THE PROPERTIES OF POLYURETHANES BASED ON SYNTHETIC POLYHYDROXYBUTYRATE FOR MEDICAL APPLICATION

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ABSTRACT

Polyurethanes belong to materials commonly used in many different fields of our life. Very important part of their application is using as biomaterial. But their properties have to be appropriate for contact with living organisms. Using in synthesis of polyurethanes the synthetic analog of natural polyhydroxybutyrate - telechelic atactic poly[(*R,S*)-3-hydroxybutyrate] (a-PHB) could make them more biocompatible.

The investigated polyurethanes were synthesized with aliphatic and aromatic diisocyanate. Their soft segments were built with polycaprolactone and a-PHB. The physical, mechanical, thermal properties and degradability of obtained polyurethanes were estimated. The polyurethane samples were also checked for medical application. After the extraction in hexane and sterilization their influence on blood parameters and on pathogenic microorganisms were estimated.

The obtained polyurethanes were amorphous and hydrophilic but the amount of absorbed water was small and is on the same level for both materials. Sterilization of aliphatic polyurethane samples insignificantly changed the mechanical properties whereas aromatic polyurethane seemed to be more sensitive to conditions of sterilization. The results of polyurethane degradation indicated higher influence of oxidative than hydrolytic mechanism. The presence of aliphatic polyurethane decreased the growth of some pathogenic microorganisms. Both kinds of polymers did not change the parameters of whole blood.

In conclusion it could be stated that aliphatic polyurethane based on atactic poly[(*R,S*)-3-hydroxybutyrate] displayed the properties appropriate for further investigations for medical application without the risk of generation the cancerogenic degradation products which could be formed from polyurethane obtained with aromatic diisocyanate.

Keywords: aliphatic polyurethane, aromatic polyurethane, atactic polyhydroxybutyrate, biomaterial

INTRODUCTION

One of the most important materials for medical applications are polyurethanes (PUR). They can be used both as degradable and non-degradable implants (Chan-Chan 2010). Their unique properties are a consequent of their segmented (hard and soft domains) structure (Gaymans 2011, Resiak 2000). Regularity and ordering of domains determine the mechanical and physical properties of PURs and influence on blood compatibility what is extremely important.

The hard segments are products of the reaction of diisocyanate and chain extender, and soft segments are built with one or more oligomers. Kind of oligomer is a major feature influencing polyurethane biodegradability. Polycaprolactone (PCL) is the oligomer used in synthesis of degradable PUR. It is known that products of PCL degradation in living organism are non-toxic (Gorna and Gogolewski 2002).

The latest investigations show that very promising oligomer for obtaining biocompatible material is the chemically synthesized substitute of natural polyhydroxybutyrate - atactic poly([R,S]-3-hydroxybutyrate) (a-PHB). Product of its degradation – 3-hydroxybutyric acid – is a common metabolite in human blood. It is produced in ketone bodies of mammals during the prolonged starvation (Piddubniak 2004). Moreover 3-hydroxybutyric acid belongs to short-chain fatty acids and reveals antibacterial activity (Defoirdt 2009).

Usually the aromatic diisocyanate were used in synthesis of polyurethanes for medicine. However, it is known that potential product of their degradation under conditions of environment of living organism could be an aromatic diamine which revealed cancerogenic properties (Guelcher 2007, Santerre et al. 2005). To avoid this, the aromatic diisocyanates are replaced by aliphatic compounds very often.

The aim of work is to estimate selected physical, mechanical and thermal properties of aliphatic polyurethane which could be used for obtaining degradable scaffolds for cell growth of soft tissues. According to potential application its degradability and the influence on blood parameters and on pathogenic microorganisms growth are also investigated. The similar properties of aromatic polyurethanes are estimated for proving that aromatic diisocyanate can be substituted by aliphatic compounds.

