Biocompatible Blends Based on Polyvinyl Chloride and Natural Polymers for Medical Device Fabrication

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New polymeric blends were prepared by mixing some receipts of polyvinyl chloride (PVC) for medical use with natural polymers (hydrolyzed collagen and a hydrolyzed collagen/elastin mixture), without the use of intercoupling agents. The mixtures were processed through rolling-pressing and extrusion. The new materials have physical and mechanical properties that allow their processing by using classical methods, specific for synthetic polymers. In vitro biocompatibility studies were performed on a primary culture of human dermal fibroblasts by the MTT test. The biocompatibility of the materials was tested in vitro by determining cell viability in a culture of human dermal fibroblasts by using the MTT method. The physical and mechanical properties and also the biocompatibility properties of the new materials recommend them to be used in the manufacture of medical devices.

Key words: synthetic polymers, natural polymers, blends, polyvinyl chloride, collagen, elastin, biocompatibility, cell viability.

The polymer blends or the polymer multi-component systems present a particular interest and a challenge for the scientific research. As new mixtures were developed, new materials were obtained with high performance properties in terms of physical and mechanical characteristics, of machinability, of biodegradability and biocompatibility [1].

In the recent decades, there is an increasing focusing on the replacement of artificial materials used in human or veterinary medicine with the so-called bioartificial materials. These materials contain at least one natural component (natural polymer), in order to enhance the biocompatibility degree of the concerned material.

The natural component of the bioartificial materials may be a protein (collagen, fibronectin, elastin), a polysaccharide, as those in the glycosaminoglycan class (heparin, heparin sulfate, hyaluronic acid), a peptidic sequence with a role in cell recognition or in the adhesion process. These components are most often macromolecules of the extracellular matrix from several connective tissues (dermis, tendon, etc). The bioartificial materials, containing a natural component extracted and purified usually from connective tissues, have a greater biocompatibility degree and can be maintained for a longer period of time in contact with the organism [2].

In order to be effectively used in medicine, a polymeric material must meet the following conditions: not to be toxic, to be well tolerated by the body, to provide stability in the biological environment. These are features that collagen and elastin proteins meet, as a series of collagen biomaterials used as implants, as hemostatic agents or as supports for active drug principles are being known [3-5].

Synthetic polymers are characterized by elasticity, mechanical resistance, thermal and biological environments activity stability. The mixtures of synthetic polymers and biological macromolecules are bioartificial polymeric materials, with improved functional properties and convenient characteristics of biodegradability and biocompatibility, at a relatively low price [6].

The polyvinyl chloride (PVC) is used for many medical devices, in some applications showing disadvantages mainly due to its poor biocompatibility, which can be increased by incorporating the natural polymer [7]. A solution to improve the PVC biocompatibility is getting blends of PVC/natural polymer. We obtained mixtures with a very good biocompatibility from PVC receipts by bioactivation with collagen and by using intercoupling agents [8-10].

Collagen is a protein used to obtain composite materials, such as collagen-synthetic polymer mixtures for a variety of medical uses, including dialysis membranes, dressings for wounds and artificial skin [11, 12]. Collagen is known as a biopolymer that allows the cellular adhesion and the enzymatic degradation [13].

Elastin is an essential constituent protein of the extracellular matrix from elastic connective tissues, and has been lately studied for its ability to associate intimately with other natural and synthetic polymers [14, 15]. The *in vitro* tests have shown that the elastin-derived peptides led to the improvement of both cellular adhesion, in the case of endothelial cells, and also to improve the resistance of the new created material. Despite its remarkable properties and its low thrombogenicity [16], elastin was little used in the manufacture of biocompatible composite materials [17].

The aim of this paper was the preparation of new bioartificial materials obtained by mixing some receipts of PVC of medical use with natural polymers (hydrolyzed collagen and a hydrolyzed collagen/elastin mixture), without the use of intercoupling agents, in order to be used for medical devices manufacture. They were characterized in terms of physical, chemical and mechanical properties and of their *in vitro* biocompatibility.

Experimental part

Polymer preparation

PVC for medical use was obtained by polymerization in suspension (OLTCHIM Ramnicu Valcea, Romania) and has the following characteristics: Kw 70; 112% plasticizer absorption; 3 mL of oxido-reducing substances expressed in mL of 0.01 N $KMnO_4$ solution/100 mL aqueous extract; 0.1% water and volatile substances; no ash; 0.05 - 0.25 mesh grain; residual monomer (vinyl chloride) ≤ 5 ppm. A plasticizer receipt was achieved with di-(2-ethyl hexil)

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Table 1
THE COMPOSITION OF BINARY PVC/HC MIXTURES AND TERNARY PVC/ HC/E MIXTURES

Sample	PVC (parts by weight)	HC (parts by weight)	E (parts by weight)
1.	100	10	-
2.	100	20	-
3.	100	30	-
4.	100	10	1

phthalate and dry PVC in the form of dry powder, in a proportion that will lead to a material hardness of 55 -60o Shore. The receipt was thermally stabilized with a mixture of calcium and zinc stearates. We used epoxided soybean oil to improve the machinability (> 1%). Other additives have been incorporated in quantities of less than 1%.

