylene content: 40 mol %)

were supplied by Solvay & C.i.e., Italy.

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2.2. Films preparation

For each type of HYAFF and for each type of Clarene, a 1% (w/v) solution in dimethylsulfoxide (DMSO) was prepared. The formulated solutions were mixed at 80 °C to obtain blends having the following composition in polymer weight ratio: 20/80, 50/50, 80/20 (the first number refers to HYAFF content). The blends obtained were as follows:

HYAFF11/Clarene L6 (20/80, 50/50, 80/20)
HYAFF11/Clarene P10 (20/80, 50/50, 80/20)
HYAFF11/Clarene R20 (20/80, 50/50, 80/20)
HYAFF11p75/Clarene L6 (20/80, 50/50, 80/20)
HYAFF11p75/Clarene P10 (20/80, 50/50, 80/20)
HYAFF11p75/Clarene R20 (20/80, 50/50, 80/20)

After stirring, films were obtained by solution casting at 60 °C. HYAFF and Clarene homopolymers film were also prepared by the same method. The films were stored in distilled water until use. The thickness of the water-swollen membranes was measured using a micrometer with an accuracy of $\pm$ 1 μm. The average thickness of the wet films was about 20 μm.

3. Apparatus and techniques

3.1. Thermal analysis

Differential scanning calorimetry (DSC) measurements were performed with a Perkin Elmer DSC-7, in a N_2 atmosphere with a heating rate of 10 °C/min in the range 30 to 190 °C. The thermal properties of the blends and homopolymers were analysed in one heating scan. The calorimetric melting temperature, T_m, and the fusion heat, ΔH_f, of Clarene were determined from the maximum and the area of the melting peak, respectively. Within the explored temperature range the biopolymer does not show any relevant thermal transition.

3.2. Morphological analysis

The surfaces and the cross-sections of the films were analysed with a Jeol T 300 scanning electron microscope (SEM). The films were freeze-fractured in liquid N_2 and sputter-coated with gold before SEM analysis.

3.3. Permeability measurements

Permeability measurements were performed at 37 °C using solutes with different molecular weight, including sodium chloride NaCl (MW 58 D), vitamin B12 (MW 1355 D) and bovine albumin (MW 69 000 D). The experimental apparatus used for solute permeability measurements is described in details elsewhere [3]. It essentially consists of a stirred diffusion cell made up of two chambers separated by the membrane to be tested. The solute concentration on the permeate side of the membrane gradually increased until it became constant (i.e. until steady-state conditions were obtained). When the steady state is reached, the flux of solute across the membrane, J_s, can be written in terms of solute concentrations in the two chambers of the diffusion cell as follows [4]:

$$J_s = \frac{PA}{h}(C_d - C_r)$$

where P is the solute permeability, A is the membrane area, h is the swollen membrane thickness, C_d and C_r are the solute concentrations in donor and receptor chambers of the diffusion cell, respectively. The above equation has been used to calculate the solute permeability, P, provided that the concentration gradient, the flux under steady-state conditions and the swollen membrane thickness are known.

3.4. Haemocompatibility analysis

The activation of human plasma prekallikrein (PKK) to kallikrein (KK), induced by the contact of blood with foreign materials, is a useful *in vitro* haemocompatibility test [5]. The activation of PKK to KK is determined by the proteolytic reaction between KK and the chromogenic substrate H-H-Pro-Arg-pNa (S-2302 Kabi Diagnostica). The test was carried out on the following materials: borosilicate glass (as a high-activation reference material), silicone (as a low-activation reference material), ethylene-vinyl alcohol copolymers (Clarene L6, Clarene P10, Clarene R20), benzyl hyaluronic acid esters (HYAFF11 and HYAFF11p75) and HYAFF/Clarene blends.

