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Oxygen plasma modification of polyurethane membranes

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Polyurethane membranes were prepared under nitrogen atmosphere by using various proportions of toluene diisocyanates (TDI) and polypropylene-ethylene glycol (P) with addition of no other ingredients such as catalysts, initiator or solvent in order to achieve medical purity. Effects of composition on mechanical properties were examined. In general, modulus and UTS values demonstrated an increase and PSBR demonstrated a decrease as the TDI/Polyol ratio of the polymer increased. Elastic modulus, ultimate tensile strength (UTS) and per cent strain before rupture (PSBR) values were found to be in the range of 1.4–5.4 MPa, 0.9–1.9 MPa, and 60.4–99.7%, respectively. Surfaces of the membranes were modified by oxygen plasma applying glow-discharge technique and the effect of applied plasma power (10 W or 100 W, 15 min) on surface hydrophilicity and on the attachment of Vero cells were studied. Water contact angle values of the plasma modified surfaces varied between 67° and 46°, demonstrating a decrease as the applied plasma power was increased. The unmodified material had 42–45 cells attached per cm^2. It was observed that as the applied power increased the number of attached cells first increased (60–70 cells/cm^2 at 10 W) and then decreased (27–40 cells/cm^2 at 100 W). These demonstrated that surface properties of polyurethanes can be modified by plasma-glow discharge technique to achieve the optimum levels of cell attachment.

Introduction

Polyurethane elastomers are the reaction products of organic isocyanates, high molecular weight polyols and low molecular weight chain extenders. Chemical factors such as composition, molecular weight of the soft segment, type and length of hard segment, addition of chain extenders and physical factors such as the fabrication method are used to alter the structure and morphology of phase separation in bulk or on the surface of the polymer. These factors, in turn, determine the performance in biomedical applications [1]. They could be synthesized in very different forms with different properties ranging from very hard and porous structures to elastomeric, smooth membranes. Ease of preparation, the wide range of physical and mechanical properties, stability during sterilization, and ease of adaptation to the biological environment make them desirable biomaterials and they are widely used in medical applications as prosthesis and supporting materials of organs. Because of their excellent blood and tissue compatibility their most common biomedical application areas are cardiovascular devices such as heart valves, artificial veins and artificial skin grafts [2]. For these applications, mechanical strength and surface biocompatibilities of polyurethanes are very important.

Polyurethanes are inherently resistant to cell adhesion and proliferation. When blood cells do not adhere, they do not accumulate and cause undesired thrombi formation, thus making them hemocompatible [3]. Another area for PU application is wound healing. In wound healing applications attachment of cells to polymeric materials and their proliferation in their structure is desired [4]. In order to modify the surface chemistry of PU and promote rapid endothelialization and tissue repair, various methods, such as plasma surface treatment [5, 6], have been developed. In the literature application of plasma both to increase and to decrease cell attachment to the polymeric surfaces were reported [7–9]. Plasma treatments (application of discharge in the presence of gases or monomers) are able to alter the surface energy of most polymers, changing their surface polarity, wettability, and adhesiveness [10–13].

In this study, polyurethane elastomeric membranes were prepared in medical purity without addition of any other ingredients except the diisocyanate and polyol

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components with different proportions. Mechanical properties were examined and effect of composition on tensile strength, percent elongation and elastic modulus were compared. The surfaces of the membranes were modified by treatment with oxygen plasma applying 13.56 MHz radiofrequency at 10 or 100 W for 15 min at low pressures and the effect of plasma on surface hydrophilicity and attachment of Vero cells were studied.

Experimental

Materials

Toluene diisocyanate (TDI) was obtained from Dow Chemical Co. (USA) as a mixture of 2,4- and 2,6-toluene diisocyanate in the ratio of 80 : 20. Polyol (polypropylene-ethylene glycol) was also obtained from Dow Chemical Co. (Voranol 3322 polyol; molecular weight: 3400–3500; hydroxyl value: 46–48 mg KOH/g; viscosity at 25 °C: 510 MPa.s; water content: 0.1% (w/w); flash point: 234 °C). Dulbecco's Modified Essential Medium (DMEM) (Biochrom KG, Cat.No: T-041-10) was used for the cultivation of Vero cells in the cell adhesion experiments. Vero cell line was initiated from the kidney of a normal adult African green monkey by Drs Y. Yasumura and Y. Kawakita (Chiba University, Chiba, Japan).

