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The affinity for dialysate species of thermally modified titania nanotubes under static and dynamic conditions

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Purpose: During the dialysis process, hemolysis is the most frequently occurring problem to solve. Titanium dioxide nanotubes (TNTs) can be considered as a material preventing hemodialysis or blood species deposition thanks to their unique properties, i.e., hydrophilicity, smooth surface, and antibacterial. The purpose of this work was the electrochemical, chemical, and morphological characteristics of the TNTs and the evaluation of the possibility of using them as filter parts in dialysis techniques. *Methods*: The tests were carried out on as-formed TNTs with a diameter of 50 ± 5 nm and 1000 ± 100 nm in height, and TNTs thermally modified in air atmosphere temperatures ranging from 350 to 550 °C. Electrochemical and microscopic analyses were performed both in the static and dynamic system of dialysis fluid (flow rate: 250 cm^3/min). Additionally, deposition or damage of blood cells was specified during the ex vivo dialysis experiment. *Results*: Obtained results proved relationship between electrochemical properties of TNTs and the method of their modification. The results demonstrated that the TNTs annealed at 450 °C TNTs can be potentially applied for constructions dialysis membrane in the hemodialysis area due to their most stable stationary potential in dialysate, the highest value of impedance modulus, and the most favourable electrokinetic properties. Additionally, it was confirmed that annealed process causes improvement of corrosion resistance and protective properties for TNTs in the dialysis fluid. *Conclusions*: The result allowed for the conclusion that annealing is responsible for reduction of adsorption properties of TNTs, though this titanium dioxide nanotube still can be used as filter part in haemodialysis.

Key words: *nanotubes, titanium dioxide, haemodialysis, annealing, renal replacement techniques*

1. Introduction

Kidney diseases recognized as non-communicable illnesses have replaced communicable diseases (such as tuberculosis, malaria, or AIDS) as the most common causes of premature death worldwide. The global incidence of chronic kidney disease (CKD) is increasing among individuals of all ages. The US Renal Data System has documented an increase of patients with kidney failure requiring dialysis therapy or transplantation from 340,000 patients in 1999 to 785,883 patients in 2018. Other sources provide information that about 10% of the population worldwide is affected by chronic kidney disease (CKD), and millions die each year because they do not have access to affordable treatment. What is more, over 2 million people in the world currently receive treatment with dialysis or a kidney transplant to stay alive.

Nevertheless, emerging data suggest that the kidney is an important injury site after chemical exposure [19]. During hemodialysis, diffusion of solution between the blood and a dialysis fluid removes metabolic waste products and replenishes body buffers

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[26], [34]. Therefore, the dialysis process can lead to worse health conditions [17], whereas renal anaemia affects mostly patients with chronic kidney disease, which amounts to about 90% of patients. Complications that may occur include damage of the cardiovascular system, respiratory system disorders, pancreatitis and hypertension, nausea, pain (abdominal, chest, back), and dyspnoea [33]. The primary cause of anaemia in hemodialysis patients is the treatment's erythropoetin deficiency, iron, and blood loss. The reason for that complication is hemolysis [10].

The most crucial element in the hemodialysis system causing the hemolysis is membranes, consequently, the materials used for their construction. Hemolysis results from resistance or obstruction to blood flow through the extracorporeal circuit caused by membrane pores, leading to hemoglobin's destruction. The surface configuration of dialysis membrane fibers is also contributed to the risk of hemolysis. The membrane fibers are set into a potting material and cut to produce a smooth and flat surface, reducing hemolysis risk [33]. The aim of the exchanges through dialyzer membranes is the removal of uraemic solutes that are retained because of renal failure (e.g., urea) and the restoration of depleted compounds (e.g., bicarbonate) [9], [16]. Therefore, permeability and biocompatibility are the two significant features of dialysis membranes that may be implicated in the outcome of patients. The materials used in construction in dialysis membrane can be listed as follows: coprophage, cellulose acetate, and polymethylmethacrylate [27], cellulose triacetate (CTA), ethylene vinyl alcohol (EVAL), polyacrylonitrile (PAN), polyester polymer alloy (PEPA), polyethersulfone (PES), polymethylmethacrylate (PMMA), and polysulfone (PS) [1]. Most of the materials listed have a weakness, mainly causing protein accumulation, which may activate the immune system [27]. Cuprophane membrane may activate the complement system. Because of the chemical ester configuration, cellulose esters do not survive steam sterilization procedures [11]. The hydrophobic nature of polyethersulfone contributes to membrane fouling [27].