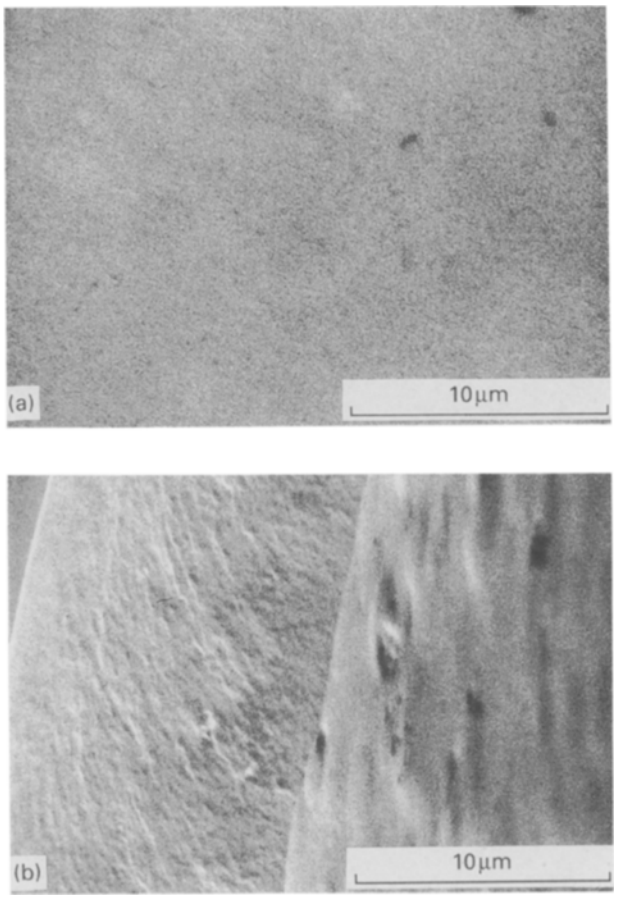

Figure 1 SEM images of HYAFF11p75/Clarene L6 80/20 film (a) surface and (b) cross-section.

4. Results

4.1. Thermal characterization

The Clarene thermal transition varied with the composition of the blends and with the degree of esterification of HYAFF. The HYAFF homopolymer films showed no significant transition in the explored temperature range (30–190 °C). The blends showed no variations with regard to the melting point, T_m, of the crystalline polymeric component, while the enthalpy of fusion ΔH_f decreased with increasing HYAFF content. Maybe this is due to morphological effects [6–8] that reduce the crystallinity degree of the synthetic polymer.

4.2. SEM characterization

SEM images showed structures gradually changing from homogeneous (Fig. 1a, b) to heterogeneous, with evident phase separation (Fig. 2a,b) dependent on the content of Clarene. Homogeneous structures, as shown in Fig. 1, refer to blends with higher values of ΔH_f; heterogeneous structures, as shown in Fig. 2, refer instead to blends with lower values of ΔH_f indicating poor miscibility between the two components in the blend.

4.3. Permeability measurements

The permeability coefficients of HYAFF/Clarene membranes are shown in Fig. 3, and Fig. 4 for NaCl, vit. B12 and bovine albumin. The permeability values, P (cm/s), are normalized to a thickness of 20 μm. The results are compared with those obtained using commercial products such as Cuprophane and polyacrylonitrile (AN69) membranes. These results show that pure HYAFF11p75 and HYAFF11 membranes have

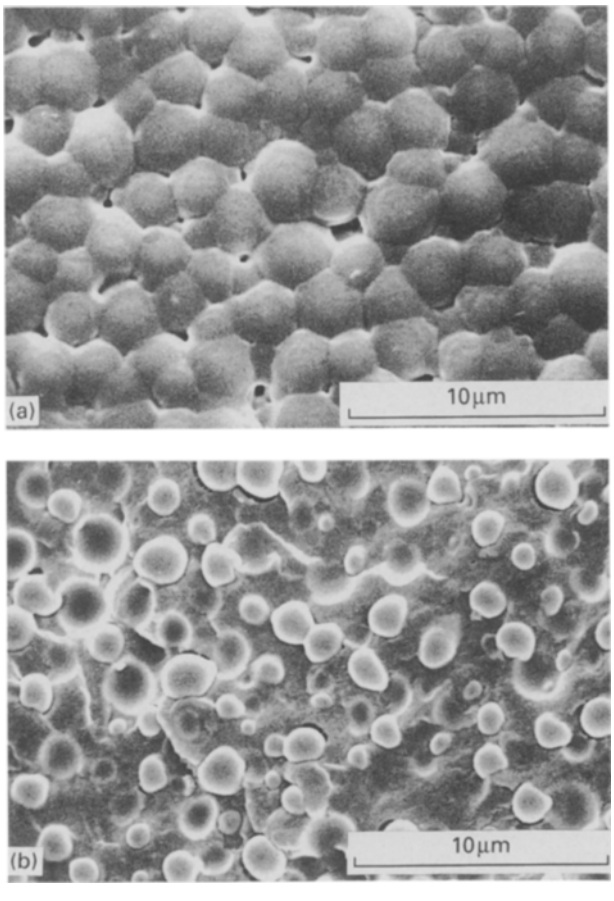

Figure 2 SEM images of HYAFF11p75/Clarene L6 20/80 film (a) surface and (b) cross-section.