1. EXPERIMENTAL

1.1. Materials

Two kinds of polyurethanes are investigated. Polymers are synthesised in a two-step reaction as described previously (Brzeska et al. 2010, Brzeska et al. 2011). The soft segments of PURs are built with the mixture of polycaprolactone and atactic

poly([R,S]-3-hydroxybutyrate). Hard segments of PURs are synthesized in reaction of 1,4-butanediol and diisocyanate. 4,4'-methylenedicyclohexyl diisocyanate (H_{12}MDI) is used for PUR-H_{12}MDI (Brzeska et al. 2011), and 4,4'-diphenylmethane diisocyanate (MDI) is used PUR-MDI (Brzeska et al. 2010).

1.2. Methods

Differential scanning calorimetry (DSC)
Thermal properties of polyurethanes are determined using of DuPont 9900 thermal analyser. The specimens are heated in sealed aluminium pans and scanned from -80°C to 200ºC. All experiments are done in a flow of dry N_2.

The tensile strength is evaluated according to PN-ISO 37:2007 and ***hardness*** in Shore A is evaluated according to PN-C-04238:1980.

Density of polyurethanes is estimated according to PN-92/C-89035. The polymer samples are weighted in air and in deionized water at room temperature.

The oil sorption of polyurethanes is estimated by immersing of polymer samples in sunflower oil at 37ºC (physiological temperature of human body) for 24 hours. Subsequently the samples are taken away from oil and after cleaning with paper sheets and then weighted (Masiulanis et al. 2000).

Hexane extracts are prepared according to Polish Pharmacopeia (Polish Pharmacopeia 2002): 1 g of investigated polyurethane samples are immersed in 50 ml of boiling hexane for 4 hours. The dry mass of residue is estimated after solvent evaporation from 25 ml of hexane solution.

The wetting angle before and after the H_2O_2 plasma sterilization is estimated using SEO Phoenix Mini Portable Contact Angle Analyzer by immersion of water drop on polymer sample at room temperature.

The water sorption is estimated by controlling mass changes after 1 hour, and next after 1, 2, 3, 7 and 14 days of incubation in deionized water at 37ºC (Glarner and Gogolewski 2007).

Solubility of polyurethanes is investigated by immersion of PURs samples in chloroform ($CHCl_3$), N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), ethanol and in ethanol/acetone.

Degradation in buffer solution
The samples of polyurethanes are immersed in phosphate buffer solution (pH=7.4) for 4, 12, 24 and 36 weeks. The pH of the ageing medium is checked every 2 weeks and replaced if the pH dropped more than 0.5 (Glarner and Gogolewski 2007). The rate of degradation is estimated by the weight changes of polyurethane samples.

Degradation in oxidative solution
Oxidative degradation of polyurethane samples is carried out for 2, 4, 12, and 16 weeks, using oxidative solution of 20% hydrogen peroxide in 0.1M cobalt chloride(II)

(Christenson et al. 2006). Solutions are changed every week to maintain a relatively constant concentration of radicals (Feng and Li 2006). The rate of degradation is estimated by the weight changes of polyurethane samples.

Zone of inhibition measurements

Zone of inhibition measurements are performed on circle PUR-H_{12}MDI samples (8 mm of diameter), to evaluate their influence on typical for biomaterial infections microorganisms: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Candida albicans* (derivated from clinical material) (Brzeska et al. 2011 B). The average distance between the edges of the polymer material and the beginning of the bacterial and fungal lawn is determined (Kébir N. et al. 2007)

Hemocompatibility of obtained polyurethanes is estimated by observations of changes in morphology and coagulation parameters of whole blood after direct contact with polymer samples (Brzeska et al. 2011 A). The polyurethane samples (sterilized using H_2O_2 plasma) are immersed in separate test tubes containing fresh blood and incubated at 37ºC for 15 minutes and 4 hours. Morphologic parameters, using flow cytometry method (hematologic Cell Dyn 3500 analyzer), are estimated. Whereas coagulation parameters are estimated by measuring fibrinogen concentration (photooptical method, ACL 9000 analyzer) in blood plasma.

2. RESULTS AND DISCUSSION

The selected properties - important for material which could be used for producing synthetic implant are determined for polyurethanes based on aromatic and aliphatic diisocyanates.

