OPTOELECTRONICS AND ADVANCED MATERIALS – RAPID COMMUNICATIONS Vol. 3, No. 12, December 2009, p. 1336 - 1342

Compatibility and cytotoxicity testing of some new biomaterials based on polyurethane and hydroxypropylcellulose blends

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The aim of the present paper is to study the compatibility and biocompatibility of new polyester urethane with hydroxypropylcellulose (HPC) blends by FT-IR spectroscopy, contact angle measurements. It has been established on the basis of the results obtained and specific interactions that the optima compatibility compositions which assure the good properties of biomaterials is 60% HPC–20% HPC/40% PU–80% PU. The biocompatibility and cytotoxicity of novel materials has been also assessed. The biological analysis showed that the new blends present a good biocompatibility, fibroblast cells cultured in the presence of polymers maintaining their normal cellular phenotype. The obtained blends are not cytotoxic and exhibits good surface properties and could be candidates for medical and pharmaceutical applications.

(Received November 24, 2008; accepted November 23, 2009)

Keywords: Hydroxylpropyl cellulose, Polyurethane, Biocompatibility, Fibroblasts

1. Introduction

Polymer blending constitutes one of the most useful methods for the improvement or modification of the physicochemical properties of polymeric materials without altering the structure and function of individual polymers. An important property of a polymer blend is the miscibility of its components, because it affects the mechanical properties, morphology, permeability and degradation. The blends between biopolymers and synthetic polymers are of particular significance because they can combine the biocompatibility with good processability and mechanical resistance and can be used as biomedical and biodegradable materials. [1]

We were interested in determining the behaviour of multi-component systems containing cellulose derivatives which are known by their applications in many dosage forms of drugs. [2] Since pharmaceutical formulations often include different polymeric components, an understanding of the fundamental interactions that occur in these systems provides a basis by which the properties of the formulations can be tailored.

Hydroxypropyl cellulose (HPC) is a cellulose derivative employed as coatings, excipients, encapsulations, binding materials, foaming agents, protection colloids, flocculants, a wide variety of applications in food, drugs, paper, ceramics, plastics, etc. [3] HPC in a mixture with a homo- or copolymer of acrylic acid or a pharmaceutically acceptable salt has appropriate oral mucosal adhesion properties and controlled drug-release features and is therefore used as a major excipient for drug tablets intended for bucal and sublingual administration. [4] In Japan, tablets named "Aftach," based on the aforementioned formulations for applications in the treatment of aphtha, [5] are now commercially available.

Polyurethanes (PUs) are widely used as binder in coatings because of their excellent mechanical properties like good hardness, high abrasion, and chemical resistance. [6] Polyurethanes are known to exhibit excellent mechanical properties [7, 8] and a good thromboresistance. [9] These properties are explained in terms of their microphase separated structure. [10]

Baron et al. report a contribution in which correlate polymer structure and properties of segmented polyurethanes focussing on its potential interest as pressure-sensitive adhesive nanomaterial. [11]

A few research papers are devoted to combine polysaccharides as cellulose and cellulose derivatives with polyurethanes properties. [12, 13]

The assessment of the biocompatibility and non-toxicity is an important request for developing and application of new materials, especially for those destined to medical applications.

Testing the *in vitro* biocompatibility on cell cultures offers precious information on *in vivo* behaviour. [14] Some of newly employed cell cultures reproduce *in vivo* conditions that permit to normal cells to grow in medium culture. A polymer can be used in medicine or/and pharmacy if it is biocompatible with blood and tissues, is

well tolerated by organism, is physically and chemically stable in biological medium, and it is not toxic. [15]

The aim of the present study is to evaluate the compatibility and biocompatibility of the blends made from a new polyester urethane and HPC in solid state by FT-IR spectroscopy, contact angle measurements to establish the optima composition range which assure a maximum compatibility degree. The cytotoxicity assessment was also done.

2. Experimental

2.1 Materials

The (hydroxypropyl) - cellulose (HPC) Klucel samples were kindly supplied by Aqualon. The HPC sample (HPC EF) was used as received without any further purification.

