International Journal of Biological Macromolecules 52 (2013) 32–37

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

# Evaluation of polyurethane based on cellulose derivative-ketoprofen biosystem for implant biomedical devices

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ARTICLE INFO

*Article history:*
Received 23 August 2012
Received in revised form
18 September 2012
Accepted 25 September 2012
Available online 2 October 2012

*Keywords:*
Polyurethane biosystem
Ketoprofen
Cytotoxicity
*In vivo* biocompatibility
Water sorption/desorption

ABSTRACT

A polyether-urethane based on polytetrahydrofuran containing hydroxypropyl cellulose for biomedical applications was tested for its biocompatibility. Ketoprofen was incorporated (3% and 6%) in the polyurethane matrix as an anti-inflammatory drug. Dynamic vapour sorption method was employed for testing the water sorption/desorption behaviour of these materials with the determination of the surface isotherms, surface parameters and the kinetic curves of sorption/desorption processes. Cytotoxicity testing *in vitro* for quantifying cell proliferation was employed, and the results evidence noncytotoxicity for the studied polyurethane–drug systems. *In vivo* biocompatibility study was performed on 200 g weight male rats. It was found that after implantation of the polyether-urethane samples a reduced acute inflammation occurred, especially for polyurethane samples with added ketoprofen.



## 1. Introduction

Segmented polyurethanes have gained considerable scientific and technical interest as useful biomaterials for implants or biomedical devices [1–4]. This class of polymers have been extensively used for various commercial and experimental blood contacting and tissue-contacting application, because of their generally excellent surface physical properties, together with their fairly good biocompatibility and haemocompatibility characteristics [5–7]. Cytotoxicity tests are important for the investigation and assessment of biocompatibility of implantable biomaterials, and cytotoxicity testing represents the initial phase in testing biocompatibility of prospective medical devices [8–10]. The biocompatibility of a material regarded as the answer of cells in contact with a material or with its degradation compounds, can be identified with the characteristics of degradation and toxicity. There are standardized methods for quantifying cell proliferation, by counting the number of cells in cultivation at different intervals after seeding. After cytotoxicity testing *in vitro* and the direct contact of the testing systems with cells in order to establish their profile when in contact with target tissue/organ, the next step in the evaluation of the biocompatibility and functionality of the proposed system constitutes the *in vivo* testing.

Polyester- and polyether-urethanes have been modified [11–13], with hydroxypropyl cellulose to improve their surface characteristics and to generate biomaterial qualities. Platelet adhesion test has been carried out *in vitro* and it was found that the cellulose component in the polyurethane matrix reduces the platelet adhesion.

The biological characteristics in contact with blood and tissues for long periods, in particular good antithrombogenic properties, recommend the use of extracellular matrix components such as collagen, elastin and glycosaminoglycans (GAG) for obtaining biomaterials [14–18]. As to biomaterials, segmented block-copolyurethanes are synthesized, starting from a polyol, which is the soft segment, a diisocyanate and a chain extender. The combination of the chain extender and the diisocyanate components is referred to as the hard segment of the polymer. It was soon realized that biocompatibility is intimately related to their microphase separated structure composed of hard and soft segment domains.

The water vapour or moisture sorption properties of polyurethane biomaterials are known important factors in evaluating their surface properties and determination of interfacial response in living organism. Moisture sorption properties are routinely determined for many biomaterials and have traditionally been evaluated by using "classical" methods.

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0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.09.026

In this paper the dynamic vapour sorption (DVS) method was applied for polyurethane materials with and without ketoprofen having different compositions and water affinity. In order to obtain a reproducible isotherm, the time pre-established for equilibration at each RH step should be long enough. In the case of adsorption water equilibrium content increased with the increase of RH, and opposite trend was observed in the case of desorption.

For estimation of the haemocompatibility properties of the obtained materials, water sorption was determined as well as the amount of fibrinogen adsorbed from solution, the amount of fibrinogen adsorbed from blood plasma, and the time of prothrombin consumption [12].

Functional longevity achievement is an important task of tissue or blood-device performing. Under normal physiological conditions, after material–tissue interaction, the body reacts in order to protect the body from the foreign implanted object. The reacting mechanisms are nonspecific one and imply immune and inflammatory cells recruitment at the implanted site. The phenomenon is termed as foreign body reaction. In the present work preliminary *in vitro* test and *in vivo* biocompatibility study of polyether-urethane containing different percentages of ketoprofen is presented.

## 2. Experimental

### 2.1. Materials

Polytetrahydrofuran (PTHF, $M_n$ = 2000 g/mol) was purchased from BASF; 4,4′-diphenylmethane diisocyanate (MDI, Merck) was distilled, prior to utilization, under reduced pressure; hydroxypropylcellulose LF (HPC, Klucel) and ethylene glycol (EG, Merck) were used as received. Ketoprofen is commercial product purchased from Sigma–Aldrich. Ketoprofen, is a non-steroidal anti-inflammatory drug with analgesic and antipyretic effects.