Table 1. Thermal properties of polyurethanes

PUR	Glass transition temp. Tg[°C]	Melting temp. of soft segments Tm_1[°C]	Melting enthalpy ΔHm_1 [J/g]	Melting temp. of hard segments Tm_2 [°C]	Melting enthalpy ΔHm_2 [J/g]
PUR-MDI	-26.4	–	–	162.5	7.8
PUR-H_{12}MDI	-32.8	50.0	2.1	117.0	1.4

Melting enthalpy both soft (ΔHm_1) and hard (ΔHm_2) segments of obtained polyurethanes are very small what indicates that these polymers are amorphous. The soft segments of aliphatic polyurethane PUR-H_{12}MDI are more separated what is seen in lower glass temperature (Tg) in comparison to PUR-MDI.

A very small crystallinity in PUR-H_{12}MDI structure observed at 50°C (Tm_1) is a consequent of the ordering of oligoesters chains. On the other hand aliphatic diisocyanate H_{12}MDI contains the mixed isomers what causes that the hard segments cannot crystalize as hard segments in PUR-MDI.

Table 2. The density of polyurethanes and their mechanical properties

PUR	Density [g/cm^3]	Hardness [°Shore A]	Tensile strength R_r [MPa]		Elongation at break ε_r [%]	
			before sterilization	after sterilization	before sterilization	after sterilization
PUR-MDI	1.19±0.01	81	9.8±0.3	7.8±0.8	532±40	537±18
PUR-H_{12}MDI	1.15±0.01	86	8.3±1.5	9.4±1.5	361±80	287±99

The hardness of polyurethanes is similar to typical polyurethane elastomers used in medical application (Gogolewski 1989).

The relatively small tensile strength (R_r) and quite high elongation at break (ε_r) suggests that obtained polyurethanes could be used for reconstruction of soft tissue.

Very important parameter for qualification of material as biomaterial is its sterilizability. The tensile strength and elongation at break of PUR-H_{12}MDI samples are changed insignificantly after the sterilization with H_2O_2 plasma whereas PUR-MDI seems to be more sensitive to conditions of sterilization.

Table 3. The wetting angle, oil absorption and mass residue after hexane extraction of polyurethanes

PUR	Wetting angle [°]		Oil sorption [%]	Mass residue after hexane extraction [mg]
	before sterilization	after sterilization		
PUR-MDI	61.4±5.2	66.0±2.8	0.7±0.05	1.50
PUR-H_{12}MDI	63.6±0.6	67.5±1.1	0.7±0.01	0.60

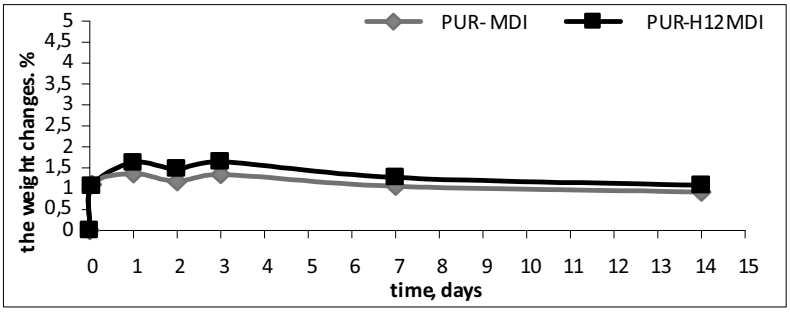

Fig. 1. Water uptake during incubation of polyurethanes in deionized water

The wetting angle of polymer surface suggests that polyurethanes are hydrophilic but the amount of absorbed water is small and is on the same level for both materials (Fig. 1). Probably the macrochains of polymers are tightly arranged in polymer structure. A bit higher water uptake in case of PUR-H_{12}MDI is connected with their lower density in comparison to PUR-MDI (Table 2).

The sterilization of polyurethanes samples using H_2O_2 plasma causes a very small increasing of wetting angle both for polyurethane based on aromatic and aliphatic diisocyanate.

