Applied Surface Science 270 (2013) 553–560

Contents lists available at SciVerse ScienceDirect

Applied Surface Science

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

Surface properties of polyurethane composites for biomedical applications

Ida Dulińska-Molak[a], Małgorzata Lekka[b,*], Krzysztof J. Kurzydłowski[a]

[a] Faculty of Materials Science and Engineering, Warsaw University of Technology, Wołoska 141, 02-507 Warsaw, Poland
[b] The Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Radzikowskiego 152, 31-342 Kraków, Poland

ARTICLE INFO

Article history:
Received 20 April 2012
Received in revised form 10 January 2013
Accepted 11 January 2013
Available online 24 January 2013

Keywords:
Polyurethane
Composites
Calcium carbonate
Adhesion force
Surface free energy

ABSTRACT

The development of tissue engineering in the field of orthopedic surgery is now booming. Biocompatibility is one of the most important characteristics of a biomedical polymer and composites material whose surface is required to interact with a biological system. Since proteins are viewed as the primary and the most important substrate in mediating polymer–organism interactions, the status of the proteins on a material surface is believed to determine the ultimate biocompatibility of a given biomaterial. In order to achieve specific responses between biomaterial surfaces and the adjacent cells, the principles for designing biocompatible materials are brought forth decorating polymer surfaces with bioceramic particles (aragonite and calcite) to induce specific protein adsorption and cell responses. In this work, we describe the adhesion properties of polyurethane/calcium carbonate composites. An understanding of the phenomena of cell adhesion and, in particular, understanding of the proteins involved in osteoblast adhesion on contact with the materials is of crucial importance, the adhesion between fibronectin (FN) and composites PUR/$CaCO_3$ surfaces were examined using atomic force microscopy (AFM). Moreover, it is found that is correlation between the detachment force and surface free energy (SFE). At the end, in order to estimate the cellular biocompatibility, the human bone derived cells (HBDC) were cultured on PUR/$CaCO_3$ composites.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Among polymers selected for clinical and surgical applications, polyurethanes (PUR) represent a very important class of materials due to their high biocompatibility and processibility [1,2]. The domain structure of polyurethanes enables to produce materials in wide range of physical and mechanical properties. In particular the biodegradable poly(ε-caprolactone) urethane elastomers are commonly used for production of scaffolds in bone tissue engineering [3–5]. PURs have tunable mechanical properties. The addition of fillers and fabrication of polymer-ceramic composites. Such materials combine advantages of the individual components: selection elasticity of polymers with excellent biocompatibility of ceramics filler [6]. Depending on the type of filler used and on its spatial distribution, the composite materials introduce large possibilities of tailoring both mechanical and biological properties behavior of such implant. For example addition of calcium carbonate (both as: aragonite and calcite) allows to stimulate the growth of bone tissue [7–10]. Such fillers in contact with body fluids form a layer of hydroxyapatite on their surface stimulating a permanent connection to the bone in a living organism [11].

The use of a composite materials for fabrication of implants depends on the physico-chemical properties of their surface, mainly on surface free energy (SFE). SFE has been also reported to be an important surface characteristic influencing the adhesion strength and proliferation of cells [12,13]. In view of this the studies reported in this paper were focused on characterization of surface properties of polyurethane/calcite and polyurethane/aragonite composites. The analyses started from the characterization of calcium carbonate particles (both calcite and aragonite) used as fillers. Then, their adhesion has been analyzed using contact angle measurements and atomic force microscopy. Moreover, study of the adsorption proteins was carried out, this phenomena plays a vital role in determining the nature of the tissue–implant interface. At the end, in order to estimate the cellular biocompatibility, the human bone derived cells (HBDC) were cultured on polyurethane/$CaCO_3$ composites. Finally the results are discussed in terms of cellular growth on the composites.

2. Materials and methods

Three types of polymeric based samples were prepared: (1) pure polyurethane, (2) polyurethane/calcite, and (3) polyurethane/aragonite composites. The polyurethanes were synthesized using the following reactants: 4,4′-dicyclohexylmethane diisocyanates (HMDI), polycaprolactone diol (PCL diol) with

* Corresponding author. Tel.: +48 126628271.
E-mail address: malgorzata.lekka@ifj.edu.pl (M. Lekka).

