



# Comparative assessment of the effect of carbon-based material surfaces on blood clotting activation and haemolysis

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## ABSTRACT

This work presents results of haemolytic reaction and activation of blood clotting in contact with various carbon materials. Synthetic graphite (SG), glass-like carbon (GLC), pyrolytic carbon (LTI), pyrolytic graphite (PG) and diamond-like carbon (DLC) were investigated. Haemolytic reaction was determined by the assessment of haemolytic index (HI), haemolysis percentage and by the morphological evaluation of erythrocytes. The results indicated that, independently of the methods used and the materials studied, values of haemolysis and morphological appearances of erythrocytes were in the range of standards. It was found that LTI carbon surface prolongs the most effectively clotting activation among the carbon materials studied. The most distinct changes in haemolysis were noted for synthetic graphite, while the smallest ones for LTI carbon. Interfacial bonding energy between GLC surface and human fibrinogen was slightly lower than that for LTI carbon, whereas its total surface energy reached the highest value among the carbon materials studied. The LTI and GLC samples were shown to be the most effective in preventing thrombus formation and in prolonging the clotting time as compared with the other carbon surfaces.

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## 1. Introduction

Soon after high blood compatibility of carbon coatings was discovered, medicine considered applying this type of material to various domains of cardiosurgery [1]. It is well known that carbon materials may occur in different lattice forms, from amorphous through intermediate to crystalline represented by hexagonal or regular lattice diamond [2–4]. All these forms of carbon can be produced by pyrolysis of solid, liquid and gaseous carbon precursors. The pyrolysis methods applied to carbon compounds allow obtaining of carbon in the form of a thin film or thick layers, solid pyrolytic carbon, powders and in the form of fibres. At present, among pure forms of carbon, the pyrolytic carbons ( $sp^2$ -bonded carbon; pyrolytic carbon [LTIC—low-temperature isotropic carbon], ULTIC—ultra low-temperature isotropic carbon) and carbon coatings ( $sp^3$ -bonded carbon; diamond-like carbon [DLC—diamond-like carbon], NDC—nanocrystalline diamond carbon, ta-C—tetrahedral carbon) on various substrates have found clinical applications in cardiovascular, orthopaedic and dental areas, as well as long-term transcutaneous implants [5–11]. The films made of tetrahedral carbon (ta-C) have attracted attention as wear-resistant coatings for biomedical application due to their high hardness, low friction coefficient and a high chemical inertness [9].

The primary application of carbon coatings is in the area of orthopaedics, and particularly in cardiosurgery. The materials interfaced

with the blood can trigger a series of processes that are significantly more complex and more rapid than reactions seen for materials implanted in soft and hard tissues. The mechanism of blood coagulation is controlled by blood chemistry and the nature of the material surface [12,13]. Carbon as a biomaterial has many advantageous biological, physical and chemical features to be used as a component for the manufacture of an implant for direct contact with blood. Medical devices in contact with blood should be haemocompatible. Haemocompatible biomaterials should perform a minimum haemolytic activity, without damaging or changing the configuration, morphology and stability of the morphotic blood components. The basic blood biocompatibility tests of artificial materials in vitro condition rely on the haemolytic activity study. They allow evaluation of the toxic effect of the materials and make a preliminary selection before clinical trials. The haemocompatibility of artificial materials is determined by their surface properties. To avoid the activation of the clotting process via trapped corpuscular blood components the artificial surfaces should be smooth enough. Clotting takes place as a result of the coagulation process cascade, which is a chain of biochemical reactions and leads to the formation of insoluble fibrin polymer networks, trapping blood cell counts and causes clog of the wound and inhibits the flow of blood [14]. Various materials designed for contact with blood, depending on their surface chemical state, roughness as well as electrochemical properties affect the clotting system differently [13]. Other parameters that characterised carbon materials including diamond-like carbon, i.e. hydrogen content,  $sp^3$  content, optical gap, refractive index, hardness,

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elastic modulus, surface energy and friction are also considered for the evaluation of haemocompatibility [12].

These contributions to the thrombus formation should be taken into consideration in the design of a device for use in contact with the blood. In this work, the major concern to be dealt with is the blood interaction involved with the initial contact with the surfaces of carbon materials. Due to the many techniques of manufacture and various sources of carbon precursors, the potential impact of carbon materials seems to be still large and materials scientists are searching continually for better carbon-based coatings with improved biological and fatigue properties.

The purpose of this study was to determine the influence of the carbon material surfaces differing in structural and microstructural characteristics on initial interaction with blood. The study focuses on the evaluation of the influence of various carbon materials on the activation of the coagulation system and haemolysis process.

