JOURNAL OF OPTOELECTRONICS AND ADVANCED MATERIALS Vol. 11, No. 3, March 2009, p. 356 - 365

New biocompatible materials by PVA / HC and biological polymers

F. CRIVOI*, L. STEFAN[a], L. MOLDOVAN[a], C. VASILE[b]
"Gr. T. Popa", Medicine and Pharmacy University, Physics Department, 16 University Street, Iasi, 700064, Romania
[a]*National Institute of Research and Developing of Biological Sciences, 296 Splaiul Independentei Street, 6 Sector, 060031, Bucharest, Romania*
[b]*"P. Poni" Institute of Macromolecular Chemistry, Physical Chemistry of Polymers Laboratory, 41 A Gr. Ghica Voda Alley, Iasi, 700487, Romania*

This paper deals with obtaining and characterization of some bioartificial materials based on polyvinyl alcohol(PVA) with hydrolyzed collagen (HC) and small amounts of natural polymers such as: elastin (ELS), hyaluronic acid (HA) and chondroitin sulphate (CS). The film samples have been obtained by mixing of the solutions of polyvinyl alcohol and hydrolyzed collagen of 10 wt% concentration and the natural polymers in different proportions. By a complex of physico-chemical, structural and biological methods such as SEM, DSC, thermogravimetry, IR-spectroscopy, MTT test, etc., the compatibility, interactions between components and cytotoxicity upon cells has been studied. On the basis of the obtained data can be concluded that all samples are compatible and the higher biocompatibility degree was recorded in the presence of glycosaminoglycans containing materials.

(Received July 15, 2008; accepted March 19, 2009)

Keywords: Polyvinyl alcohol, Hydrolyzed collagen, Glycosaminoglycans, Blends, Compatibility, Cytotoxicity

1. Introduction

The blends of polyvinyl alcohol (PVA) with other polymers are low cost materials, accessible and easily processable, with good mechanical, thermal and surface properties and are recyclable. OH groups favour physical and chemical interactions with functional groups of other polymers.

The biocompatibility of the polymer blends, that is necessary in biomedical applications, can be increased by the incorporation of biologic polymers in blends, that will conferee superior properties and will reduce the failure of implants and adverse reactions.

In our previous papers the interactions between the functional groups and the optima compatibility of the binary blends in proportions: 90-70 wt % PVA / 10-30 wt % hydrolyzed collagen (HC) have been evidenced through DSC, thermogravimetry and IR spectroscopy. The human dermal fibroblasts proliferation analysis in the presence of PVA/HC binary blends at different cultivation times, evidenced a cell viability higher than 70 %, the normal cell phenotype and the proliferation capacity are retained of the majority of human dermal fibroblasts cultivated in the presence of all the blends, that indicates the fact that they do not produce cytotoxic effects upon the studied cells. [1, 2, 3] The biocompatibility of these blends must be increased in order to utilize them in contact with living tissues. A solution is to introduce biologic polymers such as: elastin (ELS), hyaluronic acid (HA) and chondroitin sulphate (CS) in binary blends composition, as it was demonstrated by a few results mentioned in literature as follows:

PVA/collagen and PVA/hyaluronic acid (HA) blends were prepared under sponge form and hydrogels and were doped with growing hormone (GH), and the releasing of hormone was studied "*in vitro*". Releasing of GH from sponge samples was proportional with natural polymer concentration. GH releasing from PVA/HA hydrogels was linear in the first three days and than the releasing rate was very slow. In both cases there were obtained good effects for cellular proliferation. [4]

From blends that contain biodegradable polymers, for example, poly(lactic-co-glycolic) acid, (PLGA), gelatin and α-elastin in 3:1:2 and 2:2:2 (v/v/v) ratio, fibres for tissular scaffolds have been obtained. The fibres were homogenous and thinner than those obtained from natural materials; after the hydratation of PLGA-gelatin-elastin (PGE) their diameter increases for ~ 3 times without disintegration. PGE scaffolds are stable in aqueous medium without being crosslinked and are more elastic than those obtained from pure elastin. [5]

Collagen/elastin (ELS) solutions are less sensitive at UV irradiation towards the elastin hydrolysates; the elastin hydrolysates absorbance in solution increase through the UV irradiation and they have higher values than the absorbance for elastin/collagen blends; so, these blends may find applications in cosmetics. [6]

An HA based blend, biological component and an association of PVA-borax (PVAs), synthetic component, presents rheological properties dependent on dissolving temperature of PVA during sample preparation. This property is successfully used to obtain polymeric bioartificial systems with good mechanical characteristics, and more important, they are biocompatible. [7, 8]

The purpose of this study is to establish by physico-chemical methods the compatibility of complex blends PVA/HC/ELS/HA/chondrotin sulphate (CS) sustained by biological testing regarding their influence upon cellular parameters as proliferation and cellular morphology; and also, the influence of glycosaminoglycans on biocompatibility.

2. Experimental

2. 1. Materials

1) **The polyvinyl alcohol** (PVA) is a hydrosoluble polymer, biocompatible, biodegradable, thermal stable, resistant to organic solvents and oils, does not presents electrical charge, is non-toxic and non- carcinogenic, it shows a good adhesion to organic and inorganic materials, it forms films with high mechanical resistance. PVA is used to product capsules for drugs, fibres for surgical interventions, and also, to get a retard effect the drugs are bonded chemically by PVA or entrapped in the polymer matrix. In aqueous solutions the polymer becomes biodegradable. The solubility and degradability of PVA increase by the decreasing of the number of acetate groups in the molecule. The addition of additives that easily decompose, for example, starch, cellulose or collagen, determines the obtaining of materials with a higher degradation rate. [9, 10]

PVA with a number-average molecular weight of 71000 Dalton, a polymeric degree of 1600 and a degree of saponification of 95.8 mg KOH/g obtained from SA Romacril Râşnov- România, was used in this study.