0169-4332/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsusc.2013.01.085

molecular weight 530 Da, purchased from Aldrich Chemical Co. (Germany). Polyol was dehydrated during mixing under vacuum conditions for two hours at a 120 °C. Ethylene glycol (EG) (POCH, Gliwice) was dried under a molecular sieve.

The calcium carbonate ($CaCO_3$) calcite and aragonite powders were prepared at the Institute of Ceramics and Building Materials (ICiMB, Warsaw, Poland). Microscopic observations of their morphology and chemical composition analyses were performed using the scanning electron microscopy (S-2600, HITACHI) equipped with energy dispersive spectroscope (EDS). Specimens for SEM characterization were coated with a thin film of gold and observed at an accelerating voltage of 15–20 kV. X-ray diffraction (XRD, powder diffractometer, PHILIPS) was applied to identify the phase composition of the calcium carbonate powders.

To study the adhesion forces that occurs between the composite's surface and proteins, both fibronectin and fibrinogen were deposited on the prepared surfaces. The deposited fibronectin (FN) originates from human plasma (F2006, Sigma). It is a multifunctional, extracellular matrix glycoprotein composed of two nearly identical polypeptides, attached together by disulfide bonds, each of molecular weight 220 kDa.

Human fibrinogen fluorescently labeled with Alexa Fluor 488 was delivered by Molecular Probes (F-13191). It is a key component in the blood clotting process and can support both platelet–platelet and platelet–surface interactions by binding to the glycoprotein IIb-IIIa (GPIIb-IIIa) receptor.

The PBS buffer (i.e. phosphate buffered saline, Sigma), used in the experiments contained 147 mM and 27 mM of NaCl and KCl, respectively. It was used a buffer for fibronectin, fibrinogen, and glutaraldehyde (2.5%, POCH, Gliwice, Poland) solutions.

2.1. Synthesis of polyurethanes and composites

Segmented polyurethanes with molar ratio of HMDI/PCL/EG 2:1:1 and a constant isocyanate index 1.02 were synthesized in molds by the pre-polymer method. Soft segments based on a poly(ε-caprolactone)diol and hard segments were composed of 4,4′-dicyclohexylmethane diisocyanates and EG – the synthesized polyurethanes contain about 52 wt.% of the hard segments (contents of 52% of hard segments delivers the highest cell viability and proliferation rate). The PCL diol was mixed under vacuum for 2 h at 120 °C. Next the HMDI was added and the reaction was carried out at a 60 °C for 50 min. After that EG was added and the substrates were mixed for 15 min. Finally the mixture was kept at a temperature 110 °C for 16 h to complete polymerization.

Polyurethane/$CaCO_3$ composites were prepared by in situ polymerization. Synthesis was performed with a pre-polymer method. PCL diol and calcium carbonate were mixed under vacuum conditions for two hours at a temperature 110 °C. Afterwards, the synthesis proceeded in the same way as for PUR. The fillers (either calcite or aragonite) were added to the polyurethane matrix in the quantity yielding their weight content equal to 10 wt.%. Poly(ε-caprolactone)urethane and the composites were synthesized without solvents and catalysts. Before cell research the obtained polyurethane and PUR composite samples were sterilized using ionizing irradiation (25 kGy). The sterilization method was based on earlier results published in [14].

