The Effects of Polyethylene Terephthalate Surface Treatment by SO_2 Plasma on the Polymer Hemocompatibility

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Abstract

Polyethylene terephthalate polymer is a member of the polyester polymer family that has high mechanical and chemical resistance. The use of artificial vessel prostheses made of polyethylene terephthalate with acceptable physical and biological characteristics is a suitable replacement for damaged vessels. The aim of this study is to investigate the effects of modifying the surface of polyethylene terephthalate with SO_2 plasma on the hemocompatibility of the polymer.Polymer films were exposed to SO_2 gas plasma. In order to evaluate surface chemistry changes, FTIR infrared spectroscopy test was performed. 3D imaging with atomic force microscope (AFM) was performed to examine the structural changes and MTT assay and platelet adhesion tests were carried out to investigate the changes in cell activity and coagulation.The results of infrared spectroscopy in the sample treated by plasma with SO_2gas confirmed the presence of peaks related tothe symmetrical bonds of SO_2in SO_3 or SO_4 in the sample. AFM images showed the surface structure changes. The MTT assay test proved the non-toxicity of the SO_2gas plasma surface modification method. Adhesion and cell and platelet activity tests also showed the anti-clotting effect of the modified polymer.The use of plasma method with SO_2gas is a suitable method to modify the surface and to increase blood compatibility of polyethylene terephthalate polymer, and probably can be used for making artificial blood vessels.

Keywords: Polyethylene Terephthalate, Surface Modification, SO_2Plasma, Hemocompatibility.

1. Introduction

Polyethylene terephthalate is one of the most well-known thermoplastic polymer resins, belonging to the polyester family with a chemical formula. This polymer is also known by commercial names such as Dacron. The applications of polyethylene terephthalate can be categorized based on the different grades of this widely used polymer. Its use in the form of fibers or yarns is common in the production of plastic threads and fabrics, while the bottle grade is utilized for manufacturing plastic containers for beverages and food due to its resistance to gas permeation and its non-reactivity with food materials, playing a protective role in preserving beverages and food. Due to its thermoplastic nature and flexibility, this polymer is also used in combination with glass fibers in engineering composites [1].Artificial veins made from finely woven and knitted polyethylene terephthalate fibers exhibit suitable mechanical properties and biocompatibility. They serve as a viable alternative for damaged veins in individuals facing challenges such as diseases and old age, where replacing veins from other parts of their bodies is not feasible. While artificial veins with larger diameters (10-40 mm) have been successfully used, the clinical success rate is significantly lower for prostheses with smaller diameters, especially below 5 millimeters, due to clinical issues such as localized clotting in these prostheses, ultimately leading to blood flow obstruction [2]. One of the reasons for the lack of success in the construction of prostheses with small diameters, such as Dacron, is the inadequate surface properties of the polymers used in these prostheses.

These properties, when in contact with blood, trigger platelet activation and lead to complications such as blood clotting, dilation or aneurysm formation, and failure to establish proper communication with endothelial cells on their surface. Consequently, there is a need to develop new materials with surface properties that are blood-compatible, although the processes are cost-intensive and time-consuming for the fabrication and subsequent testing of new materials [1,3]. Therefore, surface modification of existing materials used in artificial vein construction, while preserving their overall properties that were previously accepted, appears to be a suitable and logical solution[4].Today, various methods are employed to induce physical and chemical changes on the surface

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of materials. Chemical methods involving the use of chemical solutions, radiation methods such as laser and gamma rays, and cold plasma are examples of methods used to modify the surfaces of polymeric materials.

Plasma treatment, due to its minimal generation of pollutants compared to chemical solution methods and its low risk in comparison to high-energy radiation sources like gamma rays, is considered a cost-effective, environmentally friendly, and compatible method [5].

The prominent feature of surface modification in polymers using plasma is the creation of desired changes on the surface in dimensions ranging from 10-50 A○ from the polymer surface, simultaneously maintaining the bulk properties of the polymers. This method is introduced as a suitable technique for surface modification of polymeric materials [6]. Research has been conducted on the surface modification of polymers used in the construction of artificial vascular prostheses, with the aim of creating changes that enhance the biocompatibility and blood compatibility of these materials.

