Acta of Bioengineering and Biomechanics
Vol. 17, No. 3, 2015

Original paper
DOI: 10.5277/ABB-00076-2014-02

Haemocompatibility and cytotoxic studies of non-metallic composite materials modified with magnetic nano and microparticles

MARIA SZYMONOWICZ[1]*, ZBIGNIEW RYBAK[1], ANETA FRACZEK-SZCZYPTA[2], DANUTA PALUCH[1], AGNIESZKA RUSAK[1], KATARZYNA NOWICKA[2], MARTA BLAZEWICZ[2]

[1] Department of Experimental Surgery and Biomaterials Research, Medical University, Wrocław, Poland.
[2] Department of Biomaterials, AGH University of Science and Technology, Kraków, Poland.

Purpose: Preventing the formation of blood clots on the surface of biomaterials and investigation of the reasons of their formation are the leading topics of the research and development of biomaterials for implants placed into the bloodstream. Biocompatibility and stability of a material in body fluids and direct effect on blood cell counts components are related both to the structure and physico-chemical state of an implant surface. The aim of this study was to determine haemocompatibility and cytotoxicity of polysulfone-based samples containing nano and micro particles of magnetite (Fe_3O_4). Methods: The polysulfone-based samples modified with nanometric and micrometric magnetite particles were examined. Physicochemical properties of the composites were determined by testing their wettability and surface roughness. The action of haemolytic, activation of coagulation system and cytotoxicity of composites was evaluated. Results: Wettability and roughness of materials were correlated with nanoparticles and microparticles content. In the tests of plasma coagulation system shortening of activated partial thromboplastin time for polysulfone with nano magnetite and with micro magnetite particles was observed in comparison with pure polysulfone. Prothrombine time and thrombine time values as well as fibrinogen concentration were unchanged. Haemolysis values were normal. Morphology and viability of cells were normal. Conclusions: Composites made from polysulfone modified with nanoparticles and microparticles of magnetite cause neither haemolytic nor cytotoxic reaction. These composites evoke plasma endogenous system activation.

Key words: *nanocomposite, magnetite particles, polysulfone, haemolysis, coagulation system, cell viability*

1. Introduction

Nanomedicine is a new domain of medicine dealing with the methods of design and manufacturing of nanomaterials designed for diagnosis and the treatment of diseased tissues [1], [11]. The world of medicine has high hopes connected with that field, especially due to the potential possibilities for the treatment of diseases, regarded as incurable so far. At present, many centres in Poland and in the world develop research devoted to nanomaterials for medical applications. Research is being conducted on the development of a new generation of materials for the treatment and diagnosis of diseases and enforcement of mechanisms reconstructing and regenerating diseased or damaged human tissues [10].

Composites based on polymers and magnetic particles can be obtained in various forms depending on their use and type of tissue. In the case of elements for contact with blood, coatings for artificial heart valves or stent surfaces, are the appropriate forms.

The introduction of the magnetic component causes changes of physicochemical properties of the surface of the obtained material composite, including wettability, surface energy, roughness and mechanical properties as well. Magnetic particles modifying biostable polymer matrices improve their visualization

* Corresponding author: Maria Szymonowicz, Department of Experimental Surgery and Biomaterials Reasearch, Medical University, ul. Poniatowskiego 2, 50-326 Wrocław, Poland. Tel: +48 71/7840135, e-mail: maria.szymonowicz@umed.wroc.pl
Received: April 7th, 2014
Accepted for publication: October 1st, 2014

in MRI (Magnetic Resonance Imaging), and such materials can be used for preparation of the implants, the position of which is monitored in tissue magnetic resonance (coating, layer, fibers) [13], [25]. Such particles have also received attention for applications in magnetic drug targeting, contrast enhancement in MRI, and hyperthermia treatment [5], [8].

Biomaterials for temporary and permanent contact with the organism should be biocompatible and possess optimal physicochemical properties matched to replaced or reconstructed tissue. Blood in the living body is the most complex dynamic biological system. Athrombogenity, haemocompatibility, absence of cytotoxic action of materials in contact with blood are prerequisites for the proper functioning of such a system [23], [24], [14].

The aim of this study was to determine haemocompatibility and cytotoxicity of various composite samples in the form of thin foils obtained from biostable polysulfone containing magnetite nano and micro particles.

Polysulfone is known to be a biocompatible polymer widely used for the manufacture of membranes, for haemodialysis and also for water purification [2]. Polysulfone-based biomaterials were studied in contact with bone tissue cells. Their potential as drug carriers or in therapies of neoplasms was also analysed [15], [20], [21]. An addition of biocompatible and bioactive fibers or inert ceramic particles allowed a composite material with improved mechanical properties to be created [9], [12]. However, polysulfone haemodialysis membranes can lead to hypertension, neutropenia or oxidative stress [21]. Chemical modification of polysulfone membranes leads to improvement of their biocompatibility. Polyethylene glycol, d-a-tocopheryl polyethylene glycol 1000 succinate or vitamin E enhanced biocompatibility of membrane for haemodialysis *in vitro* and *in vivo* studies [3], [4], [17].

