1026-1265/2001

Reactive Ester-urethane Prepolymer as Bioadhesive: Synthesis and Evaluation*

Nasrin Sheikh[1], Hamid Mirzadeh[2(**)] and Ali Akbar Katbab[3]

(1) Gamma Irradiation Centre, Atomic Energy Organization of Iran, Tehran, I.R. Iran

(2) Department of Polymeric Biomaterials, Iran Polymer Institute, P.O. Box 14965/115, Tehran, I.R. Iran

(3) Polymer Engineering Department, Amir Kabir University, Tehran, I.R. Iran

Received 30 September 2000; accepted 4 February 2001

ABSTRACT

Reactive urethane prepolymer, as a base material for the process of gluing in medicine, has been studied. For this purpose castor oil and toluene diisocyanate (TDI) were used as polyol and diisocyanate components to synthesize the isocyanate terminated urethane prepolymer. The molecular structure of this prepolymer was investigated by infrared spectroscopy (FTIR) and vapour pressure osmometry (VPO). The obtained infrared spectrum confirmed the presence of free NCO groups bonded to the prepolymer backbone. The wetting behaviour of the synthesized prepolymer was evaluated by surface tension measurement. The surface behaviour of the cured thin films of the prepolymer was characterized using contact angle measurements. The cytotoxicity of the cured prepolymer film was evaluated by in vitro cell culture experiment. Results showed a compatible cell response with no signs of toxicity.

Key Words: bioadhesive, contact angle, surface tension, wettability, cytotoxicity

INTRODUCTION

The most common and popular procedures, which have so far been employed by the surgeons to fasten soft body tissues, are surgical suturing and stapling. Both of these surgical techniques have been reported to have numerous drawbacks such as wound infection [1], sinus and granuloma formation, resulted from the suture degradation. Moreover, there are many cases where mechanical fastening and suturing might not be possible (space restriction) or not adequate to prevent fluid or blood leakage especially in cases of cardiovascular surgery.

As a result of these shortcomings, surgeons have thought of alternative means of fastening tissue such as the use of medical tissue adhesives. An adhesive used for wound healing and tissue bonding must meet a number of clinical requirements. The

(*) Part of this paper was introduced in the Fifth Iranian Seminar on Polymer Science and Technology, 12–14 Sep., 2000, Tehran.

(**) To whom correspondence should be addressed.

adhesive must hold the tissue together till the tissue regrow to heal the wound and then subsequently to degrade to biocompatible products. It must also be easy to handle and be used by the surgeon.

Cyanoacrylate adhesives have been considered and used in most surgical disciplines [2–4] for tissue bonding. However, all cyanoacrylate adhesives have been reported to degrade in aqueous media to formaldehyde, which causes inflammation and eventual scarring of the tissue [5]. The lack of bond flexibility after being polymerized can also cause problems in the applications where flexibility and softness are critical.

Among other synthetic materials, urethane based adhesives have been considered to be the most suitable which is connected to their biocompatibility, biodegradability and controlled microstructure and properties [6–8].

This article focuses on synthesis and preliminary evaluation of NCO-terminated urethane prepolymer in order to make clear its potential as adhesive for soft tissue.

EXPERIMENTAL

Materials

Castor oil (Aldrich) was used as polyester polyol and dried at 80 °C under vacuum. 2,4 Toluene diisocyanate (TDI) supplied by Riedel-de Haen Co. was used as received.

Synthesis

Castor oil and TDI were used for preparing of reactive urethane prepolymer. The synthesis procedure was carried out on the basis of prepolymer method. A weighed quantity of dried castor oil was charged into a flask equipped with stirrer, thermometer, dropping funnel, gas inlet and air condenser with a drying tube. During flushing with a slow stream of nitrogen the mixture was heated and stirred. Then an excess of TDI was added to the flask with stirring so that the NCO/OH ratio of 2 was obtained. The reaction was carried out at a proper temperature under nitrogen blanket and continued to obtain the theoretical value of NCO content based on the mentioned NCO/OH ratio in the prepolymer. For this purpose, NCO content was determined by titration method using di-*n*-butylamine according to the ASTM D-2572. Finally a purification process was carried out on the product so that a clear yellowish viscous liquid without unreacted TDI was obtained.