Preparation of polyurethane membranes

The polyurethane membrane preparation set up was composed of two parts connected to each other: the polyol container constituted the lower part that was connected to a vacuum pump and immersed in an oil bath, and the TDI container (the upper part) which was kept under nitrogen atmosphere and connected to a burette via connectors. The lower container, with polyol (20 ml) inside, was heated to 90 °C and connected to vacuum pump for one hour in order to remove the volatile chemicals and water. The stoppers to the connectors were closed and desired amount of TDI (according to Table I) was added dropwise to the hot polyol. The polymerization mixture was heated and stirred for 6 h at 90 °C. The highly viscous solutions were poured into the glass Petri plates, placed into a vacuum oven and then heated at 90 °C for a further 6 h. The samples in the Petri plates were later stored under vacuum for curing and completion of the polymerization process. Completion, as judged by the formation of solid films, was achieved in about a week.

TABLE I Compositions and modification conditions of polyurethane membranes

Sample code	Compositions	Power applied (W)
PU2	2 ml TDI + 20 mL polyol	None
PU4	4 ml TDI + 20 ml polyol	None
PU8	8 ml TDI + 20 ml polyol	None
PU210	2 ml TDI + 20 ml polyol	10
PU410	4 ml TDI + 20 ml polyol	10
PU810	8 ml TDI + 20 ml polyol	10
PU2100	2 ml TDI + 20 ml polyol	100
PU4100	4 ml TDI + 20 ml polyol	100
PU8100	8 ml TDI + 20 ml polyol	100

Mechanical tests

A mechanical tester (Lloyd LRX 5K, Lloyd Instrument, UK), which was controlled by a computer running program (WindapR), was used to measure the mechanical properties. For this purpose, polyurethane membranes (thickness: 1.00 ± 0.05 mm, width: 15.0 ± 0.05 mm, length: 50.0 ± 0.05 mm) were attached to the holders (gage length: 10 mm) of the machine. A constant extension rate of 25 mm/min was applied and the computer continuously recorded the tensile load on the specimen. The load deformation curve was printed for each specimen. The ultimate tensile strength was obtained from the equation: $\rho = F/A$, where ρ is the tensile strength (MPa), F is the maximum load applied (N) just before rupture and A is the initial area (m^2) of the specimen. The load deformation curve was converted to stress strain curve. Where stress is the load applied to per unit area (F/A as pascal) and strain is the deformation per unit length given as $(l - l_0)/l_0$ (where l is the extended length and l_0 the initial length of the specimen). Slope of the straight line (in elastic region of the stress–strain plot) is accepted as the elastic modulus of the specimen. For each type of sample, at least eight experiments were achieved and the average values of elastic modulus, tensile strength and per cent elongation values were calculated.

Surface modification with plasma

Polyurethane membranes were placed in the reaction chamber of the glow-discharge reactor by taking precautions to achieve exposure of both sides to the plasma. The chamber was flushed with dry nitrogen and then the gas inside was pumped out (pressure ca. 0.01 torr). Oxygen was introduced with the constant flow rate of 50 ml/min and plasma was created by the application of power (10 or 100 W, adjusted via matching network) for 15 min at a radio frequency of 13.56 MHz. The gas flow was continued for 10 more minutes after the power was turned off in order to let the radicals created on the polymer surface to complete their reaction with oxygen. Then the gas flow was stopped, the pressure in the reactor was brought up to the atmospheric pressure by backfilling with nitrogen, and the samples were taken out.

Contact angle measurements

Water contact angle values of polyurethane membranes were measured by captive bubble contact angle method. The container was filled with triple distilled water at 25 °C and the sample was immersed in it. An air bubble was formed on the surface of the membrane and the bubble was photographed. The equilibrium contact angle (θ) values were calculated from the height (h) and the width (d) of the air bubble by using the Equations 1 and 2. The mean values were obtained from at least five (mostly nine) measurements.

$$\theta = \cos^{-1}[(2h/d) - 1] \quad \text{for } \theta < 90^\circ \text{C} \qquad (1)$$

$$\theta = 180 - 2\tan^{-1}(2h/d) \quad \text{for } \theta > 90^\circ \text{C} \qquad (2)$$

Cell adhesion experiments

Vero cells (*Cercopithecus aethiops*), which are morphologically fibroblast-like, were used in cell adhesion studies. For cell adhesion studies, discs (diameter 1 cm) were cut from the polyurethane membranes, sterilized by UV radiation and placed into the bottom of 24 well tissue culture plates. 200 µl of cell suspension with a cell concentration of about 3.0×10^6/ml was added to each well. Plates were put in incubator at 37 °C. After 2 h of incubation, 1 ml DMEM solution with 10% inactivated fetal calf serum was added into each well and incubated under 5% CO_2/ 95% air atmosphere at 37 °C for 24 h. The cell morphology and distributions were examined and photographed by a phase contrast microscope (Olympus 202909, Japan). In order to detect the number of adhered cells, polyurethane membranes were taken out, placed in clean plates, rinsed with PBS-VT solutions for 3 min to detach the cells which were then counted by using a Thoma counting chamber. There were nine groups of samples including controls and plasma treated samples. For each group, eight experiments were carried out and the average values of the number of cells attached on the surface of the membranes were determined.