The subject of these studies varies for different polymers and is based on controllable variables in plasma phase creation, including the gases used, intensity of the flow, input pressure of these gases into the plasma, and the power applied for plasma generation by different plasma-generating devices [7,8]. Many studies have focused on graft polymerization by plasma and the attachment of biomolecules such as heparin and albumin on the surface of polymers to increase the biocompatibility and blood compatibility of the polymers.

This process, in fact, multi-stage surface modification, requires analysis and assurance of the covalent bonding of these coatings, which is essential for considering a substance as a coating on another[9].

Due to the presence of sulfur-containing groups in heparin, surface modification of polyethylene terephthalate with SO_2 gas can create sulfur-containing functional groups [10], mimicking the biomolecular structure of heparin.This aims to enhance the biocompatibility of the polymer surface. Since the binding of proteins and platelet activity on the surface of vascular prostheses leads to the formation of blood clots and subsequently results in the occlusion and thrombosis of veins, the creation of sulfate groups in the chemical structure of polyethylene terephthalate, with a biomimetic behavior from the chemical structure of heparin, by altering the energy and surface charge of the polymer, can induce greater blood compatibility to the mentioned polymer.Based on these considerations, the present study investigates the effects of surface modification of polyethylene terephthalate with SO_2 plasma on the blood compatibility of the polymer.

2.Materials and Methods

2.1.Sample Preparation

Polyethylene terephthalate films from Radmehrplast company were obtained and cut into dimensions of 10 by 20 Cm^2 using a cutter to fit into the vacuum chamber of the RF plasma device. To allow the films to hang in the chamber, small openings were created in a section of the sheets for hooks. After the cut films, alcohol and acetone were used for cleaning to remove any surface contaminants such as oils and microorganisms. The surface modification was performed using an RF plasma processing system under vacuum conditions (GC-B00RF model, manufactured by BasafanAvaran Nasir).The operating conditions involved a pressure of 10 standard cubic centimeters per minute, power of 50 watts, and a duration of 10 min. During this process, atoms and particles attached to the inside of the chamber, which were previously connected to the chamber surface, were removed to prevent the surface modification process from being influenced by these particles already present in the device [11,12].

2.2.Placing and Characterization of Polymer Samples in Sulfur Dioxide Plasma

Polymer film samples were placed inside the plasma device with a vacuum chamber and exposed to sulfur dioxide gas plasma for durations of 2 min, 1 min, 30 sec, and 15 sec with a nominal power of 150 watts. Surface modification, involving the presence of sulfur and oxygen-containing molecules or chemical groups, was analyzed using attenuated total reflection Fourier-transform infrared spectroscopy (ATR-FTIR) with a Bruker vector33 model from Germany in the wave number range of 400-4000 cm^{-1}, following previous study protocols [13]. To observe changes in the topography of the sample surfaces, a scanning probe microscope (Model 95-50E, DME) with a measurement accuracy of up to 1.0 nm was used.

2.3.Cell Toxicity Assessment

Cell toxicity assessment was conducted qualitatively by observing cell morphology using an inverted light microscope and quantitatively using the MTT colorimetric assay. After sterilizing the samples with UV light, each sample was placed in direct contact with L929 mouse fibroblast cells. Microscopic images were taken after 24 h, and cell toxicity or non-toxicity was determined based on cell morphology. In the MTT assay, living cells produce a purple-colored formazan precipitate when in contact with the MTT solution. After dissolving this precipitate in isopropanol or dimethyl sulfoxide, the density of the samples was determined using an ELISA reader at a wavelength of 570 nm [14].

2.4.Cell Adhesion Analysis with Morphology Study of Mouse Fibroblast Cells

To understand the amount of cellular adhesion to polymer film samples and the difference between control and samples exposed to sulfur dioxide plasma, L929 mouse fibroblast cells were cultured on the polymer films to assess the quantity and quality of cell attachment to the samples. The interaction between cell surface receptors and ligands on the surface, determining the cell response to biomaterials, was studied.

2.5.Platelet Adhesion Test

The primary experiment related to blood compatibility of biomaterials is the platelet adhesion test. The results of this test, after blood contact with materials, are quantitatively and qualitatively measured by counting or staining enzymes attached to platelets. For this purpose, the number of platelets in both dry and wet conditions was measured after preparing them in advance by adding deionized water for 24 h.

3. Results and Discussion

Surface modification of the polymer with the presence of molecules or chemical groups, including sulfur and oxygen, was demonstrated by ATR-FTIR spectroscopy. The results showed that SO_2 and SO groups from sulfur compounds create very strong absorption bands in the range of 1000 to 1400 cm^{-1} (Fig. 1.).

