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Original Article

Comparison of the Effect of Hydrophilicity on Biocompatibility and Platelet Adhesion of Two Different Kinds of Biomaterials

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Abstract

Determination of blood compatibility is an important problem in blood contacting devices. In this study, two classes of materials including polyurethane (based on polyethylene glycol and poly tetrametylene oxide) and polyvinyl alcohol samples, with different hydrophilicity properties were synthesized and their physico-chemical properties were compared. Water uptake ratio, FTIR spectroscopy, and contact angle measurement were conducted. *In vitro* biocompatibility experiments were undertaken using L-929 fibroblast cell lines which demonstrated desired cell viability for all samples after 7 days. The adhesion of platelets from human plasma was studied by optical microscopy. Blood coagulation time were also determined which revealed polyurethane based poly tetramethylene oxide has better interaction by blood elements among all samples.

Keywords: Biomaterial; Hydrophilicity; Platelet adhesion; Polyurethane; Polyvinyl alcohol.
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1. Introduction

Polyurethanes are groups of polymers consisting of a chain of organic units joined by urethane links. They are widely used as prostheses due to their good biocompatibility and mechanical properties [1, 2]. A wide range of chemical compositions are used to fabricate these polymers [3]. Polyeurethanes were used in catheters, pacemaker leads, heart valves and artificial hearts [4-7]. These polymers are also candidate for biodegradable applications such as tissue engineering and drug delivery systems [8, 9]. On the other hand, polyvinyl alcohol (PVA) is a hydrophilic polymer which is produced by the hydrolysis of polyvinyl acetate [10]. PVA is a semi-crystalline polymer with high crystalline content and clear melting behavior (T_m, 230 °C) as well as glass transition temperature (T_g, 80 °C) [11]. PVA shows excellent chemical resistance and biocompatibility; however, few studies have been focused on the blood compatibility of PVA hydrogels [12]. This polymer has been examined as artificial cartilage, tendon, ligament, cornea, lens, skin and intervertebral disc [13-21]. It is known that

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cell attachment has a correlation with protein adsorption and surfaces that are more likely to resist protein adsorption will resist cell attachment. Exceptions exist, but generally, hydrophilic surfaces are more likely to resist protein adsorption and cell adhesion [22]. So hydrophilicity is an important factor for evaluation of biomaterial and cell interactions.

In this study, polyurethane elastomers and polyvinyl alcohol hydrogel with different hydrophilicity were synthesized and their physical properties as well as their interaction with blood cells (platelets and erythrocytes) were compared.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (Mw=72000 gr/mol) with 99.8% hydrolysis, polyethylene glycol (PEG, Mw=1000), polytetrahydrofuran (PTHF, Mw=1000), 4,4'-methylenediphenyl diisocyanate (MDI), 1,4-butanediol (BD), tetrahydrofuran (THF) and dimethyl sulfoxide (DMSO) were purchased from Merck. PEG, PTHF and BD were dried in a vacuum oven under 50 mbar pressure at 100 ºC for 24 h. Other chemicals were analytical grades and used as received.

2.2. Polyurethane synthesis

Two different polyurethane elastomers were synthesized by a two step polymerization in which 1 mole of PEG or PTHF (as described in Table 1) were mixed with 40 ml of THF and placed into a four necked reactor purged with N_2. The reactor was immersed into an oil bath at 70 ºC. Two moles MDI with functionality of 2.3, were dissolved in 20 ml of THF and were taken in a dropping funnel. This solution was added drop wised to the reactor which was stirred with the speed of 400 rpm for 2 h. Then 1 mole of BD was added and the reaction was continued for 2 h. Finally the product was cast and left for 24 h and then washed with distilled water several times.

2.3. Polyvinyl alcohol synthesis

At first 20 percent solution of PVA in DMSO were prepared using stirrer at 90 °C. After 6 h the solution was poured into a petri dishes and hold for 24h in an oven at 70 °C. Then three freezing-thawing cycles were applied to the sample as 15h in -25 °C and 9h in 25 °C.

