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Original Article

Preparation and Evaluation of Blood Compatibility of Novel Epoxy-Modified Polyurethanes

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Abstract

In order to prepare polyurethane elastomers with acceptable physical properties and good biocompatibility, novel polyurethane networks were synthesized via curing reaction of epoxy-terminated polyurethane prepolymers (EUPs) with hexamethylene diamine. EUPs were prepared from reaction of glycidol with NCO-terminated polyurethanes (ITPs). ITPs were also synthesized from reaction of one equivalent of either poly(tetramethylene ether)glycol or poly(ethylene glycol) with hexamethylene diisocyanate. Cytotoxcicity and blood compatibility were evaluated. All of the prepared polymers via this novel and simple method showed nontoxic behavior and acceptable blood compatibility.

Keywords: Biocompatibility; Biomaterials; Blood compatibility; Polyurethanes.
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1. Introduction

In modern medicine, the use of polymers for permanent or temporary implantation in the human body has become well established [1]. Biocompatibility can be considered in terms of blood compatibility and tissue compatibility [2]. Blood compatibility is less well defined than biocompatibility, and there is no widely accepted definition. It is often defined as what should not occur including thrombosis, destruction of formed elements and complement activation. Cytocompatibility encompasses the lack of toxicity, and excessive tissue growth around an implant. It is unlikely that there will be one biomaterial that will work equally well in all applications [3].

Among different class of polymers used in biomedical field, polyurethanes are very attractive ones [4-6]. This is because of their relatively good biocompatibility, their excellent physical and mechanical properties, and the ease of tailoring them for special end use.

Commercial medical-grade polyurethanes, such as Biomer®, Elasthane™, and ChronoFlex® AR [7], are typically synthesized from 4,4'-methylenebis(phenylisocyanate) (MDI). Carcinogenic and mutagenic aromatic diamines have been reported as degradation

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products from polyurethanes incorporating aromatic diisocyantes, however, the question of whether the concentrations of these harmful degradation products attain physiologically relevant levels is currently unresolved and strongly debated [8]. To avoid the potential release of toxic degradation products to the extracellular matrix, it is desirable to synthesize new medical-grade polyurethanes from less toxic intermediates.

The aim of the present work was to synthesize polyurethane elastomers with acceptable physical, mechanical properties and good cyto- and blood compatibility. For fulfilling this purpose, epoxy-terminated polyurethane prepolymers based on poly(ethylene glycol) (PEG) and poly(tetramethylene ether glycol) (PTMEG) and HDI were prepared and cured with equivalent amount of hexamethylene diamine (HAD). It was expected that increased hydrophilicity due to presence of extra hydroxyl groups generated from ring opening of epoxy groups via reaction with HDI could improve surface hydrophilicity and increase blood compatibility. Increasing surface and bulk hydrophilicity of PTMEG based prepolymers via addition of PEG based epoxy-terminated polyurethane was another topic that was considered. All of the materials were characterized by conventional methods and through study of physical, mechanical, and thermomechanical properties as well as cells and platelets adhesion assays was established. The results showed that prepared polyurethanes may be used for biomedical applications.