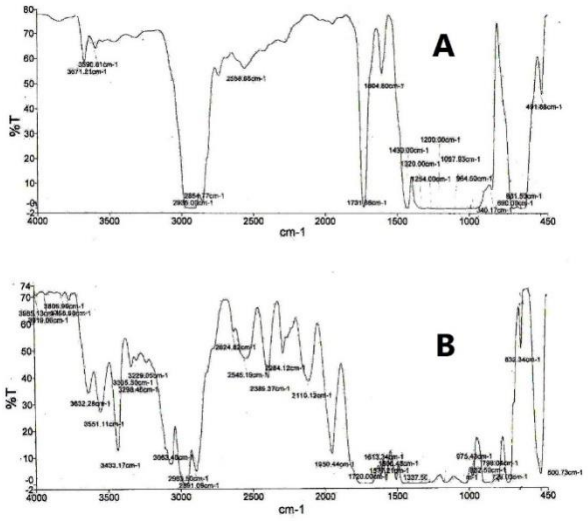

Fig.1. Part (A) corresponds to the control sample, where no specific peak is observed between wave number values of 1000 to 1500 cm^{-1}. Part (B) However, in the plasma-exposed sample, peaks related to symmetric stretching bonds of SO_2 in SO_3 or SO_4 at 1180 are observable.

Additionally, the results related to three-dimensional imaging of the sample surface with an atomic force microscope showed that after 15 seconds, the formation of particles in the form of droplets is visible on the smooth surface (Fig. 2.).

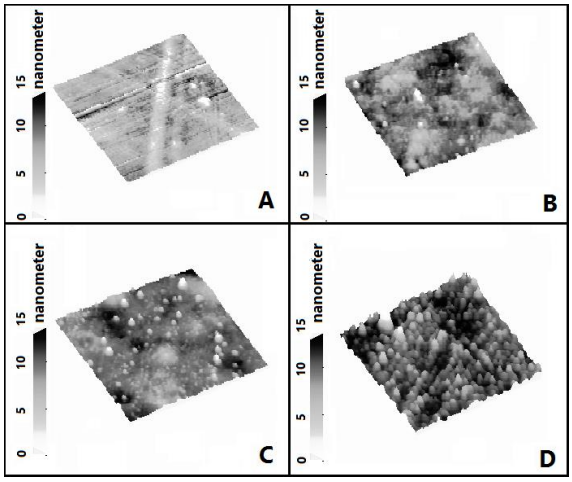

Fig.2. Three-dimensional AFM analysis images in dimensions of 5x5 micrometers. (A) Control sample, (B) 15 sec, (C) 30 sec, and (D) 2 min.

As shown in Fig.2, after 15 sec, the initiation of particle formation in the form of droplets on the smooth surface became noticeable. At 30 sec, particles with a height of less than 1 micrometer and lateral dimensions approximately equal to 1 micrometer were clearly visible. After 2 min of exposure to sulfur dioxide (SO_2) gas plasma, particles with the mentioned dimensions became observable, covering the entire surface of the sample.Cell toxicity tests were conducted for 24 h on samples exposed to argon gas plasma, sulfur dioxide gas plasma for durations of 15 se, 2 min, and the untreated plasma sample. The results indicated a cell viability of approximately 92% for the control sample. For the 15-sec and 2-min samples, viability values of 87% and 81%, respectively, were observed. According to existing standards, it can be stated that samples with viability percentages above 70% are considered safe in terms of biocompatibility. In fact, considering the slight decrease in the viability percentage of L929 mouse fibroblast cells, the surface modification process with SO_2 plasma can be reported as biocompatible (Fig. 3. and Fig. 4.).

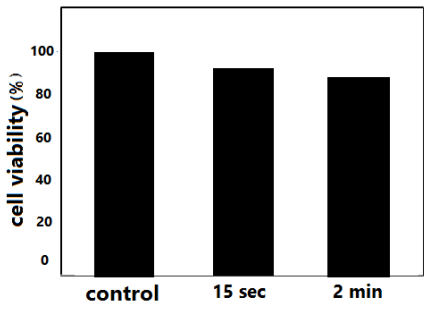

Fig. 3.Cell viability percentages of fibroblast cells in the control sample and after 15 sec and 2 min of treatment.

