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BIOCOMPATIBLE EVALUATION OF BIOMATERIALS USED IN THE NEW POLISH EXTRACORPOREAL PULSATILE HEART ASSIST DEVICE RELIGAHEART EXT

BIOKOMPATYBILNA OCENA BIOMATERIAŁÓW STOSOWANYCH W NOWEJ POLSKIEJ POZAUSTROJOWEJ PULSACYJNEJ PROTEZIE SERCA RELIGAHEART EXT

The innovative extracorporeal heart support device ReligaHeart (RH EXT) has been developed, based on POLVAD ventricular assist device clinical experience, collected in more than 300 patient applications. The innovative surface engineering technologies are applied in ReligaHeart EXT device. The pump is manufactured of new generation, modified surface structure, biocompatible polyurethanes, and equipped with original tilting disc valves, Moll type. The valve ring is made of titanium alloy, TiN+Ti_2N+αTi(N) diffusive layer modified, produced with glow discharge at plasma potential, in order to obtain the lowest thrombogenicity. The valve disc is made of polyether ether ketone. The complex *in vitro* and *in vivo* biological evaluations were performed, confirming both biomaterials biocompatible properties and device biocompatibility, proved in 30 days animal heart support.

Keywords: biomaterial biocompatybility evaluation, ventricular assist device, polyurethanes, diffusive layers, titanium nitride

Na podstawie doświadczeń klinicznych protezy serca POLVAD, zastosowanej u ponad 300 pacjentów, opracowano zmodernizowaną pozaustrojową pompę wspomagania serca ReligaHeart EXT (RH EXT). W protezie RH EXT zostały zastosowane innowacyjne technologie inżynierii powierzchni. Pompa wykonana jest z nowej generacji biozgodnych poliuretanów o modyfikowanej strukturze powierzchni i jest wyposażona w oryginalne zastawki dyskowe typu Moll. Pierścień zastawki jest wykonany ze stopu tytanu z dyfuzyjną warstwą TiN+Ti_2N+αTi(N) wytwarzaną w procesie obróbki jarzeniowej na potencjale plazmy, dla osiągnięcia niskiej trombogenności. Dysk zastawki jest wykonany z polieteroketonu. Wykonano kompleksową ocenę biozgodności *in vitro* i *in vivo*, potwierdzając biozgodne własności biomateriałów i protezy RH EXT, także w czasie 30 dniowego wspomagania serca w modelu zwierzęcym.

1. Introduction

Based on POLVAD ventricular assist device clinical experience, clinically used in over 300 patients, with the longest assistance duration lasting 650 days, the innovative extracorporeal pulsatile heart support device, called ReligaHeart EXT (RH EXT), has been developed in Artificial Heart Laboratory, Zabrze, Poland (Fig. 1a) [1-3].

The numerous construction improvements have been applied in the ReligaHeart EXT blood pump (70 ml stroke volume displacement device) including pump house construction optimized regarding blood flow architecture, low profile blood pumping membrane system, and mechanical system avoiding an incidental cannulas disconnection. The blood pump is equipped with the originally developed in Poland tilting disc valves, called Moll Valves [4,5] which consist of original valve ring, free of pivot element crossing the blood stream (Fig. 1b).

The innovative surface engineering technologies have been applied in RH EXT device. The pump is manufactured of the new generation biocompatible polyurethanes: Biospan and Bionate (DSM Biomedical, USA), with modified surface structure reducing polymer surface embolization risk and increasing long term blood contact durability. The polyurethane RH EXT elements are manufactured utilizing automatized injection moulding process (WADIM PLAST Narojek SP.J., Reguły, Poland), in order to achieve the best surface material properties directly after injection process – without any post-process surface modifications or improvements [6]. The Moll