2.4. Characterization

FTIR spectroscopy was performed with BOMEM MB100-Canada; contact angles were measured by Olympus AF (Zoom 7.1-21.3 mm C 5050, Japan) with 1 microliter water drop and UV spectrophotometer Milton Roy 601-USA was used for determination of hemoglobin absorption in 540nm.

In vitro biocompatibility experiment was undertaken using L-929 fibroblast cell lines and platelet adhesion was conducted by human platelet rich plasma. Blood coagulation time was also determined according to ASTM-F756 standard.

The water uptake measurements were done according to ASTM-D570 procedure. The samples were immersed in deionized water and were kept there until equilibrium is attained at room temperature. The relative degree of swelling was determined by weighting the specimen at different times until constant weight was achieved. Swelling ratio in water was obtained from equation (1):

$$\text{Water uptake} = [(W_t - W_o)/W_o] \times 100 \qquad (1)$$

where w_t is the weight of swollen sample at time t and w_o is the initial mass of the sample.

2.5. In vitro cytotoxicity

In vitro cytotoxicity of the samples was assessed as per ISO-10993-5. The mouse L929 fibroblast cells were used as a test model in this study. The cells were maintained in

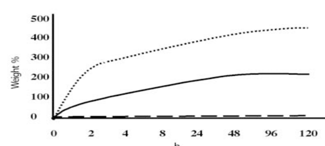

Figure 1. Water swelling of PU1 (broken line), PU2 (solid line) and PVA (dotted line) after 120h.

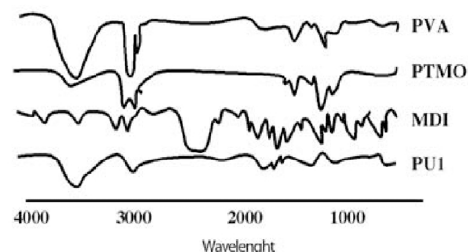

Figure 2. FTIR spectra of PTMO, MDI, PU1 and PVA.

Roswell Park Memorial Institute (RPMI)-1640 growth medium, supplemented with 100 IU/ml penicillin, 100 g/ml streptomycin, and 10% fetal calf serum. A routine subculture was used to maintain the cell line. The cells were incubated in a humidified atmosphere of 5% CO_2 at 37 °C. After 1-week incubation, the monolayer was then harvested by trypsinization. The cell suspension of 4×105 cells/ml was prepared before seeding. The samples were washed with PBS and rinsed with distilled water and then sterilized in an autoclave at 120 ºC and placed in a multiwell tissue culture polystyrene plate (Nunc, Denmark) with 5 ml cell suspension, with one well kept as a negative control, and then maintained in the incubator for one weak. After incubation, the samples were examined by optical microscopic examinations.

2.6. Platelet adhesion test

Platelet reach plasma (PRP) was supplied from Iran Blood Transfusion Organization. A quantity of 3 ml PRP was mixed with 27 ml phosphate buffer saline (PBS) and 1 ml of mixture was loaded to each cell culture plate and incubated at 37 °C for 2 h. Samples were rinsed three times with PBS to remove unattached platelets and observed by optical microscopy.

2.7. Assessment of hemolytic properties

Specimens with a determinable surface area will be used at a ratio of 3 cm^2 surface area to 1 ml of test blood solution. When red blood cells are exposed to any artificial material, excessive dynamic stresses are performed to their membranes. To investigate the effect of such stresses on the RBCs, hemolysis tests were done following exposure of blood to the samples. Blood coagulation time was determined according to ASTM-F756 standard. In this procedure 0.2 ml fresh human blood was added onto each piece of samples, followed by adding a 20 µl solution of $CaCl_2$ (0.2 mol/L) onto each one. Samples where put into distilled water (50 ml) after 10, 30 and 45 min. and the free hemoglobin were diffused into the water. The solution was tested by a spectrophotometer at 540 nm and the results were compared with each other. The higher absorption intensity represents the greater free hemoglobin concentration and indicates higher cell lysis [23].

3. Results and discussion

3.1. Swelling measurements

The water equilibrium swell is a very useful technique since it assesses compounds hydrophilicity, and also its stability and degradability in aqueous environment [24]. The water swelling percents of the macromers are presented in Figure 1.