Hydrolyzed collagen (HC) was obtained by acid hydrolysis of bovine dermis, using 3% HCl, a pressure of 1.5-2 atm and a hydrolysis temperature of 120-130 °C. These conditions have allowed us to obtain a mixture of collagen polypeptides, having an average molecular weight of 7000 Da, resistant to a temperature of 180-200°C, used in the bioactivation of the synthetic polymer.

k-Elastin (E) was prepared from insoluble elastin powder (calf ligament, Sigma) by stirring in 1M KOH ethanolic solution, at 30 °C, for 48 h. The mixture of elastin-derived peptides was neutralised with acetic acid and dialysed against double distilled water [18]. The final solution had 1.3 % dry content by weight and 68.7 % protein content.

PVC/HC and PVC/HC/E blends fabrication.

The quantities of HC entering the receipt ranged from 10 to 30 weight parts per 100 weight parts of plasticizer PVC for medical use. The dosage of the components was performed with a balance type MB-H-09/02 with an accuracy of ± 0.5 g. The mixing of the components was executed on a laboratory mixer with a speed of 60 rpm, for 20 min.

The rolling parameters were: the rolling temperature of 135-140°C and the friction coefficient of 1/1,5. The pressing parameters were: the temperature of 150 °C, the preheating time of 10 min; the pressing for 10 min; the working pressure of 300 kgf / cm^2; the cooling time 25 min; the removal temperature 50°C. The pressed plates had dimensions of 300x300x1 mm.

Three blends of PVC/HC and a mixture PVC/HC/E were done, according to table 1.

Blends machinability testing

The blend machinability was determined using the Brabender Plastograph (Duisburg, Germany). The torque diagrams for torsion force-time were obtained by mixing the blends on the 30 cm^3 vat, at various temperatures and rotation speeds. The mixing energy, absorbed in the machining process is given by the area between the torsion curve and the abscissa. The determining of the amount of energy was made by weighing recording paper cut on the contour area above. The coefficient of equivalence energy – recording paper weight is : 1 Kgfm = 0.1515 g.

Physical and mechanical characterization of the blends

The *density* determination was performed according to SR ISO 1183-94, on the punched proof samples from the rolled-pressed plate, with a regular geometric shape (discs with a diameter of 5 mm and thickness of 1 mm). The device used was the analytical balance.

The determination of the *hardness* was carried out according to SR ISO 868-95 on the rolled-pressed plate with the thickness of 4 mm. The device used was a Shore A type hardness tester.

The traction resistance and the elongation at break

The determination was performed according to the SR ISO 527-200, on proof samples type 5A (for flexible materials, plasticized), punched out of the plate with the thickness of 1 mm, rolled-pressed. The working speed has been 200 mm/min. The device used is a dynamometer type Zwick FP 10, Germany.

The determination of water absorption was performed according to STAS 5690-80, in accordance with the ISO 62-93, method A (24 h immersion in the distilled water at the room temperature) on proof samples of regular geometric shape (discs with the diameter of 50 mm and the thickness of 1 mm), obtained by punching from the rolled-pressed plate.

The determination of plasticizer loss was performed according to the method from STAS 6216-80.

Human dermal fibroblast culture

A primary culture of human dermal fibroblasts was obtained from human dermis explants and was used at passage 4. Materials were cut to 10-mm discs and sterilized by exposure to ultraviolet light in an UV sterilization cabinet (Scie-Plas, England) for 4 h. They were fit on the bottom of a 24-well tissue culture polystyrene plate and 50 µL of cells in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10 % fetal bovine serum (Sigma) were added, at a density of 1 x 10^5 cells/mL. The plate was kept in an incubator (37 °C, 5 % CO_2), for 3h. Then, another 450 µL culture medium (DMEM) were added to each well and the plate was incubated for 48 h.

Cell viability was assayed by measuring the mitochondrial dehydrogenase activity using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, which is based on the conversion of soluble tetrazolium salts to formazan by NAD and NADH dehydrogenases from the viable cells [19].

Results and discussion

Physical and mechanical properties of the polymeric blend variants

The main pressing parameters (temperature, pressure, time) were determined for the two operations of processing the PVC/HC and PVCHC/E mixtures, as well as the extrusion parameters on Brabender Plastograph, the thermodynamic stability and the mixing torque values.