## 2. Materials and methods

The following carbon materials were used in this study:

1. SG: Synthetic graphite; the samples were supplied by SGL-Carbon, Poland. This is a fine, high density polycrystalline graphite obtained by temperature treatment of calcined coke in composition with a pitch-based binder above 2500 °C. This type of material was also used in this study as a substrate for preparation of pyrolytic carbon coatings.
2. PG: laboratory manufactured pyrolytic graphite by the CVD method on the graphite substrate (SG) at 2300 °C. Graphite coating was obtained by pyrolysis of methane.
3. GLC: Glass-like carbon; the samples were laboratory manufactured by the controlled pyrolysis of phenolic resin. On heating to 1200 °C the resin was transformed into a glass-like carbon structure retaining the shape of the original resin and having the sp<sup>2</sup> hybridised carbon elements corresponding to amorphous carbons (near range ordering).
4. LTI: Carbon coating on graphite substrate (SG); laboratory obtained by the CVD method. Carbon coating was obtained by pyrolysis of methane at 1000 °C.
5. DLC: laboratory obtained by means of a planar magnetron gun WMK–1000 with carbon target. The process was conducted in the presence of methane. The films were deposited on carbon substrates made of synthetic graphite (SG). The IR analysis showed that carbon films were made of amorphous carbon containing sp<sup>2</sup> and sp<sup>3</sup> hybridised carbon atoms.

SG and GLC samples in the form of plates were cut from the block whereas PG, LTI and DLC samples were deposited in the form coatings on graphite substrate (SG).

All samples were used without additional polishing. Only synthetic graphite as the substrate for LTI, DLC and PG coatings was preliminary polished using standard silicon carbide polishing papers of varying grit size. Each graphite plate was polished with a series of papers (600–1000) and then polished on a special textile cloth immersed in water, without any powder. The initial step in the testing of the carbon plates was the cleaning of the surfaces. The samples were first cleaned in an ultrasonic cleaner using anhydrous methyl alcohol and methyl chloroform solutions. After several minutes of cleaning, the samples were rinsed vigorously in distilled water. Thereafter, the samples were heated in a furnace to 150 °C, under vacuum, for 24 h. The final step was to pack the samples into the special plastic bags, under vacuum, and finally sterilized by means of the H<sub>2</sub>O<sub>2</sub>–plasma technique (Sterrad 120, ASP, Johnson& Johnson, USA).

The samples of two sizes were prepared of each carbon material, namely—(5 × 5) mm, and (10 × 10) mm.

X-ray diffraction (XRD) patterns of the samples were collected with the D5000 Siemens diffractometer equipped with a graphite secondary monochromator, and using Cu K<sub>α</sub> (λ = 1.54 Å) radiation. Tungsten powder of purity better than 99.9% and grain diameter smaller than

1 μm was mixed with samples and used as an internal standard for XRD measurements. Based on these measurements, the structure and microstructure parameters of carbon materials have been determined. Roughness of the surface samples was determined by the surface profilometry technique (Hommel Tester T1500). The thickness of the carbon coatings on the graphite substrate was measured using the scanning electron microscope (SEM) microphotographs and atomic force microscopy (AFM) (Explorer ThermoMicroscopes, Veeco) in a contact mode.

The physical and structural parameters of carbon materials used in the study are gathered in Table 1.

The thicknesses of carbon coatings for DLC and LTI were substantially similar, and their surface topography and morphological characteristics were almost identical (roughness) to the substrate material, i.e., synthetic graphite. Due to the procedure of samples manufacturing, three carbon samples, namely DLC, LTI and SG, represent relatively similar surface topography. The film thicknesses for DLC and LTI were determined to be approximately 300 nm and 400 nm, respectively [15]. The GLC sample was manufactured directly from solid carbon precursor and its surface topography distinctly differs from the other samples; the surface is very smooth with a very low roughness parameter. Moreover, this kind of carbon contains very low porosity (less than 0.5%) with a closed pore system.

Contact angle measurements were made to determine surface energies and interfacial bonding between the materials surface and whole blood, fibrinogen and albumin. The procedure involved measuring the contact angle of the two liquids of known surface tensions (water and diiodomethane) on the carbon sample surfaces. Both components of surface energy, i.e., dispersive and polar as well as interfacial bonding, were determined using Owens–Wendt methods. Values of polar and dispersive energies for water are γ<sup>p</sup> = 51 mJ/m<sup>2</sup> and γ<sup>d</sup> = 21.8 mJ/m<sup>2</sup>, respectively, for diiodomethane γ<sup>p</sup> = 2.3 mJ/m<sup>2</sup> and γ<sup>d</sup> = 48.5 mJ/m<sup>2</sup>, respectively, for albumin are γ<sup>p</sup> = 33.62 mJ/m<sup>2</sup> and γ<sup>d</sup> = 31.38 mJ/m<sup>2</sup>, respectively, for fibrinogen are γ<sup>p</sup> = 40.5 mJ/m<sup>2</sup> and γ<sup>d</sup> = 24.72 mJ/m<sup>2</sup>, respectively, and for whole blood are γ<sup>p</sup> = 36.30 mJ/m<sup>2</sup> and γ<sup>d</sup> = 11.20 mJ/m<sup>2</sup>, respectively [9,16]. Table 2 shows the determined values of surface energies and interfacial bond energies between carbon surfaces and whole blood as well as fibrinogen and albumin. The interfacial bond energy was determined from the following formula [9,16]:

$$\gamma_{S,B} = \left[ \left( \sqrt{\gamma_B^p} - \sqrt{\gamma_S^p} \right)^2 + \left( \sqrt{\gamma_B^d} - \sqrt{\gamma_S^d} \right)^2 \right]^2$$

where:

γ<sub>B</sub><sup>p</sup> and γ<sub>B</sub><sup>d</sup> are dispersive and polar energy for blood [mJ/m<sup>2</sup>];  
γ<sub>S</sub><sup>p</sup> and γ<sub>S</sub><sup>d</sup> are dispersive and polar components of the carbon surface energy [mJ/m<sup>2</sup>].

The same equation was used to calculate interfacial bond energy between carbon materials and albumin and fibrinogen.

**Table 1**  
Characteristics of carbon materials.

Material	Mass density g/cm <sup>3</sup>	Interplanar distance d <sub>002</sub> , nm	Apparent crystallite size, L <sub>c</sub> nm	Surface roughness, nm	Coating thickness, nm
SG	1.75	0.336	18.7	61 ± 3	–
PG-coated SG	2.11	0.337	16.7	42 ± 3	1000
DLC-coated SG	–	–	<1.0 <sup>a</sup>	53 ± 4	300
GLC	1.42	0.371	1.2	4.0 ± 0.5	–
LTI-coated SG	1.89	0.339	1.8	64 ± 4	400

<sup>a</sup> The films perform an amorphous diamond-like structure.

**Table 2**

Surface energies (polar and dispersive), interfacial bond between carbon surface and blood ( $\gamma^{m,b}$ ) and between carbon surface and human fibrinogen ( $\gamma^{m,f}$ ), albumin ( $\gamma^{m,a}$ ).

Material	Surface energy, mJ/m <sup>2</sup>		Interfacial bond, ( $\gamma^{m,b}$ ), mJ/m <sup>2</sup>	Interfacial bond, ( $\gamma^{m,f}$ ), mJ/m <sup>2</sup>	( $\gamma^{m,a}/\gamma^{m,f}$ )
	Dispersive component, $\gamma^d$ , mJ/m <sup>2</sup>	Polar component, $\gamma^p$ , mJ/m <sup>2</sup>			
SG	34.49	0.16	38.02	36.38	0.803
PG	37.86	0.02	42.50	40.12	0.805
DLC-coated SG	58.56	5.54	43.56	39.78	0.792
GLC	37.64	2.98	26.25	22.86	0.738
LTI-coated SG	26.13	1.26	27.15	27.49	0.804

### 2.1. Biological methods and characteristics

Research was performed on human blood **0 (zero no O)** Rh + preservative fluid collected on CPD (sodium citrate, citric acid, glucose, sodium dihydrogen phosphate), Research Ethics Committee, Medical University (No. KB-338/2008). Subsequently, blood was mixed with a haematologic oscillatory stirrer with a frequency of 20 rpm for 180 s and kept before testing no longer than 24 h at 5 °C [17–19].

### 2.2. Evaluation of haemolytic action

The experiments were performed on human blood taken for sodium citrate (1:10, V:V) and the proportions of the material to blood were chosen experimentally.

### 2.3. Haemolytic action with the use of whole blood

Citrate blood dilution with the carbon material (0.25 g/5 ml) and without material (control) was incubated over 4 h at 37 °C. Next, the blood was centrifuged (1500 rpm for 10 min.) and the supernatant was separated from the blood elements. The haemoglobin concentration was determined in the plasma with the cyanomethaemoglobin method. To measure the haemoglobin concentration, a lysing agent is added to a sample of diluted blood; the lysing agent disrupts all the red cells in the sample and releases the haemoglobin into the fluid so that the resulting sample consists of a solution of haemoglobin. The haemoglobin is converted into a form called cyanomethaemoglobin and the concentration is read by a spectrophotometer with the wavelength set at the peak absorbance of cyanomethaemoglobin.

The calculated concentration of haemoglobin in the plasma and the concentration of haemoglobin in the blood were used to determine the haemolytic index.

Haemolytic index (HI) has been determined from the following formula:

$$HI = \frac{Hb_s \cdot 100}{Hb_c}$$

where:

$Hb_s$ —haemoglobin concentration in the plasma [g/dl]

$Hb_c$ —total haemoglobin concentration in blood [g/dl]

HI for all carbon samples has been evaluated in accordance with the following numerical grade given in Table 3.

Based on the calculated HI, damaging effects of carbon materials on human red blood cells were determined. Haemolytic index for a haemocompatible material should be ranged from 0 to 2 [19,20].