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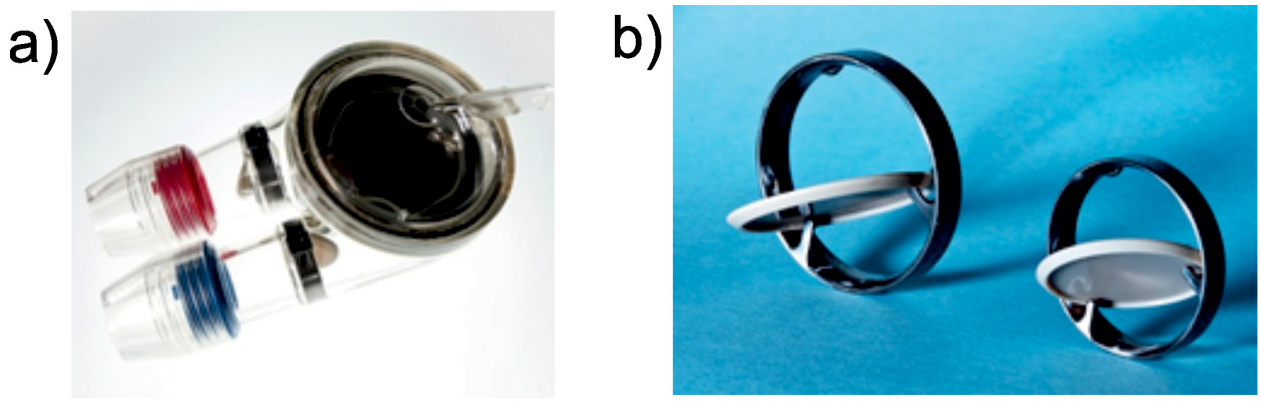

Fig. 1. ReligaHeart EXT ventricular assist device (a), Moll tilting valves (b)

valve ring is made of titanium and titanium alloy Ti6Al4V with TiN+Ti_2N+αTi(N) diffusive layers produced with glow discharge in so called plasma potential. The surface modifications of titanium and its' alloys influence surface layer structure and morphology, adhesion and inside layer residual stresses. That creates total implant stability in live organism habitat as well as improves biocompatibility, especially good blood compatibility [7-10]. The valve disc is made of innovative biopolymer: polyether-ether-ketone PEEK-OPTIMA (Invibio, USA). The pump cannulas are made of biocompatible, high durability polymer Tygon ND100-65 (Saint-Gobain, France). The required biocompatible properties of ReligaHeart EXT biomaterials confirmation were necessary to qualify the device into clinical trials.

2. Materials and methods

Selected biomaterials, polymers and titanium materials, are used in different RH EXT device parts (Table 1).

TABLE 1

ReligaHeart EXT material construction

Biomaterial	Device element	Technological process
Bionate II (90A) polyurethane	Blood chamber, membrane rings	Injection process
Bionate II (55D) polyurethane	Pneumatic chamber, inflow and outflow connectors	Injection process
Biospan (70A) polyurethane	Membranes	Lamination process
Titanium grade 2 and titanium alloy Ti6Al4V both with TiN+Ti_2N+αTi(N)	Valve ring	Machining + glow discharge process
PEEK-OPTIMA polymer	Valve disc	Machining
Tygon polymer	Cannulas	Extrusion + polymer gluing

The polymers of various hardness are utilized in the blood pump elements. There are hard polymer parts as pneumatic chamber, connectors as well as valve disc, and soft polymer parts as blood chamber and membrane. The polymer parts are produced in the following technological processes: dipping process for soft membranes and injection process for hard shell elements: chambers and connectors mainly [6]. The titanium valve rings and polymer valve discs are manufactured utilizing in numerically controlled machining process [6].

The titanium nitride layers were formed on titanium surface, utilizing glow discharge in plasma space, developed and introduced into use at the Faculty of Materials Science and Engineering of the Warsaw University of Technology. The process temperature is about 700°C, the treating time – about 4 hours. The coating layers have a nanocrystalline structure and uniform low roughness [7-10].

RH EXT device, designed for long term application with permanent blood contact (up to one year), belongs to the group of implantable medical devices, compatible with the European Standard for Medical Devices: ISO 13485. The complex biocompatible evaluation was performed including *in vitro* and *in vivo* study, in compliance with ISO standard 10993. Biomaterials were examined after selected technological processes, in order to confirm their biocompatible properties stability after technological treatment, required for ventricular assist device application. After detailed state of the art and risk analysis, the list of necessary biocompatible examinations was developed. The polyurethanes used in RH EXT device were examined *in vitro* first (in order to establish haemolytic, thrombotic and cytotoxic properties), and then *in vivo* (in order detect inflammatory reaction after implantation), after different technological processes utilized for RH EXT device production. Titanium layers were examined, especially in the aspect of thrombogenicity. The biocompatibility of RH EXT device as the final medical product was examined the fields of: haemolysis, thrombogenicity, cytotoxicity, inflammatory reaction induced by implantation procedure, irritation, skin sensitization, and systemic toxicity, in 30 days animal study of mechanical left ventricle support.