2. Materials and methods

2.1. Research material

Polysulfone (PSU) from Sigma-Aldrich and two types of powders of magnetite differentiated with particle size (Sigma-Aldrich Company) were used to manufacture the composite samples. Nano-sized materials consisted of grain fraction less than 50 nm, and micrometric powders contained grain fraction below 5 μm. The composite samples containing nanoparticles were marked in the work with symbol (N), whereas the samples with micorparticles with symbol (M). TEM microphotographs of magnetite particles in the micro and nanometre scale are shown in Fig. 1. The polymer compositions of magnetite were prepared as follows: first, the appropriate amount of magnetite particles was mixed in dichloromethane (DCM, POCH) using an ultrasonic bath (Ultrasonic Cleaner Polsonic) for 15 min., then 10% solution of the polymer in the same solvent was added to create a suspension of magnetite in DCM, and the mixture was stirred with ultrasonic stirrer for 24 h. The whole was homogenized for another 15 min. After this time the samples were poured into Petri dishes and left for the solvent to evaporate in a fume hood for 24 hours at room temperature and a further 24 h in a vacuum oven 313 K (40 ± 1)°C. Four types of composites with different amounts of magnetite were obtained using this procedure. Then, the samples were subjected to radiation sterilization with the dose of 25 kGy. The following types of samples in the form of foils were prepared for the study:

As-received pure polysulfone sample (reference) – denoted in the work as PSU, polymer sample containing 0.25% or 1% nanometric magnetite (Fe_3O_4) – denoted as PSU-0.25N and PSU-1N. Polymer sample containing 0.25% or 1% micrometric magnetite (Fe_3O_4) – denoted as PSU-0.25M and PSU-1M.

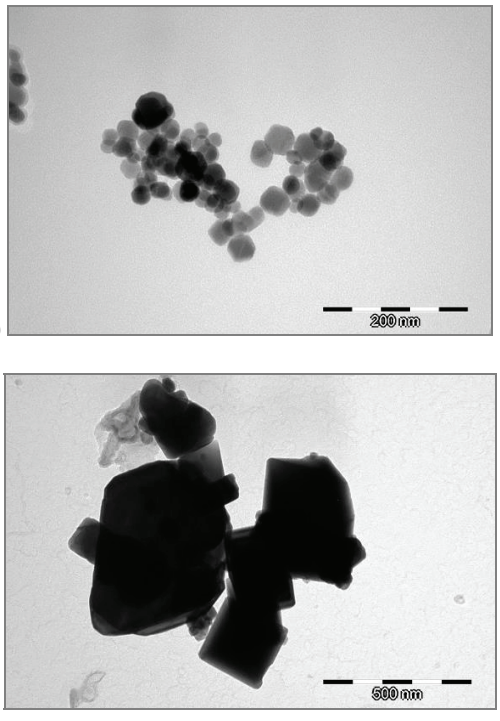

Fig. 1. TEM microphotographs of nanometric magnetite Fe_3O_4(N)

Materials were designed and manufactured at the University of Science and Technology, Faculty of Materials Science and Ceramics, AGH, Kraków, Poland.

2.2. Physicochemical tests

Roughness of the surface samples was determined by the surface profilometry technique (Hommel Tester T1500). Morphology of magnetites was determined using transmission electron microscopy (TEM, Jeol). The contact angle of polymer composites containing magnetite was measured by sessile drop method using an automatic drop shape analysis system DSA 10 Mk2 (Kruss, Germany). UHQ-water (produced by Purelab UHQ, Elga, Germany) drops of the volume of 0.2 μl were put on each sample and the contact angle was calculated by averaging the results of 10–11 measurements. The material surfaces were evaluated with contrast reverse-phase microscope CKX 41 (Olympus).

2.3. Biological tests *in vitro*

The biological examinations of the PSU-based samples modified with nanometric and micrometric magnetite particles comprised the assessment of haemolytic action, plasmatic activation of coagulation system and cytotoxic reaction [6], [7], [16].

The tests were performed on a nameless human blood group 0Rh+ taken for preservative fluid CPD (citrate-phosphate-dextrose). Consent of Bioethical Commission of Wrocław Medical University was obtained for the tests (No.KB-199/2011). Investigation was conducted at the Department of Experimental Surgery and Biomaterials Research, University of Medicine, Wrocław, Poland.

2.3.1. Haemolytic action tests

0.2 cm^3 full citrate blood was taken for the material sample area of 1 cm^2 and incubated for 30 min at 310 K (37±1) °C. The solution of 4 cm^3 of PBS was then added and incubated for another 60 min at 310 K(37 ± 1) °C. In parallel with the samples tested the following samples were set: control (PBS), and water for injection – 100% haemolysis, to which 0.2 cm of full blood was added. Then, the samples were centrifuged (652 g/10 min). Absorbance at a wavelength of 540 nm was measured.

The percentage of haemolysis (% H) was calculated for the following formula

$$\% H = [(AB - AK) \times 100]: (A100 - AK) \quad (1)$$

where

AB – absorbance of the sample tested, AK – absorbance of the control sample,

A100 – absorbance of the sample with 100% haemolysis.

For materials intended for contact with blood, haemolysis value should not exceed 1% [18].