The water uptake data after 120h, showed 5%, 220% and 450% weight increasing for PU1, PU2 and PVA, respectively. Polyvinyl alcohol as a hydrogel with a lot of OH groups in its structure swelled 450% of its initial weight, which showed its higher hydrophilicity in comparison with the

Table1. The amount of samples composition.

Sample	MDI (gr)	PTHF (gr)	PEG (gr)	BD (gr)
PU1	4.54	10	-	0.9
PU2	4.54	-	10	0.9

polyurethane samples. PU2 had more hydrophilicity than PU1 due to hydrophilicity of polyethylene glycol in its composition. After finishing the experiments, PU samples were dried for 48h and weighted in order to show there is no difference between the first and the last weight.

Water swelling results showed that all of the samples were crosslinked and can be swelled without degradation. There is no chemically crosslinking agent used for PVA preparation and only 3 cycles of freezing and thawing procedure were performed after applying at 70 °C. This method addresses toxicity issues because the risk of chemical residues is removed [11]. Such physically crosslinked materials also exhibit higher mechanical strength than PVA gels crosslinked by chemical agents, because the mechanical load can be distributed along the crystallites of the three dimensional structure. The characteristic of crystallite formation depends on the properties of polymer (percent of hydrolysis and molecular weight) and the number of freezing and thawing cycles [11]. These crystallites perform as a crosslinking point and prevent solution of the polymers.

On the other hand, using liquid MDI, with the mixing functionality of 2 and 3, in the synthesis of polyurethane samples, causes to crosslink the polymer just in the time of polymerization.

3.2. FTIR spectroscopy

FTIR spectroscopy was used extensively in the characterization of polyurethanes. Figure 2 compares the spectra of raw materials (MDI, PTMO and PEG) and the final polyurethane products. The presence of the

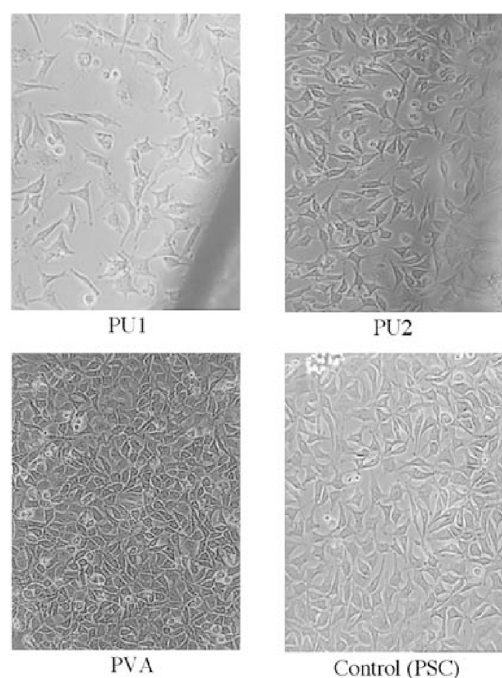

Figure3. Cytotoxicity results for PU1, PU2, PVA and control (×400).

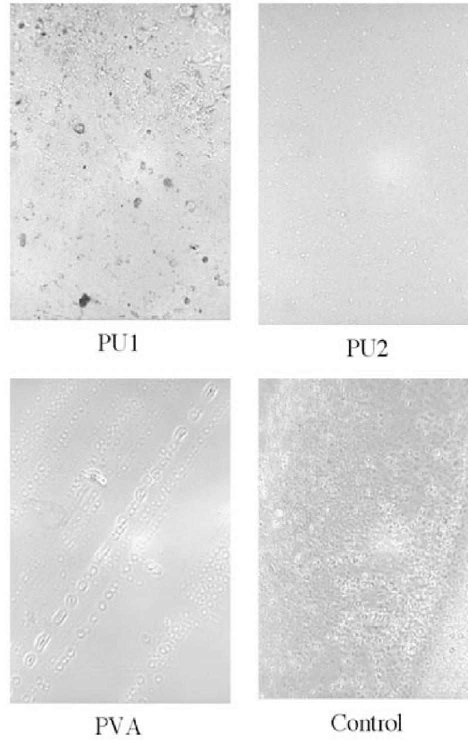

Figure 4. Platelet adhesion on PU1, PU2, PVA and glass as a positive control (×400).