### 2.4. Haemolytic reaction with the use of erythrocytes

Haemolysis was determined according to the following procedure; the carbon materials (0.5 g) in the form of thin plates were placed

in polystyrene tubes of 10 cm<sup>3</sup> volume each. Next, 5 ml (150 mol/l) of NaCl isotonic solution was added. As controls two solutions were used, namely containing 5 ml NaCl isotonic solution and 5 ml of water for injection, where 100% haemolysis occurs. Then, condensed erythrocytes (20  $\mu$ l) were added to samples and incubated at 37 °C, for 6 h. Subsequently, the samples were centrifuged and absorbance measurements were made for the supernatants taken from the erythrocyte sediments. Absorbance at 530 nm was determined in a MARCEL s 330 spectrophotometer against water blank as control.

Haemolytic percentage was determined from the following formula:

$$\%H = \frac{(A_b - A_k) \cdot 100}{(A_{100} - A_k)}$$

where:

$A_b$ —test sample absorbance

$A_k$ —control absorbance

$A_{100}$ —test absorbance with 100% haemolysis.

The value of haemolytic percentage cannot exceed the value of 3% [21,22].

### 2.5. Evaluation of the shape of red cells

To prepare the samples for microscopic analysis, the suspension of red cells of 0.02 ml, after being in contact with carbon materials and after incubation in 150 mmol/l of sodium chloride solution, was placed on a base glass and covered with a cover glass. In the same way, blood suspensions without any material contact were prepared. The shape of cells for non-stained samples was analysed by optical microscopy under 1400 $\times$  magnification.

Images were documented photographically.

### 2.6. Study of blood coagulation

Activation of blood coagulation was determined by assessment of the clotting times (CT) on the surface and in whole contact of carbon samples with blood.

### 2.7. Study of blood coagulation on materials surface

A 0.02 ml volume of the citrated blood was deposited on a material surface in the form of a 1 cm<sup>2</sup> plate followed by observation of its shape. After 120 s 0.02 ml of CaCl<sub>2</sub> (25 mmol/l) was added, and clotting time was measured. The measurement was stopped when the first fibrin strand was formed [22].

### 2.8. Study of blood coagulation upon volume contact with materials

Carbon samples in the form of plates having dimensions of 0.5  $\times$  0.5  $\times$  0.05 cm surface area were placed in a polystyrene tube and incubated with 0.5 ml citrate blood at 37 °C, for 2 h. Subsequently, 0.5 ml of CaCl<sub>2</sub> (25 mmol/l) was added and CT was measured. The measurement was finished when the first fibrin filament was formed [22].

**Table 3**

Numerical scale of haemolytic index (HI).

0–2	Lack of haemolysis
2–10	Slight haemolysis
10–20	Mild
20–40	Moderate
>40	Acute

## 2.9. Statistics

Statistical analysis was done with the use of the Statistica 8.0 program. Mean values and standard deviations were calculated. Statistical comparisons between LTI and other samples were determined by *T* test for independent attempts. *p* values  $\leq 0.05$  were considered significant.

## 3. Results

The mean values of HI and haemolytic percentage are shown in Figs. 1 and 2. It can be seen that HI of all samples is below the limit of 2, which indicates that the materials tested do not provoke any haemolytic reaction. The lowest value of this index was noted for PG and DLC samples, whereas the highest one was found for synthetic graphite. The HI value of LTI was significantly lower (29%,  $p < 0.01$ ) in comparison to SG samples and simultaneously significantly higher in comparison to PG (54%,  $p < 0.001$ ) and GLC.

Haemolytic percentage of all materials tested didn't exceed the value of 3% and ranged from 0.77% to 0.90% (Fig. 2). The lowest value of HI was obtained for GLC sample, and the highest one for SG sample.

Haemolytic percentage for LTI samples statistically decreased by about 136% ( $p < 0.001$ ) in comparison to SG samples and, on average of 14% compared to PG, whereas it increased significantly by about 89% ( $p < 0.001$ ) compared to GLC.

The red cells after incubation in isotonic extract from carbon samples were found to have normal size (Fig. 3B–F) in comparison to cells after incubation in isotonic NaCl solution used as control (Fig. 3A). Some differences can be observed by analysing the morphology of these cells. The morphology of the red cells in control was typical as for in vitro test (erythrocyte with short cytoplasm pseudopodia distributed regularly). Similar morphology of red cells was observed for PG, DLC and LTI samples, although cytoplasm pseudopodia for PG were shorter, and for LTI longer as compared to control. Additionally, for the PG sample and to a lesser extent for LTI and DLC single spherical cells (spherocyte) were observed (Fig. 3C, D, F, arrows). The vast amount of spherical shaped cells was observed for the GLC sample (Fig. 3E). The spherical shaped red cells were not found in normal peripheral blood smear. Their presence may indicate a greater suspicion of haemolytic anaemia and reduction of the ability of gas exchange across the cell membrane [23]. Distinct changes in erythrocyte morphology were observed after incubation in isotonic extract from SG sample (Fig. 3B). The cells have the spiked shape with more elongated cytoplasm pseudopodia as compared to red blood cells in the control (Fig. 3B). Due to abnormal thorny projections this type of cells, known as acanthocyte, in human biology and medicine, refers to a form of red blood cell that has a spiked cell membrane. The cells can be found in case of excessive haemolysis and may indicate the lack of plasma lipoproteins, or haemolytic anaemia