2.3.2. Test of the plasmatic coagulation system activation

Citrate plasma was obtained from human full blood after centrifugation (1467g × 10 min) and separation from the morphotic elements of blood. The proportions of material for plasma volume and the contact time were chosen experimentally. Low platelet plasma with test polymers (1 cm × 1 cm/0.8 cm^3) and without material (control plasma) were incubated for 2 h at 310 K (37 ± 1) °C.

In the plasma partial thromboplastin time after activation (APTT, s) was determined as well as prothrombin time (PT, s) for which prothrombin index (PT %) and prothrombin ratio (international normalized ratio) (INR) were specified. Thrombin time (TT, s) and fibrinogen (Fb, g/l) concentration were marked. The tests were performed on a coagulometer Coag Chrom 3003, at 310 K (37±1) °C at a wavelength of 405 nm [24], [6].

2.3.3. Cytotoxicity tests

Mouse fibroblast cells L-929 (NCTC clone 929: CCL 1, American Type Culture Collection ATCC®), were cultured in culture medium composed of Eagle's minimum essential medium (EMEM) with L-glutamine (ATCC®) and supplemented with 10% foetal bovine serum (FBS, Lonza®). The tests using extracts prepared form culture medium with serum were performed.

Extract preparing

The following extracts were prepared: negative control – high density polyethylene (HDPE, U.S. Pharmacopeia – Rockville, MD, USA) and samples of polysulfone with the surface area of 6 cm/1 cm^3 of culture medium, positive control sodium lauryl sulfate (SLS, SIGMA-ALDRICH®) in medium with the following concentrations: (0.15, 0.1, 0.05) mg/cm^3. In order to conduct the *in vitro* cytotoxicity assessment, the samples of polysulfone extracts were prepared with the following concentrations: (100, 50, 25,

12.50) %. In addition, a blank test was used, i.e., medium with serum without the sample.

MTT test

L-929 cells were seeded in 96-well flat-bottomed cell culture plates (at 1×10^4 cells in 100 µl of medium per well), incubated at 310 K (37 ± 1)°C for 24 h to allow attachment and then the medium was replaced with 100 µl of test extract or control group extract. Cytotoxicity was assessed after further 24 h of incubation. The changes in cell morphology were evaluated with the use of the inverted microscope (CKX 41, Olympus®). The viability of cells was assessed with the use of the MTT assay (1 mg/1cm³ MEM without phenol red, without glutamine and without $NaHCO_3$, SIGMA-ALDRICH®). To perform MTT 50 µL of MTT reagent was added to each well and plates were incubated at 310 K (37 ± 1) °C in 5% CO_2 for 3 h. Then, the MTT solution was discarded and 100 µl of isopropanol, analytical grade (STANLAB®) was added in each well. After 30 min, the absorbance values were recorded at 570 nm using Epoch Microplate Spectrophotometer (BioTek®).

Cell viability was calculated according to the formula

$$\text{Viab. \%} = (\text{A: S}) \times 100 \qquad (2)$$

where

A – is the mean value of the measured optical density extracts of the test sample,

S – is the mean value of the measured optical density of the blanks.

Evaluation of the cytotoxity

The evaluation of cytotoxity effect was performed after 24 h incubation of the cells in the extracts of materials. The morphological changes upon contact with the materials were observed in contrast reverse-phase microscope CKX 41 (Olympus). Cell viability was evaluated with MTT assay. The degree of toxicity of the materials was evaluated on the basis of changes in cell morphology and cell viability. According to the 4-degree scale changes in the cell cultures higher than 2 degrees and a decrease in cell viability greater than 30% are considered to be caused by the cytotoxic effect [7].

2.3.4. Statistical analysis

The results were statistically analyzed using the Statistica 8. Arithmetic mean (X), standard deviation (±SD) level of significance (p), and the reference range of values were counted. Significant differences in mean values were determined by T-test for independent samples. It was assumed that the correlation coefficients are significant at $p < 0.05$.

3. Results

The surface roughness measured by the Ra parameter depends on the size and concentration of magnetite powder in polymer matrix (Table 1). For sample containing 0.25% of Fe_3O_4 (N) the roughness was almost the same as for pure PSU sample. For samples containing 1% of Fe_3O_4 (N) and 0.25% of Fe_3O_4 (M) this parameter was higher than for PSU sample and amounted to 40 nm. The highest surface roughness was observed for composite samples containing 1% of magnetite in microscale (Fe_3O_4 (M)). For these samples the roughness was significantly higher (almost 70%) in comparison with PSU sample. These data indicate that the greatest impact on the changes of the surface roughness has a dimension of magnetite particles. Magnetite in micrometric scale has a distinct influence on the surface roughness of the composite samples. Despite the differences in the roughness, all the samples show homogeneous surface.

Table 1. Surface characteristics of pure PSU and magnetite-modified PSU samples

Samples	Surface roughness [nm]	Contact angle [°]
PSU	30.00 ± 1.00	90.90 ± 1.90
PSU-0.25N	30.00 ± 1.00	79.70 ± 2.20
PSU-1N	40.00 ± 1.00	71.10 ± 1.80
PSU-0,25M	40.00 ± 1.00	89.70 ± 1.20
PSU-1M	50.00 ± 1.00	85.00 ± 2.50

The surface wettability of samples depends on the concentration and particle size of the magnetite in polysulfone (Table 1). The surface wettability of the composites modified with magnetite powder showed a tendency to decrease, particularly in the case of PSU-1N. For this sample the contact angle decreases over 20% as compared to PSU. Generally, magnetite particles in the nanometric scale have a more significant impact on hydrophilicity of composite samples. It is likely that for these samples the ceramic component is better dispersed in polymer matrix reducing the direct impact of the polymer on the contact angle value. Moreover, a higher surface area of magnetite in

nano scale (>60 m^2/g) in comparison to micrometric magnetite also affects the surface wettability of the composites.

