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Surface analysis of long-term hemodialysis catheters made of carbothane (poly(carbonate)urethane) before and after implantation in the patients' bodies

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Purpose: The vascular cannulation is associated with a number of complications. The aim of this work was to study the composition and distribution of the film covering the surfaces of Mahurkar Maxid and Palindrome catheters, which were removed from the body of long-term hemodialysis patients. Moreover, the roughness and contact angle of the catheters were evaluated. *Methods*: Two brand new (as a reference) and thirty used catheters were the subject of the study. Their implantation period lasted from 4 months to a year and the reason for removal was the production of another vascular access or obstruction. Surfaces were analyzed by scanning electron microscope, atomic force microscope and goniometer. *Results*: The inner surfaces of the used catheters were covered with a film of various complexity which includes a plurality of protein, blood cell counts and the crystals. The closer to the distal part the film becomes more complex and multi-layered. Even the surfaces of brand new catheter were not completely smooth. The only significant difference between analyzed models was the presence of thrombus in the distal part of Mahurkar Maxid catheters, not in the Palindrome. *Conclusions*: The distal part of the catheters is the place most exposed to obstruction and infection, which may be due to not reaching the anticoagulant agent into this part. Not only the occurrence of side holes affects the formation of thrombus, but also their quantity, geometry and distribution which effect on fluid mechanics. The surface of the catheters needs to improvement to minimize the occurrence of defects and cracks.

Key words: *surface roughness, atomic force microscopy, scanning electron microscopy, Catheter-tissue contact, film*

1. Introduction

In the world a steady increase in the number of dialyzed patients with vascular catheters can be observed. It is estimated that in the US they are used for almost 300 million per year, which puts them in first place in the ranking of the most frequently used medical tools [22]. However, the use of catheters is associated with many complications. Their usage increases the risk of death to 2 or even 3 times and the occurrence of serious infections 5–10-times, compared to the access which uses the arteriovenous fistula [17]. In addition, relatively to the general population, the dialyzed patients have a 100-times greater risk of death associated with sepsis and other infections. The catheter-related infections are the most common complications which occur in dialyzed patients and represent about 80% of all vascular access infections [11].

The above complications are the effect of continuous contact of the catheter with the tissues and the patient fluids resulting in the formation of a film composed of constituents of body fluids on its surface, which is a perfect environment for the development of infection.

The first step in the contact of the catheter with blood is the adhesion of the plasma components to its

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surface, including fibrinogen, globulins, albumins and other proteins as well coagulation factors. These components have the ability to attract numerous blood cells because the protein attached to the catheter vessel change the conformation, which helps platelets stick together and activate them. It is proven that the same protein which is not adsorbed on the surface of the catheter does not have the ability to activate them. From the blood vessel the collagen fibers that make up the fibrin sheath may be also detached. These factors and blood clotting inside the catheters between hemodialysis sessions contribute to reducing the patency of the catheters [1], [7], [18], [20]. On the surface of catheters components of anticoagulants agents which protect them between treatments may be also embedded [12]. The catheter introduced into the vessel is also exposed to the mechanical effect of blood flow [9], [13].

Bearing the above-mentioned phenomena in mind, it seems advisable to assess the structure of catheters as a result of prolonged contact with the flowing blood and tissues of the body. The paper also identifies which elements of body fluids show the highest affinity for the catheter surface and the distribution of these components along the entire length of the catheter. Moreover, the effect of the catheter tip shape which influences the patency and the roughness and contact angle of the brand new and used catheters were evaluated.

2. Materials and methods

Two brand new and thirty long-term hemodialysis catheters removed from the patients were the subject of the study. Their implantation period lasted from 4 months to a year and the reason for removal was the production of another vascular access or obstruction (blood collection and donation disorders of the functional type – to reduce the flow rate – or mechanical – total or partial obstruction). Between dialysis sessions they were filled with anticoagulant which was a saline solution containing heparin. None of the patients had a catheter infection.

Two types of dual lumen catheters produced by Covidien company were tested. Catheters were built with circular VasCath type cross-section (or D-lumen) including a partition which divides the catheter into two identical parts. They differed from each other with the shape of the distal end, layout and the number of side holes. Both catheters were made of carbothane – an aliphatic poly(carbonate)urethane. The catheters were sterilized using ethylene oxide gas. Physical and mechanical properties of poly(carbonate)urethane are shown in Table 1.