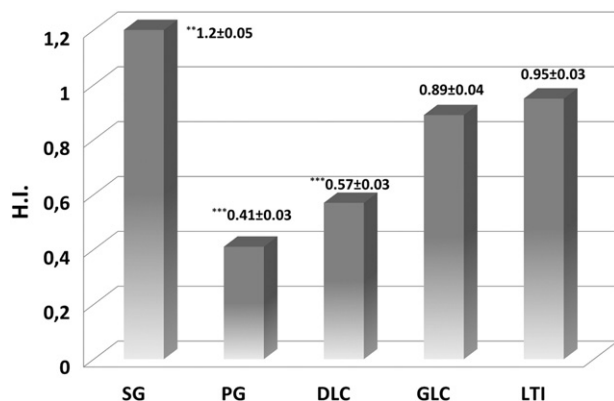


Fig. 1. Haemolytic index for carbon materials, \*\* $p < 0.01$ , \*\*\* $p < 0.001$ —in comparison to LTI sample.

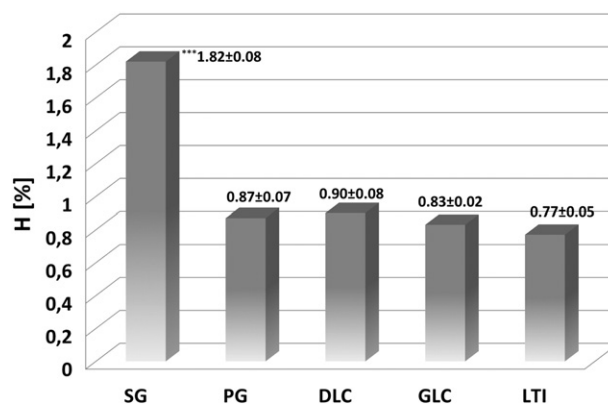


Fig. 2. Values of haemolytic percentage for carbon materials, \*\*\* $p < 0.001$ —in comparison to LTI sample.

[24]. The unfavourable change in shape of red blood cells for synthetic graphite was also confirmed by high value of the haemolytic index (HI = 1.2).

The results of the coagulation processes determined by assessment of the clotting times on the surface carbon samples and in the volumetric contact with blood are shown in Figs. 4 and 5, respectively. Fig. 4 compares the CT of blood on the surface of all examined carbon samples. It can be noted that the surface of LTI carbon prolongs CT by 37% ( $p < 0.01$ ) in comparison to DLC, by 27% ( $p < 0.01$ ) compared to PG and by 26% ( $p < 0.01$ ) compared to SG samples. On the contrary, the CT on the LTI surface is almost similar to the time for the GLC surface (increase by 5%). The times of forming a blood clot determined in the tubes after full immersion in the blood of SG, PG, DLC and GLC are compared to the clotting time of the LTI (Fig. 5). As shown in this diagram the LTI material significantly prolongs blood CT in comparison to SG by 42% ( $p < 0.001$ ), PG by 26% ( $p < 0.01$ ), to DLC by 39% ( $p < 0.001$ ), and GLC by 31% ( $p < 0.001$ ).