The influence of lipids, which can be found in natural fluids of living organism, on polymers can be simulated by absorption of sunflower oil or by hexane extraction. The value of absorbed oil is small and identical for both polyurethanes (Table 3). According to Polish Pharmacopeia (Polish Pharmacopeia 2002) the mass residue after hexane extraction of polymer sample for medical application cannot be higher than 15 mg. The residue masses, obtained after the hexane evaporation from polyurethanes extracts, are much smaller (Table 3).

The solubility in different solvents is a very important parameter of material for medical implant (for example manufactured by electrospinning).

Table 4. Solubility of polyurethanes in organic solvents

PUR	DMF	DMSO	$CHCl_3$	Ethanol	Ethanol/acetone
PUR-MDI	+	/+/	389±31% (degree of swelling)	–	–
PUR-H_{12}MDI	+	//+//	+	–	–

„+" means soluble at room temperature, „/+/" means soluble at 37°C, „//+//" means soluble at 50ºC, „–" means insoluble

Both investigated polymers are soluble in DMF, DMSO and they are insoluble in ethanol and mixture of ethanol/acetone. In $CHCl_3$ PUR-MDI is swollen.

The potential application of obtained polyurethanes could be degradable scaffolds for soft tissue rebuilding. The estimation of their degradability is necessary. In vitro degradation is the first step in investigation of material sensitivity to influence environmental conditions.

The hydrolysis is a very important mechanism of degradation of implanted material because a human body contains 50–70% of water. On the other hand after the immersion of implant in living organism the natural mechanisms of immunology generate oxidative compounds.

The obtained polyurethanes are degraded in hydrolytic and oxidative solutions.

Presented in Fig. 2 and 3 results of polyurethane degradation indicates higher influence of oxidative than hydrolytic mechanism. The weight reducing polymer samples after incubation in oxidative solution is higher in case of polyurethane based on aromatic diisocyanate.

Biomaterials implanted into living body are in contact directly with blood ingredients. The presence of biomaterial should not induce any changes in components of blood and in their concentration.

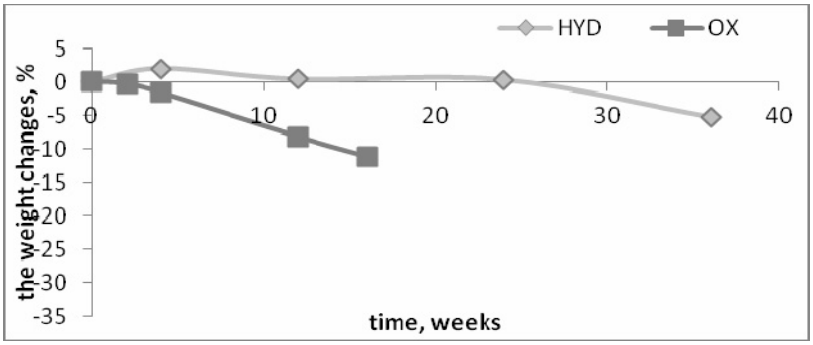

Fig. 2. The weight changes of aliphatic PUR-H_{12}MDI during incubation in hydrolytic and oxidative solutions

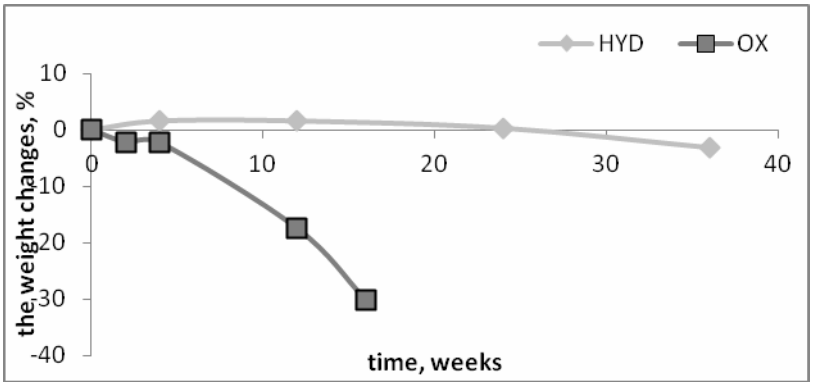

Fig. 3. The weight changes of aromatic PUR-MDI during incubation in hydrolytic and oxidative solutions