The parameters which showed an interest were the mixing energy at 10 min and the thermodynamic stability. The diagrams torque of torsion forces–time, for the binary blends PVC/HC studied are shown in figure 1.

The thermodynamic stability of the mixtures is higher than 15 min, so they are stable during machining. The machinability characteristics of the binary mixtures are presented in table 2.

The values of the parameters involved in the process of mixing remain within the limits in which the mixture is

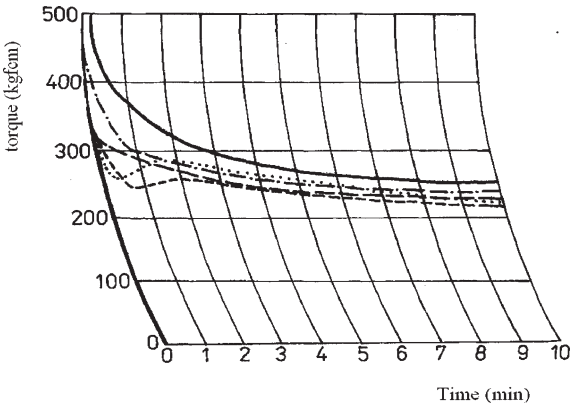

Fig. 1. The curves representing variation of shearing forces torque in time for PVC/HC binary mixtures and PVC/HC/E ternary mixture. (PVC ___; 100PVC/10HC_ _ _; 100PVC/20HC _._._; 100PVC/30HC- - - - - -; 100PVC/10HC/1E.…….)

Table 2
FEATURES OF MACHINABILITY FOR PVC/HC AND PVC/HC/E MIXTURES

No.	Mixture composition (parts in weight)	Mixing force at 4 minutes (Kgf)	Mixing force at 10 minutes (Kgf)	Torsion module (Kgfm)	Mixing energy at 10 minutes (Kgfm)
1.	PVC	1.425	1.225	240	2.33
2.	100PVC/10HC	1.225	1.125	220	1.98
3.	100PVC/20HC	1.300	1.175	240	2.08
4.	100PVC/30HC	1.200	1.050	210	1.90
5.	100PVC/10HC/1E	1.325	1.112	220	2.02

machinable by classical methods. They were similar to the values obtained for synthetic polymer (PVC) machining. The decrease of the mixing forces values with increasing amount of collagen is due to the low molecular weight of HC.

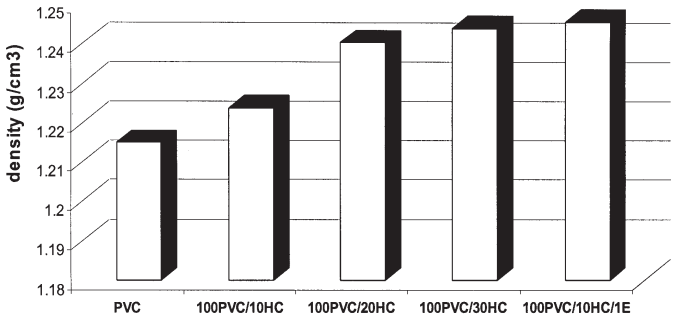

Fig. 2. Density variation for PVC/HC binary mixtures and PVC/HC/E ternary mixture

The properties of the mixtures depend on the content of natural polymers. The density increases slightly at the ternary mixtures compared to the binary mixtures (fig. 2).

The Shore hardness increases and the strength and elongation at break decrease, even with values of more