All the biomaterials and RH EXT devices were prepared to biocompatibility examinations in the compliance with the final medical device validated procedure, ethylene oxide (EO) sterilization utilizing EOGas 4 sterilizer (Andersen Products, USA) for 12 hours sterilization cycle at temperature of 30°C.

The polymer samples and RH EXT devices were aerated for 28 after sterilization.

Biomaterial thrombogenicity investigations, utilizing model of shear stress induction for the blood cells by the biomaterial surface, were performed *in vitro* with the use of Impact-R device (DiaMed, Switzerland). The Impact-R method determines platelets activation during experiment of citrated fresh human blood flow above the biomaterial surface in physiological-like conditions. The 14,5 diameter biomaterial samples were exposed to 130 µl of blood, rotating with the speed of 720 rpm within 300 seconds. The goal of the study was to evaluate the platelets activation, aggregation and adhesion of different biomaterials surfaces. The blood, collected after biomaterial examination, was investigated with cytometry (Beckman Coulter FC500 analyser, USA). The biomaterials surface was evaluated using fluorescent microscopy (Axio Observerver – Zeiss, Germany). The leucocytes and platelets activation and aggregation were evaluated utilizing CD45 and CD62 antibodies.

The biomaterials haemolysis examinations were performed *in vitro* in compliance with ISO 10993-4 and ASTM F 756 – 00. The 0,8 cm diameter biomaterial samples were exposed to human blood (collected from Regional Centre of Blood Donors and Blood Health Care) for 8 and 24 hours, in free movement conditions at temperature of 37°C. Haemolysis index, free haemoglobin concentration (fHGB) and cells morphology were examined. Haematological parameters were evaluated by: MCV (average erythrocyte volume), MCH (average haemoglobin mass in erythrocyte), MCHC (average haemoglobin concentration in erythrocyte), HGB (haemoglobin concentration) and RBC (erythrocytes number). The haemolytic index was calculated. The level of free hemoglobin in blood plasma was measured utilizing haemolytic analyser (HemoCue). Cell morphology was evaluated utilizing May-Grunwald and Giemsa reagents. Erythrocytes indicators were measured with the use of haematological analyser (Mindray).

Biomaterial cytotoxicity examination was performed *in vitro* in compliance with ISO 10993-5 using the direct method. The 1,4 diameter biomaterial samples were exposed to the mouse fibroblasts (L 929 American Type Culture Collection) for 48 hours. Then the fibroblasts were incubated in temperature of 37°C with 5% CO_2 flow, coloured utilizing propidium iodide, and counted by fluorescent microscope.

Inflammatory reaction after biomaterials implantation was evaluated *in vivo* in compliance with ISO 10993-6. The flat biomaterials foils (5×5×1 mm) were prepared and EO sterilized for implantation. The three group of small animals (rabbits) were observed: animals after PU samples implantations and two control groups – animals with surgical procedures only and animals free of any procedures. The antibiotic protection was applied for 5 days after implantation. During 30-day observation of the rabbits the scar look was controlled microscopically and the animal's behaviour was monitored. After animals euthanasia at the end the experiment period, the autopsy examination of collected tissue samples: muscle sections with implant, lymph nodes, as well as internal organs (heart, thymus, liver, spleen and kidneys) was performed.

Tests for irritation, skin sensitization and systemic toxicity were performed *in vivo*, during RH EXT device implantation in short and long term animal trials, utilizing the pig model. The RH EXT pump had the direct contact with the animal shoulder skin, blood and tissues. The 6 animals were observed for 7 days and 4 animals were observed for 30 days. The additional intradermal allergic examinations were carried out for TYGON cannulas – the histopathological evaluation of cannulas wounds was done after animal autopsy. The device sub-acute toxicity was evaluated, as RH EXT internal surface examination after continuous contact with circulating animal blood during *in vivo* trials. The laboratory blood parameters during animal heart assistance were examined (haematocrit, white blood cells, red blood cells, platelets count, free haemoglobin, bilirubin, creatinine levels, and many more), and organ samples were microscopically analysed after autopsy.

2. Results and discussion

The *in vitro* biocompatible examination of polyurethanes used in RH EXT device confirmed its required properties for long term implantation: haemolytic, thrombogenic and cytotoxic properties.

