THE BIOCOMPATIBILITY IN VITRO STUDY OF AN INNOVATIVE ELASTOMER FOR HEART ASSIST DEVICES CONSTRUCTION

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Introduction

The progress in modern medicine would not have been possible without advanced medical devices application, basing on development of new biomaterials with improved biocompatibility properties. The new elastomeric biomaterial have been developed as a part of WPTU PI BMT Dept. study, dedicated to be used as a construction material of polish pulsatile extracorporeal ventricular assist device ReligaHeart EXT. The material is a co-polymer, basing on poly(terephthalate ethylene) (PET) modified by dimerized parts of fatty acids (DLA)- PET/DLA. The essential part of the new biomaterial investigation is its biocompatibility assessment in compliance with PN EN ISO 10993 [1]. The PET/DLA biocompatibility evaluation was performed for two biomaterial forms: the raw material and for the material technologically processed of high-pressure injection moulding.

Materials and Methods

The reaction of petroleum ether cleaned biomaterial PET/DLA with red blood cell components and fibroblasts was investigated in compliance with PN EN ISO 10993-1,4-5 [1]. Biomaterial was assessed in two forms: granulate before technological processing, and 8 mm diameter discs, manufactured using high-pressure injection moulding process. The biomaterial samples were prepared for study in accordance with guidelines defined for applied investigation technique, and in form representative for final medical device shape. The sterilization process of the PET/DLA biomaterial discs has been performed as 12 hours cycle of ethylene oxide exposure at temperature of 30°C, utilizing sterilization cabinet EOGas 4 series Andersen Products. After the sterilization process, the biomaterial has been submitted to a natural airing process for 28 days. The biomaterial granulate was indirectly contacted with the biological medium, while the biomaterial discs were tested using direct methods. The blood haemolysis examinations have been performed after biomaterial samples incubation with CPDA preserved Whole Blood, with increased temperature and natural slow mixing conditions, applied for period of 8 and 24 hours [1-4]. The following parameters were analyzed: blood morphology, concentration of free plasmatic haemoglobin (fHGB), red cells osmotic resistance. The Haemolysis Indexes (IH) have been calculated in compliance with ASTM 756 00. The cytotoxicity evaluation was performed on fibroblasts line - clone 929 L, treated with 5% CO_2 flow and temperature of 37°C for 48h [1]. The propidium iodide coloured cells have been investigated using fluorescence microscope.

Results

The free plasmatic haemoglobin (fHGB) concentration at the blood after contact with PET/DLA was retained on the level 0,5 g/L for both biomaterial forms: the raw material and material after technological processing (FIG. 1). The red blood cells parameters and osmotic resistance values were close to the preserved Whole Blood value and oscillated within referential values interval.

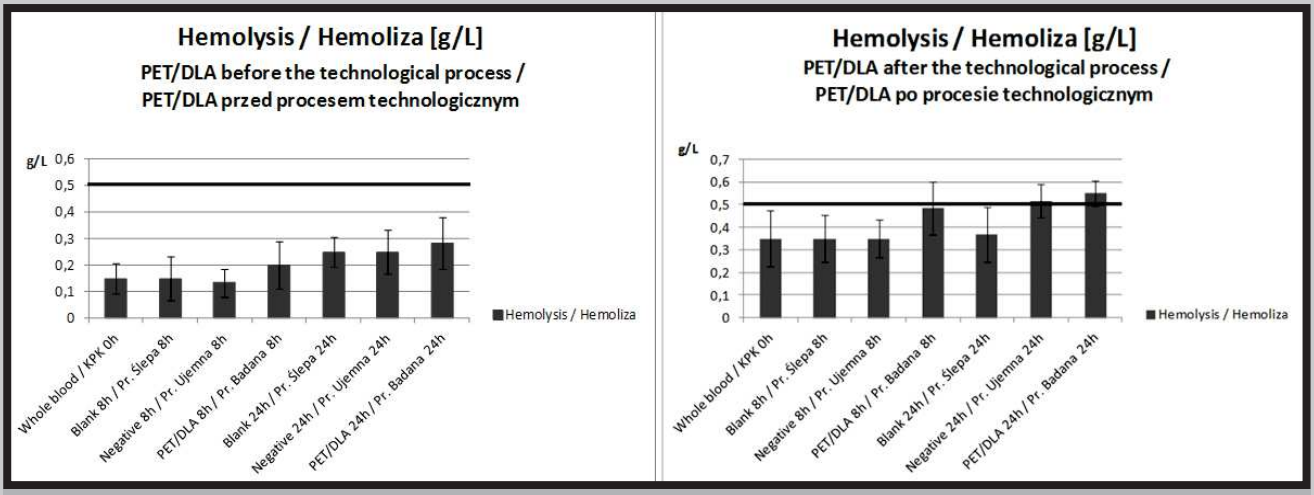

FIG. 1. Concentration of free osmotic haemoglobin for PET/DLA which were contacted with the preserved Whole Blood a) direct b) indirect. Legend: for the studied research groups:
• Whole Blood - the preserved Whole Blood; • Blank – Whole Blood standing at room temperature for 8 and 24 h, not subjected to the conditions of the experiment; • negative control - Whole Blood without contact with the test materials, subjected to the conditions of the experiment for 8 and 24 h (subject to natural slow mixing); • study group - tested biomaterials PET / DLA contacted with the preserved Whole Blood directly and indirectly, at the experimental conditions for 8 and 24 h.