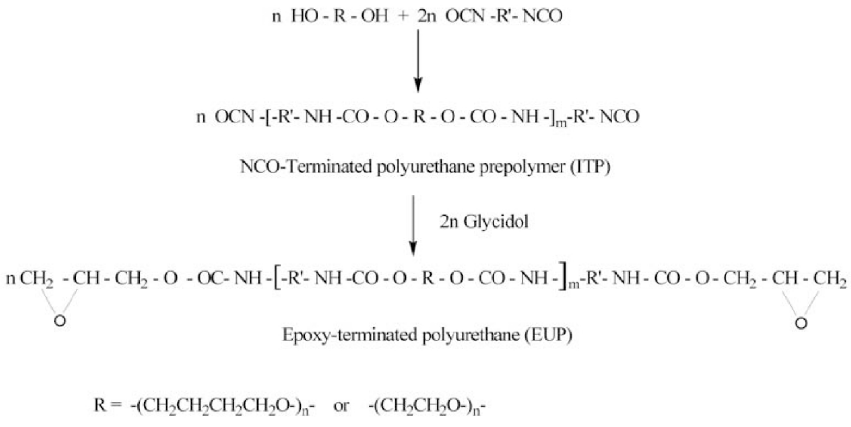
2. Materials and methods

2.1. Materials

PTMEG, molecular weights 1000 from Aldrich, was dried at 90 ºC under vacuum. PEG, molecular weight 1000 from Merck was freed from moisture by an azeotropic distillation with toluene prior to use. HDI from Merck was purified via vacuum distillation. 2,3-Epoxy-1-propanol (glycidol) from Aldrich was vacuum distilled before use. Hexamethylene diamine (HMDA) from Aldrich, streptomycin from Gibco BRL Laboratories, Germany, L929 fibroblast cells from Pasteur Institute of Iran, fetal calf serum from Gibco, and platelet- rich plasma (PRP) from Iran Blood Donation Center were used as received.

2.2. Synthesis of epoxy modified polyurethane based on PTMEG (EUP1)

Into a four-necked reaction kettle equipped with mechanical stirrer, heating mantle, reflux



Scheme 1. Synthetic route to epoxy-terminated polyurethanes.

Table 1. Chemical composition of epoxy-modified polyurethanes.

Code	Type of polyol (molecular weight)	Type of diisocyanate	Weight of constituent (g)			NCO content (%) of intermediate ITP		Epoxy content of EUP (mol epoxy / kg polymer)	
			Polyol	Diisocyanate	Glycidol	Theo.	Exp.	Theo.	Exp.
EUP1	PTMEG(1000)	HDI	100	33.6	14.8	6.2	6.1	1.3	1.2
EUP5	PEG(1000)	HDI	100	33.6	14.8	6.2	6.1	1.3	1.2

condenser, dropping funnel and N_2 inlet and outlet was placed PTMEG and the temperature was increased to 60 °C. Then HDI was added dropwise to the reactor at a rate that the reaction temperature would not surpass 70 °C. The temperature was then increased to 85 °C and the reaction was continued till the NCO content reached to the theoretical value as determined by dibutyl amine titration. Then the reaction kettle was cooled to 40 °C and glycidol was added dropwise through dropping funnel and mixed. The temperature was increased slowly and maintained at about 70 °C to allow the termination reaction to take place. The reaction was continued until NCO peak at 2270 cm^{-1} was disappeared totally at the FTIR spectra of samples taken from the reaction kettle every 0.5 h.

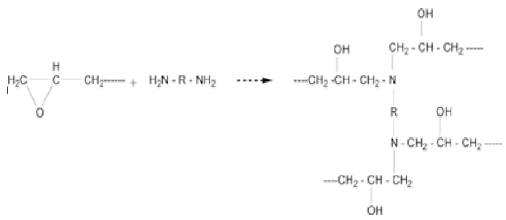
2.3.Synthesis of epoxy modified polyurethane based on PEG (EUP2)

Into 250 ml a three-necked, round-bottomed flask equipped with a Dean-Stark trap, a condenser, a N_2 inlet tube, a thermometer, an oil bath and a magnetic stirrer was placed appropriate amount of PEG and dry toluene (200 ml). The reaction mixture was heated to 115 °C for 5 h with continues stirring. The water was removed from the reaction mixture by azeotropic distillation. Then the reaction temperature was raised to 120 °C to remove most of solvent. The flask was cooled to 50 °C and Dean-Stark trap was replaced by a reflux condenser as fast as possible. Dry tetrahydrofuran (THF) (200 ml) was added and after complete dissolution of polymer, HDI was added to the flask dropwise over a period of 2 h. The temperature was then increased to 85 °C and the reaction was continued till the NCO content reached to the theoretical value as determined by dibutyl amine titration. Then the reaction flask was cooled to 40 °C and glycidol was added dropwise through dropping funnel over a period of 30 min. The temperature was increased slowly and maintained at about 70 °C to allow the termination reaction to take place. The reaction was continued until NCO peak at 2270 cm^{-1} was disappeared totally at the FTIR spectra of samples taken from the reaction kettle every 0.5 h. The product was dried for 2 days in a vacuum oven at 50 °C. The chemical compositions of the EUPs are presented in Table 1.

Required amount of EUP or EUPs mixture and appropriate amount of curing agent (1:1 molar ratio of epoxy:NH groups) were placed in a beaker according to formulation depicted in Table 2. The beaker content was stirred vigorously for 1 min. and placed into a vacuum desiccator in order to remove air bubble from bulk of mixture. Then the homogeneous mixture was poured slowly into a clean Teflon mold and cured at 60 °C for 5 h. The thickness of the films was adjusted to 1 mm and cut to the desired shape for further experiments.