In the study of haemolytic action it was observed that the mean value of the haemolysis percentage for the materials tested does not exceed the limit value by the standard, that is, 1% [18]. The mean values of the haemolysis percentage for the evaluated polymers are presented in Table 2. The haemolysis percentage determined for the PSU-0.25N (PSU-1N) slightly increased in comparison with the PSU. The values were in the normal range.

Table 2. Percentage of haemolysis

Sample	H [%]	
	X ± SD	*p*
PSU-0.25 M	0.33 ± 0.03	0.745
PSU-1 M	0.33 ± 0.02	0.743
PSU-0.25 N	0.41 ± 0.03 *	0.032
PSU-1 N	0.42 ± 0.04 *	0.024
PSU	0.32 ± 0.04	–

X – average, ± SD – standard deviation,
p – level of significance,
* $p < 0.05$ – differences in relation to the PSU.

Microscopic evaluation of the material surface using contrast reverse-phase microscope is shown in Figs. 2–4. Photomicrographs show the differences in the surface microstructure of the samples. Despite the initial homogenization of micro- and nano-powder suspensions before introducing them into a polymer matrix, single aggregates of magnetite in the composite samples can be observed. The size of aggregates occurring into the near surface region of the samples increases with increasing the fraction of magnetite in the polymer. In the case of nanocomposite samples containing nanometric magnetite with a greater fraction (1%), the photomicrographs show aggregates of elongated shape, uniformly spread on the surface samples.

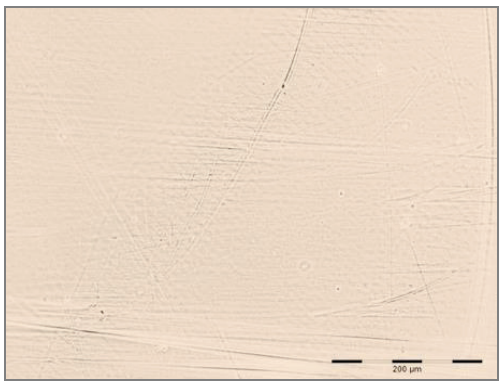

Fig. 2. TEM microphotographs of nanometric magnetite micrometric Fe_3O_4 (M)

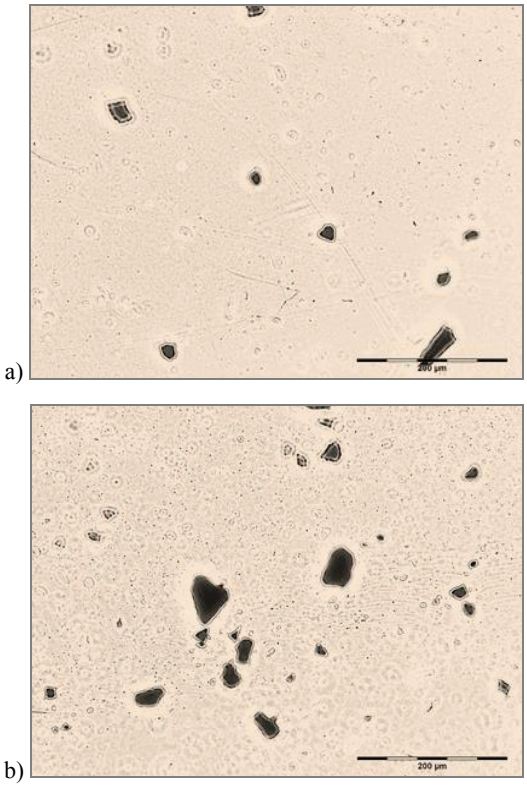

Fig. 3. Microscopic image of polysulfone with: (a) 0.25% micrometric magnetite – PSU-0.25 M, (b) 1% micrometric magnetite surface – PSU-1 M

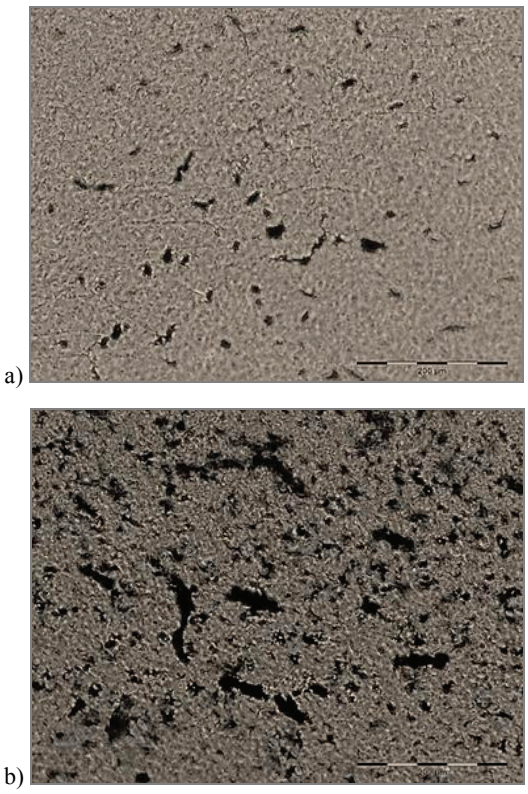

Fig. 4. Microscopic image of polysulfone with: (a) 0.25% nanometric magnetite – PSU-0.25 N, (b) 1% nanometric magnetite surface – PSU-1 N