Table 1. Physical and mechanical properties of poly(carbonate)urethane [15]

Property	Minimum to maximum value
Glass temperature	–35 °C
Thermal expansion coefficient	101–161 μstrain/°C
Heat deflection temperature at 0.45 MPa	98 °C
Heat deflection temperature at 1.8 MPa	59 °C
Water absorption	0.821–1.18%
Tensile strength	41.6–59.1 MPa
Elongation	292–498%
Flexural modulus	0.0913–1.55 GPa

The first type of analyzed catheters is a Palindrome of the same size and the same length of channels. Both of them have one side hole with a square shape (Fig. 1A).

The second is a Mahukar Maxid catheter of 4.8 mm in diameter and 28 cm in length with different arterial and venous length of channel. Arterial channel was 2.2 cm shorter and had 5 round side holes with one located on the partition of the catheter (Fig. 1B).

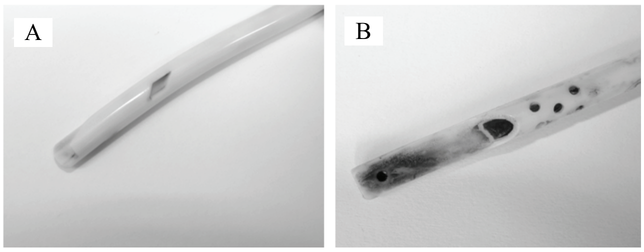


Fig. 1. A tip of: (A) Palindrome and (B)Mahurkar Maxid catheters removed from the body of the long-term hemodialysis patients

2.1. Samples preparation for microscopic analysis

Upon extraction, catheters were stored at 4 °C until being processed within a maximum of 24 hours after removal. At the beginning, the venous and arterial channels were divided from the catheter partition with a sterile scalpel. 1.5 cm pieces were cut from the distal, proximal, and middle sections of the catheters and prepared for microscopic examinations. The solution of glutaraldehyde in phosphate buffer was used for fixation of biological layer on catheters. Glutaraldehyde influences the production of crosslinks between different chemical group of the specimen and the creation of methylene bridges. As a result, the structure of this product is more bounded and stiffened [16]. Then the samples were rinsed three times for 15 min in phosphate buffer. The samples were dehydrated in acetone solutions with increasing concentrations (% v/v 10, 20, 30, 40, 50, 60, 70, 80, 90) for 10 min. The final dewatering was carried out in 100% acetone, twice for 30 min. These operations were carried out at room temperature. Further, samples were dried at critical CO_2 point using the critical point dryer CPD E3000 (Quorum Technologies Ltd.), and then coated with chromium by means of the turbomolecular-pumped coating system Q150T S (Quorum Technologies Ltd.).

2.2. Surface analysis

The morphology and chemical composition of the samples were analyzed by scanning electron microscopy (SEM JEOL 7600F) with energy dispersive X-ray spectroscopy (EDS) microanalyzer (Oxford Instruments). The roughness measurement was performed by an atomic force microscope AFM EasyScan 2 (Nanosurf). The contact angle measurement was accomplished by goniometer PG2 (Fibro Systems).

3. Results

3.1. Scanning electron microscopy analysis

The surface morphologies of two types of implantation catheters with varying distal end shape and the number of side holes have been investigated using scanning electron microscopy, and shown in Fig. 2.

As can be seen in Fig. 2C, the distal part of the catheter is covered with the most complex film presenting multi-layered structure. It consists of numerous different elements which look like: proteins, inter alia, albumin and globulin, blood cell counts and the crystals which may come from saline solution with heparin (an anticoagulant).

A layer in the middle section of catheters (Fig. 2B) is formed by crystals (from anticoagulant), single proteins and blood cell counts. The inner surface of catheter (Fig. 2A) located closely to the skin is coated only with crystals. No fibrin fibers were observed in any parts of the catheters.