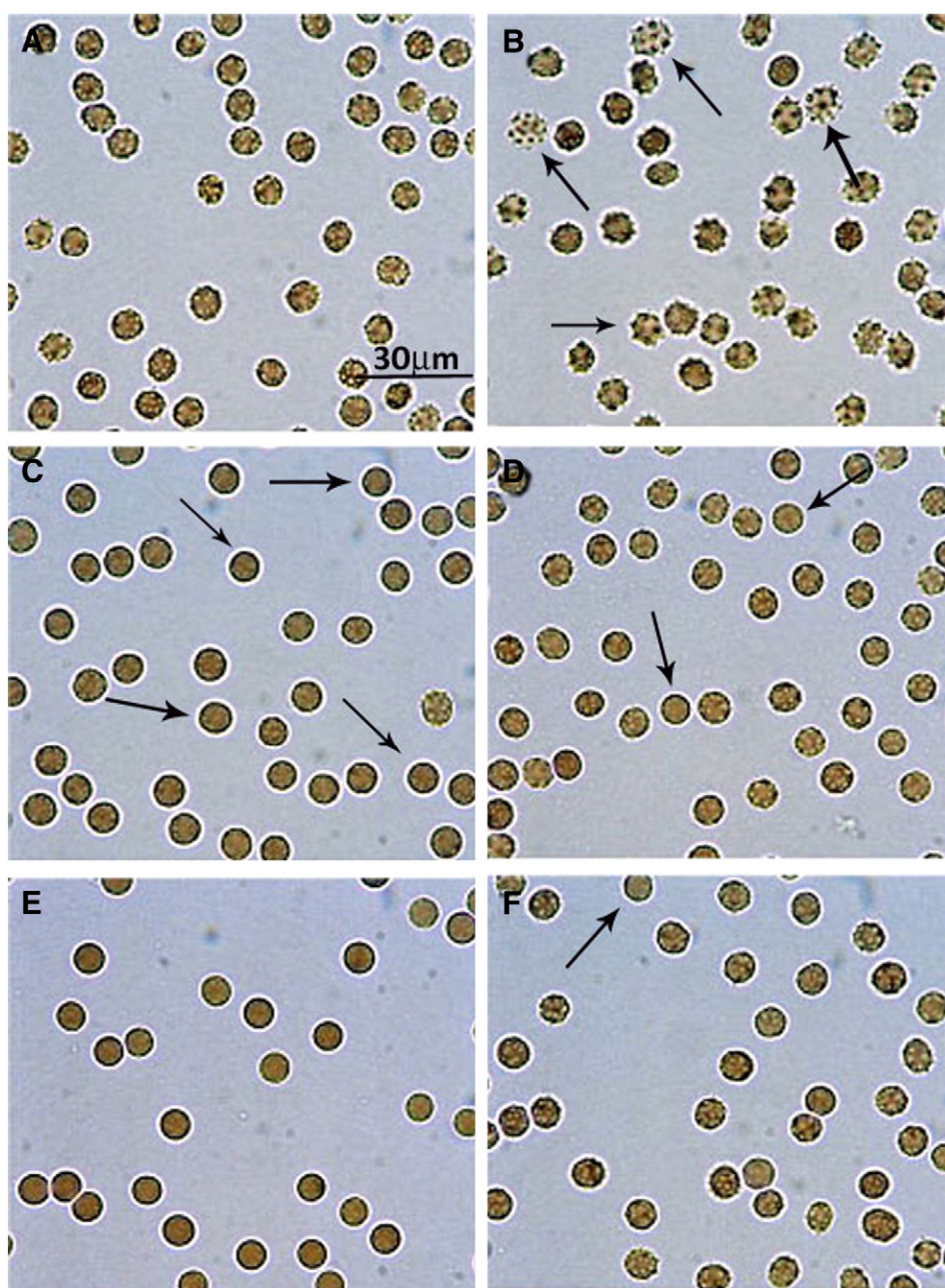
## 4. Discussion

Blood in living organisms is the most complex dynamic biological system. One of the important in vitro indicators for compatible synthetic materials with blood is the haemolytic reaction and clotting activation [25]. This study has indicated that all investigated materials present a relatively low diversification in reaction with red cells. The most significant changes of HI, haemolytic percentage and morphology were found for synthetic graphite.

The haemolytic index for all carbon samples was found to be in the range of 0 to 2. The lowest value of HI was noted for PG and DLC coating, whereas the highest values were obtained for synthetic graphite. The haemolysis percentage didn't surpass the acceptable level of 3%. The lowest H [%] was found for glass-like carbon (GLC), and the highest one for SG. Very close values of H [%] (0.77–0.90%) were obtained for PG, DLC and LTI. Also, a weak effect of the carbon surfaces on red cell morphology was noted in comparison to control sample. Only cytoplasm pseudopodia for PG were shorter, and for LTI longer in comparison to control. Moreover, single cells in spherical shape (spherocyte) especially for PG sample and to a lesser extent for LTI and DLC were observed (Fig. 3C, D, F, arrows). On the contrary, the most significant changes in HI and H [%] were observed for erythrocytes in contact to SG surface. For this sample, acanthocyte red cells were found, and HI and H [%] were 1.2 and 1.82, respectively. These values are still below the acceptable limit ( $< 2$ ).