2) The collagen and its hydrolysates (HC) are used in surgical applications as coverage of orthopaedic implants; as sutures, for treating the burns as transdermal patches, as cellular substratum; controlled release systems; and also in cosmetics due to its increased biocompatibility, in comparison with other biopolymers. [11]

The hydrolyzed collagen (HC) was supplied by the National Institute of Research and Development for Biological Science – Bucharest, Romania. It has been obtained by acidic hydrolysis of bovine derma. The powdered sample was obtained by a spray-drying process with a BÜCHI 190 MINI SPRAY DRYER. It was a type I and III collagen and it consists of a mass of peptides with the following elemental composition: 42.7 wt% C, 10.8 wt% H, 12.2 wt% N and 34.3 wt% O and has a number average molecular weight determined by GPC on solution in dimethyl formamide of 99 000 Da and a polydispersity of 1.66. It is thermally resistant up to 180 ºC.

3) The elastin (ELS) Elastin consist of amino-acids as: glycine, valine, alanine and proline. Elastin is formed by soluble tropoelastin molecules bonds, by reactions catalysed by lisine oxidase, which forms massive insoluble crosslinked formations. The amino-acid involved in this reaction is the lisine. The elastin is rich in apolar amino-acids having reduced possibilities to form intermolecular bindings. The structure is highly amorphous. Due to its remarkable characteristics: elasticity, long term stability, biological activity, the elastin presents multiple applications as biomaterial in tissular engineering. The elastin can be used in different forms as: fibres of insoluble ELS, hydrolysed, soluble ELS, tropoelastin, block copolymers of elastin and combinations with other polymers. [12]

k-Elastin used in the study was a mixture of peptides with different molecular weights as 16 000, 80 000 and 120 000 Da. It was obtained by alkaline hydrolysis at room temperature of natural elastin.

4) The hyaluronic acid (HA) helps in the acceleration healing because acts as an antioxidant that removes the radicals formed in the wound limiting the inflammation. Incorporation of HA in tissular scaffolds determine a good biocompatibility evidenced by cellular growth, and permit water and nutrients passing through the scaffold, for example, in the case of poly(lactide-co-glicolide)/HA blend. [13] Being used as a biomaterial, almost all its applications use its high viscosity and its capacity of gel forming, so, it is successfully used in ophthalmology, in cataract and retina reattachment surgery, for the protection of corneal endothelium. [14]

HA used in the study has the following composition: uronic acids 31.5 wt%; total nitrogen 3.7 wt% and hexozamine 28.7 wt%.

5) Chondroitin sulphate (CS) in a sulphated glycosaminoglycan that consists in an alternant monosaccharide chain (N-acetyl-D-galactosamin and D-glucuronic acid). Usually it is attached to a protein, as a part of a proteoglycan. A CS chain may have over a hundred individual saccharides, each one may be sulphated in different positions and quantities. Each monosaccharide may by unsulphated, simple sulphated or double sulphated. Sulphatation that appears in different positions confers specific biological activities to the chondroitin sulphate chain, the most common situation is the sulphated of 4 or 6 position in N-acetyl-D-galactosamine. The most used forms are bovine and porcine cartilages extracts (bovine trachea, pigs ears and noses), but may be used also sharks extracts, other fishes, or birds cartilages. [15]

Condroitin sulphate used in this study was obtained from bovine tracheas by digestion with papaine in 0.05 M phosphate buffer at pH = 6.5 in presence of EDTA-Na and cisteine hydrochloride for 48h at 60 ^{o}C and extraction with a 0.5 M NaOH solution at 4 ^{o}C. After purification by ethanol precipitation and drying, the product was physico-chemical characterized, and the following composition was found: uronic acids 28,7 wt%; total nitrogen 4,1 wt%; hexozamine 21,2 wt%, sulphur as sulphate 5,7 wt% and a average number molecular mass of 38000 Da.

2. 2. Blends preparation

Two solutions have been obtained of polyvinyl alcohol and hydrolyzed collagen of a 10 wt% concentration. PVA completely dissolution, the solution was heated in thermostated bath at 60-70 ºC for 5 hours, under continuous stirring. By cooling, a white viscous solution was obtained. This was centrifuged and the resulted solution was completed at a constant volume with twice distilled water. The concentration was evaluated by evaporation of a small volume of solution and dried substance was weighted up to a constant mass.

The HC resulted solution is brown-yellowish and weakly viscous.

By mixing of the resulted solutions in various proportions the blends in the mixing ratios presented, in Table 1 were obtained.

Table 1. Blends composition and their notation

Blends composition	Notation
90 wt% PVA/10 wt% HC	1
84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS	2
84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA	3
84.6 wt% PVA/7.4 wt% HC/ 7.4 wt% ELS/0.7 wt% CS	4

The blends were stirred for 15 minutes and left to room temperature for 48 hours. After this period of time, the blends were homogeneous, they present neither separation nor color changes. After 24 h mixing, the blends were again stirred and films have been made from these solutions by solvent casting method. The solution has been laid on polymethyl methacrylate (PMMA) plates of 10 x 15 cm, dried at 60-70 ºC for one hour, and then kept under vacuum at room temperature and films were obtained. Films are easily removed from plates; they are transparent and homogenous, this being the first proof of the components compatibility.

2. 3. Investigation methods

2.3.1. SEM

The study of the polymeric blends by scanning electronic microscopy (SEM) has been done by means a TESCAN VEGA II microscope, at an acceleration tension of 30 kV. The film sample was activated at room temperature with colloidal carbon, after on the thin carbon layer and three silver layers have been deposited following different directions by means of a device of cathodic sputtering. The magnification is indicated on each picture.