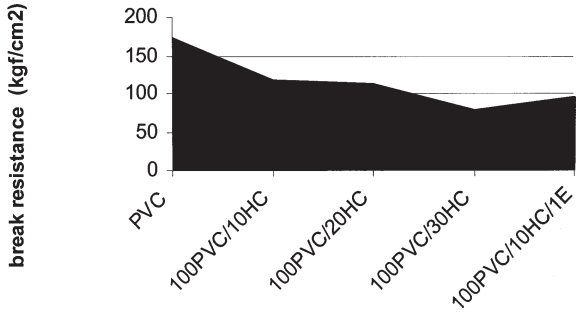

Fig. 3. Break resistance variation with the content of natural polymers

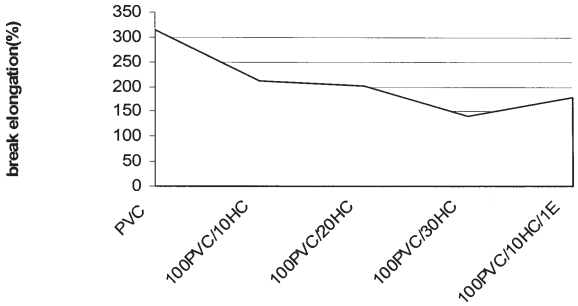

Fig. 4. Break elongation variation with the content of natural polymers

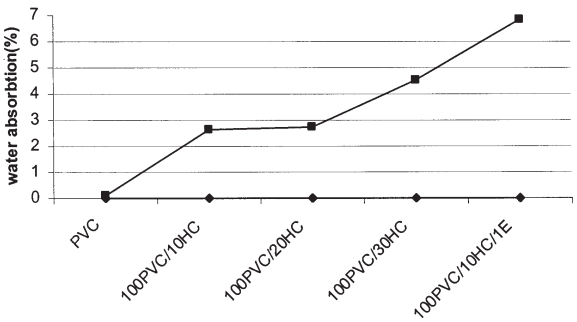

Fig. 5. Water absorbtion variation for PVC/HC binary mixtures and PVC/HC/E ternary mixture

than 30% compared with the corresponding values of the plasticized PVC (fig. 3). The Shore hardness of the ternary mixtures is close to that of the binary mixtures.

Strength at break and elongation have higher values in the ternary mixtures than in the binary ones, a significant increase being observed at the elongation at break (fig. 4). Also, when the collagen content was higher, the hydrophilia of the mixtures increases (fig. 5). The loss of plasticizer from the mixtures, as it migrates, is lower in the mixtures with collagen than in the plasticized PVC and is very little influenced by the presence of elastin in the mixture (fig. 6).

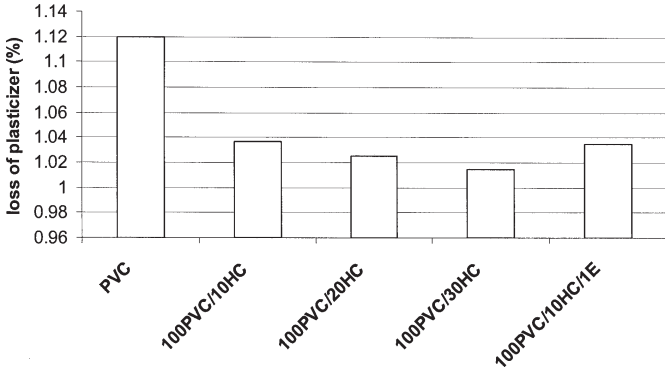

Fig. 6. Loss of plasticizer variation for PVC/HC binary mixtures and PVC/HC/E ternary mixture

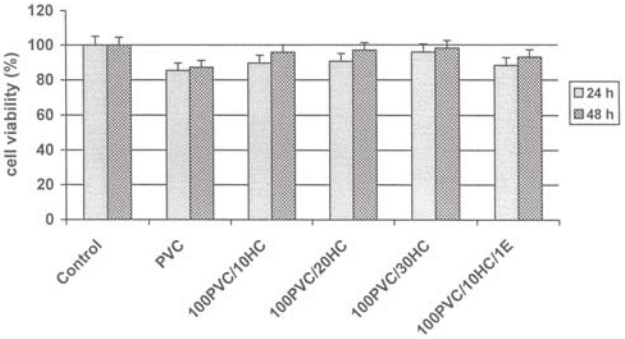

Fig. 7. Human dermal fibroblast viability on PVC-based material variants after 24 h and 48 h of cultivation. The results are based on MTT absorption and reflect the mitochondrial activity in viable cells

Several parameters like molecular weight, composition and structure influence the tensile strength and elongation at break of plastic materials [20].

In vitro biocompatibility of the polymeric blend variants

Human dermal fibroblasts were cultured on the PVC composite materials for 24 h and 48 h. After each period of time, the cell viability was assessed by the mitochondrial activity assay. The results were expressed as percentage from the control cell culture (cells cultivated on the plastic well) (fig. 7). Results represent mean of 3 determinations ± S.D.

The calculated values showed that PVC and PVC-based polymers mixed with natural ones were biocompatible (the percentage of cell viability was over 80 %). No significant differences were found at 24 h of cell cultivation on polymeric variants. All materials supported cell proliferation, with fibroblast growing more extensively on the PVC/HC than on PVC/HC/E and PVC sheets, at 48 h after cell seeding. The highest dermal fibroblast viability value was obtained from PVC/30HC polymeric sheet.

The benefit of blending the PVC with HC and HC/E, respectively, was demonstrated in our study, by higher values of fibroblast viability (assessed as mitochondrial activity) for PVC-biopolymer composites than for PVC alone.

Conclusions

It was demonstrated that blends of PVC with different natural polymers (HC, E) could be prepared and processed as biomaterial sheets in the described conditions. The new materials were characterized through physico-mecanical parameters and cellular growth assessments, properties required for medical applications. Our results propose them as biomaterials for medical device fabrication. Future investigations regarding *in vivo* biocompatibility are necessary.

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