The main chain of the HPC is constituted of glucopiranosyl units linked to (1-4) β position. [16] The weight average molecular weight of HPC EF is $\bar{M}_W$ ~ 1.000.000, the molecular substitution MS is 3.7, particle diameter ~ 78 μm, and solution viscosity (1 g/dL in water) ~ 2400 cps.

It is possible that the hydroxypropyl substituents exist as a unit or as short side chains containing one to six hydroxypropyl units. [17]

The polyurethane used in this study was synthesized in our laboratory and has the following structure:

$$[PEGA - MDI - (EG - MDI)_4]_n$$

Fig. 1. The structure of polyurethane.

where:

PEGA is $HO - [(CH_2) - OCO - (CH_2)_4 - OCO]_n - (CH_2)_2 - OH$; n = 10,3

MDI is $OCN - C_6H_4 - CH_2 - C_6H_4 - NCO$

EG is $HO - (CH_2)_2$ - OH

PEGA is poly(ethylene glycol)adipate;

MDI is 4,4' – methylene diphenylene diisocyanate;

EG is ethylene glycol.

Poly (ethylene glycol) adipate (PEGA) is a commercial product purchased from Fibrex SA, Savinesti, Romania (M_n = 2000 g/mol, purity 97 %).

Diphenylmethane 4,4'-diisocyanate (MDI), a commercial Merck product, was distilled prior to utilization under reduced pressure.

Ethylene glycol (EG) is a commercial product purchased from Fibrex SA, Savinesti, Romania (purity 95 %).

The poly (ester urethane) was obtained from aromatic diisocyanate, MDI, with PEGA and EG as chain extender, in a two-step polyaddition process, in N,N-dimethylformamide (DMF), according to the procedure previously described for other kinds of polyurethanes. [18] In the first stage of the reaction the NCO-terminated prepolymer was formed. PEGA was dehydrated for 3h at 120 ^oC, followed by addition of MDI. The reaction mixture between diisocyanate and macrodiol was kept 1h under nitrogen atmosphere, at 90 ^oC. The amounts of diisocyanate and PEGA was controlled at a NCO:OH molar ratio of 5:1. The second step involved the reaction of the free isocyanic groups with chain extender (EG). The reaction temperature was controlled at 60 ^oC when the chain extender was added. The polymer was precipitated in water and dried under vacuum, for several days.

The weight average molecular weight of PU is M_w ~ 150.000; the obtained PU is soluble in DMF, DMSO and it is a film forming polymer.

2.2 Films' preparation

The HPC and PU solutions (10 % wt) were prepared in dimethyl formamide (DMF). The blended solutions of HPC and PU in DMF were prepared by mixing the solutions in different proportions at room temperature by magnetically stirring for 1h. The obtained HPC EF/PU ratios are: 100/0, 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, 30/70, 20/80, 10/90, 0/100; the entire composition range being covered. The films have been obtained by casting method followed by prolonged drying in a vacuum oven at 70 ^oC and p = 950 mbar. It was observed that the most films are homogeneous, transparent, while those with prevalent HPC content are brittle.

2.3 Methods of investigation

IR analysis. ATR FT-IR spectra of the HPC/PU film samples were recorded with a Bruker VERTEX 70 Spectrophotometer. Spectra were recorded from 4000 to 500 cm^{-1} averaging 64 scans with a resolution of 2 cm^{-1}.

Contact angle measurements. The contact angle measurements were determined by "sessile" drop method using a CAM-PLUS – 200 Micro instrument. In order to obtain the components of the free surface energy of the solid material and the total free surface energy, the contact angle at equilibrium between the studied surface and three pure liquids (from which, at least two must be polar), needs to be measured.

The droplets of the different pure liquids like: twice distilled water, formamide, α-bromonaphtalen, are placed on the solid surface – Table 1. After 30 seconds from placing the droplet on the solid surface, time which is consider to be long enough for the droplet to make an equilibrium contact angle and also short enough for the droplet to do not start the evaporation process, it is measured the base diameter (d) and the height of the

droplet (h). The contact angle is determined with the equation:

$$tg\frac{\theta}{2}=\frac{2\cdot h}{d} \quad (1)$$

where θ is the contact angle formed between droplet and the surface, h is the height of the droplet and d is the droplet diameter.