2.3. Determination methods

FTIR spectra were obtained on a Bruker IFS 48 instrument. Mechanical properties



Scheme 2: Cross-linking reaction of terminal epoxy groups of EUP with N-H groups of curing agent (representative structure)

Table 2. Different formulations of final cured samples.

Code	Type of EUP	Weight of EUP (g)	Weight of HMDA (g)	Gel content (%)
CEUP1	EUP1	5.0000	0.1740	99
CEUP2	EUP2	5.0000	0.1740	92
CEUP3	(EUP1 + EUP2)	(3.5000 + 1.5000)	0.1740	97
CEUP4	(EUP1 + EUP2)	(2.5000 + 2.5000)	0.1740	92
CEUP5	(EUP1 + EUP2)	(1.5000 + 3.5000)	0.1740	91

including tensile strength, initial modulus, and elongation at break were determined from stress-strain curves with MTS tensile tester model 10/M at a strain rate of 20 mm/min. The measurements were performed at 25 °C with a film thickness of about 1 mm and stamped out with an ASTM D638 Die. Surface hydrophilicity of films was determined by measurement of water droplet contact angel. The contact angel was determined via running Image-Pro Plus, version 3.1 software on the digital pictures taken from interfaces of films and droplets. The data presented were average of four measurements. Scanning electron microscopy was performed using a Steroscan 360 model 1992, Cambridge, equipped with EDXA (Oxford, with Si/Li crystal and EXL program).

Gel content measurements were performed in a Soxhelt extractor using acetone as solvent. NCO content of polyurethane prepolymers were determined according to procedure reported in ASTM D-2572 and epoxy content of prepolymers were determined by method reported in reference [9] and presented as mol epoxy per kg polymer.

In order to water uptake evaluation, the dried films were weighted and immersed in purified water. After 24, 48, and 72 h, the films were taken out from the water, wiped dry with tissue paper, and weighted again immediately. The water contents (expressed as a percentage) were calculated using the following formula:

$$\text{Water uptake \%} = \frac{m_w - m_d}{m_d} \times 100$$

where m_d and m_w are the masses of dry and wet samples, respectively.

Cell culture assays in contact with samples did according to ISO10993.

Platelet adhesion test was performed The PRP (platelet rich plasma) and PPP (platelet-poor plasma) were prepared from the blood of a healthy human. The platelets were adjusted to150,000 platelets/mm^3 by adding PPP to PRP. PRP (1 ml) was placed on each of the samples in a vial and allowed to stand for 1 h at 37 °C. The films were then rinsed with PBS. For SEM evaluation of adhered platelets, the films treated with 2.5% glutaraldehyde for 30 min. at room temperature, then rinsed with PBS and dehydrated by systemic immersion in a series of ethanol-water solutions [50, 60, 70, 80, 90, 100% (v/v)] for 30 min. and allowed to evaporate at room temperature. The platelet-attached surfaces were coated with gold and scanned by SEM.

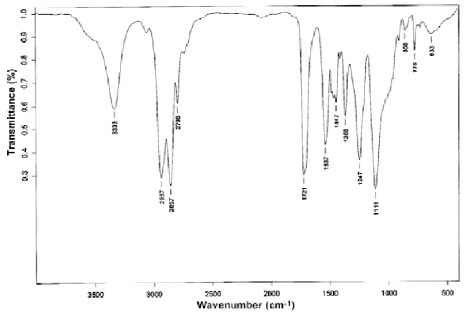


Figure 1. FTIR spectra of EUP1.

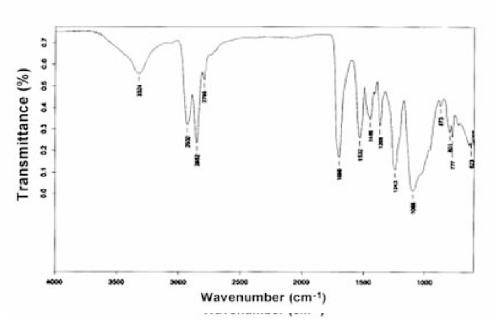


Figure 2. FTIR spectrum of CEUP1.