Due to the defects mentioned above, it has become important to look for new materials for dialysis membranes. Titania nanotubes (TNTs) are a modern and excellent example of the material for dialysis membranes. According to Gao et al. [14], TNTs in biomembranes acquire hemocompatibility, making them good material for medical applications. They are a biocompatible inorganic material, inexpensive, environmentally benign, and chemically and thermally stable [18]. The large surface-to-volume ratio, porosity, and high homogeneity of TiO_2 nanotubes make it promising material in medicine and biotechnology. TNTs are considered a significantly important material due to their promising applications in many fields ranging from energy harvesting to sensors and implantology [4]. TiO_2 nanotube arrays have demonstrated many important applications, including gas sensing [41], solar cells, photocatalysts, tissue engineering and biosensors [4], [6]. Moreover, TNTs have antibacterial properties, which were proven, amongst others, by Arkusz et al. [8]. Considering the above-mentioned biomedical applications, TNTs were characterized in Ringer solution [29], simulated body fluid (SBF) [20], phosphate buffered saline (PBS), artificial saliva [2]. Titanium nanotubes have been characteristic also in artificial urine [15]. However, no one has decided to examine them in the dialysis solution.

One of the most frequently chosen methods of improving the properties of TNTs is a thermal treatment, which is helpful in many biomedical applications because as-formed TNTs have poor thermal and mechanical stability. At the same time, the crystalline structure provides better thermal and mechanical properties and improved electrical, optical, and catalytic behaviour [21]. For example, the interfacial shear strength for unannealed TNTs was estimated at 163.3 MPa, after annealing at 250 °C at 370.2 MPa, and at 400 °C at 684.5 MPa, while the fracture toughness was estimated at: 0.996, 1.433 and 2.803 MPa × $m^{1/2}$, respectively [42]. Generally, the annealing of TNTs is carried out in a wide range of temperatures from 300 to 1200 °C, whereas the temperature around 600 °C causes the deformation of TNTs, and its further collapses. Finally, the nanotubular structure disappears completely [5]. Hence, the use of temperatures below this limit appears justified when using TNTs as a filter medium.

The aim of the study was the electrochemical characterization of unmodified and thermally modified titanium dioxide nanotubes, as well as the assessment of the possibility of their use as a filter medium in dialysis techniques. TiO_2 nanotubes were annealed in air at temperatures ranged from 350–550 °C. Before and after electrochemical measurements in static and dynamic conditions (flow of 250 cm^3/min), SEM-EDS analysis was performed, which determined the affinity of electrolyte elements to the TNTs surface. The novelty of the work is the performance of electrochemical measurements in the dialysis solution and the assessment of absorption properties in *ex vivo* conditions. For this purpose, the chemical and physical in-use stability in dialysis fluid was determined.

2. Materials and methods

Titanium foil (purity of 99.7%, thickness of 0.25 mm), platinum mesh (99.9%), ethylene glycol (99.8%), and ammonium fluoride (NH_4F, ≥98.0%) acetone (≥99.5%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dialysate (pH 8.532 ± 0.109) was prepared by mixing two concentrates: acid and bicarbonate in standard ratio 1:34 with purified water obtained from Fresenius Medical Care (Bad Homburg, Germany). The elemental composition of dialysis fluid is presented in Table 1. Dialysis fluid was employed for all electrochemical experiments as an electrolyte.

Table 1. Composition of dialysate in mmol/dm^3

Element	Acid concentrate [mmol/dm^3]	Bicarbonate concentrate [mmol/dm^3]
Na^+	138	1000
K^+	2.00	–
Ca^{2+}	1.25	–
Mg^{2+}	0.50	–
Cl^-	105.50	–
HCO^{3-}	32.00	1000
CH_3COO^-	6.0	–
Glucose	1.00	–

2.1. Measuring apparatus

A potentiostat/galvanostat PGSTAT302N from Autolab (Metrohm, Herisau, Switzerland) was used to prepare the TNTs, static and dynamic electrochemical measurement processes. All measurements were carried out using the standard three-electrode configuration with TNTs electrode as the working electrode, a platinum mesh as the auxiliary electrode, and the standard silver chloride electrode ($E_{Ag/AgCl}$ = 0.222 V vs. Standard Hydrogen Electrode) by Metrohm as the reference electrode.