2.3.1. DSC analysis

The differential scanning calorimetry (DSC) analysis were achieved by means of a Perkin Elmer 12E Thermal analysis instrument. Before starting measuring, the calorimeter was calibrated with indium, which has T_m =156.6 ºC and melting heat ΔH_m = 28.43 J/g. Samples were thermal scanned in the temperature interval: room temperature 220 ºC, with a heating rate of 10 ºC/min. The samples having masses comprised between 6 and 8 mg were put into aluminium crucibles. A nitrogen flow of 30 mL/min was passed through the calorimeter to assure the inert atmosphere.

2.3.2. TG / DTG curves were registered by means a Paulik-Paulik-Erdey derivatograph type, MOM-Budapest, in following conditions: 50 mg film, heating rate 12°C/min, temperature interval: 20 - 600 °C, air flow of 30 mL/min.

Two curves were recorded for each sample. Actual (β) values were obtained from the temperature-time curve and the calculated (β) values were further employed in the evaluation of the kinetic parameters. Three or four repeated readings (temperature and weight loss) were performed on the same TG curve, each of them having at least 15 points. The kinetic parameters have been evaluated by integral Coats-Redfern (CR) [16] and Reich-Levi (RL) [17] or differential Swaminthan- Modhavan (SM) [18] methods.

In the evaluation the general form of the conversion function was used as:

$$\beta\, d\alpha /dT = Ae - E/RT\, [\alpha^m (1- \alpha)^n [-\ln(1- \alpha)]^p]$$

where: $\alpha = w_t/w_\infty$ is the convertion degree (ratio of the weight loss at time „t" and at the end of process); *T* is the temperature in K, *A* is the pre-exponential coefficient, *E* is the activation energy and *n* – reaction order, *R* is the gas constant. The exponents *m, n, p* may take different values with respect to the reaction mechanism or physical processes occuring during decomposition such as diffusion and transport phenomena.

$f(\alpha) = [\alpha^m (1- \alpha)^n [-\ln(1- \alpha)]^p]$ is the differential form of the conversion function, *n* is the reaction order while *m* and *p* are other exponents of the differential conversion function. From the mathematical point of view both positive and negative values of *A, E* or of the exponents can describe with enough accuracy the TG or DTG curves, but not every value has a kinetic significance. Positive values of the kinetic parameters, *A* and *E*, must be used as a selection criterion for „the most probable kinetic parameters". As additional criteria used in our studies it could mention: the good reproductibility of kinetic parameters obtained from different readings of TG data, maximum values of correlation coefficient or minimum values of average square errors for each experiment point of the DTG or TG curves with respect to the calculated ones using the obtained kinetic parameters, etc. The global kinetic parameter values are used for comparative purposes, being evaluated in the same conditions for all studied samples.

2.3.3. FT-IR spectra of the blend films were recorded at 4 cm^{-1} resolution by means of a DIGILAB Scimitar Series FT-IR spectrometer (USA). Processing of the spectra was done by means of Grams/32 program (Galactic Industry Corp.).

2.3.4. Biologic study

Polymers biocompatibility was evaluated by extract method in accordance with European Standard ISO 10993-5, by qualitative methods (cytochemical cells colouring with Giemsa) and quantitative methods (MTT assay).

Cellular culture

Tests were done on cellular line MRC5 (from human foetal lung) obtained from The European Collection of Cell Cultures (ECACC) and maintained in MEM supplemented with 10% foetal bovine serum (FBS), 1% PSN (penicillin, streptomycin and neomycin) and incubated at 37 ºC in a humidified atmosphere of 5% CO_2. The morphology and growth of cells were monitored with a phase contrast microscope.

Extracts preparation

Samples were inserted into 35 mm diameter dishes in MEM with 10 % foetal bovine serum (SFB) and were incubated at 37 ± 1 ºC for 24h ± 2h, the extraction ratio

from sample surface and fluid volume being 6 cm^2/mL, in accordance to ISO standard.

MTT assay

Cell viability was measured by MTT test. Spectrophotometric method is based on the conversion of 3-(4,5-dimethiltiazol-2-il)-2,5-difeniltetrazolium bromide (MTT) to insoluble blue crystals of formazan by mitochondrial succinate-dehydrogenase in viable cells.

For the experiment, cells were seeded onto 24-well plates at a density of $2,5x10^4$ cells/mL, and after, there were incubated for 24 h at 37ºC in humid atmosphere with 5% CO_2 to allow the cells to adhere. After the incubation period the culture medium was discarded and replaced with extract medium. At 24 h and 48 h, of cultivation in the presence of the extracts, medium was discarded and replaced with extract medium, 50 µl of MTT solution (0.25 mg/mL) dissolved in the culture medium were added in each well and then, cells were incubated at 37ºC for 3h at dark. Later, water-insoluble dark blue formazan crystals formed in viable cells were solubilised with isopropanol and the absorbance of the dissolved colouring was measured at 570 nm using Tecan reading plates.

Concentration of converted dye directly correlates to the number of metabolically active cells in the culture. Cell viability was calculated by comparison with control sample (fibroblasts cultivated in absence of polymers), considered to be 100% viable. The extracts were triplicate analysed.

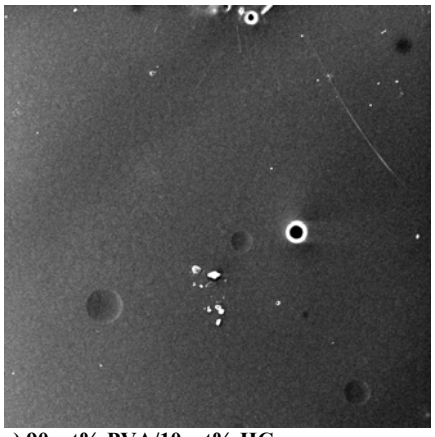
Cellular morphology

To analyze cell morphology, dermal fibroblasts cultivated in presence of polymeric blends, for 48 h, were washed with PBS, fixed with cold methanol (-20 ºC), stained with Giemsa solution and photographed using an inversed-phase microscope Zeiss Observer D1. In all experiments the control sample was represented by dermal fibroblasts cultivated in the absence of polymers.