#### 2.1.1. Preparation of polyurethane

The polyurethane was prepared by conventional two-step solution polymerization using N,N-dimethylformamide (DMF, Fluka) as solvent. First, the NCO-terminated prepolymer was prepared by dehydrating the macrodiol for 3 h at 90 °C under vacuum. Then MDI was added to the vigorously stirred macrodiol. The reaction between diisocyanate and macrodiol was carried out for 1.5 h under nitrogen atmosphere at 90 °C. After lowering the temperature to 70 °C the ethylene glycol was added. The reaction continued for 1 h, at 70 °C and then a solution of hydroxypropylcellulose in 10 ml DMF was added and the stirring continued for another 0.5 h. The resulting polymer was precipitated in water and dried under vacuum for several days. The synthesis route of polyetherurethane containing HPC is presented in Scheme 1.

The number-average molecular weights, $M_n$, and the dispersity of the polyurethane (polymer solution in DMF, 1 g/dL), was determined by using a GPC PL-EMD 950 evaporative mass detector instrument. It was found $M_n$ = 70,291; $M_w/M_n$ = 1.59; composition PTHF/MDI/EG/HPC, wt%: 52.24/36.57/7.27/3.92.

#### 2.1.2. Sample preparation for DVS measurements

Solutions in N,N-dimethyl formamide of polyurethane solely and with added ketoprofen (3 wt% and 6 wt%) were prepared and homogenized under vigorously stirring followed by solvent casting under high vacuum.

The polymeric materials were processed as thick films and small fragments of these films were cut and placed in the stainless steel sample bucket of fine mesh. The amount of sample is dictated by the capacity of the microbalance and the vapour adsorption capacity of the sample.

#### 2.1.3. Human dermal fibroblast (HDF) isolation

HDF were isolated by explant method, using human skin resulted from abdomenoplasty surgery. Briefly, 1 $cm^2$ of human skin was sterilized using 3-step washing procedure in the Dulbecco's Modified Eagle Medium (DMEM – Sygma) with decreasing antibiotic concentration as follows: 1st step – 10 min vortexing in 4% penicillin/streptomycin/neomycin (PSN) solution (Sygma) in DMEM; 2nd step – 10 min vortexing in 2% PSN solution and 3th step – 10 min vortexing in DMEM without PSN. After the last washing step, the hypodermic part of the skin was removed, whilst dermis and epidermis were cut in small pieces (1–2 $mm^2$) and then plated with the dermis-side down on the thin layer of the bovine foetal serum (BFS – Sygma) by adding small amount of DMEM containing 10% BFS and 1% PSN. After 2–3 weeks of culturing at 37 °C and 5% $CO_2$ atmosphere, with each 3-day medium refreshing, cells were passed on the 25 $cm^2$ surface area culture flask (Nunc) and cultured until the confluent cell shit were reached.

#### 2.1.4. Sample preparation and cell plating

Before performing cell proliferation assay, polyurethane samples was sterilized for 15 min in 70% ethanol solution, washed in sterile phosphate buffer (PBS – Sygma) and then incubated in DMEM at 37 °C and 5% $CO_2$ atmosphere for 48 h for equilibration. After equilibration, polyurethane samples were transferred on the 24-well cell culture plate. One sample was used for each plate well in triplicate mode. For each polymeric sample an initial concentration of 40,000 HDF cells/well was added and incubated for 24, 48 and 72 h chosen as end point for MTT assay performing.

### 2.2. Methods

#### 2.2.1. Determination of the water sorption/desorption isotherms

It was employed the fully automated gravimetric analyser IGAsorp (Hiden Analytical, Warrington (UK)) equipped with an ultrasensitive microbalance (0.1 μg resolution for 100 mg range and a 200 mg capacity) for measuring the weight change as the humidity is modified in the sample chamber under constant temperature. Level of humidity is controlled to desired RH set-point by mixing wet and dry gas ($N_2$) streams. The weight signal is accurately analysed to determine when the uptake has reached the new equilibrium value. Once the equilibrium has been attained, the next desired humidity level is set. This process is automatically performed for desorption isotherm measurement. The relative humidity measurement ranges between 1 and 95% (accuracy of ±1% for 0–90%RH and ±2% for 90–95%RH). The measurement range of temperature is between 5 and 80 °C (accuracy of ±0.05 °C). The sample container is a gas permeable micromesh stainless pan for solids or a Pyrex bulb for liquids and fine powders.

The samples placed in the container were placed on the microbalance and dried at 25 °C under flowing nitrogen until the weight of each sample is in equilibrium at RH < 1%. The obtained value is considered the dry mass. After drying, the absorption curve is determined. After the maximum level for RH has been attained, desorption steps can be achieved.

#### 2.2.2. In vitro biological evaluation test

A systemic toxicity test method was applied according to standard method ISO 10993 – Biological evaluation of medical devices. In our laboratory it is employed for isolation of neutrophils HBSS (Hank's balanced salt solution), without calcium and magnesium, with phenol red, from Sigma.

MTT cell proliferation assay was done using primary human dermal fibroblasts in contact with polyurethane surface. The dimensions of the film samples were 5 mm × 5 mm and thickness is 1 mm.