Coagulation of blood was studied directly on carbon surfaces and after full immersion of the materials in blood. Activation of clot forming on LTI and GLC surfaces and clotting times was prolonged in comparison to other carbon materials. The highest shortening of clotting formation was noted for SG surfaces. Generally, the clotting process on the surface





**Fig. 3.** Microphotographs of red cells after incubation in: isotonic NaCl solution-control (A) (red cells with short regular cytoplasm pseudopodia); isotonic extract from SG samples (B) (red cells with regular elongated cytoplasm pseudopodia); isotonic extract from PG samples (C) (red cells with very short, regular cytoplasm pseudopodia); in isotonic extract from DLC samples (D) (red cells with short regular cytoplasm pseudopodia); isotonic extract from GLC (E) (spherical red cells with faint cytoplasm pseudopodia); isotonic extract from LTI samples (F). Non-stained sample, mag. 1400 $\times$ .

samples and after whole immersion in blood (volumetric contact) shouldn't differ significantly in relation to the same type of carbon. In both experiments LTI carbon was found to prolong the most effectively CT among the carbon materials studied. Some discrepancies in CT measurements on the material surfaces and by volumetric blood measurement can be explained by the fact that during volumetric contact, the blood also interacts with the side surfaces of carbon plates (SG-based plate, 1 mm thick) used in this study. In the case of LTI samples, the pyrolytic layer entirely covers the plate, including side surfaces of the sample. The SG samples have non-uniform lateral surfaces due to the presence of small pore defects.

Based on these experiments, all materials studied may be considered to be haemocompatible.

Considering possible reasons of the observed differences in CT for various carbon materials, two parameters in particular appear to be important, namely surface structure and its topography (roughness). The surface structure of the samples may be analysed in view of their crystallinity and the surface's energy. The surface topography of carbon materials was characterised by their surface roughness (see Table 1). It is interesting to note that although the GLC surface is much smoother than other samples the CT value determined by volumetric measurement is lower in comparison with LTI and PG and almost comparable with, e.g., DLC sample.

The changes of CT and apparent crystallite sizes for various carbons studied in this work are shown in Fig. 6. Crystallinity of carbon materials reflects their surface structural state and should be decisive in the

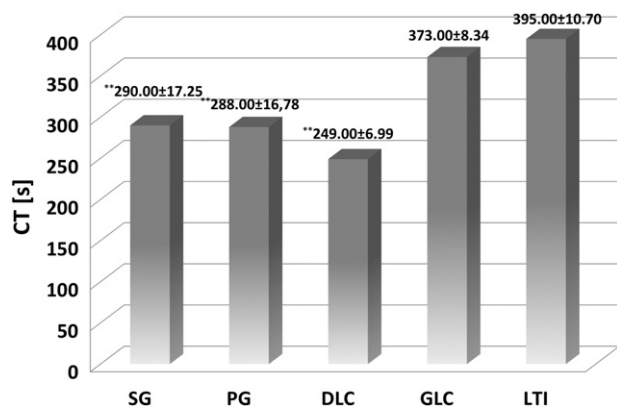


Fig. 4. Clotting times of blood in contact with various carbon material surfaces (surface contact) \*\*  $p < 0.01$  –in comparison with LTI sample as the reference.

interaction with blood and clotting mechanism. The carbon samples with a high crystallite size (PG, SG) are in contrast to the low-crystalline carbon samples, which demonstrate the longest CT. However, DLC values (shown in this figure separately) show the lowest value of CT, although its crystallite structure represents a small range of ordering.

As seen from data gathered in Table 2, the correlation between the total surface energy and compatibility of different carbon surfaces with blood, as shown by CT, is rather poor. A slightly better correlation was obtained between the CT of carbon materials and the dispersion energy component. Clearly, for carbon materials representing a lower value of dispersion energy component, prolonged CTs were found.

The presence of low size crystallites near the surface region of the carbon sample is likely to affect the clotting mechanism, inhibiting the formation of fibrinogen.

A probable explanation for the slight increase in clotting time of the whole blood in contact with LTI in comparison to GLC is that LTI total surface energy is smaller. The LTI surface exhibits the smallest value of the surface energy among the all investigated carbons. On the contrary, the total surface energy for GLC carbon is higher than other  $sp^2$ -based carbon materials, while its CT value is very close to LTI (5% difference). Notwithstanding its high surface energy, GLC shows the lowest value of interfacial bond energy with HFG ( $\gamma^{m,f} = 22.86 \text{ mJ/m}^2$ ). For materials in direct contact with whole blood, a key issue is the ability of a material surface to prevent thrombus creation. Taking into consideration a simple mechanism of clotting process during the initial time in static blood contact, the fibrinogen is a main protein interacting with the material exposed to the blood environment. It is also known from the literature that a high surface energies ratio of the proteins albumin/

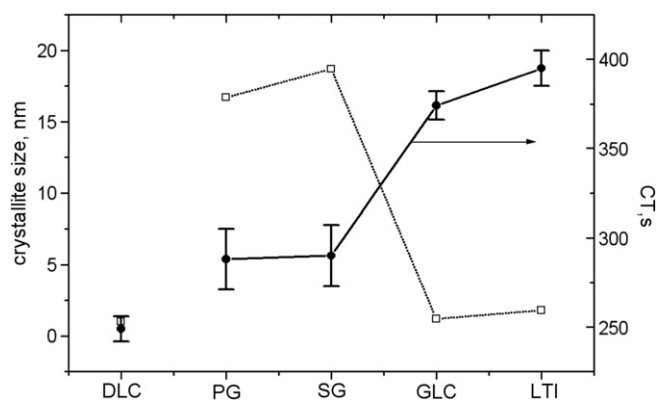


Fig. 6. Clotting time and apparent crystallite sizes for various carbon surfaces.