Analytical Measurements

The chemical structure of the prepolymer was characterized using a Brucker FTIR model IFS 45 spectrophotometer. The number average molecular weight of the prepolymer was determined by a Knauer vapor pressure osmometer (VPO).

Surface tension of the prepolymer was measured by a Kruss K12 tensiometer. For this purpose a vessel with the prepolymer lifted up to the lower edge of a platinum plate until the prepolymer liquid enters into contact with the plate. The pulling force of the prepolymer at the plate is a linear measure of the surface tension of the prepolymer. The surface tension is given by:

$$\sigma = F_w / L.\cos\theta \qquad (1)$$

where, F_w: Wilhelmy force, L: wetted length, and θ: contact angle between prepolymer liquid and platinum plate.

With the use of a platinum plate it can be assumed that the contact angle is 0°, and therefore the $\cos\theta$ becomes one.

Film Preparation

For the preparation of the polyurethane films, the liquid prepolymer was cast onto the surface of a glass plate and then left to be cured under ambient condition. The cured prepolymer films were then ultrasonically rinsed in distilled water/acetone and stored in a dessicator prior to use in experiments.

Surface Properties

Water compatibility of the cured uncatalyzed prepolymer film was determined by measuring static and dynamic water contact angles using the sessile drop and the Wilhelmy plate techniques. In sessile drop

technique, a drop of distilled water was put on the surface of the cured prepolymer film and then the angle between water drop and film surface was measured by a Kruss G10 goniometer.

But in Wilhelmy plate technique, the glass microscope coverslips coated with prepolymer were immersed into and drawn out of distilled water at a rate of 21 mm/min and a Kruss K12 tensiometer was used to calculate the advancing and receding contact angles.

Evaluation of Cytocompatibility

The cytocompatibility of the cured noncatalyzed prepolymer film was evaluated by in vitro cell culture test using L929 cell line. The cell suspension of 4×10^5 cells/mL was prepared before seeding. The cured urethane prepolymer film (PU) and control films, latex and tissue culture polystyrene (TCPS) were punched into discs of 15 mm diameter and then sterilized by gamma radiation method (25 kGy). Polymeric discs well -were individually placed into the well of a multi plate. A volume of 5 mL cell suspension was seeded into each well. These cultures were placed in a CO_2 controlled incubator at 37 °C for 1 and 5 days and then the media were decanted. The samples were washed with phosphate buffer saline solution (PBS), their attached cells were fixed by ethanol solution and stained with crystal violet or giemsa solution as staining agents. All air-dried samples were examined by light microscopy.

RESULTS AND DISCUSSION

Reactive urethane prepolymer was synthesized by the reaction between a polyester polyol and an excess of diisocyanate (Figure 1). We have obtained an optimization by different assessments. This ratio was found to be 2 according to the molecular weight of polyol and kind of diisocyanate used, which leads to a specified percent of free NCO groups. These free NCO groups are responsible for prepolymer curing, its adhesion to tissue model and also its biocompatibility. Below this percent of free NCO groups the curing of the prepolymer was observed to be very slow even though a catalyst was being used. Above this percent of free NCO groups, the prepolymer was found to be undesirable for the medical purpose due to high reactivity of NCO groups, because no chemically reactive substance is reported which can lead to adverse tissue reaction in the body [9]. As the medical use of the prepared prepolymer is intended, the thin layer evaporation method as a purification process was carried out so that the thin layer chromatography (TLC) results showed the absence of unreacted TDI [10].

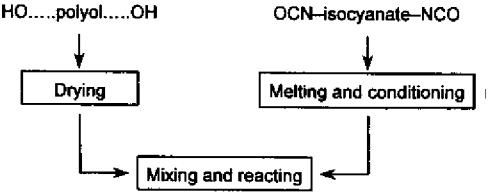

Figure 1. The stages for the synthesis of reactive-urethane prepolymer.