The haemolysis the examined polyurethanes did not exceed the required standard level (Table 2). In the microscopic evaluation the proper erythrocytes shape was observed in blood after contact with all the examined biomaterials. MCV, MCH, MCHC, RBC, HGB and fHGB did not exceed the clinical accepted values (Fig. 2).

TABLE 2

Haemolysis examination results of polyurethanes examination

Biomaterial	Blood exposure time	Haemolytic index	Haemolysis*
Bionate II90A	8 hours	0,08%	Not haemolysed
Bionate II 55D	8 hours	0,11%	Not haemolysed
Bionate II90A	24 hours	0,16%	Not haemolysed
Bionate II 55D	24 hours	0,04%	Not haemolysed

* in compliance with ASTM F 756 – 00

The platelets circulating in the blood over the examined sample were activated with share stress and partially adhered to the sample surface. To study the level of induced platelets activation and the ratio of platelets adhesion to the surface, the following measurements were performed in the blood circulating over the sample: number of platelets, number of platelets aggregates, (including small and big aggregates) percentage of platelets-monocytes and platelets-granulocytes aggregates, platelet activation ratio determining with change of IIb/IIIa integrin receptor conformation, platelet activation ratio determining with P-selectin expression. The examination was performed in comparison to the reference material – polystyrene (PS). The highest leucotycet activity (the percentage of CD 45 receptor activity) was observed in the blood after contact with the reference biomaterial – polystyrene (Fig. 3).

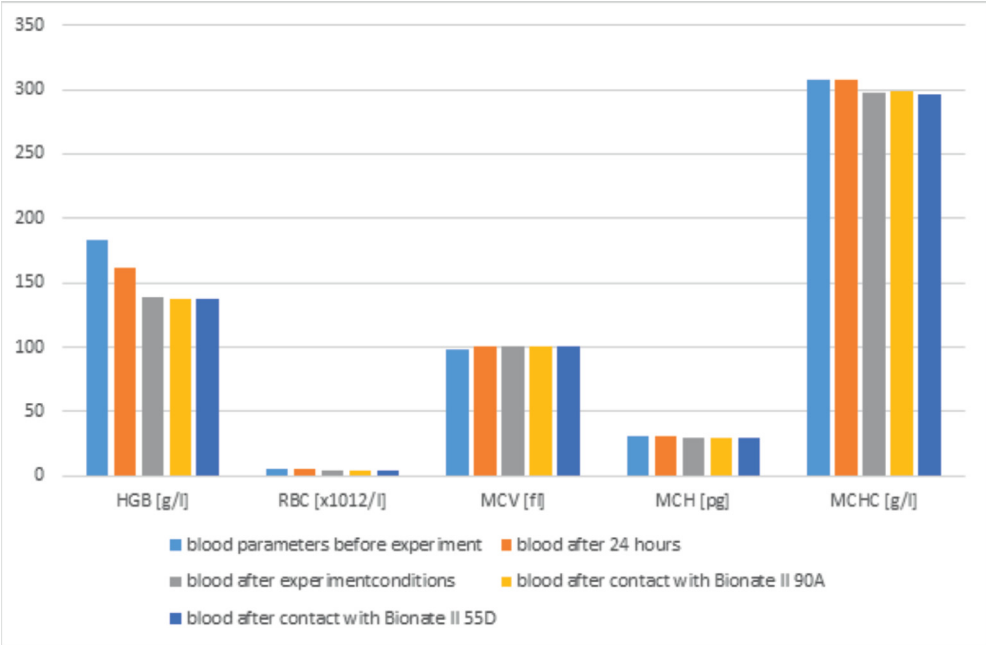

Fig. 2. Morpfhollologic blood properties after 24 hours contact with examinated polyurethanes