Table 3. Surface and bulk hydrophilicity data of samples.

Code	Contact angle	Water uptake (%)		
		24h	48h	72
CEUP1	68±0.5	7.30	7.35	7.42
CEUP2	44±1.0	189.32	193.49	193.56
CEUP3	64±0.5	37.50	42.07	43.50
CEUP4	57±1.0	98.51	100.46	102.79
CEUP5	51±0.4	137.93	138.85	143.09

3. Results and dissections

3.1. Synthetic route

Scheme 1 outlines the synthetic route designed for preparation of our samples.

3.2. FTIR results

The EUPs were characterized by conventional spectroscopic methods. FTIR spectra of EUPs showed characteristic bands of urethane groups at 3337- 3333 cm^{-1} (N-H stretching), 1721-1717 cm^{-1} (NHCOO stretching),1537-1533 cm^{-1} (C-N stretching, combined with N-H out of plan bending) (Figure 1). Etheric bands stretching vibration arose from polyol parts of EUP appeared at 1111-1109 cm^{-1}.The peaks of epoxy groups were also appeared at 953-851 cm^{-1}.

Transformation of EUPs to crosslinked networks was performed by reaction of terminal epoxy groups with HMDA (Scheme 2). The amounts of HMDA and EUP were adjusted in the way that a 1:1 molar ratio of epoxy and NH groups was established (Table 2). The investigation of gel content measurement data (Table 2) indicated complete curing of the prepared films.

FTIR spectra of cured samples showed characteristic peak of urethane carbonyl and etheric bands at 1698 and 1098 cm^{-1}. The red shift of urethane carbonyl and etheric bands of cured films in comparison to uncured EUP is a result of extra hydrogen bonding of urethane and etheric groups with amine groups present in cured films. Also, the intensity of epoxy groups peaks at ca 900 cm^{-1} diminished considerably, indicating complete reaction of epoxides. Representative example is shown at Figure 2.

The surface and bulk hydrophilicity of biomaterials are important issues for biocompatibility. CEUPs surface hydrophilicity, as characterized by static water contact angle, is reported in Table 3. Considerable decrease in contact angle value for CEUP2 in comparison to CEUP1 is an effect of PEG presence in the polymer backbone with high ability to interact with water molecules through hydrogen bonding. For samples (CEUP3-5) the value of contact angle was a function of EUP2 content. The contact angel value decreases as EUP2 content increases. Water absorption was measured to determine polymer bulk

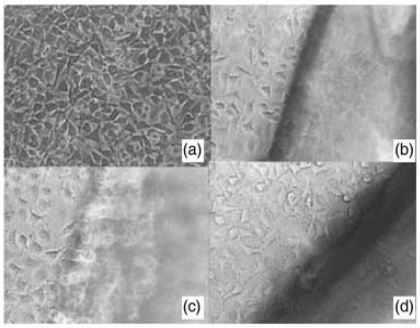


Figure 3. Photographs of L-929 cells interaction with samples film. a) negative control; b) CEUP1; c) CEUP2; d) CEUP3.

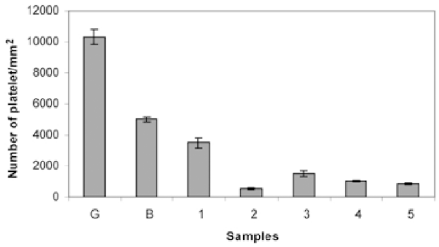


Figure 4. Number of platelets attached onto the different samples surface derived from optical micrographs of samples.

hydrophilicity. Water uptake as a function of time and type of samples are collected in Table 3. The trend of water up take for different samples is the same as contact angel values. In comparison to similar polymers based on PTMEG polyol with the same amount of hard segment content [10] the prepared samples show higher ability to absorb water. It seems extra hydroxyl groups produced via ring opening of terminal epoxy groups are responsible for increased hydrophilicity of samples.