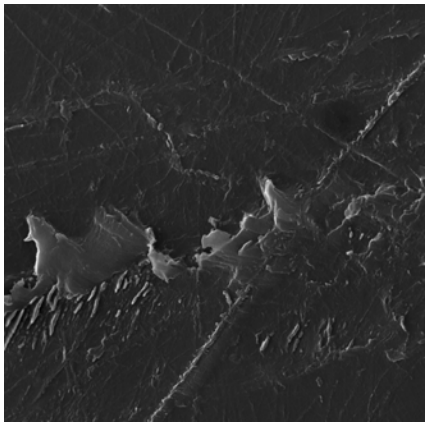
3. Results and discussion

SEM results

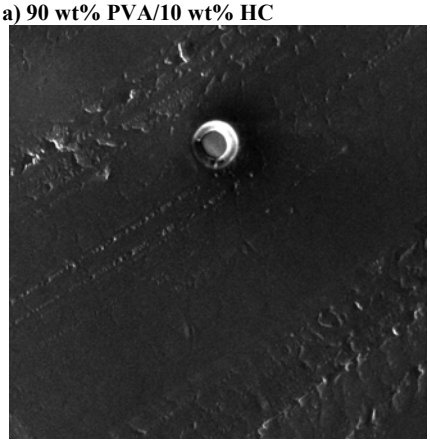
Microscopic aspects of the films from complex blends and those of the binary blend are given in Fig. 1a, 1b, 1c and 1d. In the binary blends, HC domains appear separate under corpuscle forms that are susceptible to the attack by surrounding medium (even for the electron beam used in method SEM) (Figure 1a). In the presence of biological polymers the films aspect is much homogenous, HC particles being no longer visible. Elastin presence determines the appearance of some fibrous formations that are more pronounced in the 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS and 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blends (Fig. 1b and 1d).



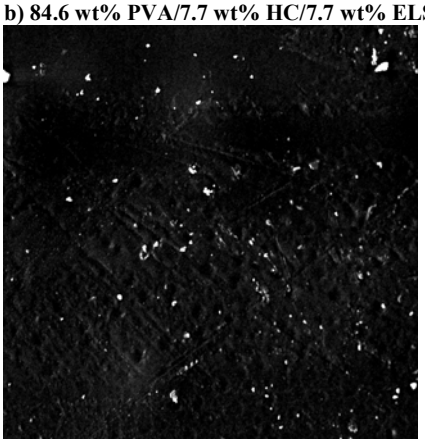
a) 90 wt% PVA/10 wt% HC



b) 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS



c) 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA



d) 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS

Fig. 1. Microscopic aspects of PVA/HC/Biological polymers films

DSC curves are usefully to evidence the first and the second order transitions in the blends. They present the same shape, but the peaks form is characteristic for every blend. The DSC curves presented in Figure 2, evidence the recorded transitions temperatures and the endothermic melting. The DSC results are summarized in Table 2.

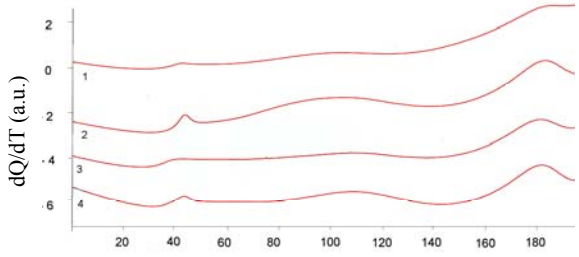


Figure 2. The DSC curves of the binary and complex blends recorded during heating

Table 2. The DSC results for the PVA/HC and PVA/HC/biological polymers blends.

Sample	T_g (ºC)	ΔC_p (J/g*ºC)	T_m (ºC)	ΔH_m (J/g)
1	36.62	0.392	179.77	7.92
2	37.96	0.544	181.27	18.95
3	34.60	0.663	179.33	17.79
4	36.23	0.594	180.08	19.62

T_g – glass transition temperature, ΔC_p – difference in heat capacity, T_m , ΔH_m – melting temperature and melting heat

Second order transition temperature (T_g) presents a maximum value of 37.96 ºC for the ternary blend, and the blend that contain hyaluronic acid shows the smaller value of 34.60 ºC.

Transition temperature modification may be attributed to some interactions between components of the blend with elastin and also maybe to the plasticizer role of HA. The elastin/chondroitin sulphate combination does not modify the transition temperature. (Table 2)

Specific heat capacity variation presents the higher values in case of ternary and quaternary blends, the modifications being more obvious in this transition region. In case of the 3 blend a maximum value of specific heat variation, $\Delta Cp = 0.663$ J/g*°C was obtained, the transition between two conformations being more pronounced. This fact means that the biological polymers increase the chain mobility. (Table 3)

The melting endhoterm for the binary blend increase in intensity whith the number of components increases. Melting temperature remains practically unchanged but the melting enthalpy increases, the system being more ordered. The melting enthalpy presents a maximum value in the case of the 4 blend, of 19.62 J/g. (Table 2)

IR spectral analysis

IR spectra are presented in figures 3 and the results are summarized in Table 3.

The band that appears in the binary blend spectra at 3308 cm^{-1}, assigned to the –OH group, is moved to higher values in glycosaminoglycans containing blends spectra which means a stronger interactions between components of these blends.

The bands at 2943 cm^{-1} and 2868 cm^{-1} in the blends spectra assigned to C-H bond are slightly shifted to smaller values in case of the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA blend.