The ASTM F 756-0 norm defines three intervals of haemolysis levels: IH from 0 to 2% - haemolysis level = non-haemolytic, IH from 2 to 5% - haemolysis level = slightly haemolytic, IH > 5% - haemolysis level = haemolytic. The Haemolysis Indexes of exanimated biomaterial have not exceeded 2% (FIG. 2). Pursuant to IH values, the assessed PET/DLA has been determined as non – haemolytic in both forms: row biomaterial and injection moulding processed.

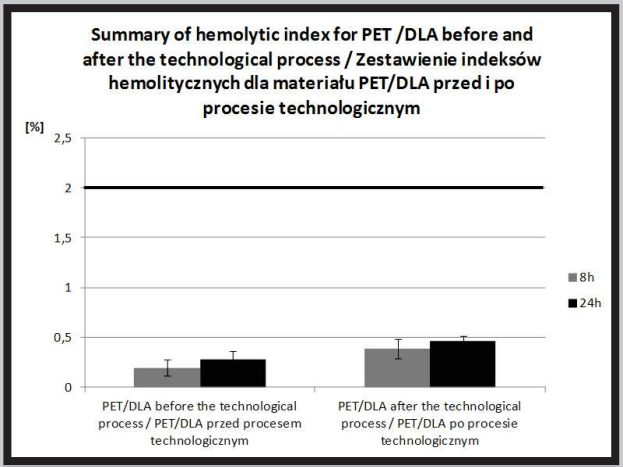

FIG. 2. Haemolysis Index for preserved Whole Blood after a contact with the PET/DLA in forms: granules (row material before technological process), and disc samples (after technological process).

Observation of culture for both: cells supplemented with extract of row material granulate, and cells after direct contact with injected biomaterial discs, haven't show the essential cytotoxicity feature. The number of necrotic cells has remained at the level from 0 to 0,83%. The remaining cells were positive in the presence of fluorescein diacetate (FDA) and classified as alive. Most of alive cells shown normal morphology, typical for fibroblasts. According to cytotoxicity assessment scale of ISO 10993-5 standard, there have been assigned: cytotoxicity level equal to 0 with no reactivity for studied extracts of row biomaterial PET/DLA before technological process, and cytotoxicity level equal to 1 with slightly reactivity for PET/DLA after the technological process.

TABLE 1. Results of fibroblasts cytotoxicity evaluation within indirect contact of PET/DLA before technological process (granulate of row material) and within direct contact of PET/DLA biomaterial injection moulded.

Biomaterial PET/DLA	Before technological process	After technological process
Cytotoxity Grade	0	1
Reactivity	None	Slight

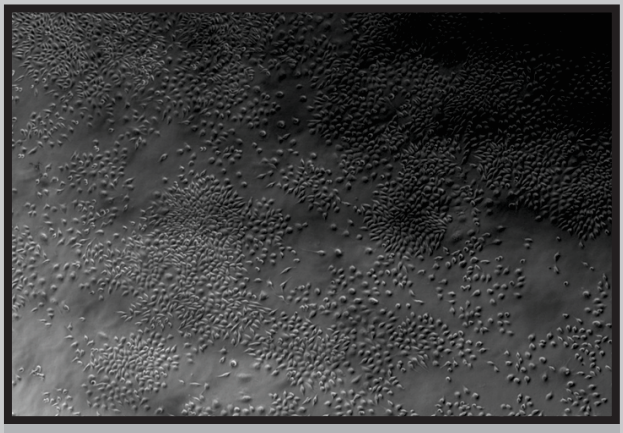

FIG. 3. Fibroblasts sample image after cytotoxicity tests for PET/DLA extract.

Conclusions

The performed initial biocompatibility investigations of biomaterials, in accordance with the Polish standard PN-EN ISO 10993, have shown that exanimated biomaterial PET/DLA is biocompatible in aspect of blood haemolysis and cytotoxicity - as well as a raw material and after technological process. Neither of investigated biomaterials have not negatively affected on morphological blood elements and have not caused cells cytotoxicity. The study will be continued in order to carry out the complete biocompatibility in vitro and in vivo evaluation of PET/DLA biomaterial after a technological injection moulding process, to confirm the full range of biomaterial biocompatible properties, essential for its application in the construction of heart prosthesis.

Acknowledgments

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References

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