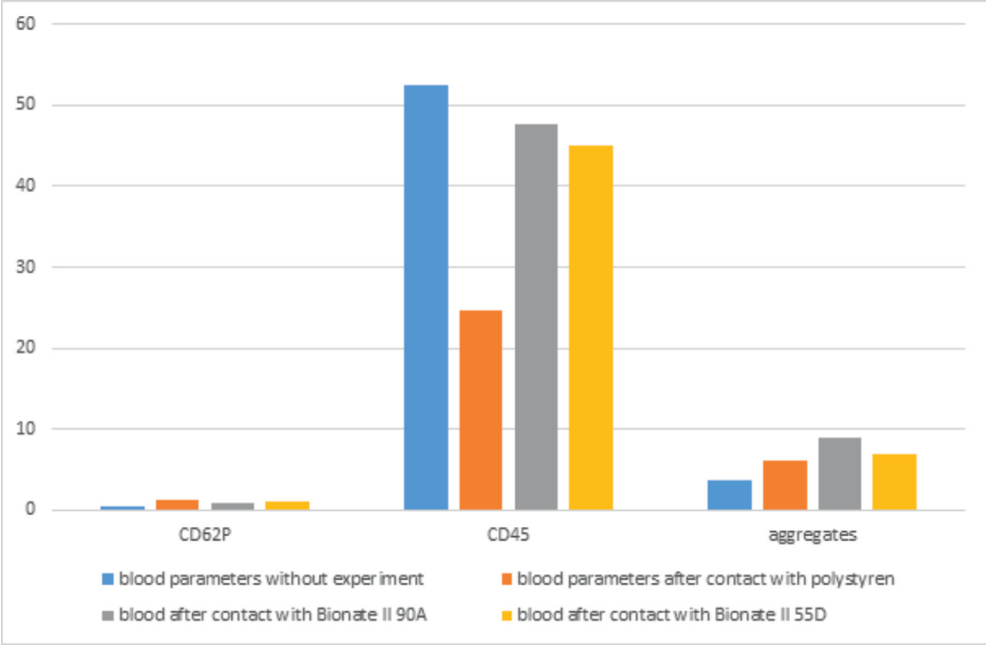

Fig. 3. Thrombogenicity properties of blood after contact with examinated polyurethanes

The lowest leucotycet activity was observed in the blood after contact with Bionate II 90A. The platelets activation (the percentage of CD 62P receptor activity) was low in the blood after contact with all examined polyurethanes.

The thrombogenicity evaluation results of titanium layers are presented in Fig. 4-6.

The examination was performed in comparison to the following materials: PS – polystyrene; PU – polyurethane used before in POLVAD device; Ti6Al4V – titanium alloy; TiN – titanium nitride layer. The induced platelets activation is presented together with results for blood not exposed to shear stress: BAS – blood preserved in static condition and ADP – blood with clothing activated by adenosine diphosphate (ADP) and preserved in static condition. The blood contacted with TiN layer has demonstrated the smallest percentage of created platelet aggregates (5,19%) – while 24,09% of aggregates were formed on titanium alloy, respectively.

The parallel analysis of a number of platelets circulating in the blood over the sample after shear stress exposure with level of these platelets activation, showed that the biggest percentage of platelets had circulated in the blood after shear stress exposure to TiN surfaces and these platelets were also not activated. While,

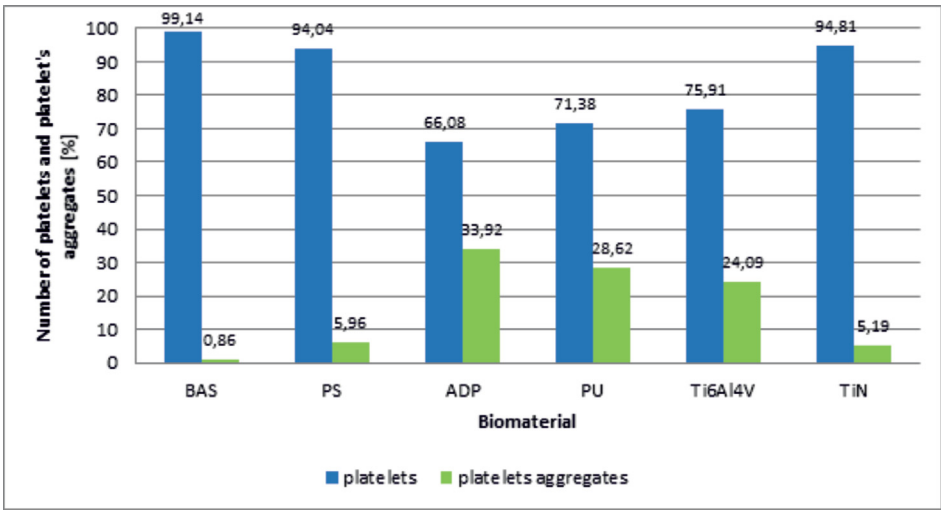

Fig. 4. Number of platelets and platelet's aggregates in the blood after contact with examined biomaterial