The band at 2747 cm^{-1}, is very weak, and appears only in 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend spectra.

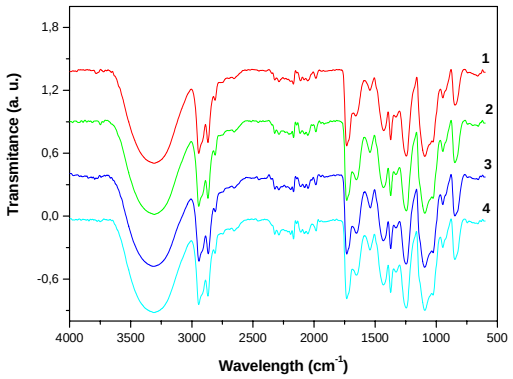
Bands in the 2800 – 2000 cm^{-1} spectral region have different shapes, specific for each blend, that should be attributed to different chemical groups of components. In case of 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS blend appear a maximum at 2205 cm^{-1}, of weak intensity, that does not appear for any other blends spectra.

Band at the 1659 cm^{-1} in the binary blend spectrum appears also in other spectra, and it could be assigned to end groups interactions, and it is shifted towards smaller wavelengths in the case of multicomponent blends and shows the higher intensity for the proteoglycans containing blends.

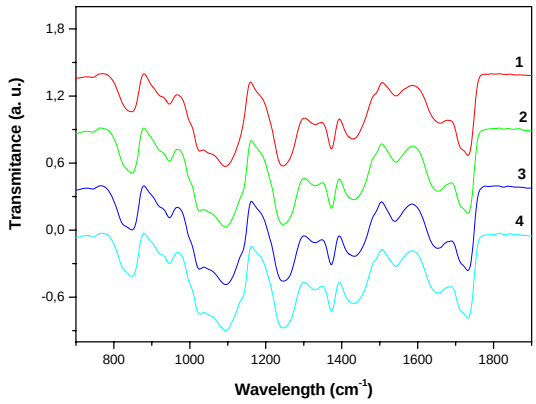
The band at 1543 cm^{-1} wavelength corresponds to HC, and it has higher intensity and it is sharper for the multicomponent blends. It presents maximum intensity and is shifted to a smaller wavelengths in case of 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA blend.

Table 3. IR peaks position for the PVA/HC and PVA/HC/biological polymers samples

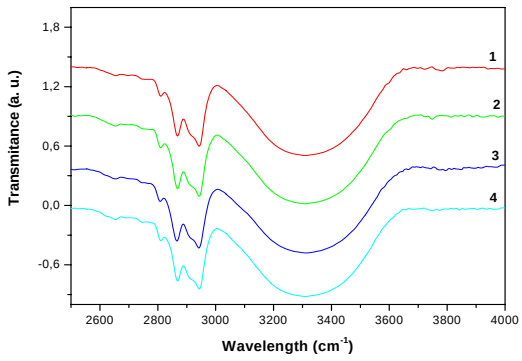
Sample	**Peak position (cm^{-1})**										
1	3308	2868	-	-	2321	-	1984	1659	1543	1430	-
2	3307	2868	2810	-	2322	2205	1984	1653	1542	1432	1330
3	3311	2866	-	-	2320	-	1985	1652	1539	1433	1329
4	3310	2868	2810	2747	2321	-	1983	1653	1543	1432	1328



a



b



c

Fig. 3. IR spectra of the PVA/HC and PVA/HC/biological polymers blends a) 500-4000 cm^{-1} spectral region; b) 700-1900 cm^{-1} spectral region; c) 2500-4000 cm^{-1} spectral region.

The band at 1430 cm^{-1} in the binary blend spectrum is shifted towards higher wavelengths for all the multicomponent blends spectra.

The 1330 cm^{-1} band is present in all complex blends spectra but it does not appear in the binary blend spectrum.

Thermogravimetry

The curves TG/DTG – Figure 4 shape of the complex blends is alike to that of the binary blend. In the 0 – 500 °C interval temperature, they present three thermogravimetric stages. DTG peaks corresponding to the water desorption is situated around 100 °C, and has higher peak temperature values (T > 165 °C) for the HA and CS containing blends, and those corresponding to the decomposition are shifted towards lower temperatures.

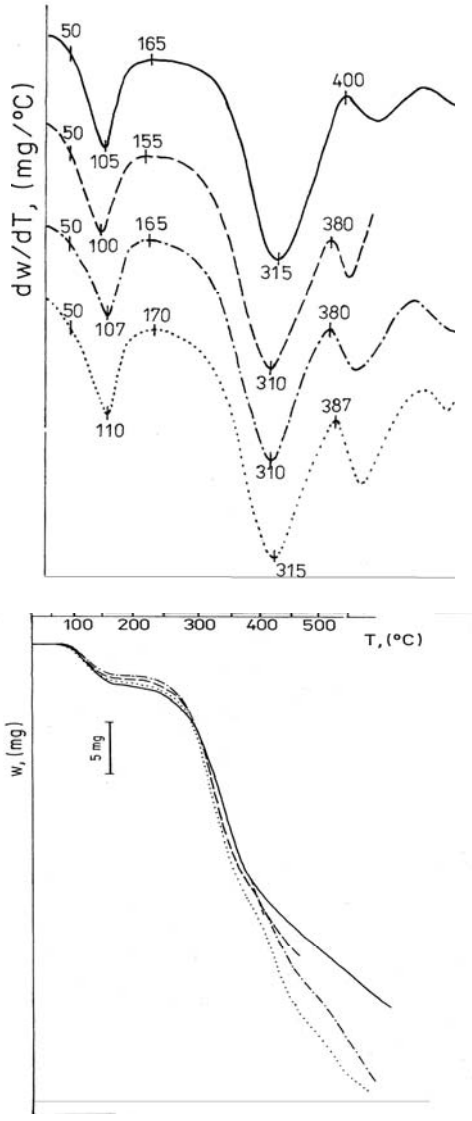


Fig. 4. TG/DTG curves for the blends (— 1; – – – 2; –·–·– 3; ····· 4)

In the first thermogravimetric stage, the mass losses are comparable, being of 5-8 wt%, the smaller mass loss of 5.4 wt%, being recorded in the case of the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA blend.