The FTIR spectrum of the prepolymer product is presented in Figure 2. The presence of NCO groups in the structure of the prepolymer is observed (2272 cm^{-1}) which probably enables a chemical bond to be formed between prepolymer layer and the adherend. Also, the spectrum of prepolymer contains polyurethane characteristic bands in the regions of 1540 cm^{-1} (amide), 1700 cm^{-1} (ester) and 3300 cm^{-1} (NH).

The number average molecular weight $\overline{M}_n$ of the prepolymer was found to be 1300 g/mol from

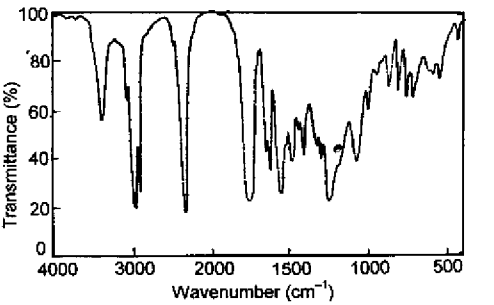

Figure 2. FTIR Spectrum of the synthesized prepolymer.

Table 1. Dynamic and static contact angles of water on urethane prepolymer film.

Angles (degrees)		
Dynamic	(Advancing & Receding)	Static
θa	θr	θ
85.3	49.3	67.8

vapor pressure osmometer.

The wetting behaviour of the synthesized prepolymer was evaluated by measuring surface tension, which was found to be about 21.7 mN/m. This value indicates good wetting properties for the liquid prepolymer.

The results of the water contact angle as the average of three measurements for the cured prepolymer films are presented in Table 1. The uncatalyzed cured prepolymer film had 85.3° and 49.3° as the advancing and the receding contact angle, respectively. The advancing and receding contact angles related to the hydrophobic and hydrophilic feature of film surface [11].

However, the presence of hard and soft segments on the surface of the urethane film can have profound effect on the water contact angle and therefore surface wettability. The result of contact angle measurements showed that the polyurethane surface is a substrate with moderate hydrophilicity.

Results obtained from in vitro cell culture are presented in Figures 3_a–3_d. For this type of test, a material is considered to be biocompatible if it supports cell attachment and growth [12]. The positive control (latex) is a toxic material therefore only few detached cells are observed on its surface (Figure 3_a). But the negative control (TCPS) supports cell adhesion and growth because it has no toxicity (Figure 3_b). In the case of the polyurethane film (Figure 3_d) similar behaviour has been shown for