To investigate the chemical composition and surface morphology, scanning electron microscope (SEM) equipped with energy-dispersive X-ray spectroscopy (EDS) were used after preparation of TNTs, thermal annealing, static and dynamic electrochemical measurements. The microscopic observation of the samples covered with biological material required preparation procedures according to [26]. First, TNTs were immersed in a 25% solution of glutaraldehyde in a phosphate buffer (pH = 7.2) and then rinsed three times in deionized water. Secondly, samples were dehydrated using 10 ml of acetone-water solutions with a 10% to 100% concentration and dried in air at room temperature. Thirdly, TNTs surface was covered with a 5 nm layer of chromium.

2.2. Preparation and thermal modification of TNTs

The titanium foil was sonicated in distilled water and acetone and afterward dried in nitrogen. The formation of the nanotube oxide layer was carried out at room temperature by electrochemical anodizing in 85% ethylene glycol solution with 0.65% wt. NH_4F in a two--electrodes system. A platinum foil was used as a counter electrode. The anodization of Ti foils was performed in a two-step process:

1) potentiodynamic, where the anodization voltage was increased from 0 to 17 V with the rate 0.05 V/s;
2) the potentiostatic, where the anodization voltage (17 V) was kept for 3750 s.

After the anodization process, the Ti foil was washed by distilled water and dried in nitrogen.

The TNTs were annealed in an air atmosphere at 350, 450, and 550 °C, respectively, for two hours with a heating and cooling rate of 6 °C/min using an AMP furnace (AMP, Zielona Góra, Poland).

2.3. Electrochemical measurements

The measurements were divided into three groups:

1) electrochemical measurements were performed in static mode, using the standard three-electrode configuration were in dialysate solution at 36.5 ± 0.5 °C;
2) electrochemical measurements were performed in dynamic mode, using the standard three-electrode configuration with a 250 cm^3/min dialysis fluid flow at 36.5 ± 0.5 °C;
3) electrochemical measurements were performed in dynamic mode, using the standard three-electrode configuration with swine blood (flow rate: 250 cm^3/min) at 36.5 ± 0.5 °C.

Each step consisted of open circuit potential (OCP), electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) measurements. OCP measurements were performed for 4 hours. EIS tests were carried out in the frequency range of 0.1–10^5 Hz with a signal amplitude of 10 mV. CV spectra were recorded in the potential range of –1–1 V with a scan rate of 0.05 V/s. The flow was caused by the Fresenius hemodialysis machine 4008S (Fresenius Medical Care, Bad Homburg, Germany). Each measurement was repeated three times.

3. Results

3.1. Microscopic analysis of annealed TNTs

In Figure 1, the SEM micrographs of TNTs with a height of 1000 ± 100 nm and 50 ± 5 nm in diameter before and after thermal treatment in temperature of 350 °C, 450 °C, and 550 °C are shown. Prepared TNTs were vertically oriented, and the nanotubes were hollow and cylindrical with smooth walls. After thermal treatment, any significant changes in height and diameter value or any visible crack of the titanium nanotube layers were observed.

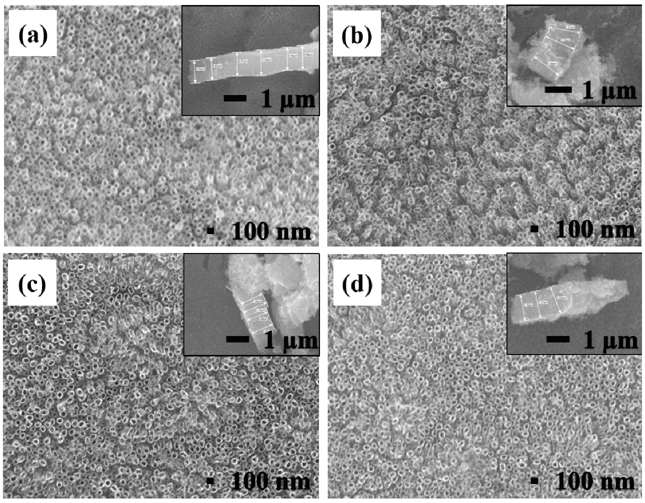

Fig. 1. SEM top-view and cross-sectional images of unmodified (a) and annealed TNTs layers in temperature of 350 °C (b), 450 °C (c), and 550 °C (d)

Table 2. Chemical composition of unmodified and annealed TNTs

TNTs	Unmodified	350 °C	450 °C	550 °C
Titanium [% wt.]	72.15 ± 2.4	70.22 ± 0.4	64.83 ± 1.9	63.04 ± 3.9
Oxygen [% wt.]	18.03 ± 1.7	29.87 ± 0.3	35.17 ± 1.2	36.96 ± 3.9
Fluorine [% wt.]	9.82 ± 0.3	–	–	–

The chemical composition of TNTs before and after thermal modification is listed in Table 2. It can be seen that the thermal treatment in the air atmosphere resulted in the removal of fluoride ions and an increase in the oxygen content in the structure with the increase of the annealing temperature.