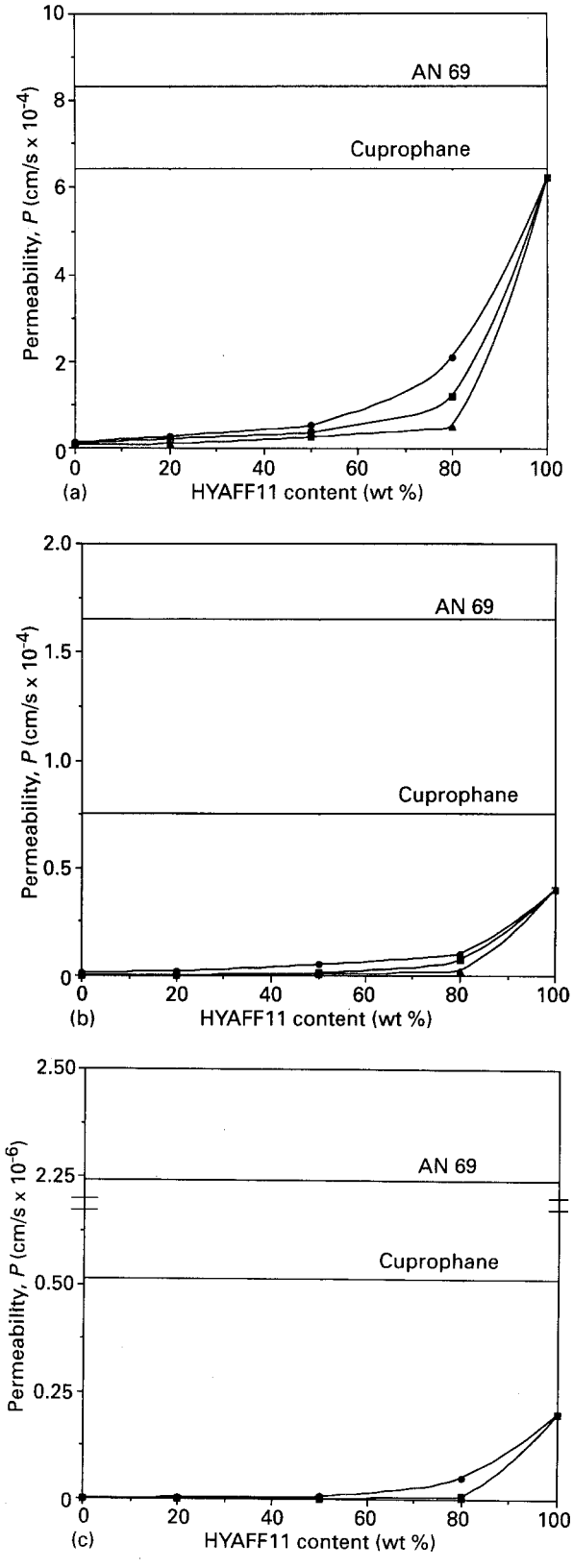

Figure 3 Permeability of HYAFF11/Clarene blend membranes versus percentage weight of HYAFF11 in the blend, for (a) NaCl, (b) vit. B12 and (c) bovine albumin. (● HYAFF11/Clarene L6, ■ HYAFF11/Clarene P10; ▲ HYAFF11/Clarene R20).