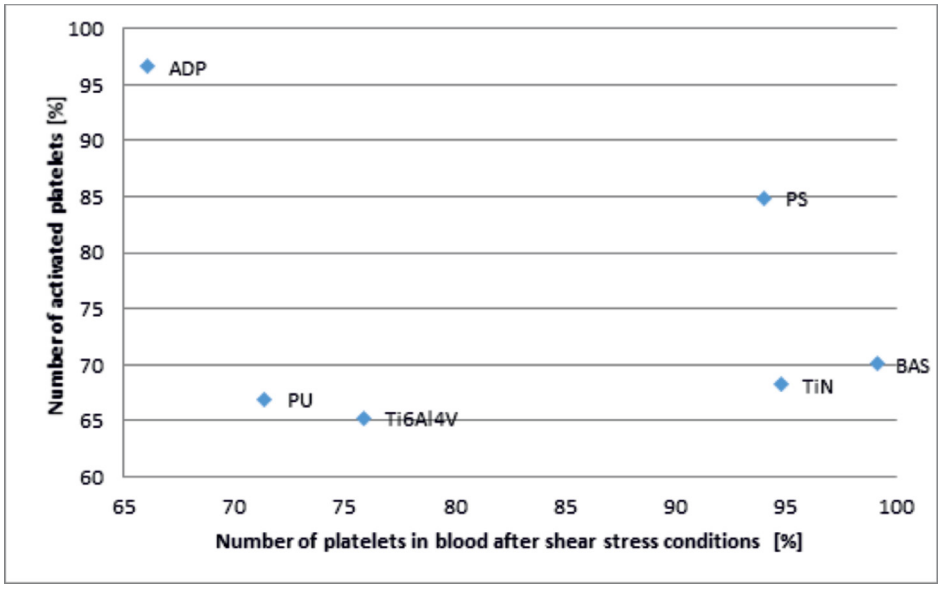

Fig. 5. The parallel analysis of activated platelets and platelets still circulating in the blood – GPIIb/IIIa receptor examination

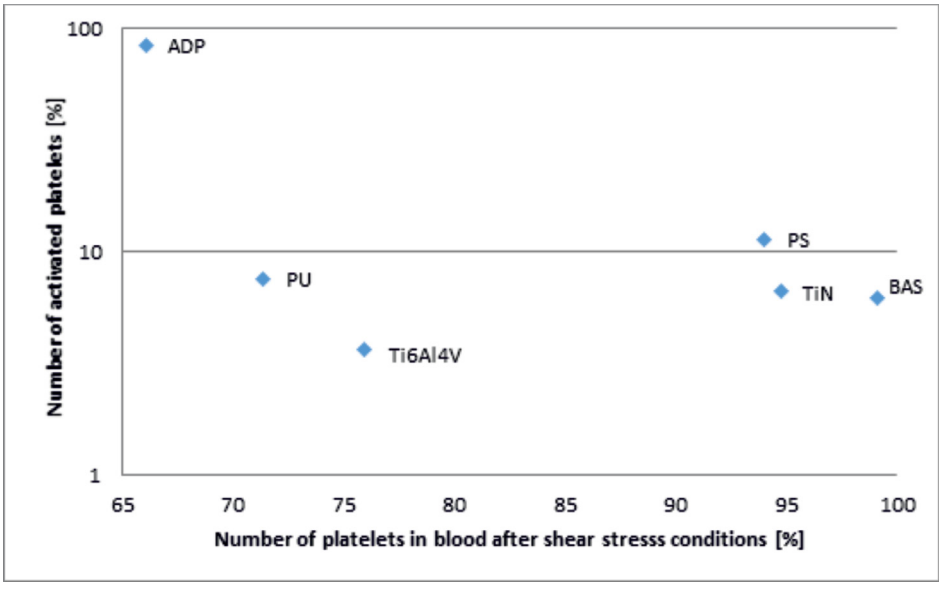

Fig. 6. The parallel analysis of activated platelets and platelets still circulating in the blood – P-selectin examination

platelets circulating after exposure to pure PU or Ti6Al4V were activated at the similar level as for TiN, the number of circulating platelets was much smaller. Similar results has been showed by measurements of GPIIb/IIIa receptor expression (Fig. 5) and P-Selectin expression (Fig. 6).