peaks at 2850-2950 cm^{-1} is related to CH_2 band of the synthesized polymers. As can be seen the initial isocyanate band (2270 cm^{-1}) has been removed, the urethane band (1715 cm^{-1}) increased and NH band (3420 cm^{-1}) formed. In PVA spectra, bands at 3450-3490 cm^{-1} (O-H stretching), 2850-2980 cm^{-1} (C-H stretching), 1450 cm^{-1} (C-H bending) and 1090 cm^{-1} (C-O stretching) can be observed.

3.3. Contact angle measurements

Table 2 compares the contact angles of the samples. PVA has the lowest contact angle, due to its more hydrophilicity and hydrogen bonding can occur in the surface of this polymer between free OH groups and H_2O molecules. On the other side, PU1 has the highest contact angle because of its hyrophobicity quality.

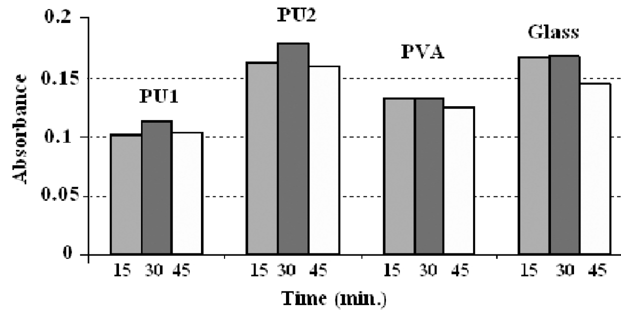

Figure 5. The results of hemolysis experiments for samples and control (absorption at 540 nm).

Table 2. Results of contact angle measurements.

Sample	Contact angle (°C)
PVA	12±2
PU1	68±3
PU2	29±2

3.4. Cytotoxicity test

In vitro biocompatibility experiments were undertaken using L-929 fibroblast cell lines. Figure 3 shows the comparison between the samples and control which demonstrated desired cell viability for all samples. The response of the cells to the PVA sample is even better than negative control (PTSC).

3.5. Platelet adhesion

Platelets poorly adhered to the PU1 films whereas more platelets seemed to attach to the PU2 samples. The only difference in polyurethanes structures referred to the difference between polytetra hyrofuran and polyethylene glycol which has a large difference in their hydrophilicity. Thus hydrophilicity may increase the adhesion of platelets in the PU samples.

As can be seen in Figure 4, PU2 has the highest range of platelet adhesion on the surface between all the samples, but polyvinyl alcohol is more hydrophilic than PU2. So it can be concluded that, hydrophilicity is not the only factor that can affect platelet adhesion. There are also chemical factors and morphology of surfaces should be considered.

3.6. Assessment of hemolysis

Hemolysis test was done based on the fact that destruction of erythrocytes resulting in the liberation of hemoglobin into suspension medium. Figure 5 gives the results of hemolysis experiments. The y-axis represents absorbance values at 540 nm. It has been shown that glass resulted in a very high absorbance and hence can be classified as a poor material and positive control [23]. At a sodium chloride concentration of 0.2 mol/L after 45 min., PU1 exhibited a value of 0.105 while, PVA displayed a value of 0.125. Among all of the samples, PU2 has the highest values in all the times that resembled the more cell lysis than the other. The results also showed that, the cell lysis may decline with increasing in the time of exposure as deduced from diagram, the amount of absorbance decreases from 30 to 45 min. in all samples. The outcome of this procedure demonstrated that the cell lysis decreased in order of PU2>PVA>PU1.

4. Conclusions

In this study three different samples with different hydrophilicity were examined for *in vitro* cytotoxicity and blood interactions. PEG based PU, due to its higher hydrophilicity had higher swelling ratio and lower contact angle than PTHF based PU. *In vitro* biocompatibility experiments showed appropriate cell morphologies. Platelets from human plasma poorly adhered to the PTHF based PU whereas more platelets seemed to attach to the PEG and PVA samples probably due to more hydrophilicity.

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