Contact angles were measured at room temperature averaging the contact angles over ten measurements.

In order to establish the biocompatibility of the polymers with blood, need to be taking into account, not only the surface properties of the biomaterials but also those of the blood. For this reason, it was defining the interfacial tension blood – biomaterial by the following equation: [21]

$$\gamma_{SL}=\left[\left(\gamma_L^{p}\right)^{1/2}-\left(\gamma_S^{p}\right)^{1/2}\right]^2+\left[\left(\gamma_L^{d}\right)^{1/2}-\left(\gamma_S^{d}\right)^{1/2}\right]^2 \quad (2)$$

where $\gamma_{S,L}{}^{p}$ and $\gamma_{S,L}{}^{d}$ are the polar component and dispersive component, respectively of the biomaterial (S)/blood (L) surface.

Table 1. The values of the components of the interfacial tension liquid-vapour in three liquids. [19, 20].

Liquid	γ_{LV} (mN/m)	γ_{LV}^{d} (mN/m)	γ_{LV}^{p} (mN/m)	γ_{LV}^{+} (mN/m)	γ_{LV}^{-} (mN/m)
Twice distilled water	72.8	21.8	51	25.5	25.5
Formamide	58.0	39.0	19.0	2.28	39.6
α-bromonaftalen	44.4	44.4	0	0	0

The *in vitro cytotoxicity* of polymeric blends was evaluated on the basis of cell proliferation, viability and morphology. Fibroblasts primary culture obtained from human dermis explants were used. The samples analyzed were: 90HPC/10PU (powder); 30HPC/70PU (film); 10HPC/90PU (film); 100PU (film) .The dimension of the studied films was 5x5 mm. All the samples were sterilized with UV radiation for 8h.

Cell proliferation and viability in contact with polymeric samples. To analyze cell proliferation human dermal fibroblasts (3.5 x 10^4 cells/ml) were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 UI/ml penicilin, 100μg /ml streptomycin and 50 μg/ml neomycin into 35 mm diameter culture dishes. After 24h of incubation at 37 ^{0}C the culture medium was replaced with a fresh one and the samples were added into the dishes. Cells were allowed to grow for another 24h and 48h, respectively. After that, fibroblasts were trypsinised, stained with a 0.4 % Trypan blue solution and the viable cells were counted using a Burker Turk hemocytometer. Trypan blue negative cells indicated viable cells.

MTT assay. Cell viability was spectrophotometrically measured by MTT test. Fibroblasts were seeded into 96-well plates at a density of 3.5 x 10^4 cells/ml, and after 24h of incubation at 37 ^{0}C the polymeric samples were added. After 24h and 48h, the culture medium was discarded, 50 μl of MTT solution (5 mg/ml) dissolved in the culture medium were added in each well and then, cells were incubated for 3h. Water-insoluble dark blue formazan crystals formed in viable cells were solubilized with isopropanol and the absorbance of every well was measured at 570 nm using an UV-VIS spectrophotometer (Jasco V-650, Japan). Concentration of converted dye directly correlated to the number of metabolically active cells in culture. Cells viability was calculated by comparison with control samples (fibroblasts cultivated in absence of polymers), considered to be 100 % viable cells.

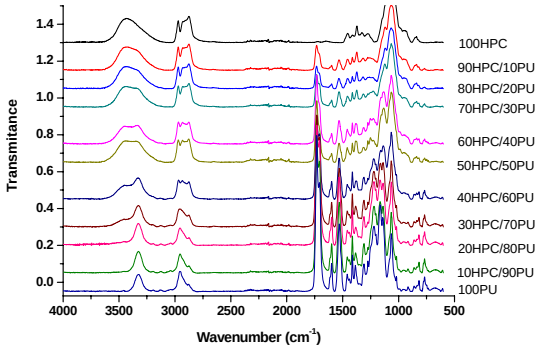
Cell morphology. Fibroblasts morphology was assessed by light microscopy. Human cells were incubated at 37 ^{0}C for 24h. After this period of time, the blends were added in cell culture. Fibroblasts cultured

in the presence of polymeric samples for 48h were washed with PBS, fixed in methanol, stained with Giemsa solution and pictures were taken using an inversed-phase microscope (Nikon Japan). In all experiments the control sample was represented by dermal fibroblasts cultivated in the absence of polymers.