The parameters of plasma coagulation system for the control plasma, and after contact with the polymers are presented in Table 3. APTT, PT, TT and Fb values obtained for plasma after contact with the modified polysulfones were compared with the values for plasma after contact with pure polysulfone and with the value of the material-free plasma (control).

Partial thromboplastin time after activation (APTT) of blood plasma after 120 min contact with PSU-1M was significantly ($p < 0.001$) shortened, while for PSU-0.25M it was comparable to the APTT value observed for PSU. APTT value observed for PSU-1N was significantly ($p < 0.05$) shortened and for the PSU-0.25N close to the value observed for the PSU. APTT values observed for the polymers under study

Table 3. Activated partial thromboplastin time (APTT) in the citrate blood plasma control and after contact with magnetite-modified PSU samples

Sample	APTT [s]		
	X ± SD	*p*	*p*1
PSU-0,25 M	38.26 ± 0.25 ###	0.162	0.0008
PSU-1 M	34.53 ± 0.38*** ###	0.0007	0.0008
PSU-0,25 N	38.00 ± 0.11 ###	0.448	0.0007
PSU-1 N	36.80 ± 0.40 * #	0.037	0.024
PSU	37.80 ± 0.40 #	–	0.003
Control plasma	35.57 ± 0.45	–	

Reference range APTT: 29.20 s– 36.50 s – 43.80 s,
X – average, ± SD – standard deviation, *p* – level of significance,
* $p < 0.05$, *** $p < 0.001$ – differences in relation to the PSU,
# $p < 0.05$, ### $p < 0.001$ – differences in relation to the control plasma.

Table 4. Prothrombin time (PT) in the citrate blood plasma control and after contact with the magnetite-modified PSU samples

Sample	PT								
	s			INR			PT, [%]		
	X ± SD	*p*	*p*1	X ± SD	*p*	*p*1	X ± SD	*p*	*p*1
PSU-0,25 M	12.20 ± 0.20	0.272	0.411	0.93 ± 0.02	0.411	0.411	107.33 ± 3.51	1.000	1.000
PSU-1 M	15.06 ± 0.06	0.482	0.562	0.94 ± 0.01	1.000	1.000	108.33 ± 2.08	0.587	0.624
PSU-0,25 N	12.40 ± 0.30	0.656	0.748	0.94 ± 0.02	0.829	0.829	107.00 ± 3.00	0.882	0.889
PSU-1 N	12.13 ± 0.05	0.488	0.386	0.93 ± 0.02	0.411	0.411	109.66 ± 2.51	0.283	0.319
PSU	12.30 ± 0.20	–	0.829	0.94 ± 0.02	–	1.000	107.33 ± 2.08	0.052	1.000
Control, plasma	12.33 ± 0.15	–		0.94 ± 0.02	–		88.33 ± 0.57	–	

Reference range: PT: 10.60 s – 13.20 s – 15.80 s; PT INR: 0.78 –0.99 – 1.20; PT%: 84.00 – 102.00 –125.00,
INR – prothrombin ratio (international normalized ratio),
PT% – prothrombin index,
X – average, ± SD – standard deviation, *p* – level of significance.

Table 5. Thrombin time (TT) and fibrinogen concentration (Fb) in the citrate blood plasma control and after contact with magnetite-modified PSU samples

Sample	TT [s]			Fb [g/l]		
	X ± SD	*p*	*p*1	X ± SD	*p*	*p*1
PSU-0,25 M	11.03 ± 0.06	0.518	0.068	2.05 ± 0.06	0.169	0.278
PSU-1 M	11.07 ± 0.06	0.101	0.102	2.02 ± 0.03	0.137	0,426
PSU-0,25 N	11.17 ± 0.05	1.000	0.348	2.04 ± 0.03	0.405	0,313
PSU-1 N	11.07 ± 0.12	1.000	0.144	2.01 ± 0.04	0.183	0,808
PSU	11.07 ± 0.10	–	0.101	2.06 ± 0.04	–	0,557
Control, plasma	11.26 ± 0.15	–		2.06 ± 0.04		

Reference range TT: 10.30 s – 12.50 s – 15.50 s Fb: 2.55 g/L – 3.19 g/L – 3.82 g/l,
X – average, ± SD – standard deviation, *p* – level of significance.