3.2. Characterization of thermally modified TNTs in dialysis fluid and static conditions

The next stage of the research was the evaluation of the electrochemical properties of unmodified and annealed TNTs at the temperatures of 350, 450, and 550 °C under static conditions and the SEM-EDS evaluation of the samples after the measurements.

The SEM micrographs and EDS investigation results after static electrochemical measurement of unmodified and annealed TNTs are presented in Fig. 2 and Table 3. The SEM micrographs (Fig. 2) illustrate that the surface of unmodified samples after electrochemical tests contains adsorbed elements of the dialysis fluid, the composition of which has been shown to be sodium and calcium (Table 3). Additionally, the presence of fluorine was not demonstrated in these samples (as before).

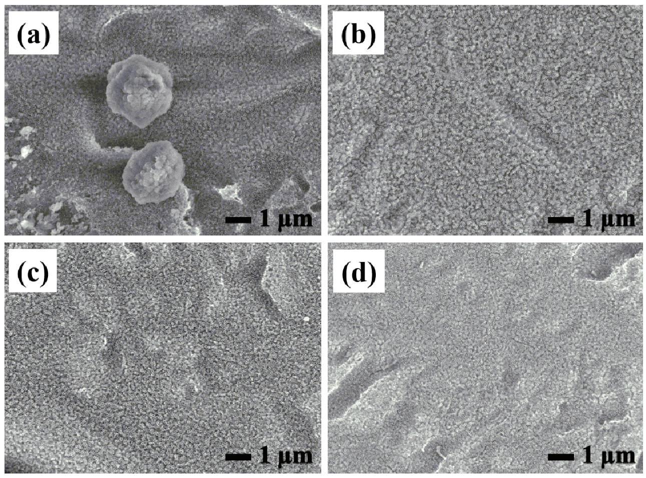

Fig. 2. SEM top-view of unmodified (a) and annealed TNTs layers in temperature of 350 °C (b), 450 °C (c), and 550 °C (d) after static electrochemical measurement

Table 3. Chemical composition of unmodified and annealed TNTs after static electrochemical measurement

TNTs	Unmodified	350 °C	450 °C	550 °C
Titanium [% wt.]	59.83 ± 18.8	69.66 ± 2.5	60.05 ± 3.2	55.31 ± 1.9
Oxygen [% wt.]	15.25 ± 9.1	30.34 ± 0.9	39.95 ± 1.4	44.69 ± 2.7
Sodium [% wt.]	24.92 ± 9.7	–	–	–
Calcium [% wt.]	44.49 ± 10.3	–	–	–

In Figure 3, the OCP curves of unmodified and annealed in 350, 450, and 550 °C TiO_2 nanotubes measured in dialysis solution, during 4 hours in static conditions are shown. All samples exhibit relative stabilization of the stationary potential. The most significant changes in the course of the curves are observed for unmodified and annealed at 350 °C TNTs samples. The highest value of OCP was recorded for TNTs annealed at 450 °C.

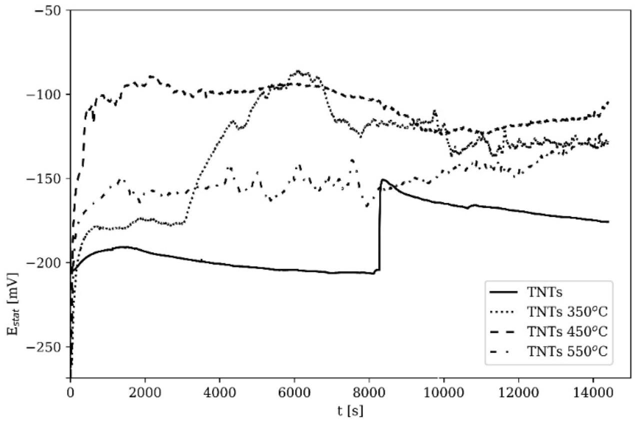

Fig. 3. Open circuit potential curves of unmodified and annealed in air TNTs layers. Spectra were recorded in the dialysis solution (pH 8.532 ± 0.109)

The EIS measurements results of the unmodified and heat-treated at 350, 450, and 550 °C TNTs are shown in Fig. 4 as Bode plots. Thermal modification causes the increase of impedance modulus and the phase angle of TNTs. The highest |*Z*| value was observed for TNTs annealed at 450 °C.