3. Results and discussion

3.1 IR results

Attenuated total reflexion fourier transform-infrared spectroscopy (ATR-FT-IR) is an analytical technique for functional group analysis that provides information about the chemical make up of materials. FT-IR technique provides a rapid means of identifying functional groups of the substances, characterizing contaminants, and comparing materials. In the present investigation, FTIR experiments were performed in order to establish if between HPC and PU, some interactions are present, which should be due to the mutual solubility of components. The ATR-FT-IR spectra for HPC PU and their blends are presented in Fig 2.



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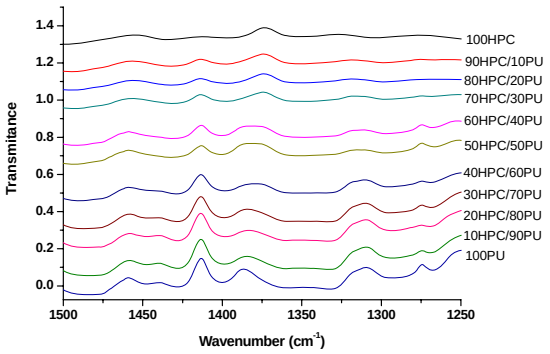

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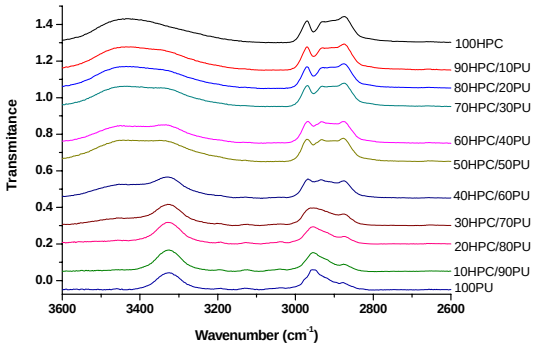


(c)

Fig. 2. ATR-FT-IR spectra of HPC/PU blends (a) entire spectral region; (b) 1500 - 1250 cm^{-1} "fingerprint" spectral region and (c) 3600 – 2600 cm^{-1} spectral region.

Table 2. The main bonds involved in intermolecular interactions in HPC/PU blends. Band position in cm^{-1}.

Sample	C-H C-N	Amide I	C=O	CH_2 symmetric	CH_2 asymmetric	CH_2 asymmetric	OH NH stretching vibration	OH NH stretching vibration
100HPC	1375.8	-	-	2874.26	2929.21	2969.51	3434.79	-
90HPC/10PU	1375.8		1731.2	2874.26	2929.21	2969.51	3438.46	3346.87
80HPC/20PU	1375.8		1731.2	2874.26	2929.21	2969.51	3434.79	3343.2
70HPC/30PU	1375.8		1731.2	2874.26	2929.21	2969.51	3438.46	3343.2
60HPC/40PU	1379.5		1731.2	2874.26	2932.88	2969.51	3445.78	3343.2
50HPC/50PU	1383.2		1731.2	2874.26	2932.88	2969.51	3445.78	3346.87
40HPC/60PU	1379.5	1709.22	1731.2	2874.26	2932.88	2969.51	3445.78	3328.55
30HPC/70PU	1383.2	1709.22	1731.2	2874.26	-	2958.52	3449.45	3328.55
20HPC/80PU	1383.2	1705.56	1731.2	2874.26	-	2954.86	-	3328.55
10HPC/90PU	1383.16	1705.56	1731.2	2874.26	-	2954.86	-	3328.55
100PU	1386.82	1705.56	1731.2	2874.26	-	2954.86	-	3328.55

The bands in ATR-FT-IR spectra have been assigned based on the literature data. [22, 23].