Table 6. Results of the in vitro cytotoxicity assessment of magnetite-modified PSU samples

	Extract	Cell viability [%]	Description	Grade
PSU	100%	94.2	Discrete intracytoplasmatic granules, no cell lysis (Fig. 2a)	0
	50%	95.2	Discrete intracytoplasmatic granules, no cell lysis	0
	25%	100.6	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
	12.5%	100.3	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
PSU-25 M	100%	95.2	Discrete intracytoplasmatic granules, no cell lysis (Fig. 2b)	0
	50%	98.2	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
	25%	97.93	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
	12.5%	99.0	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
PSU- 1M	100%	95.0	Discrete intracytoplasmatic granules, no cell lysis (Fig. 2c)	0
	50%	96.3	Discrete intracytoplasmatic granules, no cell lysis	0
	25%	95.9	Discrete intracytoplasmatic granules, no cell lysis	0
	12.5%	99.3	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
PSU-0.25 N	100%	87.9	Around 15% of the cells are round, loosely attached, Culture density lower than the negative control (Fig. 2d)	1
	50%	91.2	Around 10% of the cells are round, loosely attached, Culture density lower than the negative control	1
	25%	97.7	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
	12.5%	99.3	Discrete intracytoplasmatic granules, no cell lysis, density of culture is comparable to density of negative control culture	0
PSU-1N	100%	77.7	Around 20% of the cells are round, loosely attached, Culture density lower than the negative control (Fig. 2e)	1
	50%	77.9	Around 20% of the cells are round, loosely attached, Culture density lower than the negative control	1
	25%	88.1	Around 10% of the cells are round, loosely attached, Culture density lower than the negative control	1
	12.5%	88.7	Around 10% of the cells are round, loosely attached, Culture density lower than the negative control	1

were significantly ($p < 0.05$, $p < 0.001$) prolonged compared to the control plasma APTT values. The observed changes in the values of the APPT do not exceed the reference values.

Prothrombin time (PT), prothrombin index PT%, prothrombin ratio (INR) thrombin time (TT) and fibrinogen plasma concentrations of blood plasma after contact with the test polymers were not significantly different from control values in plasma (Table 4).

PT values and plasma concentrations of fibrinogen found for the polymers studied were not significantly different ($p > 0.05$) from the values of these parameters in plasma control. The values were comparable to each other and close to the values obtained for PSU.

The results of cytotoxicity test are shown in Table 6. PSU sample and its composites with magnetite: PSU-0.25M; PSU-1M; PSU-0.25N; PSU-1N did not induce morphological changes in cultured cells and did not reduce cell viability above 30%.

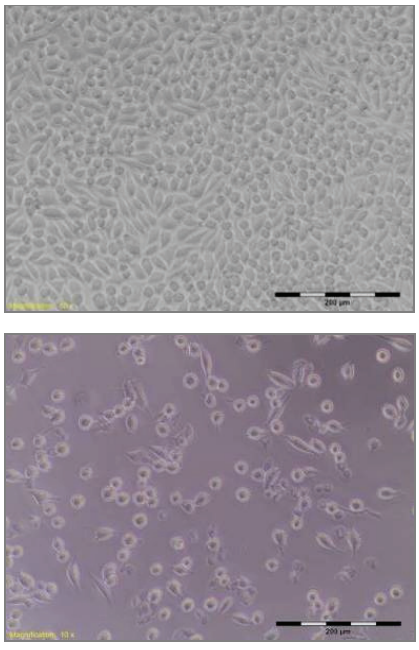

Fig. 5. Cells culture after contact with:
(a) negative control – HDPE, (b) positive control – SLS 0.15 mg/cm^3

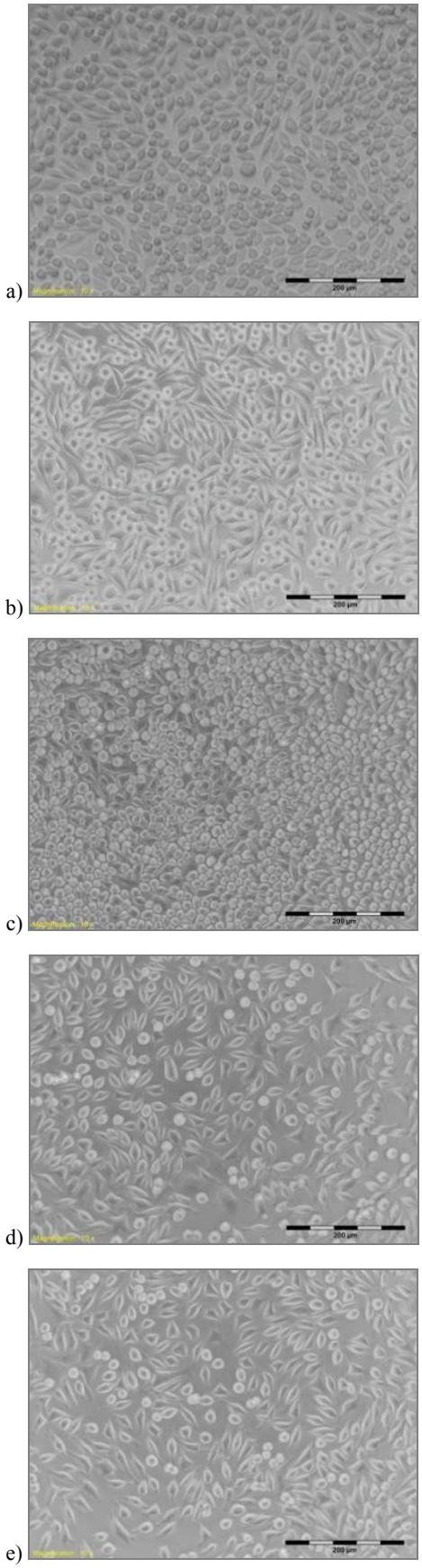

Fig. 6. Cells culture in the extract of:
(a) 100% PSU, (b) 100% PSU-0.25 M, (c) 100% PSU-1 M,
(d) 100% PSU-0.25 N, (e) 100% PSU-1 N

Larger changes in morphology and a reduction in cell viability were observed for the composites PSU-0.25 N and PSU-1 N. Morphology of cells after contact with control materials (positive and negative control) is shown in Fig. 5. Morphological changes of cells after contact with materials under investigation are presented in Fig. 6.