Statistical procedure

A one way ANOVA test was applied to determine the average, standard deviation and significance of the differences between the values obtained for mechanical properties, contact angles and cell attachment experiments.

Results and discussions

Polyurethane membranes

Polyurethane membranes have very good physical and mechanical properties, and possess high modulus of elasticity, biocompatibility, and excellent stability over long implantation periods [2]. Polyurethane elastomers are generally obtained from the reactions of organic isocyanates, high molecular weight polyols and low molecular weight chain extenders. In this study polyurethane membranes were synthesized from 2,4- and 2,6-toluene diisocyanate (TDI) and poly(propylene-ethylene) glycol. In order to achieve medical purity, no other ingredients such as chain extender, solvent or catalyst were used.

Mechanical properties

It is known that an increase in the proportion of hard segments in polyurethane elastomers leads to higher modulus materials [14, 15]. The proportion of hard segments is related to the existence of isocyanate groups which leads to further crosslinking reactions in the structure. For the prepared polyurethane membranes, elastic modulus results were found as 1.4, 1.8 and 5.4 MPa, for PU2, PU4, and PU8 samples, respectively. It was observed that when the amount of TDI is doubled (from 2 to 4 ml) the increase in modulus was slight but doubling the amount of TDI (from 4 to 8 ml) caused a significant increase (4.5 times) in modulus.

TABLE II Mechanical properties of polyurethane membranes

Sample	Modulus (MPa)	UTS (MPa)	PSBR (%)
PU2	1.4 ± 0.1	0.9 ± 0.1	90.4 ± 9.0
PU4	1.8 ± 1.0	1.5 ± 0.6	99.7 ± 10.0
PU8	5.4 ± 0.3	1.9 ± 0.1	60.4 ± 8.0

The ultimate tensile strength results were very similar to the modulus results. For the prepared polyurethane membranes, UTS results were found to be 0.9, 1.5 and 1.9 MPa, for PU2, PU4, and PU8 samples, respectively (Table II). When the proportion of hard segment (TDI) increases in the structure, UTS values also increase. In the literature, it was mentioned that the increase in NCO/OH ratio increases the intermolecular attraction of hard segments, which in turn increases the tensile strength and decreases the elongation [10]. It can be concluded that for PU2 and PU4 samples, amount of TDI is quite low and therefore crosslinkings between the isocyanates and polyols are less. As the amount of TDI increases, crosslinkings between the chains, and therefore the hard segments, increase leading to harder polymeric structures.

For polyurethanes, in general, when the amount of hard segments increase, strain before rupture (SBR) values are expected to decrease because of an increase in crosslinking density. But it was observed that, as the amount of hard segments increase, strain values show an increase first and then drop. For the samples which contain a less amount of TDI (PU2), since the hard segment content and crosslinking are quite low, samples did not demonstrate high elongation values. For PU4 samples, crosslinking density is higher and it is expected that at the given TDI/Polyol ratio, the polymer had a proper distribution of hard and soft segments leading higher PSBR values. A further increase in TDI content causes harder materials causing a drop in SBR values. PU polymers which contain 2, 4, and 8 ml TDI were found to be 90%, 100%, and 60% respectively. Mechanical test results of all samples containing the values of elastic modulus, ultimate tensile strength (UTS) and SBR are tabulated in Table II.

Contact angle results

Captive bubble contact angle method was used and the angle formed at contact between the hydrated sample surface and the air bubble was calculated from the bubble's height and diameter at the interface and was reported in degrees (Table III). In this application a greater contact angle indicates a greater hydrophobicity of the polymer surface [16, 17]. Contact angle values of unmodified (control) polyurethane membranes were found to be in the range of 61°–67°. A slight increase

TABLE III Contact angle results

Sample	Plasma treatment		
	Untreated control	10 W	100 W
PU2	61 ± 2°	58 ± 2°	46 ± 2°
PU4	62 ± 2°	59 ± 2°	53 ± 2°
PU8	67 ± 2°	59 ± 2°	46 ± 2°

was observed in the contact angles upon an increase in the TDI content. Contact angles of control, and 10 and 100 W treated PU8 polymers were found to be 67°, 59°, and 46°, respectively. Therefore, the decrease in contact angle values upon increase of the applied power indicates higher oxygenation of the surface leading to an increase in hydrophilicity.