2.2. Cells

The human bone derived cells (HBDC) were cultured on polyurethane/$CaCO_3$ composites to estimate their cellular biocompatibility. The cells were isolated from the trabecular bone tissue chips which were harvested from the bottom distal part of the long tight bone during a standard procedure of the knee joint alloplasty. The procedure was approved by the Local Ethics Committee of the Warsaw Medical University (Decision No. KB/74/2005) and the donors provided informed consent. HBDC isolation was based on the protocols described by Gallagher et al. [15], with modifications published in [16], as described in details in [17]. Cells were cultured in DMEM (Dulbecco's Modified Eagle's Medium, Gibco) supplemented with 10% (final concentration) fetal bovine serum (FBS) (Gibco), L-glutamine (1% solution in medium–based on stock solution (100×), Gibco), 1% antibiotic–antimycotic mixture (containing 10,000 units of penicillin (base), 10 mg of streptomycin (base), and 25 lg of amphotericin B ml^{-1} – Gibco BRL, Paisley, UK), and 100 μM L-ascorbic acid 2-phosphate (Sigma). They were seeded on a surface of polyurethane/$CaCO_3$ composite samples of $d = 6$ mm diameter and $h = 2$ mm height. As a reference, a surface of the bottom of one well of 96-wells microplate was used. The culture was carried out for 7 days in the CO_2 incubator (at 37 °C in 5% CO_2, humidified atmosphere). After 7 days the Alkaline Phosphatase (AP) Assay Kit (AP Diagnostic Kit 104, Sigma) was applied. This is a plate-based, colorimetric-endpoint assay for the determination of alkaline phosphatase enzyme in serum from rodents or other mammals. The amount of alkaline phosphatase was determined using ELISA reader (FLUOstar OPTIMA–BMG LABTECH) at wavelength 405 nm.

2.3. Measurements of surface free energy

The contact angle measurements were performed using the sessile drop method with PG-X (FIBRO System AB, Sweden). The image of a drop was construed with a video camera and the contact angle was calculated from the shape of the drop. Three liquids were used as probes for surface free energy calculations: di-iodomethane (Sigma), formamide (Sigma), and distilled water. Contact angles were measured at room temperature, and the drop was deposited on the surface after cleaning ultrasonically for 20 min in distilled water and drying air. For each sample 10 drops were analyzed (two different samples per surface type).

The surface free energies were calculated using two theoretical models: (i) Owens Wendt (OW) and (ii) Van Oss (VO). The OW model allows to calculate the long-range dispersion (Lifshitz–van der Waals) γ_S^D and the short-range polar (hydrogen bonding) γ_S^P components of surface free energy [18], expressed as:

$$\sqrt{\gamma_S^D} = \frac{\gamma_d \cdot \left(\cos\Theta_d + 1\right) - \sqrt{\frac{\gamma_d^P}{\gamma_w^P}} \cdot \gamma_w \cdot \left(\cos\Theta_w + 1\right)}{2 \cdot \left(\sqrt{\gamma_d^D} - \sqrt{\gamma_d^P \cdot \frac{\gamma_w^D}{\gamma_w^P}}\right)} \quad (1)$$

$$\sqrt{\gamma_S^P} = \frac{\gamma_w \cdot \left(\cos\Theta_w + 1\right) - 2 \cdot \sqrt{\gamma_S^D \cdot \gamma_w^D}}{2 \cdot \sqrt{\gamma_w^P}} \quad (2)$$

In these formula γ_d is the surface free energy of di-iodomethane, γ_w is the surface free energy of water, γ_D^P is the polar component of γ_d, γ_w^P is the polar component of γ_w, γ_d^D is the non-polar (dispersive) component of γ_d, γ_w^D is the non-polar (dispersive) component of γ_w and Θ_d and Θ_w are the contact angle of the test liquids, di-iodomethane and distilled water, respectively.

The VO approach provides estimates of the dispersive γ_S^{LW} and the polar acid–base γ_S^{AB} components. The latter is divided into two parts, i.e. acidic $\gamma_S{}^+$, $\gamma_L{}^+$ and basic $\gamma_S{}^-$, $\gamma_L{}^-$[19], according to the following equations (S and L denotes the investigated surface while L–liquid):

$$\gamma_{S,L} = \gamma_{S,L}^{LW} + \gamma_{S,L}^{AB} \quad (3)$$

$$\sqrt{\gamma_S^{LW} \cdot \gamma_L^{LW}} + \sqrt{\gamma_S^+ \cdot \gamma_L^-} + \sqrt{\gamma_S^- \cdot \gamma_L^+} = \frac{\gamma_L \cdot \left(1 + \cos\Theta\right)}{2} \quad (4)$$

Download English Version:

https://daneshyari.com/en/article/5360466

Download Persian Version:

https://daneshyari.com/article/5360466

Daneshyari.com