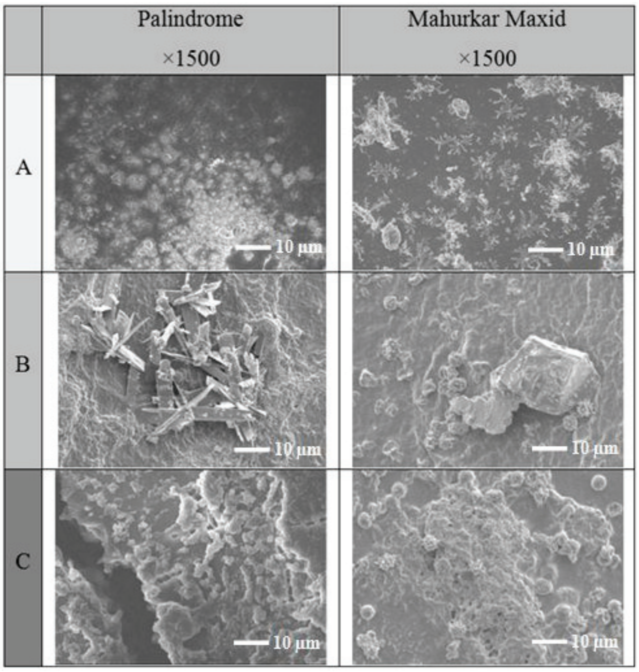
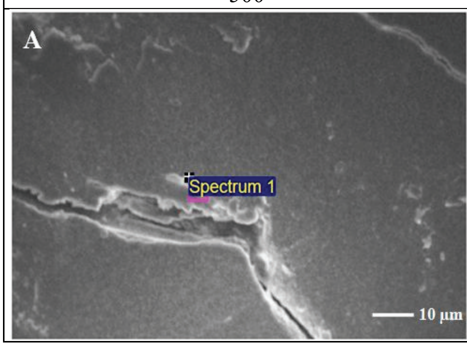
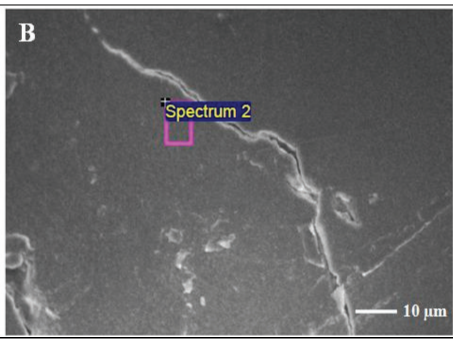


Fig. 2. SEM images of biological layer coated inner surfaces of (A) Mahurkar Maxid and (B) Palindrome catheters

Table 2. The results of EDS analysis of brand new Palindrome and Mahurhar Maxid catheters

Palindrome ×500	Mahurkar Maxid ×500	Element	% wt. A	% wt. B
		C	50.72	45.78
		O	31.05	28.18
		S	3.22	4.52
		Cr	3.11	3.07
		Ba	11.90	18.47

3.2. EDS analysis

In this study, the EDS (n = 85) analysis of new and post-implanted Palindrome and Mahurkar Maxid catheters was carried out.

The EDS analysis of the brand new catheters (Table 2) shows that inner surfaces are saturated by barium sulfate ($BaSO_4$) – contrast medium, which allows for visualization of the catheter on X-ray. In addition, pictures A and B in Table 2 show irregularities and cracks on surface of catheter. Chromium is a conductive metal needed prior to SEM imaging of non-conductive samples.

The EDS (n = 80) analysis of used (removed from the patients' bodies) Palindrome and Mahurhar Maxid catheters found that components of biological elements and crystals are composed of organic, micro- and macro-elements, such as carbon (C), oxygen (O), sodium (Na), sulfur (S), chlorine (Cl), potassium (K), phosphorus (P), calcium (Ca), silicon (Si), barium (Ba). The presence of these elements confirms that the film covering the surface of the catheter consists of components derived from body fluids and an anticoagulant agent (sodium chloride NaCl and heparin).

3.3. Roughness and contact angle measurement

The study was carried out in three randomly selected locations on the inner surface of the brand new catheters and on six used catheters in three randomly selected locations on the same part for the scan area 5 µm × 5 µm, with a resolution 256 by 256 points.