Very high intensities of the –OH and –NCO peaks are positioned at 3470–3600 cm^{-1} and 2258 cm^{-1} respectively, while that at 3314 cm^{-1} is due to the =NH group. The shoulder peak appearing at 3420 cm^{-1}, is attributed to the non-hydrogen bonded =NH groups in polyurethane. [24]

The IR stretching bands at 3440 and 3354 cm^{-1} correspond to free (NH free) and hydrogen-bonded (NH bonded) N–H in the polyurethane, respectively. [25]

All IR spectra show the bands at 3440 (free N–H stretching), 3354 (hydrogen-bonded N–H), 2935–2931 (CH_2, asymmetric stretching (polyether)), 2852-2794 (CH_2, symmetric stretching)1727 (COO of urethane), 1716 (C=O stretching, amide-I region), 1595 (N–H bending), 1529–1527 (C–N stretching and N–H bending (amide-II region)), 1209–1226 (C–N stretching (amide-III region)); 1108–1105 (C–O–C, asymmetric stretching (polyether and urethane)).

Examination of the data in Table 2 reveals important shifts in the bands position of OH and NH stretching,

amide I, and CH_2 asymmetric, CH and CN. These shifts indicate the formation of the hydrogen bonds between the components of the blends which contribute to the realization of a high degree of compatibility.

3.2 Blood-film surface tension

From the experimental values of the contact angle with twice-distilled water, formamide and α-bromonaftalen of HPC/PU films the free surface energy and its components and also the interfacial tension between blood and film surface of the blends have been evaluated – Table 3.

Generally the values of the surface tension and its components for the HPC/PU blends are average of the values of the components. However, it can be remarked the higher value of the γ_S^+ and $\gamma_{SV}^{LW/AB}$ are for the blends with 60% HPC – 20% HPC / 40% PU – 80% PU composition range which can be due to the changes in the relation between components as was also evidenced by ATR-FT-IR study.

Therefore deviation from calculated value occurs due to some interactions where mainly the acceptor groups are involved probably by hydrogen bonding (such as aromatic rings from MDI). It can conclude that this composition range correspond to the optima compatibility between HPC and PU. Moreover, the values of the interfacial tension blood – biomaterial (γ_{SL}) are situated in the range of 1.96 – 3.27 mN/m which allow us to conclude that there is a good haemocompatibility of the materials with blood, as will be also proved by some biological properties presented below.

Table 3. Surface tension, its components and the interfacial tension blood – film blends.

Sample	γ_S^{LW} (mN/m)	γ_{SV}^{AB} (mN/m)	γ_S^+ (mN/m)	γ_S^- (mN/m)	$\gamma_{SV}^{LW/AB}$ (mN/m)	γ_{SL} (mN/m)
HPC	4.41	44.35	8.8	39.52	48.78	3.05
90 HPC/10 PU	9.67	38.93	6.67	38.98	48.6	3.27
80 HPC/20 PU	11.49	36.48	8.4	32.25	47.97	2.85
70 HPC/30 PU	10.7	38.69	8.64	34.93	49.38	2.81
60 HPC/40 PU	10.7	38.69	8.3	37.7	49.38	2.81
50 HPC/50 PU	11.09	40.7	8.9	36.2	51.8	2.37
40 HPC/60 PU	11.09	40.7	9.36	35.45	51.8	2.37
30 HPC/70 PU	12.46	39.06	8.76	34.8	51.52	2.09
20 HPC/80 PU	13.91	37.21	8.36	32.85	51.12	1.96
10 HPC/90 PU	16.98	32.03	7.23	28.98	49	2.49
PU	19.9	29.6	6.63	25.53	49.5	2.93

subscript "SV" denote the interfacial surface-vapour tensions, γ_{SV} total surface tension, superscripts "LW" and "AB" indicate the disperse and the polar component (Liftschitz-Van der Waals), respectively obtained from the γ_{SV}^- electron donor and the γ_{SV}^+ electron acceptor interactions, while superscript "LW/AB" indicates the total surface tension.