4. Discussion

The surface parameters of materials such as roughness, wettability or the chemical surface state are particularly essential when designing a biomaterial with a view to its potential use in medicine. The lower the angle, the higher the hydrophilicity of the material surface [23].

Especially interaction of cells of fluid tissue like blood strongly depends on the surface properties of the material. Biomaterials designed for contact with blood should possess suitable characteristics that allow for their use without the occurrence of undesired reactions. Thrombotic occlusion is considered to be the main reason for the failure of implants for cardiosurgery. Thus, the control of the blood–biomaterial interaction is particularly important in order to minimize or even eliminate anticoagulation therapy [19], [22].

The study shows that the presence of a magnetite nanoparticle favours the increase of hydrophilicity of the surface of nanocomposite materials compared to pure polysulfone. The wettability of the material surface depends on the concentration and size of the magnetite particles in the material. Magnetite in the nanometric scale had a greater impact on hydrophilicity of composite samples, especially for samples containing 1% magnetite (PSU-1N). On the contrary, the highest wettability was found for composite samples containing 1% micrometric magnetite (PSU-1M).

The biological studies of composites comprised an evaluation of haemolytic, cytotoxic action and activation of the clotting process. Haemolytic action was determined by marking the percentage of haemolysis. The percentages for the evaluated samples were found to be in the normal range. The largest haemolysis value was observed for PSU-1N.

Activation of the coagulation system dependent on plasma contact factors (intrinsic system) denoting partial thromboplastin time after activation (APTT) has been evaluated. Dependent on the tissue thromboplastin, the activation of coagulation with prothrombin test (PT) was performed. Thrombin test (TT) was used for measurement combining the two systems. In addi-

tion, the concentration of fibrinogen was marked. Clotting activation of the composites was compared to APTT pure polysulfone expressed by shortening the PSU-1M and PSU-1N, with no changes in the values of PT and fibrinogen concentration being observed. The highest shortening or stimulation of the extrinsic coagulation factors was noted for PSU-1M. The observed changes may be attributed to higher surface roughness of this sample in comparison with other materials studied.

In assessing the morphological pictures and long-life cell culture, there were no significant changes. Only major changes in cell morphology and reduced cell viability were demonstrated for PSU-0.25 N and PSU-1N.

Blood tests of haemolytic activity, blood cell reactivity, coagulation activity and cell culture cytotoxicity as well as evaluation of topography surface of nano- and micro-engineered composite samples allowed materials with optimal biological properties to be selected. In the evaluated materials the smallest changes were observed for PSU-0.25M. This will allow us to perform further studies *in vitro* and *in vivo*. In the future, such materials may be used to design and manufacture the implants for bone tissue reconstruction and as biostable devices in contact with blood.

Polysulfone has been used for years for membranes in dialyzers, and also as a material for the construction of elements and devices in contact with blood. Surface modification of this polymer with the use of nanoparticles to alter its hydrophobicity and surface roughness may contribute to extend its range of applications in cardiac surgery. Nano-engineered sample surfaces can affect in an entirely different way an answer of proteins responsible for thrombotic processes, and consequently the haemocompatibility of the whole material.

5. Conclusions

1. Composites made from polysulfone modified with nanoparticles and microparticles of magnetite do not cause haemolytic and cytotoxic reaction.
2. These composites cause plasma endogenous system activation without an influence on the exogenous system. These changes are within referential values and thiss has a diagnostic significance.
3. Changes in coagulation activation and the morphology of the cell cultures are connected with the surface properties of the composites tested, such as wettability and roughness.

Acknowledgements

The work was supported by the project no. ST-619 of the Wrocław Medical University, Poland and the statute funds of AGH – University of Science and Technology, Faculty of Materials Science and Ceramics, Poland project No. 11.11.160.256.

References

[1] CAO Q., HAN X., LI L., *Enhancement of the efficiency of magnetic targeting for drug delivery: Development and evaluation of magnet system*, J. Magn. Magn. Mater., 2011, Vol. 323, 1919–1924.

[2] CHŁOPEK J., *Kompozyty w medycynie*, Kompozyty (Composites), 2001, Vol. 1, 50–54.

[3] DAHE G.J., KADAM S.S., SABALE S.S., KADAM D.P., SARKATE L.B., BELLARE J.R., *In Vivo Evaluation of the biocompatibility of surface modified hemodialysis polysulfone hollow fibers in rat*, PLoS ONE, 2011, Vol. 6(10), 1–9.