Obtained AFM images (Fig. 3) show that the inner surface of the brand new catheters has a high smoothness whereas irregularities and cracks can be seen at ×500 magnification (Table 2). The only difference (visible also with the naked eye) between analyzed catheters was the presence of thrombus in the distal part of Mahurkar Maxid catheters, not in the Palindrome catheters, what confirms the three-dimensional

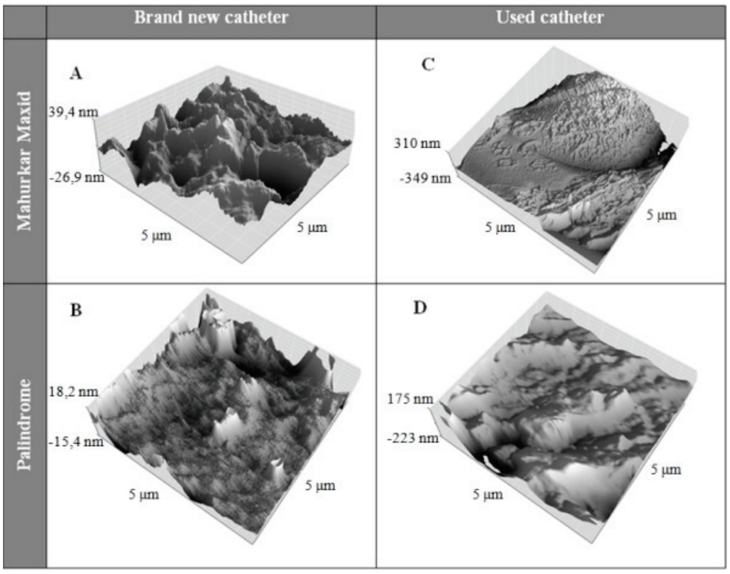


Fig. 3. The three-dimensional roughness surface charts from distal part of the brand new and used catheters (Palindrome and Mahurkar Maxid)

Table 3. The results of contact angle and roughness parameters of Mahurkar Maxid and Palindrome brand new and used catheters

Roughness parameters		Brand new Mahurkar Maxid catheter	Brand new Palindrome catheter	Used Mahurkar Maxid catheter	Used Palindrome catheter
Line	R_a [nm]	17.99 ± 5.55	6.84 ± 4.29	140.65 ± 6.44	42.15 ± 12.58
	R_q [nm]	21.23 ± 6.73	11.72 ± 5.22	165.66 ± 12.76	52.88 ± 14.78
Area	S_a [nm]	17.49 ± 3.86	8.43 ± 3.06	72.65 ± 15.55	38.89 ± 5.51
	S_q [nm]	22.06 ± 4.69	11.98 ± 3.55	106.74 ± 22.39	50.88 ± 8.16
Contact angle [°]		45 ± 3	76 ± 3	54 ± 5	80 ± 5

R_a – roughness average, R_q – root mean square (RMS) roughness, S_a, S_q – extension of R_a, R_q to a surface.

roughness surface chart (Fig. 3C), which presents the elements resembling erythrocytes and proteins in shape.

Table 3 shows Ra, Rq and Sa, Sq parameters characterizing the roughness and contact angle parameters of the catheter surfaces before and after exposure to body fluids. The results are presented as a mean and standard deviation (mean ± SD). The values of roughness parameters decrease after incubation of a catheter in patient' bodies, which may confirm the deposition of protein and other elements of body fluids on the cavities of the catheter surface. The consequence of this is the increase in the value of the contact angle of the used catheters, i.e. increased their wettability.

4. Discussion

The aim of the presented research was to determine the inner surface compositions of brand-new and used (removed from the patients' bodies) Mahurkar Maxid and Palindrome catheters and to examine the differences and causes of their occurrence depending on the catheter model. Hence, the effect of the catheter tip shape on the patency, roughness, and contact angle of these catheters was evaluated.

Performed study showed that the surface of the brand new catheter is coated by barium sulfate ($BaSO_4$) which causes numerous irregularities and cracks on its surface and may facilitate adhesion of microorganisms, so this interface layer needs improvement to minimize the occurrence of defects [4].

The inner surfaces of the long-term hemodialysis catheters removed from the patients' bodies are covered with biological conditioning layer of various complexity. The external section of the catheter positioned in the subcutaneous tissue consists mostly of precipitated crystals (NaCl and heparin), due to the closest position to the site of injection of the anticoagulant agent. The closer to the distal part (tip) of the catheter the film becomes more complex, multilayered and is composed of numerous proteins and cellular components of blood and just single crystals, which thus becomes the place most exposed to biofilm formation. Such distribution of film components suggests that anticoagulant agent does not reach the distal part of the catheter or are rinsed out by side holes. This hypothesis is confirmed by Twardowski and Moore [24] who suggest that the blockage of the catheters is due to the side holes presence. Heparin locking solutions are rinsed out by side holes and do not reach the catheter tip. The results of the last studies [20] suggest that the concept of large side hole is better because catheters with small side holes have inferior primary assisted patency (PAS) and also infection rates [14]. In addition, when multiple side holes are presented (like in Mahurkar) the first available hole is primarily used, especially from the aspiration lumen – a low flow zone at the catheter tip and blood backflow may potentially result in catheter malfunction [6].