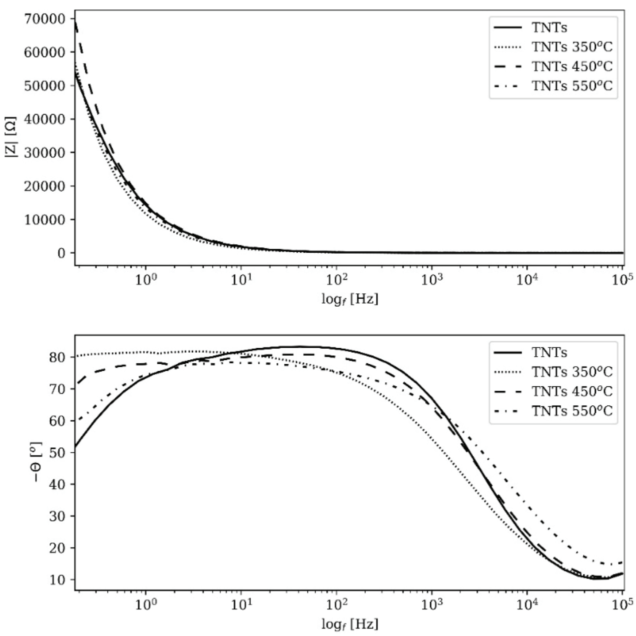

Fig. 4. Bode plots of unmodified and annealed TNTs layers. Spectra were recorded in the dialysis solution (pH 8.532 ± 0.109) in the frequency range of 0.1–10^5 Hz with an amplitude of 10 mV

In Figure 5, the cyclic voltammograms of unmodified and heat-treated at 350, 450, and 550 °C TNTs layers are shown. Current changes in the low potential range are related to the oxidation and reduction of hydrogen and at approx. 1 V to the oxidation of the TNTs surface. In the case of annealed TiO_2 nanotubes, there are much stronger peaks related to Ti^{4+} reduction at –0.5 V and Ti^{3+} oxidation at –0.3 V.

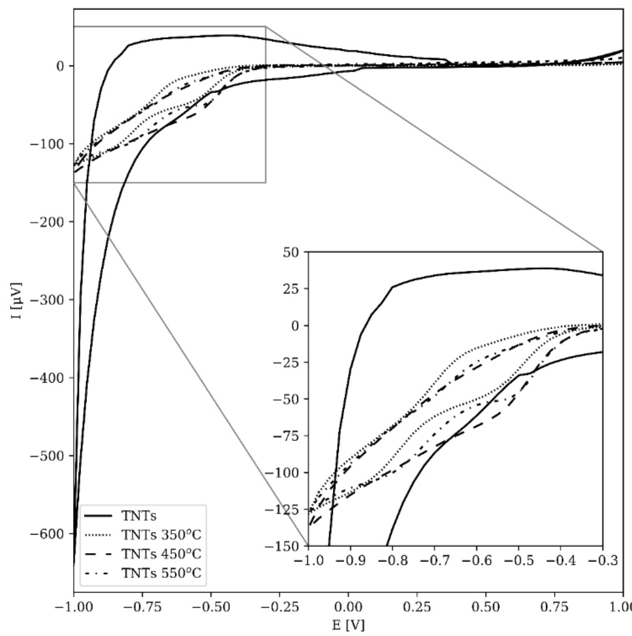

Fig. 5. Cyclic voltammograms of unmodified and heat-treated TNTs layers recorded in the dialysis solution (pH 8.532 ± 0.109) in the potential range from –1 to 1 V with a scan rate of 0.05 V/s

3.3. Characterization of thermally modified TNTs in dialysate and dynamic conditions

The next part of the studies was dynamic electrochemical tests with the dialysate flow of 250 cm^3/min using unmodified and annealed at 450 °C TNTs layers. Thermal modification in temperature of 450 °C was chosen based on the most stable OCP in dialysate, the highest value of impedance modulus, and the most favorable electrokinetic properties. Further elaboration of adsorption properties was evaluated based on OCP measurement and SEM/EDS analysis.