[4] DAHE G.J., TEOTIA R.S., KADAM S.S., BELLARE J.R., *The biocompatibility and separation performance of antioxidative polysulfone/vitamin E TPGS composite hollow fiber membranes*, Biomaterials, 2011, Vol. 32, 352–365.

[5] GUPTA A.K., GUPTA M., *Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications*, Biomaterials, 2005, Vol. 26(18), 3995–4021.

[6] ISO 10993-4: 2009, *Biological evaluation of medical devices*. Part 4: *Selection of tests for interactions with blood*.

[7] ISO 10993-5 2009, *Tests for in vitro cytotoxicity*.

[8] LAURENT S., FORGE D., PORT M., ROCH A., ROBIC C., VANDER ELST L., *Magnetic ironoxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications*, Chem. Rev., 2008, Vol. 108(6), 2064–2110.

[9] LINES S.W., CARTER A.M., DUNN E.J., LINDLEY E.J., TATTERSALL J.E., WRIGHT M.J., *A randomized controlled trial evaluating the erythropoiesis stimulating agent sparing potential of a vitamin E-bonded polysulfone dialysis membrane*, Nephrol. Dial. Transplant., 2014, Vol. 29, 649–656.

[10] LIU H., WEBSTER T.J., *Nanomedicine for implants: a review of studies and necessary experimental tools*, Biomaterials, 2007, Vol. 28, 354–369.

[11] MANGUAL J.O., LI S., PLOEHN H.J, EBNER A.D., RITTER J.A., *Biodegradable nanocomposite magnetite stent for implant-assisted magnetic drug targeting*, J. Magn. Magn. Mater, 2010, Vol. 322, 3094–3100.

[12] MAPHUTHA S., MOOTHI K., MEYYAPPAN M., IYUKE S.E., *A carbon nanotube-infused polysulfone membrane with polyvinyl alcohol layer for treating oil-containing waste water*, Scientific Reports, 2013, Vol. 3, 1–621.

[13] MCCARTHY J.R., WEISSLEDER R., *Multifunctional magnetic nanoparticles for targeted imaging and therapy*, Adv. Drug. Deliv. Rev., 2008, Vol. 60(11), 1241–1251.

[14] MODRZEJEWSKA Z., PALUCH D., *Investigation of the cytotoxic activity of chitosan membranes to be used as dressing*, Engineering of Biomaterials, 2011, Vol. 14 (103), 23–28.

[15] OH J.K., PARK J.M., *Iron oxide-based superparamagnetic polymeric nanomaterials: Design, preparation, and biomedical application*, Progress in Polymer Science, 2011, Vol. 36, 168–189.

[16] SEYFERT U.T., BIEHL V., SCHENK J., *In vitro hemocompatibility testing of biomaterials according to the ISO 10993-4*. Biomol. Eng., 2002, Vol. 19(2–6), 91–96.

[17] SHEN C., ZHANG G., MENG Q., *Regulation of epithelial cell morphology and functions approaching to more in vivo-like by modifying polyethylene glycol on polysulfone membranes*, PLoS ONE, 2012, Vol. 7(4), 1–10.

[18] SINGHAL J.P., RAY A., *Synthesis of blood compatible polyamide block copolymers*, Biomaterials, 2002, Vol. 23, 1139–1245.

[19] SPIJKER H.T.R., GRAAFF P.W., BOONSTRA H.J., BUSSCHER W., VAN OEVEREN, *On the influence of flow conditions and wettability on blood material interactions*, Biomaterials, 2003, Vol. 24, 4717–4727.

[20] STANKOVA L., FRACZEK-SZCZYPTA A., BLAZEWICZ M., FILOVA E., BLAZEWICZ S., LISA V., BACAKOVA L., *Human osteoblast-like MG 63 cells on polysulfone modified with carbon nanotubes or carbon nanohorns*, Carbon, 2014, Vol. 67, 578–591.

[21] SWIETEK M., TOKARZ W., FIGIEL H, TARASIUK J., WRONSKI S., BLAZEWICZ M., *Magnetic polymer sponge for medical applications*, Innovative technologies in biomedicine: the 1st International Conference: October 15–16, 2013, Kraków, Poland, 52–53.

[22] SZELEST-LEWANDOWSKA A., MASIULANIS M., SZYMONOWICZ M., PIELKA S., PALUCH D., *Modified Poly (carbonateurethane). Synthesis, properties and biological investigation in vitro*, Journal of Biomedical Materials Research part A, 2007, Vol. 82(12), 509–520.

[23] SZYMONOWICZ M., FRACZEK-SZCZYPTA A., RYBAK Z., BLAZEWICZ S., *Comparative assesment of the effect of carbon-based material surfaces on blood clotting activation and haemolysis*, Diamond & Related Materials, 2013, Vol. 40, 89–95.

[24] SZYMONOWICZ M., PIELKA S., OWCZAREK A, HAZNAR D., PLUTA J., *Study on influence of gelatin-alginate matrixes on the coagulation system and morphotic blood elements*, Macromolecular Symposia, 2007, Vol. 253(1), 71–76.

[25] WOJCIK M., CHMIST J., PRZEWOZNIK J., FIGIEL H., BLAZEWICZ S., *Magnetic properties of PAN-based carbon fibres modified with magnetite nanoparticles*, Carbon, 2012, Vol. 50(4), 1604–1613.