3.3 Cytotocompatibility of blends in vitro

To analyse possible release of toxic products by the blends, dermal fibroblasts were grown in contact with polymeric samples. The results of cell proliferation presented in Table 4 indicate that the grown of dermal fibroblasts in contact with blends, after 24h and 48h of cultivation was dependent of samples type. It can be observed an increase of fibroblast number at 24h and 48h in the case of all blends but cell proliferation was comparable with control sample only for 10HPC/90PU and for PU.

Table 4. Cell proliferation of human fibroblasts grown in presence of polymeric variants after 24h and respectively 48h of cultivation.

Sample	24h (cells/ml)	48h (cells/ml)
Control	22,500	51,250
90HPC/10PU	26,250	32,500
30HPC/70PU	20,000	39,000
10HPC/90PU	21,250	45,000
PU	32,500	41,250

The effect of blends on human dermal fibroblasts was evaluated by MTT test, a rapid and economical assay for measurement of cellular viability, proliferation and cytotoxicity. The method is based on the conversion of tetrazolim salt [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to purple formazan by the mitochondrial succinate dehydrogenase in viable cells – Fig 3. It can be observed from the data of Fig 3 that, with the exception of the 90HPC/10PU sample, all other studied samples have the cellular viability percentage over 80%; the highest dermal fibroblasts viability value was obtained from 10HPC/90PU polymeric film.

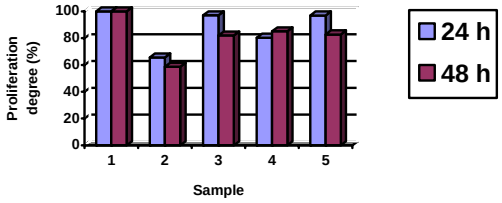
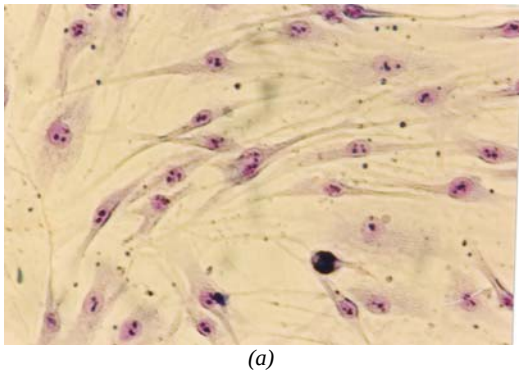


Fig. 3. Effect of polymeric blends on dermal fibroblasts viability after 24 and 48 hours of cultivation, evaluated by MTT assay (1-control; 2-90HPC/10PU; 3-30HPC/70PU; 4-10HPC/90PU; 5-PU).

3.4 Cell morphology

The cell morphology of fibroblasts was studied after 48h of cultivation. Light microscopy images showed that the presence of different polymeric blends into cell culture did not constitute a stress factor for dermal fibroblasts – Fig. 4 (e). Cells presented an elongated morphology, typically for normal fibroblasts, with euchromatic nucleus, 1-3 nucleoli, with thin cytoplasmic extensions and a fine granular cytoplasm. No differences of morphology were found between cells cultured in the presence of polymeric materials – Fig. 4 (b)-(e) and control sample – Fig. 4 (a). In the case of 10HPC/90PU – Fig. 4 (d) and PU – Fig. 4 (e) samples it was observed that the fibroblasts adhered to the surface of the polymeric films.