The cytotoxicity examination revealed that the investigated polyurethanes created a low cytotoxicity. The low number of necrotic cells was observed (Fig. 7). The living cells had the proper morphologic structure.

The proper inflammatory reaction after examined polyurethanes implantation was confirmed. The morphology, CRP and miscroscopic evaluation of rabbits blood 30 days after implantation were proper, which was confirmed by the good postoperative scar healing process. The histopathological examination of

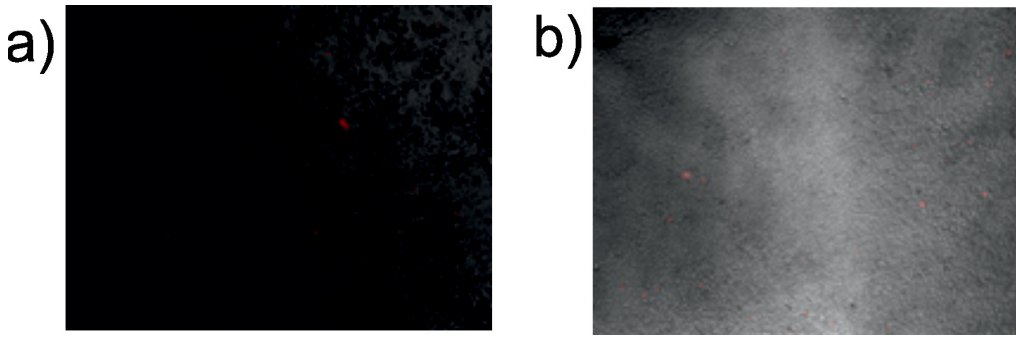

Fig. 7. Microscopic evaluation of examined polyurethanes in cytotoxicity test (single necrotic cells show red fluorescence); A – Bionate II 90 A, B – Bionate II 55D.