In Figure 6, the SEM micrographs of unmodified and thermal modified TNTs after dynamic electrochemical measurement are shown (SEM photos before electrochemical measurements were presented in Fig. 1a and 1c, respectively). The images did not show any significant changes in height and diameter value or any visible crack, only for the titanium nanotube lay-

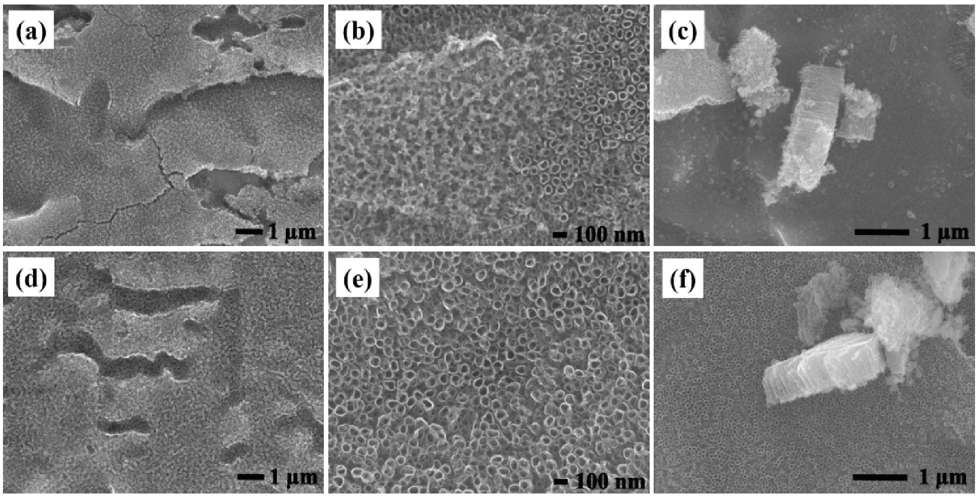

Fig. 6. SEM images of unmodified (a)–(c) and annealed at 450 °C (d)–(f) TNTs after dynamic electrochemical measurements

ers annealed in air at 450 °C. These results also show that annealed TNTs do not reveal adsorption properties for elements included in dialysis fluid, also confirmed by EDS tests (Table 4). Compared to unmodified TNTs, the dialysate flow caused numerous cracks and delamination of the nanotubular layer from titanium foil. Additionally, the EDS results confirmed the calcium adsorption onto the TNTs surface.

Table 4. Chemical composition of unmodified and thermally modified TNTs after dynamic electrochemical measurements

TNT	Unmodified	450 °C
Titanium [%]	42.25 ± 3.9	65.32 ± 1.4
Oxygen [%]	39.45 ± 2.7	38.34 ± 2.1
Calcium [%]	25.30 ± 1.3	–

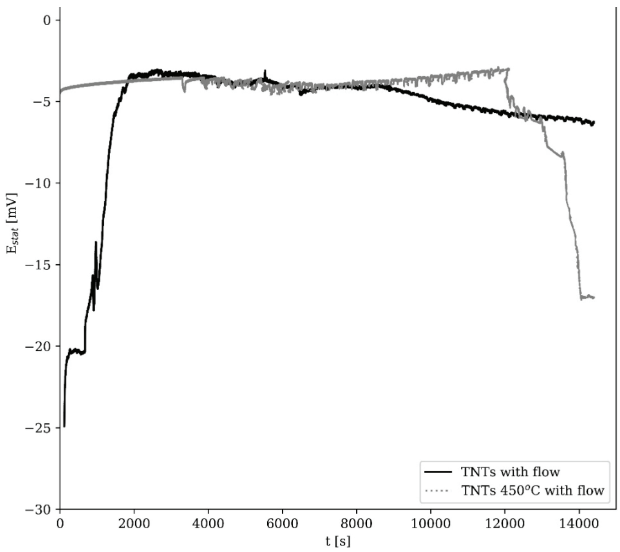

Fig. 7. Open circuit potential curves of unmodified and annealed at 450 °C TNTs layers recorded in the flow conditions. Spectra were recorded in the dialysis solution (pH 8.532 ± 0.109)

In Figure 7, the OCP curves of unmodified and annealed at 450 °C TiO_2 nanotubes measured in dialysis solution under dynamic conditions are shown. The flow caused the shift of OCP towards positive values for both samples (Fig. 3). The flow-induced oscillations of OCP during the measurement to a greater extent for annealed TNTs.