fibrinogen adsorbed on an implant surface is an indication of the ability of a material surface to inhibit thrombus formation [26]. The determined values of albumin/fibrinogen surface energy ratios ( $\gamma^{m,a}/\gamma^{m,f}$ ) in our work for most of carbon materials are ranged between 0.792 and 0.805, while for GLC samples this ratio amounts to 0.738. These results lead to the conclusion that GLC surface should favour clot formation as compared to other materials studied. However, the conclusions published in other papers are inconsistent to observations of our experiments [25,27].

The LTI surface exhibits the lowest total energy, although its interfacial bonding between whole blood and HFG is slightly higher than that of GLC. It's worth noting, however, that the GLC surface is much smoother than the LTI carbon surface (Table 1). It is a well-known fact that roughness tends to cause a material to thrombose more rapidly than the same material with a smooth surface [28,29]. The defects on the surface of GLC are in nanometric scale, while the other carbon surfaces contain the surface inhomogeneities of an order higher. The results indicate that the energetic factors seem to be even more important in the sequence of clotting events than the topography features of the carbon samples represented here by the roughness parameter. As indicated from both coagulation tests (surface and volumetric), as well as haemolytic parameters, no influence is observed between surface roughness of the samples and CT, which can probably be explained by static contact of blood with the analysed surfaces.

In summary, it can be concluded that the sequence of clotting formation on various carbon surfaces is complex, and the initiating step may result from the complex nature of the analysed surfaces. Surface energy is an average parameter characterised by the chemical state (chemical bonds, functional groups) and physical state (crystallites size, defects, surface charge, topography) of a material. To explain the observed behaviour of analysed carbon materials, both energies (total surface energy and interfacial bonding between material surface and fibrinogen) should be taken into consideration: in the case of SG, PG and DLC samples, both energy components are high, hence surface activation in the initial adsorption of fibrinogen is easier, and the protein easily adheres to the surface of the sample. On the contrary, low values of  $\gamma^{m,f}$  for GLC and LTI favour creating relatively unstable surface sites with adhering proteins. Although the total energy ( $\gamma^T$ ) for GLC is high, the bonding between fibrinogen and the surface is weak, and it is likely that fibrinogen is partially desorbed by components of the blood. This is due to specific surface morphology of this material (low roughness, very low apparent crystallite size, high interplanar graphene distance  $d_{002}$ ) and very small surface sites where fibrinogen deposits occur. It is likely, that due to low  $\gamma^{m,f}$  for GLC, the process of activation of clot formation on its surface is longer in comparison to PG and SG samples displaying higher energy between their surface and human fibrinogen (see Table 1). Only in the case of LTI carbon both energy components ( $\gamma^T$ ,  $\gamma^{m,f}$ ) are low, therefore this type of the surface is the most effective in preventing the thrombus formation.

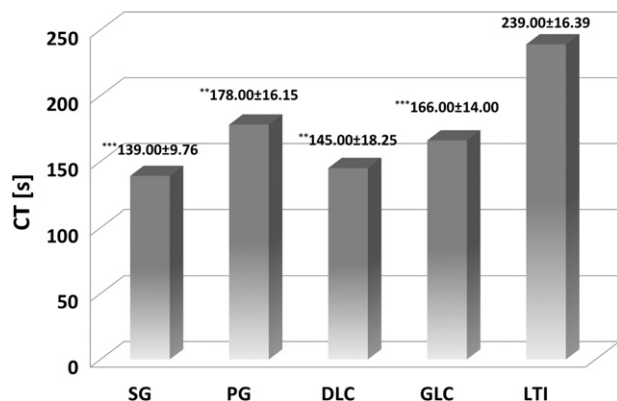


Fig. 5. Clotting times of blood in contact with carbon samples after complete immersion in the blood (volumetric contact)  $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ —in comparison to LTI sample as the reference.

## 5. Conclusions

In vitro studies in static contact with blood showed that haemolytic properties of all examined carbon materials are suitable for the development of blood-compatible medical devices. All examined materials were relatively well tolerated in contact with blood and inhibited the clotting process in comparison to the reference material. The LTI samples have shown to be the most effective in preventing thrombus formation and do not reveal any blood compatibility problems. Prolongation and shortening of the clotting time on carbon surfaces indicates possible activation and/or deactivation of blood plates and clotting factors.

## Prime novelty statement

The work indicates the main advantageous features of carbon surfaces for the improved blood compatibility taking into consideration structural, microstructural and topography parameters. Initial stages of blood coagulation in contact with differently manufactured carbon coatings were studied. Selecting of coating for blood contact biomaterials.

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