Table 5. Influence of polyurethanes sample on white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), platelets (PLT) and fibrinogen (FIB) concentration (±SD)

PUR	WBC [x 10^3/mm^3]		RBC [x 10^6/mm^3]		HGB [g/dl]		PLT [x 10^3/ml]		FIB [g/l]	
	15′	4h	15′	4h	15′	4h	15′	4h	15′	4h
0	6.47 ±1.38	6.05 ±1.76	4.33 ±0.10	4.35 ±0.10	12.43 ±0.60	12.52 ±0.52	264.22 ±52.74	246.89 ±62.30	3.44 ±0.71	3.42 ±62.30
PUR-MDI	6.32 ±1.51	6.01 ±1.54	4.35 ±0.08	4.35 ±0.08	12.49 ±0.61	12.54 ±0.55	264.0 ±48.00	255.44 ±52.15	3.22 ±0.66	3.23 ±0.77
PUR-H_{12}MDI	6.59 ±1.23	6.08 ±1.59	4.36 ±0.16	4.36 ±0.13	12.56 ±0.78	12.57 ±0.67	255.25 ±72.75	246.78 ±51.04	3.45 ±0.94	3.49 ±1.04
Referenced values	4.0–10.0		4.0–5.0		12.0–16.0		140.0–400.0		1.50–4.50	

Values of hematologic parameters (WBC, RBC, HGB) before and after incubation of polyurethanes samples are in reference ranges and they are similar to parameters of blood sample (sample "0"), which is not in contact with polymers.

Moreover, the concentration of platelets and fibrinogen did not change significantly in comparison to 0 probes. These both parameters are involved in creation of thrombus which is a common complication after the inserting of synthetic or natural implant into living organism. No changes in concentrations of platelets and fibrinogen after contact with PUR samples are the very optimistic results.

For polyurethane based on aliphatic diisocyanate the influence on some pathogenic microorganisms is estimated by observation of zone of growth inhibition around polymers sample.

Table 6. Zone of growth inhibition of Staphylococcus aureus, Escherichia coli and Candida albicans, measured as the average distance between the edges of the polymer material and the beginning of the bacterial and fungal lawn (±SD)

PUR	Zone of inhibition [mm]		
	Staphylococcus aureus	Escherichia coli	Candida albicans
PUR-H_{12}MDI	8±1.0	1.5±0.5	0

It is concluded that growth of Staphylococcus aureus and Escherichia coli is restricted (Table 6). Especially the bacteria of Staphylococcus aureus appear sensitive to contact with polymer. It is very optimistic because both kinds of species are common reason of surgery infection because of adhere ability to natural tissue and synthetic material surface.

Generally fungi are more resistant to environmental factors than bacteria, because of much complicated eukaryotic fungal cell structure in comparison to prokaryotic bacterial cells. No growth inhibition of Candida albicans is observed around polymer samples.

CONCLUSIONS

The polyurethanes based on synthetic polyhydroxybutyrate are amorphous and hydrophilic. Sterilization of aliphatic polyurethane insignificantly changed the mechanical properties whereas aromatic polyurethane seems to be more sensitive to conditions of sterilization. The results of polyurethane degradation indicates higher influence of oxidative than hydrolytic mechanism. The presence of aliphatic polyurethane decreases the growth of some pathogenic microorganisms. Both kinds of polymers do not change the parameters of whole blood.

It could be stated that aliphatic polyurethane based on atactic poly[(*R,S*)-3--hydroxybutyrate] displays the properties appropriate for further investigations for medical application without the risk of generation the cancerogenic degradation products which could be formed from polyurethane obtained with aromatic diisocyanate.

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