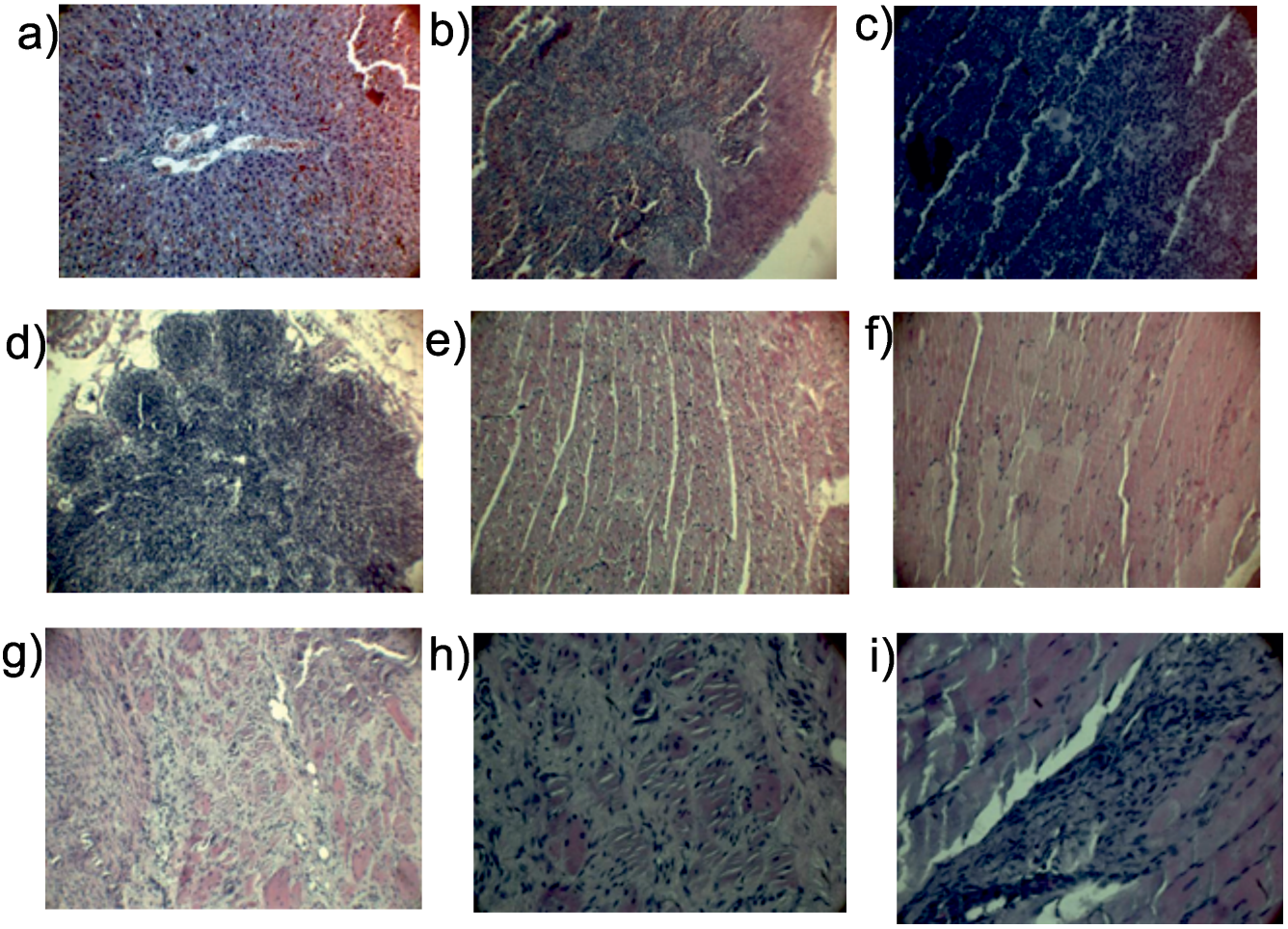

Fig. 8. Histopathological examination of rabbits internal organs after 30 days post implantation
a – correct liver structure, congestion visible; b – physiological spleen picture with correct red tissue structure; c – physiological thymus structure; d – physiological lymphatic gland structure; e – regular heart muscle structure; f – regular skeletal muscle structure; g – postoperative scar – small blood vessels and lymphatic infiltration visible; h – postoperative scar – lymphocytes, macrophages and long fibroblasts between collagen observed; i – postoperative scar with elongated fibroblasts and macrophages

rabbits internal organs also showed the proper structure (Fig. 8). No pathological properties were found in lymph nodes. Hearts, muscels and kidneys showed the proper histological structure. The small quantity of inflamantory cells was observed in the scars, but the healing process didn't differ from the proper resorption and fibrogenesis process.

As for skin sensitization and irritation reaction, no discolorations, reddening or erythema on animal's skin were observed after both the 7 and 30 days of exposition to RH EXT device. Animal autopsy results also did not show any changes on the animal skin surface. During the intradermal allergic TYGON cannulas examination, the local inflammatory condition of external skin and upper layers of subcutaneous tissue were observed. The laboratory blood parameters in the 7 and 30 day animal trials were on the normative levels, low increase of enzymes was observed on the first day after implantation due to surgical procedure and analgesic reaction. No toxic inflammatory features were observed in the histopathological animal organs examinations.

3. Discussion

The biocompatible *in vitro* evaluation showed that the examined biomaterials (polyurethanes Bionate II of 90A and 55D hardness) after technological injection process did not induce haemolytic reaction, did not change the erythrocytes morphology and did not have any influence on blood parameters, did not have significant thrombogenic activity, did not activate the platelet – leucocytes system, and were not cytotoxic. The thrombogenicity examination result analysis demonstrated that diffusive TiN + Ti_2N + αTi(N) layers produced on titanium alloy surface activate platelets less than pure titanium alloy.

The biocompatible *in vivo* evaluation showed that the examined biomaterials and the RH EXT prosthesis do not cause any acute or chronic organ failure. The inflammatory evaluation after polyurethanes implantation showed the proper healing process of implantation scar, only temporary local inflammatory reaction in the implantation area was observed, as a result of implantation tissue manipulation. After 30 days from PU implantation no global inflammatory histological symptoms were found (no inflammatory reaction was observed beyond the implantation area) and no immunological stimulation was observed. No significant irritation, skin sensitization, and systemic toxicity were observed after 7 and 30 days of RH EXT device animal trials.

4. Conclusion

The biocompatibility *in vitro* and *in vivo* examinations of polyurethanes after different technological processes and TiN diffusive layers, produced in glow discharged process at plasma potential, confirms the relevant and expected properties of new generation biomaterials, required for RH EXT ventricular assist device human application. The RH EXT device was approved for clinical trials by the Office for Registration of Medical Products in Poland.

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