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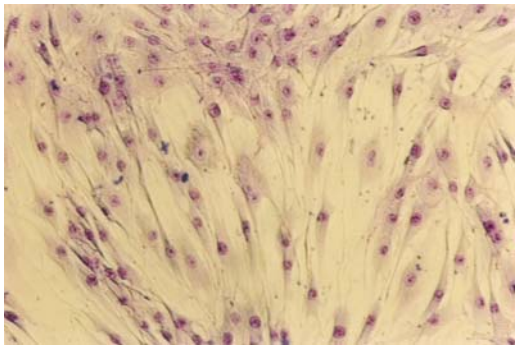

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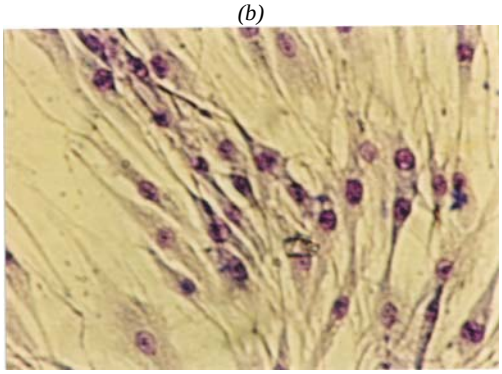

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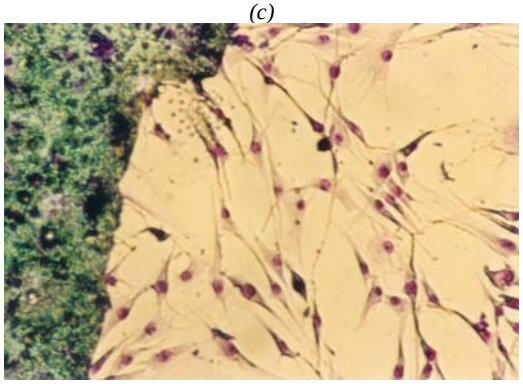
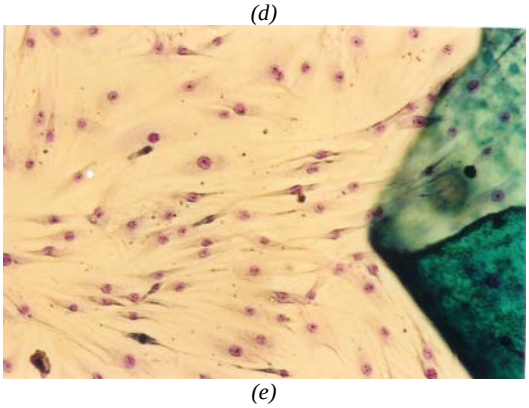


(d)



(e)

Fig. 4. (a) Morphological aspect of the control after 48h of cultivation (Giemsa, x 200); (b), (c) Morphology of dermal fibroblasts cultivated in presence of 90HPC/10PU film (x100) and 30HPC/70PU film (x200) after 48h of cultivation (Giemsa staining); (d), (e) Human dermal fibroblasts cultivated in presence of 10HPC90PU and PU blends after 48h of cultivation (Giemsa staining, x100).

The most biocompatible blends are 30HPC/70PU, 10HPC/90PU and PU. The PU sample is similar to the control. The synthesized polyurethane has antitrombogenic properties and is hemocompatible (compared to other hemocompatible materials) [26, 27] and as a result, all studied components and blends can be recommended for biomedical applications.

4. Conclusions

In this study it has been shown that a relationship between the degree of compatibility in the polyurethane/ HPC blends and their biological properties exist. It has been established that the optima compatibility compositions which assure the good properties of biomaterials is 60%HPC-20%HPC/40%PU-80%PU. The increase in the degree of compatibility is due to the creation of interactions between –OH groups from HPC and the polar group of PU.

The morphological analysis of the dermal fibroblast behaviour in the presence of the 30HPC/70PU, 10HPC/90PU and PU indicate that there is a good biocompatibility, evidenced also by a high proliferation capacity, the adhesion to the polymeric films. 10HPC/90PU and PU blends there were a good biocompatibility, evidenced by a high cell proliferation capacity, cell adhesion to the polymeric films and normal fibroblast morphology.

The obtained blends are not cytotoxic and exhibit good surface properties and hemocompatibility, therefore could be candidates for medical and pharmaceutical applications.The studied blends were tested on a human dermal fibroblast culture, and in the case of 30HPC/70PU,

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