3.4. Adsorption of blood components on TNTs

The final stage of the research was to perform a hemodialysis procedure using swine blood flow of 250 cm^3/min for 4 hours at a temperature of 36.5 ± 0.5 °C using the hemodialysis machine Fresenius 4800S. In this part, non-modified and annealed at 450 °C TNTs were tested by the microscopic (SEM/EDS) and electrochemical (OCP) analyses while the results were presented in Fig. 8, Table 5 and Fig. 9, respectively.

SEM pictures (Fig. 8) and EDS analysis (Table 5) illustrate that TNTs before and after heat treatment own adsorption properties for carbon and calcium ions included in swine blood. These ions create on TNTs surface calcium carbonate. The larger crystals were formed on non-annealed TNTs.

In Figure 9, the results of OCP values for non-annealed and annealed TiO_2 nanotubes in temperature of 450 °C measured in dialysis solution at a temperature of 36.5 ± 0.5 °C, during 1800 s after adsorption of pig blood components are shown. A higher stationary potential value was observed for annealed TNTs than non-annealed TNTs for research after submerging in blood. Therefore, the surface of annealed TNTs is more stable and resistant to corrosion. However,

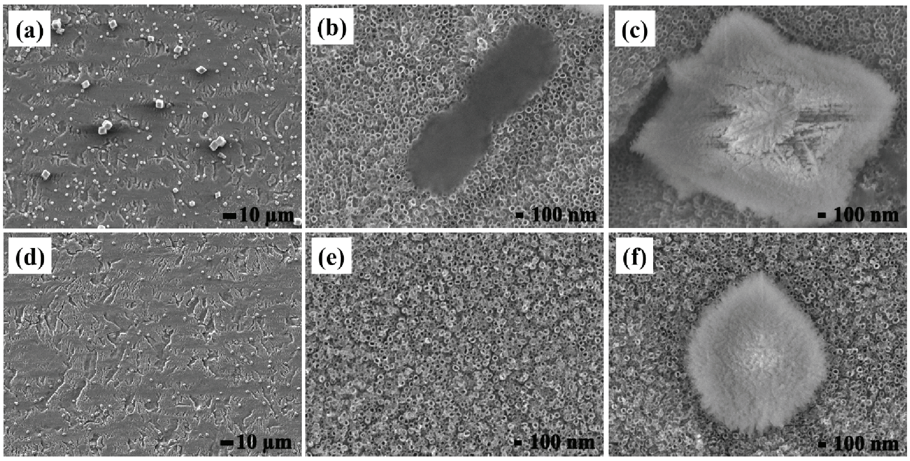

Fig. 8. SEM top-view of non-annealed (a)–(c) and annealed in temperature of 450 °C (d)–(f) TNTs after adsorption of swine blood components during a four-hour dialysis session

after adsorption of pig blood components, the OCP values are lower than OCP values measured in static and dynamic conditions after 14400 s. These results confirm adsorption ions from blood on TNTs surface (Table 5).

Table 5. Chemical composition of unmodified and heat treatment in temperature of 450 °C TNTs after adsorption of swine blood components during a four-hour dialysis session

TNTs	Unmodified	450 °C
Carbon [% wt.]	14.38 ± 1.3	12.38 ± 2.5
Oxygen [% wt.]	22.72 ± 1.8	30.45 ± 8.1
Titanium [% wt.]	62.90 ± 2.6	61.30 ± 2.6
Calcium [% wt.]	28.60 ± 0.3	26.35 ± 2.3