Examination of catheters showed greater structural complexity of layer covering both channels than the catheter partition. Increased amount of crystals and biological components observed in the catheter channels rather than in its partition may be associated with construction of these parts – the channels are semicircular in contrast to flat partition. The minimum flow rate occurs in the space adjacent to the wall of the channel. These conditions also help in deposition of biological elements.

The only difference between analyzed catheters was the presence of thrombus in the distal part of Mahurkar Maxid catheters, not in the Palindrome catheters. It can be assumed that not only the occurrence of side holes affects the formation of thrombus [21], but also their quantity, geometry, and distribution, which affects fluid mechanics. According to recent studies, the new single-large-hole construction of catheter like in Palindrome is better due to the low blood recirculation, inferior primary assisted patency and also infection rates, which results from improved catheter tip design patterns [5], [6], [14], [20].

AFM images obtained (Fig. 3) show that the inner surface of the brand-new catheters has a high smoothness. Taking the size of proteins and other body fluids components (several tens of micrometres) into consideration, the resulted smoothness should prevent their adhesion. However, the SEM images (Fig. 2) clearly indicate that the biological elements can be deposited even on very smooth surfaces. The *in vivo* studies suggest that the threshold value for Ra equal to 0.2 μm below which the adhesion of bacteria is reduced [25]. According to authors' results, the designated roughness parameters of brand new catheters are 10 times smaller than this value. However, as a result of the catheter contact with body fluids, its surface is covered by layer, what affects in the surface roughness changes (Table 3). This biological layer is also called "conditioning film", which facilitates the adhesion of microorganisms to catheter surface. This film is formed shortly after implantation of the catheter in the patient's body and can mask functional group of antimicrobial coatings which are often impregnated catheters [3]. This may be the reason why coatings are not sufficiently effective.

It is also important that the surface roughness of the brand new Mahurkar Maxid catheter is higher than brand new Palindrome (Table 3), and, therefore, deposition of biological components from body fluids, anticoagulant and thrombus is followed more easily with the surface roughness increases. This is due to the shear forces diminishing and increasing of more rougher surface area.

Another factor influencing the roughness of the catheter is hydrolytic and oxidative degradation by environmental stress-cracking and metal ion oxidation [15]. Increased surface roughness leads to enhanced bacterial colonization by providing shelter [23].

It is currently believed that the bacterial adhesion is initiated by electrostatic binding, hydrophobic interactions and Van der Waals forces between the surface of the microorganisms and the substrate material [2] but the mechanism of attachment cannot be obtained without considering the effects of the substratum, hydrodynamics and characteristics of the fluids and various properties of the microorganisms, e.g., surface energy, the presence of fimbriae, flagella, and surface-associated polysaccharides or proteins.

The values of the contact angle of brand-new and used catheters were less than 90° (Table 3), what means that the surfaces are hydrophilic and in agreement with the results of Hsu and Lin (72–82°) [8]. Differences between above-mentioned and our results may be due to a lack of standarized measurement conditions for determining surface contact angle. The obtained changes in the contact angle after inserting the catheter in the body, results in further exposure to body fluids and anticoagulant, which causes a change of the contact angle on the hydrophobic character. Several studies have shown that the hydrophilic surfaces promote bacterial colonization [3], but the overwhelming majority say that hydrophobic materials favor bacterial adherence more than hydrophilic ones [10], [19], [23].

The most common bacteria diagnosed in catheter-related infection are *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococci* and *Candida* species [10]. Many of them are hydrophobic or have hydrophobic components (at their surface), so they will preferably adhere to hydrophobic biomaterials [19].

In conclusion, the attachment of microorganisms to surfaces may be easier on surfaces that are rougher and coated by conditioning film. The highest smooth as possible of the catheter surface is required because, as shown by studies of many materials, reducing the smoothness and, thus, surface development is the decisive factor that increases the risk of blood clots forming in the vessels. It is important that all these catheter properties do not change during its stay in the body [13]. Ideally, the surface should be hydrophobic, but considering the fact that the surface properties of bacteria are a dynamic process (depending on multiple conditions), the contact angle loses significance.

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