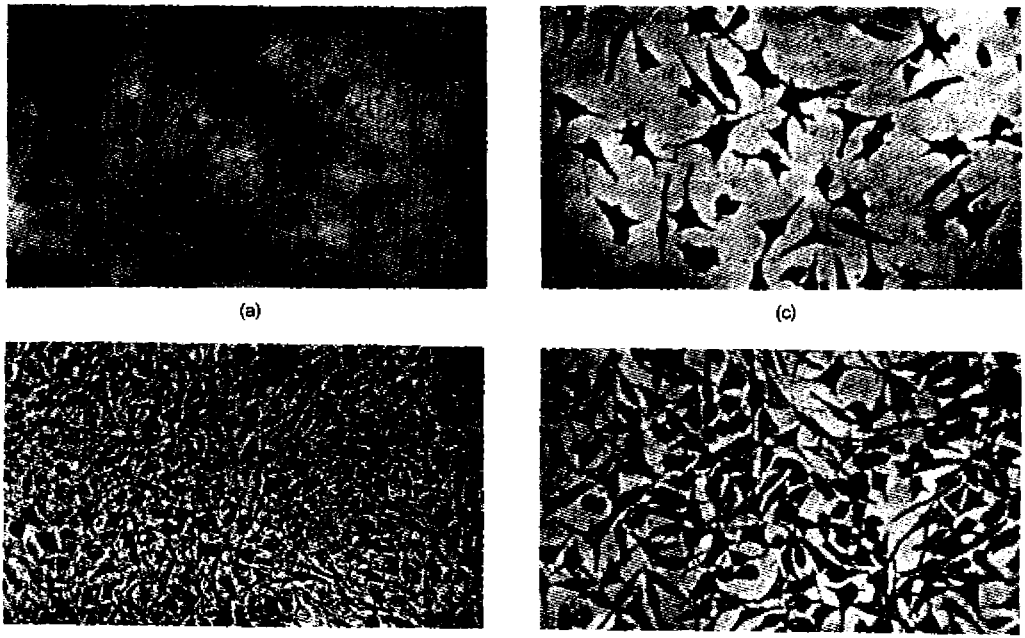

Figure 3. Cellular response of L929 fibroblast cells to the surface film (a) Latex, 5d incubation, (b) TCPS, 5d incubation, (c) PU, 1d incubation, (d) PU, 5d incubation.

the TCPS substrate. This result is in agreement with the high cell adhesion reported in the literature for a range of biomaterials possessing low to moderate hydrophilicity [13].

Comparison between the photomicrographs demonstrated in Figures 3_c and 3_d exhibits that the cell attachment and growth increases with the time of incubation. This cell response is a sign of cyto-compatibility of the urethane substrate.

CONCLUSION

NCO-Terminated urethane prepolymer in medical quality was prepared without ingredients beside monomers (TDI and Castor oil), and applying a purification process on the product. The cured prepolymer film was found to be a substrate with moderate wettability. In vitro cell culture test presented fibroblast cell attachment and growth on the cured prepolymer film without toxic responses.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support of the Gamma Irradiation Center, Atomic Energy Organization of Iran (AEOI) for this research. Also, the help providing by Cell Culture Lab. of Laser Research Center, AEOI in performing of cytotoxicity tests is gratefully acknowledged.

REFERENCES

1. Lerner R. and Binur S., "Current status of surgical adhesives", *J. Surgical Research*, **48**, 165, 1990.
2. Matsumoto T., Vienna Int. Symp., "Tissues adhesives in surgery", *Arch Surg*, **96**, 226, 1968.
3. Chivers R.A. and Wolowacz R.G., "The strength of adhesive-bonded tissue joints", *Int. J. Adhesion & Adhesives*, **17**, 2, 127, 1997.
4. Vanholder R., Misotten A., Roels H. and Matton G., "Cyanoacrylate tissue adhesive for closing skin wounds", *J. Biomaterials*, **14**, 10, 737, 1993.
5. Refojo M.F., Adhesives in Ophthalmology, In F.M. Polack (Ed.), Cornea and External Diseases of the Eye, First Inter-Am. Symp., Charles C Thomas, Springfield, I11, **183**, 1970.
6. Kutay S., Tincer T. and Hasirci N., "Polyurethanes as biomedical materials", *Brit. Polym. J.*, **23**, 267, 1990.
7. Laleh M.D. and Cooper S.L. Polyurethane in Medicine, CRC Press Inc., Florida, **57**, 1986.
8. Affrossman S., Barbenel J.C., Forbes C.D., Macllister J.M.R., Meng J., Pethrick R.A. and Scott R.A., "Surface structure and biocompatibility of polyurethanes", *Clinic. Mat.*, **8**, 25, 1991.
9. Park J.B. and Lakes R.S., Biomaterials: An Introduction, Plenum, New York, 223, 1992.
10. Sheikh N., Katbab A.A. and Mirzadeh H., "Isocyanate terminated urethane prepolymer as bioadhesive base material: synthesis and characterization", *Int. J. Adhesion and Adhesives*, **20**, 299, 2000.
11. Garbassi F., Morra M. and Occhiello E., Polymer Surfaces From Physics to Technology, John Wiley, New York, 177, 1995.
12. Pizzoferrato A., Ciapetti G., Stea S., Cenni E., Arciola C.R., Granchi D. and Savarino L., *Clinic. Mat.*, **15**, 173, 1994.
13. Lydon M.J., Minett T.W. and Tighe B.J., "Cellular interactions with synthetic polymer surfaces in culture", *Biomaterials*, **6**, 396, 1985.