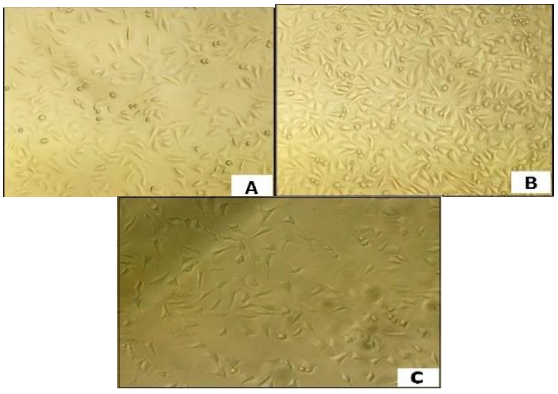

Fig. 4. Morphology of cells in the cell toxicity test on the experimental samples. The elongated shape of the cells indicates their healthy and active status.

On one hand, SEM imaging results of cell culture on the surface-modified polymer samples with sulfur dioxide plasma for durations of 15 sec, 2 min, and the control sample are shows in Fig. 5. In the images of the control sample, the clear presence of cells with attachment to the sample surface and cell spreading is evident, indicating the provision of the necessary biological conditions. In one of the images of the 15-sec sample, we observe numerous cells side by side; however, their morphology is completely rounded, indicating only initial attachment or lack of cell adhesion to the sample surface. However, in the images obtained from the 2-min sample, we hardly or never see the presence of cells on the sample surface, indicating the failure to provide suitable biological conditions.

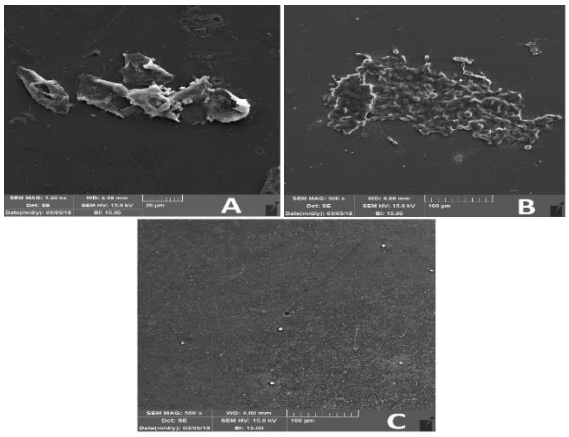

Fig. 5. SEM images of cell culture on the surface of samples A) control, B) 30 sec, and C) 2 min.

In relation to the results regarding platelet adhesion, based on Fig.6., the adhesion of platelets on the surface of untreated plasma sample, 15 sec, 1, and 2 min under sulfur dioxide plasma indicates no significant difference with increasing exposure time to plasma. This could be due to the almost constant concentration and the lack of a noticeable increase in sulfate functional groups on the polymer surface, within the time range of 15 sec to 2 min.

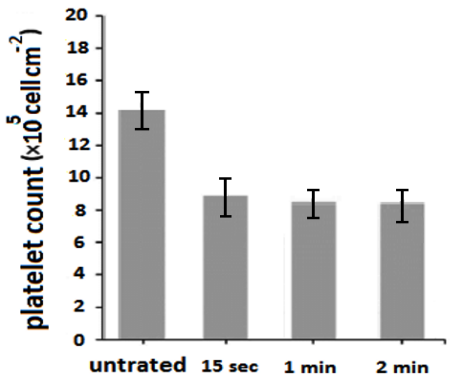

Fig.6. Number of platelets adhered to the surface of polymer samples with a counting unit of 100,000 cells per square centimeter.

While polyethylene terephthalate (PET) polymers have gained attention in the field of medical sciences, surface modification of these polymers to achieve high biocompatibility remains a significant research challenge. The results of this study indicate that surface modification of PET polymer with SO_2 plasma can enhance its biocompatibility, especially in the context of hemocompatibility. This finding suggests a potential application of this polymer in the manufacturing of prostheses, particularly in the field of blood-contacting devices. Although polymers are widely used in the biomedical industry, they are rarely used in their unmodified state. Surface modification can alter the physical and chemical properties of a polymer's surface to exhibit antibacterial or cytotoxic properties, making it suitable for various biomedical applications[15]. The use of ultraviolet radiation for surface modification of PET has been explored in previous studies, indicating its role in improving biocompatibility[16]. Additionally, the modification of the surface layers of PET by extreme ultraviolet (EUV) photons has shown to play a crucial role in enhancing the biocompatibility of PET films[17]. Furthermore, the surface modification of various engineering polymers, including polyethylene terephthalate, polyethylene, and polypropylene, using argon gas and characterization through Fourier-transform infrared spectroscopy (FTIR) and atomic force microscopy (AFM), highlights the significant role of this method in surface modification[18].