In the second thermogravimetric stage, the mass losses are different; the higher value of 45.8 wt% is recorded for 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7

wt% CS blend; and the smaller one of 42.8 wt% is in the case of 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS. For the 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS blend the onset decomposition temperature, T_i is minimum that indicates a lower thermal stability. (Table 4) For the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend, the onset decomposition temperature, T_i and the temperature that corresponds to the maximum rate of mass loss, T_m, are maximum, of 170 °C, respectivelly 315 °C, this blend being the most stable blend and that release heavier degradation products. (Table 4)

The mass losses present the higher values in the second stage. The total mass losses have the lowest value for the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA blend due to the good compatibility and of the strong interactions between the components for this composition. (Table 4)

The 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend presents the higher value of the onset decomposition temperature, of T_i = 170 °C, and also, of temperature that corresponds to the maximum mass loss rate, of T_m = 315 °C in the second stage. (Table 4) In the first stage of water loss, the mass losses decreases at the increasing of the number of components, for the blend, being recorded the lower value.

In the second thermogravimetric stage the mass losses have an increasing trend with increasing the number of component. The higher mass loss is recorded for the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend in both water loss and also, in the decomposition stage too. (Fig. 5)

Table 4. Characteristic temperatures and mass losses for thermogravimetric stages of the PVA/HC and PVA/HC/biological polymers blends.

Sample	Stage I				Stage II				
	T_i (°C)	T_m (°C)	T_f (°C)	ΔW_I (wt%)	T_i (°C)	T_m (°C)	T_f (°C)	ΔW_{II} (wt%)	ΔW_r (wt%)
1	50	105	165	7.6	165	315	400	42.4	50.0
2	50	100	155	6.4	155	310	380	42.8	49.2
3	50	107	165	5.4	165	310	380	43.2	48.6
4	50	110	170	7.0	170	315	387	45.8	52.8

T_i, T_m, T_f – onset, maximum rate of mass loss and final temperature; ΔW_I, ΔW_{II} – mass losses; ΔW_r – residual mass

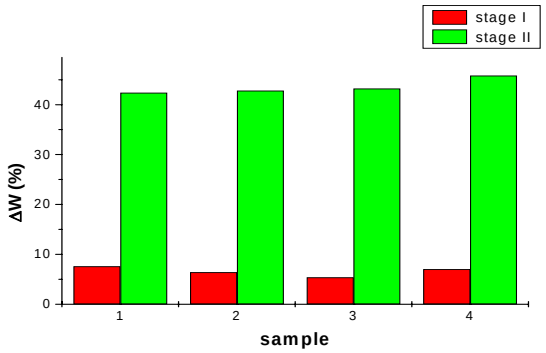


Fig. 5. Mass losses in the two thermogravimetric stages

Table 5. Kinetic parameters in the first thermogravimetric stage

Sample	E_{CR} (KJ/mol)	n_{CR}	E_{SM} (KJ/mol)	n_{SM}	$\ln A_{SM}$	$E_{RL,\alpha=0}$ (KJ/mol)
1	73.3	1.9	78.6	2.05	24.1	95.5
2	90.2	2.1	118.1	2.71	38.38	103.7
3	99.05	1.9	97.78	1.96	31.77	106.8
4	106.7	2.6	125.59	3.04	40.26	124.5

Table 6. Kinetic parameters in the second thermogravimetric stage

Sample	E_{CR} (KJ/mol)	n_{CR}	E_{SM} (KJ/mol)	n_{SM}	$\ln A_{SM}$	$E_{RL,\alpha=0}$ (KJ/mol)
1	46.5	0.0	50.3	1.8	15.3	65
2	63.51	0.5	122.8	0.0	21.7	91
3	82.6	1.2	78.4	1.08	14.54	102
4	95.1	1.4	86.4	1.3	16.09	119

Kinetic parameters overall activation energy and reaction order characteristic for the first thermogravimetric stage increase for the complex blends, the higher values being obtained for the proteoglycans containing blends, that means the water is stronger linked by these components. The same behaviour is obtained in the second stage. (Tables 5, 6)

Similar variation presents also the activation energies values obtained through Reich-Levi method, the curves corresponding to the complex blends being situated far over those corresponding to the binary blend. (Figure 6)

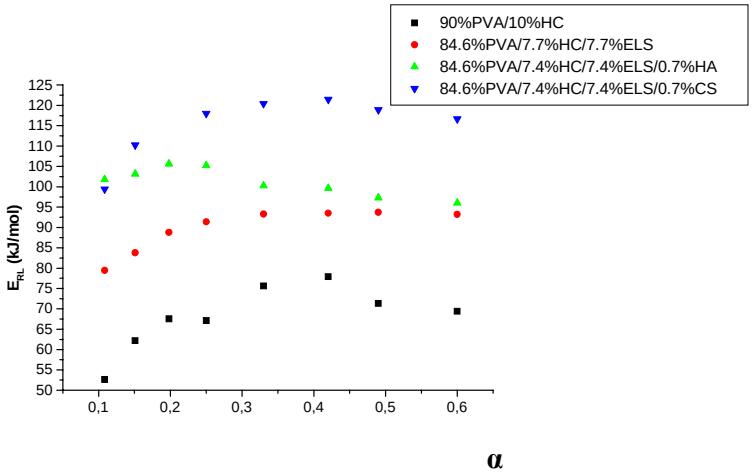


Fig. 6. Activation energy, E_{RL} evaluated by Reich-Levi methods versus conversion degree for the second stage of \ mass losses.