In the cell culture method, the performance of a cell is investigated by comparing it with a negative control. A negative control, tissue cell culture polystyrene. Figure 3 shows optical photomicrographs of L-929 fibroblast cell attachment onto the negative control, and CEUP1-3, respectively. The light microscopy evaluation of cell morphology showed cells were growing around all of the materials with spindle shape morphology, indicating no cytotoxicity. However, no cell adhesion on the surface of prepared materials was observed after staining with 5 % Giemsa. It is well documented that increasing surface hydrophilicity has considerable effect on improving biocompatibility [11] but unfortunately, significant difference on cell adhesion behavior on the surface of different prepared samples was not observed. For example CEUP2 (with contact angel 44 º and water uptake 193%) and sample CEUP1 (with contact angel 68 º and water uptake 7%contact angel) showed the same behavior. The interaction between a biomaterial and living tissue occurs in a narrow interface zone. Biocompatibility of prepared samples may largely be determined by some other important material surface properties than surface hydrophilicity, like: surface roughness, surface tension, chemical composition and electrical charge [12-14], as most of the prepared samples showed low cellular adhesion.

Blood components behavior at the interface with foreign materials is an essential problem in the biomedical applications of synthetic polymers. When blood is in contact with a foreign material surface, the absorption of plasma proteins occurs first, followed by platelet adhesion and deformation. These platelets release substance that starts the coagulation process, resulting in thrombosis. Blood compatibility is reached when there is not too much interaction of platelets at its surface. Therefore, platelet adhesion on the implants from human plasma is an important test for the evaluation of the blood compatibility.

The results of polymers response to platelets are collected in Figure 4. The number of adhered platelets on the surface of CEUP1 sample reduced slightly in comparison to Biomer®. The sample CEUP2 based on PEG also shows very good blood compatibility.

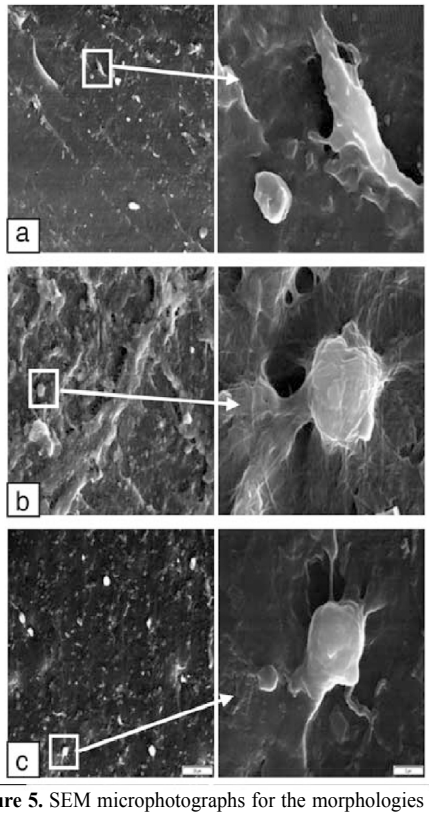


Figure 5. SEM microphotographs for the morphologies of PRP contacted surfaces (60 min., 37 ºC and 8000×). a) $CEUP_1$; b) $CEUP_2$; c) $CEUP_3$.

The increased surface hydrophilicity of PEG based polyurethane, inhibited the adsorption of plasma protein, as a first stage of platelet adhesion and deformation. Addition of different weight ratios of EUP2 to EUP1 and co-curing of these prepolymers produced polymeric films (CEUP3-5) with reduced number of surface adhered platelets in comparison to the CEUP1 sample. Again in these samples increased amount of adsorbed water on the surface of blends act as a barrier between the blood and polymers surface that can prevent platelets from having direct contact with surface.

The morphology of adhered platelets was classified into three types based on degree of deformation as I) attachment of platelets at a point of contact with substratum, II) centrifugal growth of filopodia III) cytoplasmic webbing and flattening of the central mass. In order to find better perspective concerning level of blood compatibility of adhered platelets, SEM photomicroghraghs of selected samples were studied (Figure 5). As it is obvious from the Figure 5, platelets are still in the filopodia stage and cytoplasmic webbing was not occurred. This behavior is another indication of good blood compatibility of these samples.

4. Conclusion

The objective of this work was to synthesize, characterize and evaluate novel polyurethanes for their potential use as blood contacting biomaterials. All of prepared samples were nontoxic, however the level of cytocompatibility needed to be improved via further controlling the surface characteristics of the prepared samples. Blood compatibility of samples in regard to number of adhered platelets and their morphology were studied. Most of prepared polymeric samples showed better behavior in comparison to negative controls. The blood compatibility was increased with increase of degree of hydrophilicity.

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