In alignment with the current study's findings, research has demonstrated that the application of sulfur dioxide (SO_2) plasma, either with a mixture of SO_2 and oxygen or with hydrogen, plays a crucial role in modifying the surface of polymers. Surface modification with plasma can involve the formation of sulfides or highly oxidized sulfur-functional groups, such as sulfonic acid and sulfates. Spectroscopic analyses, such as X-ray photoelectron spectroscopy (XPS) and infrared reflection-absorption spectroscopy (IRRAS), indicate that

surface modification with SO_2 plasma is an effective method[19].

Laboratory and clinical studies have indicated that improving the biocompatibility of PET vascular grafts is essential for preventing thrombosis. SO_2 plasma can play a vital role in the modification of surface properties, forming sulfate functional groups. The presence of these groups has been shown to act as anti-thrombotic agents, contributing to successful hemocompatibility.

However, the biocompatibility of such materials remains a challenging aspect[20]. In another study, samples of polyethylene terephthalate polymers were exposed to a weakly ionized gas plasma to modify the surface properties of the polymer for better cell adhesion. The gases used for treatment were sulfur dioxide and oxygen at various partial pressures. Subsequently, the samples were incubated with human umbilical vein endothelial cells. Biological experiments included the assessment of cellular metabolic activity (MTT) and toxic effects of unknown compounds (TOX) under laboratory conditions to determine biocompatibility.

The results of this study indicated that surface modification had a significant effect on enhancing the biocompatibility of the polymer[21]. However, some experiments suggest that surface modification with sulfur dioxide may induce thrombogenic effects, potentially causing blood compatibility issues[22]. Additionally, in a study where polyethylene terephthalate polymer was treated with a sulfur-containing plasma to introduce various functional groups and improve blood compatibility, the surface of the treated samples was analyzed using X-ray photoelectron spectroscopy, and surface blood compatibility was assessed by platelet adhesion tests[23].

The results revealed that treatment with sulfur dioxide increased platelet adhesion, raising questions about the blood compatibility of the polymer.

5.Conclusion

The current research illustrated that:

1. the introduction of sulfate functional groups on the surface of polyethylene terephthalate effectively prevented platelet adhesion to the polymer.
2. Additionally, the study revealed that alterations in the topography of the material, forming a specific pattern observed in three-dimensional atomic force microscopy imaging under the influence of sulfur dioxide gas plasma, could create sites for attachment of internal tissue cells of blood vessels, although it did not play a specific role in platelet adhesion.
3. Further clinical studies in this field could provide deeper insights into the role of surface modification of polyethylene terephthalate by SO_2 plasma in enhancing its blood compatibility.

In summary, the outcomes of this research suggest that surface modification of polyethylene terephthalate using SO_2 plasma, which leads to the formation of sulfate groups on the polymer surface, holds promise for preserving and enhancing blood compatibility while altering surface properties. This process could potentially play a crucial role in the development of artificial prostheses, especially artificial blood vessels.

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References

[1] He W, Benson R. Polymeric Biomaterials. Applied Plastics Engineering Handbook(Second Edition), Process., Mater., Appl., Plast. Des. Libr. 2017; 1:145-64.

[2] Chandy T, Das GS, Wilson RF, Rao GH. Use of plasma glow for surface-engineering biomolecules to enhance bloodcompatibility of Dacron and PTFE vascular prosthesis. Biomater. Res. 2000; 21(7):699-712.

[3] Ratner BD. The catastrophe revisited: blood compatibility in the 21st century. Biomater. Res. 2007; 28(34):5144-7.

[4] de Mel A, Jell G, Stevens MM, Seifalian AM. Biofunctionalization of biomaterials for accelerated in situ endothelialization: a review. Biomacromolecules. 2008; 9(11):2969-79.

[5] Holländer A, Kröpke S. Polymer Surface Treatment with SO_2-Containing Plasmas.,Plasma. Process.Polym., 2010; 7(5):390-402.