Biological study results

a. Polymeric blends biocompatibility

The effect of the polymeric blends based on PVA and HC has been evaluated by MTT test. The results for the studied samples are comparable with those for the control sample from the point of view of fibroblasts adhesion and of cellular viability. Cellular viability was similar to that of the control sample in case of the blends that contain proteoglycans, HA and CS, after 24 h and also after 48 h; and for the other two blends, the proliferation was higher than 83 %. In case of the binary blend it was obtained a minimum value for the cellular viability of 88.7 % after 24 h, and 83.13 % after 48 h of incubation. The results obtained by MTT test evidenced that all the polymeric samples were not cytotoxic (the cellular viability > 83.13%), the cellular viability biggest value, of 98.3 %, being obtained for 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend. (Fig. 7).

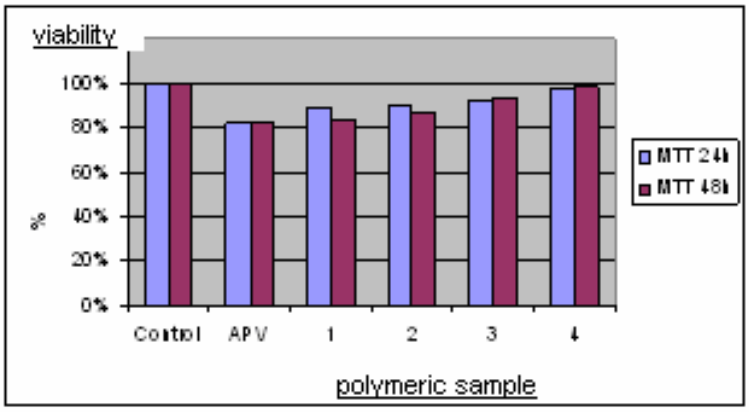
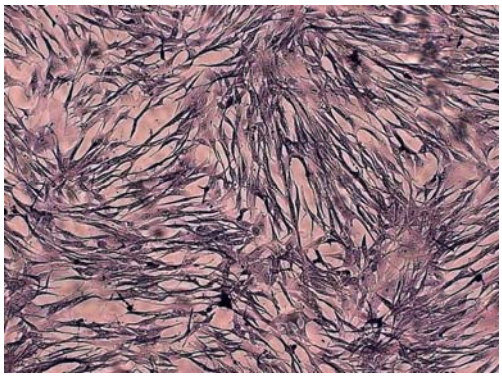


Fig. 7. Cellular proliferation degree at 24 h and 48 h evaluated by MTT test, 1 – control sample; 2 – PVA; 3 – 90 wt%PVA/10 wt%HC; 4 - 84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS; 5 - 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA; 6 - 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS.

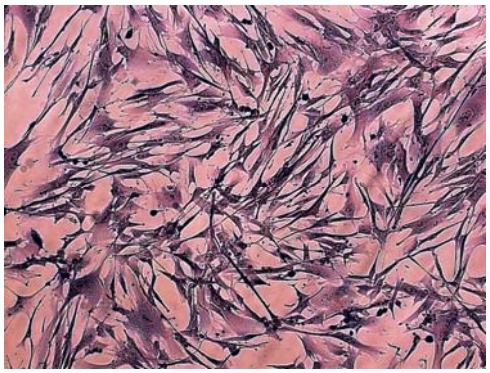
b. Cellular morphology

Polymeric biocompatibility analysis has been carried out also by cellular morphology analysis, after 48 hours of cell cultivation in the presence of the extract medium, after Giemsa colouring. The control human dermal fibroblasts cultivated presented after 48 h a normal phenotype, the cells presented an elongated morphology with a fine granular cytoplasm, euchromatic nuclei, 1- 4 nucleoli. (Fig. 8a)

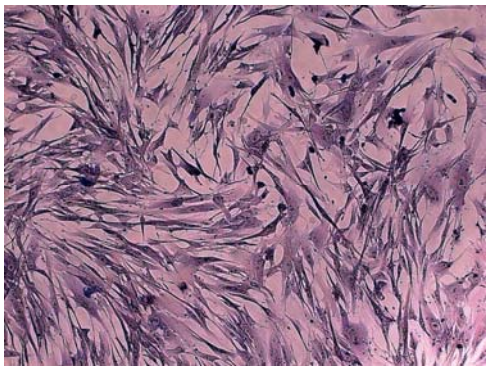
Morphological analysis of human dermal fibroblasts behaviour in the presence of the studied blends indicated in all cases a good biocompatibility evidenced by a high proliferative capacity, adherence to films and retaining the cellular normal phenotype. Cells maintained their elongated form, with thin cytoplasmatic extensions, with round nucleus, and 1-4 nucleoli. (Fig. 8 b-f)



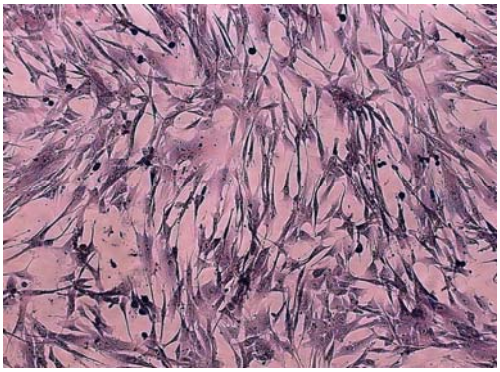
Control (cultivated cells in extracts absence) 100 X
a