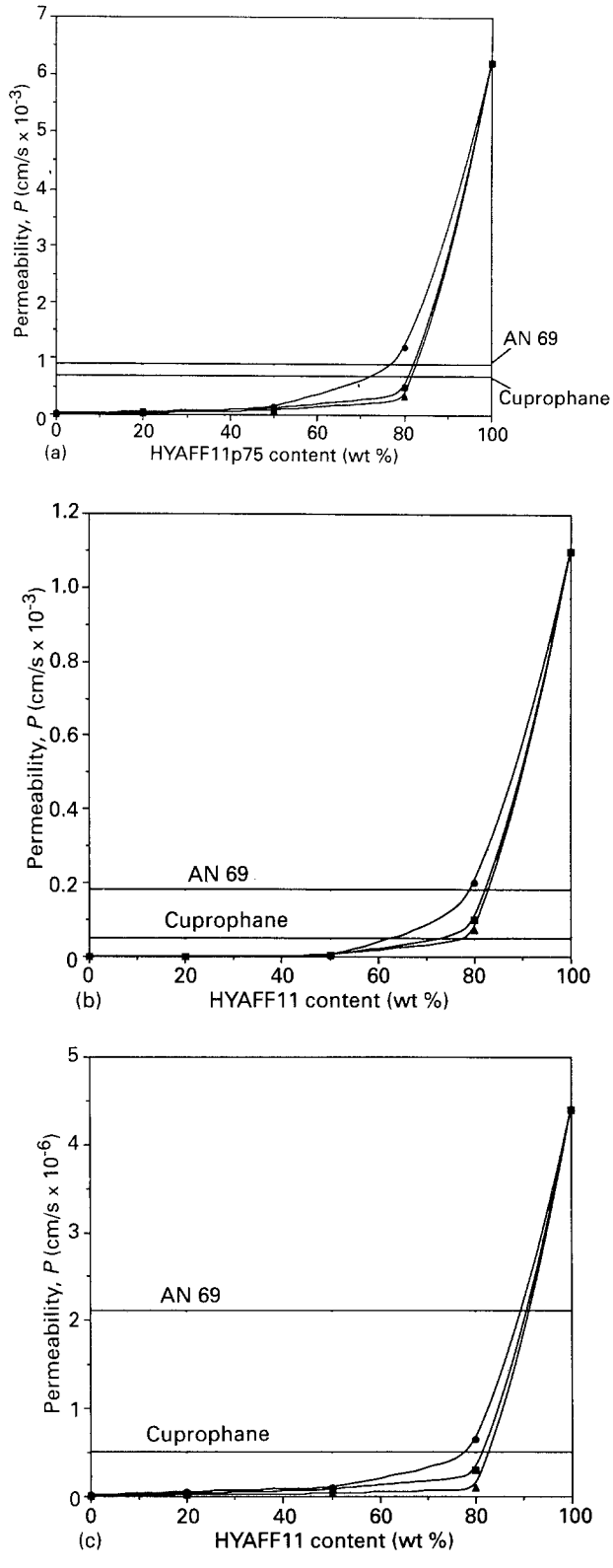

Figure 4 Permeability of HYAFF11p75/Clarene blend membranes versus percentage weight of HYAFF11p75 in the blend, for (a) NaCl, (b) vit. B12 and (c) bovine albumin. (● HYAFF11p75/Clarene L6, ■ HYAFF11p75/Clarene P10, ▲ HYAFF11p75/Clarene R20).

good permeability while pure Clarene membranes have poor permeability to all tested substances (Figs. 3 and 4). Consequently, the permeability of blends decreases drastically with increasing Clarene content, and this is due to well-known barrier properties of ethylene-vinyl alcohol copolymers.

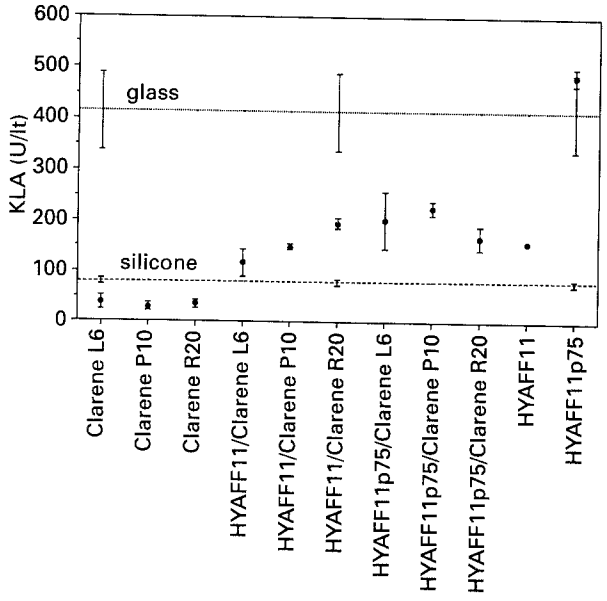

Figure 5 KLA for different materials and for HYAFF/Clarene (50/50) blends (the results are the mean ± SE for five measures). Plasma contact time 3 min at 37 °C.

4.4. Haemocompatibility characterization

Fig. 5 shows the KLA (kallicrein-like activity) induced in human plasma by HYAFF, Clarene and some blends HYAFF/Clarene (50/50), in comparison with that induced by borosilicate glass and silicone. Each point is the mean value of five measurements. The results show that the haemocompatibility of the different ethylene–vinyl–alcohol copolymers is very good and the activation induced by the various blends examined is nearer to that induced by silicone than to that induced by glass. The pure HYAFF11p75 only shows a KLA value near to that induced by glass.

5. Conclusions

HYAFF/Clarene films can be easily prepared from solutions in DMSO using a solution casting method. DSC and SEM analysis, show that there are not specific interactions between the two components. HYAFF/Clarene blends show generally good haemocompatiability but poor transport properties because of their dense structure. This is due to the casting method used in preparing the membranes. The enhancement of the permeation properties could therefore be achieved by employing different technologies, such as phase-inversion processes, in order to obtain structures with controlled porosity.

Acknowledgements

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