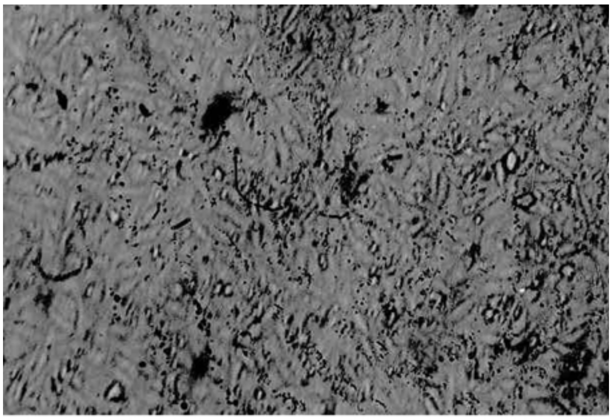
Cell attachment results

Polyurethane surfaces were modified by oxygen plasma-glow discharge application and the effect of this treatment on the number of attached cells was examined by using Vero cells. A typical cell morphology and distribution is presented in Fig. 1.

The average number of the attached cells are given in Table IV. Cell numbers were found to be in the range of 28–70 cells per cm^2 where 100 W treated PU4 (PU4100) demonstrated the lowest and 10 W treated PU4 (PU410) had the highest values. For the unmodified (control) groups PU2, PU4, and PU8, the attached cell numbers were very similar to each other (ca. 45 cells per cm^2) indicating that all these unmodified surfaces have almost similar attraction for the cells. It was, however, observed that, glow discharge applications altered the surfaces significantly affecting (both positively and adversely) the interactions of the cells. For the samples modified with 10 W plasma application, numbers of cells attached to these surfaces were found to be much higher than the others. On the other hand, for the surfaces modified with 100 W plasma application a decrease in the number of attached cells was observed. It is very possible that 10 W plasma application broke some bonds on the surface creating radicals and oxygenated groups. Higher power plasma application (100 W) although created more hydrophilic groups (lower contact angles) the arrangement of the groups on the surfaces were probably not similar to the ones modified with 10 W. Therefore, the number of attached cells decreased upon modification with oxygen plasma at 100 W.

TABLE IV Quantification of attached cells

Sample	Plasma treatment		
	Untreated control	10 W	100 W
PU2	44 ± 11	62 ± 6	36 ± 2
PU4	42 ± 6	70 ± 3	28 ± 3
PU8	45 ± 14	66 ± 2	40 ± 4


(a)

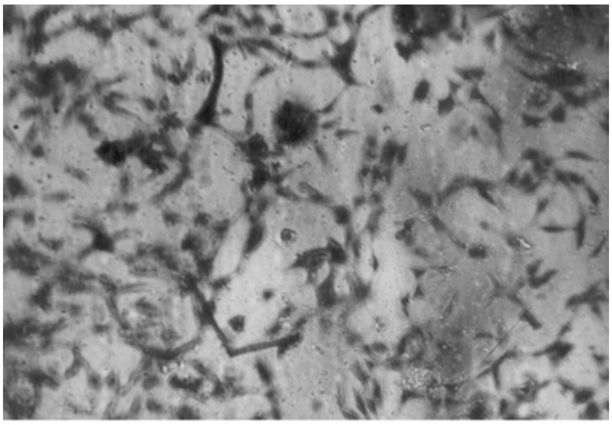

(b)

Figure 1 Morphology and distribution of Vero cells (A) PU4 control (unmodified) (× 100) (B) PU4100 (100 W oxygen plasma treated) (× 100).

Conclusions

In this study it was shown that polyurethane elastomers can be prepared from its main components, toluenediisocyanate (TDI) and polyol, without using any other ingredient such as chain extender, catalyst or solvent. The ratio of the ingredients used initially affected the mechanical properties and an increase in the amount of TDI caused stiffer membranes. The elastic modulus values varied in the range of 1.4–5.4 MPa, ultimate tensile strength values about 0.9–1.9 MPa and strain at break values in the range of 60–100%. It was observed that as the applied oxygen plasma power was increased, contact angle values decreased by 3°–20°. Plasma modification affected the number of Vero cells attached onto the surface. The highest attachment numbers were obtained for membranes modified with 10 W and were in the range of 60–70 cells per cm^2. The lowest values were obtained with samples modified with 100 W and were in the range of 28–40 cells per cm^2. Histology micrographs revealing cell morphology and distribution support the numerical results.

As a result, it can be concluded that polyurethane membranes can be prepared in medical purity without using catalysts or solvents or any other ingredients and these polymers can be modified by plasma-glow discharge application. Depending on the preparation and plasma modification conditions, it is possible to obtain surfaces with a range of hydrophilicities. Such a surface modification affects cell attachment capability of the membranes. It is therefore possible to modify a polymeric surface in accordance with the desired biomedical properties.

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