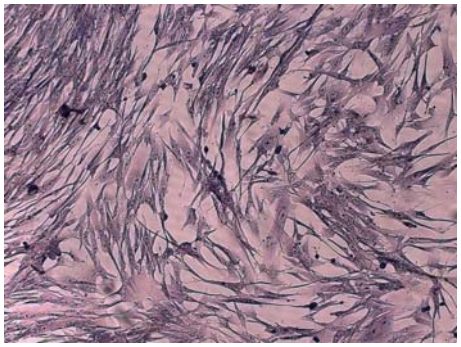
PVA, 100 X
b



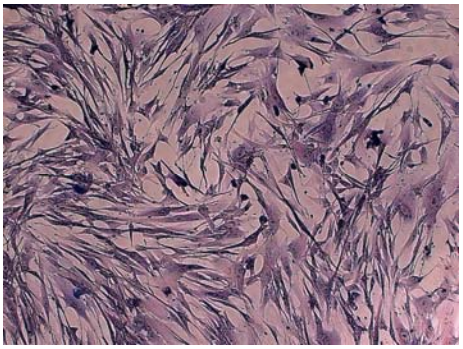
90 wt% PVA/10 wt% HC; 100 X
c



84.6 wt% PVA/7.7 wt% HC/7.7 wt% ELS; 100 X
d



84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/
0.7 wt% HA; 100 X
e



84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/
0.7 wt% CS; 100 X
f

Fig. 8. Microscopic images of the cultures after 48 hours

Light microscopy images showed that all four tested polymeric samples caused no morphological or cytotoxical modifications of human dermal fibroblasts; after 24 h of incubation, the control and cells cultivated in presence of polymeric materials have retained their specific characteristics; the higher biocompatibility was obtained in case of the samples that contain glycosaminoglycans, hyaluronic acid and chondroitin sulphate.

4. Conclusions

Both DSC and IR spectroscopy evidence specific interactions between functional groups of the components

of the PVA/HC/biological polymers blends. The second order transition temperature is minimum in the case of hyaluronic acid containing blend. The melting endhoterm for the binary blend increases in intensity by increasing of the components number in the blends. The melting temperature remains practically unchanged but the melting enthalpy increases, the system being better ordered. The 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% CS blend presents maximum values for the onset temperature, T_i, and also, for the temperature that corresponds to the maximum mass loss rate, T_m, in the second thermogravimetric stage, this being the more stable blend, that release harder degradation products.

The mass losses present the lowest values for the 84.6 wt% PVA/7.4 wt% HC/7.4 wt% ELS/0.7 wt% HA blend due to the good compatibility and of strong interactions between the components for this composition.

Human dermal fibroblasts proliferation analysis of cells in the presence of PVA and HC based blends at different cultivation times evidence a cellular viability higher than 82 % for all the samples with at least 10 % higher than the samples without biological polymers. Normal cellular phenotype and proliferative capacity are retained of the majority of the human fibroblasts that indicates the fact that these do not cause cytotoxic effects on the studied cells. Among the studied blends the higher biocompatibility degree was recorded in the presence of the glycosaminoglycans containing blends. These blends may find applications in medicine and pharmacy as controlled release matrix of drugs, bioactive substances, in cosmetics, etc.

References

[1] F. Crivoi, C. Vasile, BiomMed, 9-11 nov. Iaşi, 2006, J. Optoeletron. Adv. Mater., **9**(11), 3330 (2007).
[2] F. Crivoi, L. Stefan, L. Moldovan, C. Vasile, Roum. Biotechnol.Lett., **12**, 6 (2007).
[3] F. Crivoi, C. Vasile, Conferința Națională de Fototerapie, 14-16 mai, Iaşi, (2008).
[4] M. G. Cascone, L. Di Silvio, B. Sim S. Downes, J. Mater. Sci.: Materials in Medicine, **5**(9-10), 770 (1994).
[5] M. Li , M. J. Mondrinos, X. Chen, M. R. Gandhi, F. K. Ko, P. I. Lelkes; J. Biomed. Mater. Res. **79**(4)**,** 963 (2006).
[6] A. Sionkowska; J. Skopinska, M. Wisniewski; A. Leznick; J. Photochem. Photobiol. B, Biol., **86**(2), 186 (2007).
[7] S. H. Kim, K Hyun, T S Moon, T Mitsumata, J S Hong, K H Ahn, S J Lee, Polymer **46,** 7156 (2005).
[8] C. Weiss, In: Abatangelo G, Weigel PH, editors. New frontiers in medical sciences: redefining hyaluronan. Amsterdam: Elsevier (2000).
[9] L. R. Krupp, W.J. Jewel; Environ. Sci. Technol., **26**, 193 (1992).
[10] A. C. Palmisano, Ch.A. Pettigrew, BioScience; **42**, 680 (1992).
[11] I. Bujor Ionescu, V. Trandafir, I. Demetrescu, H. Iovu, G. Zgirian, European Cells and Materials, **5**(1), 10 (2003).
[12] W.F. Daamen, J.H. Veerkamp, J.C.M. van Hest, T. H. van Kuppevelt, Biomaterials, **28**, 4378 (2007).
[13] J. E. Scott, "The chemical morphology of the vitreous". Eye; **6,** 553 (1992).
[14] Chih-Ta Lee, Yu-Der Lee, J Mater. Sci. Mater. Med. **17**(12): 1411 (2006).
[15] J. G. Barnhill, C. L. Fye, D. W. Williams, D. J. Reda, C. L. Harris, D. O. Clegg J. Am. Pharm. Assoc. Washinghton DC, **46**(1), 14 (2006).
[16] A. W Coats, J. T. Redfern, Kinetic parameters from thermogravimetric data. Nature, London, **201**, 68 (1964).
[17] L. Reich, D.W. Levi, Makromol. Chem., **66**(102), 22 (1963).
[18] V. Swaminathan, N. S. Modhavan, J. Anal. Appl.Pyrolysis, **3**, 131 (1981).

*Corresponding author: florin_crivoi@yahoo.com