[6] Mozetič M, Primc G, Vesel A, Modic M, Junkar I, Recek N, Klanjšek-Gunde M, Guhy L, Sunkara MK, Assensio MC, Milošević S., Application of extremely non-equilibrium plasmas in the processing of nano and biomedical materials. Plasma. Sources. Sci. Technol., 2015; 24(1):015026.

[7] Hiratsuka A, Fukui H, Suzuki Y, Muguruma H, Sakairi K, Matsushima T, Maruo Y, Yokoyama K. Sulphur dioxide plasma modification on poly (methyl methacrylate) for fluidic devices. Curr. Appl. Phys. 2008; 8(2):198-205.

[8] Abbasi F, Mirzadeh H, Katbab AA. Modification of polysiloxane polymers for biomedical applications: a Rev.Polym. Int. 2001; 50:1279-87.

[9] Li YP, Li SY, Shi W, Lei MK. Hydrophobic over-recovery during aging of polyethylene

modified by oxygen capacitively coupled radio frequency plasma: A new approach for stable superhydrophobic surface with high water adhesion. Surf. Coat. Technol. 2012; 206(23):4952-8.
[10]Berteau O, Mulloy B., Sulfated fucans, fresh perspectives: structures, functions, and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. Glycobiology. 2003; 13(6):29R-40R.
[11] Novák I, Popelka A, Valentín M, Chodák I, Špírková M, Tóth A, Kleinová A, Sedliačik J, Lehocký M, Marônek M. Surface behavior of polyamide 6 modified by barrier plasma in oxygen and nitrogen.,Int. J. Polym. Anal. Charact. 2014; 19(1):31-8.
[12] Thompson M. Handbook of inductively coupled plasma spectrometry. 2nd Ed. Springer Science & . Business Media; 2012.
[13] Edelmann A, Diewok J, Baena JR, Lendl B. High-performance liquid chromatography with diamond ATR–FTIR detection for the determination of carbohydrates, alcohols and organic acids in red wine. Anal. Bioanal. Chem. Res. Chem. 2003; 376:92-7.
[14] Hersel U, Dahmen C, Kessler H. RGD modified polymers: biomaterials for stimulated cell adhesion and beyond. Biomater.Res. 2003; 24(24):4385-415.
[15] Neděla O, Slepička P, Švorčík V. Surface modification of polymer substrates for biomedical applications. Mater. Today. 2017; 10(10):1115.
[16] Bartnik A, Fiedorowicz H, Jarocki R, Kostecki J, Szczurek M, Biliński A, Chernyayeva O, Sobczak JW. Physical and chemical modifications of PET surface using a laser-plasma EUV source. Appl. Phys. A. 2010; 99:831-6.
[17] Ahad I, Fiedorowicz H, Budner B, Kaldonski T, Vazquez M, Bartnik A, Brabazon D. Extreme ultraviolet surface modification of polyethylene terephthalate (PET) for surface structuring and wettability control. Acta Phys. Pol. A. 2016; 129(2):241-3.
[18] Kostov KG, Nishime TM, Castro AH, Toth A, Hein LR. Surface modification of polymeric materials by cold atmospheric plasma jet.,Appl. Surf. Sci. 2014; 314:367-75.
[19] Holländer A, Kröpke S. Polymer Surface Treatment with SO_2-Containing Plasmas. Plasma Process.Polym. 2010; 7(5):390-402.
[20] Vesel A, Recek N, Motaln H, Mozetic M. Endothelialization of polyethylene terephthalate treated in SO_2 plasma determined by the degree of material cytotoxicity. Plasma. Med., 2017; 1(1):12-22.
[21] Recek N, Resnik M, Zaplotnik R, Mozetic M, Motaln H, Lah-Turnsek T, Vesel A. Cell proliferation on polyethylene terephthalatetreated in plasma created in SO_2/O_2 mixtures. Polym. 2017; 9(3):82.
[22] Ko TM, Lin JC, Cooper SL. Surface characterization and platelet adhesion studies of plasma-carboxylated polyethylene. J. Colloid Sciinterface. 1993; 156(1):207-17.
[23] Vesel A, Zaplotnik R, Modic M, Mozetic M. Hemocompatibility properties of a polymer surface treated in plasma containing sulfur.,Surf. Interface Anal. 2016; 48(7):601-5.