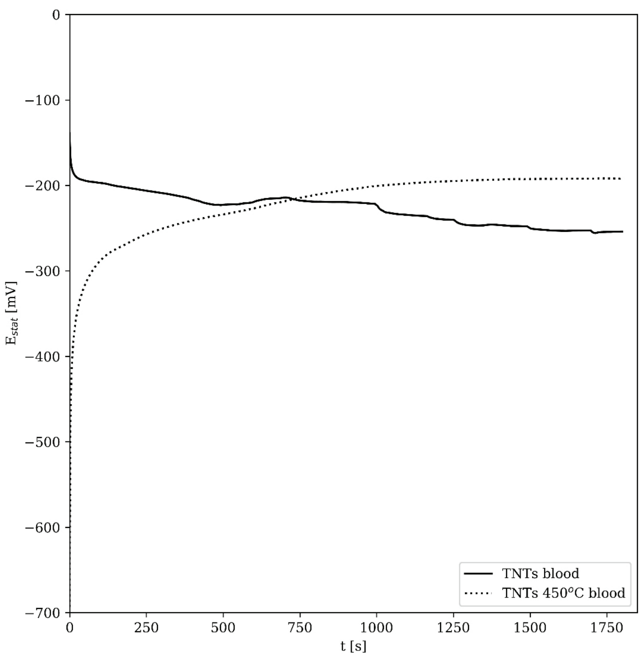

Fig. 9. Open circuit potential (OCP) of non-annealed and thermally modified TNTs temperature of 450 °C after adsorption of pig blood components. Spectra were recorded in the dialysis solution (50 ml, pH 8.532 ± 0.109) in temperature of 36.5 ± 0.5 °C

4. Discussion

4.1. Microscopic analysis of annealed TNTs

The SEM micrographs (Fig. 1) confirmed the formation of TNTs with smooth walls by using organic solutions with high viscosity (ethylene glycol) as electrolytes [25]. The smooth walls and surface of TNTs are necessary for the dialysis membrane to do not cause any damages to blood components during the hemodialysis [33]. Other anodization parameters such as time and the value of the anodizing current made it possible to receive TNTs with 50 ± 5 nm in diameter. The diameter 50 nm of TNTs is higher than the dialysate pore diameter (in the range of 10.9–15.8 nm [3]) and ensures the best protein immobilizations than smaller diameters [22].

The results of the EDS tests (Table 2) showed that the thermal treatment removes the fluoride from the TNTs structure [7]. Annealing in the air atmosphere also caused a noticeable increase in the oxygen content [37]. The Ti:O ratio decreases along with increasing annealing temperature due to the method used to investigate the chemical composition, which exceeds 1 µm of the test depth, and consequently, the increased amount of titanium may derive from the titanium foil. This ratio also may suggest that the oxide layer is not only composed of TiO_2 but also other titanium oxides and is hydrated (oxygen from water and hydrated species) [7]. Removing fluoride ions during the annealing of TNTs can be favorable due to its application as a filtering part in dialysis techniques. Patients under hemodialysis may exhibit excess ions in the blood, so they must be removed and not collected. Additionally, ions can lead to the formation of kidney stones, which causes deterioration of dialysis patient's health.

4.2. Characterization of thermally modified TNTs in dialysis fluid and static conditions

According to the human body temperature, the temperature of dialysis fluid was maintained during tests in terms of 36.5 ± 0.5 °C. Hemodialysis time (4 hours) was chosen as the time of one dialysis session according to the research of Tharmaraj and Kerr [33]. Long-term OCP measurement allows for determining changes in the stationary potential that could occur during hemodialysis due to deposition of ions from the dialysate on the sample. Differences in the amount and type of embedded ions will indicate whether the TNTs layers have good adsorptive properties. In addition, the embedded elements may influence the changes of the impedance modulus of the sample and change their oxidation and reduction abilities.

Scanning electron microscopy (SEM) micrographs (Fig. 2) and Energy Dispersive Spectroscopy (EDS) results (Table 3) confirm that unmodified TNTs adsorbs sodium and calcium included in dialysis fluid, in contrast to annealed TNTs. Park et al. [28] observed the deposition of calcium on TNTs only after ten days in the environment of the simulated body fluid. In these studies, adsorption also occurred on the TNTs surface annealed at 500 °C, which was not observed in our study (Table 3). This divergence of the results can be a consequence of using different liquids and various duration of the tests. After electrochemical tests, no fluorine content was noted in unannealed TNTs samples. Therefore, it can be assumed that fluorine was present in the form of ions physically adsorbed to TNTs, which were rinsed out during the long-term measurement of OCP. In present literature reports [12], a result similar to the one discussed in this paper did not appear. Moreover, the samples showed a much higher oxygen content, probably due to the adsorption of water or OH- groups during the measurements.

The annealing process caused a shift of open circuit potential (OCP) values (Fig. 3) towards positive values, which means that the surface of annealed TNTs is more stable and corrosion resistant. The same conclusions were occurred by Mazare et al. [24]. The annealed TNTs at temperature of 450 °C have the highest value of OCP potential, which means that they have the best corrosion resistance and are the most accurate material for dialysis membranes. Literature reports also confirm the increase in the TNTs stationary potential after annealing. According to Arkusz et al. [7], TiO_2 nanotubes stationary potential increases after heat treatment, and its value for non-annealed TNTs have a value of –220 mV in phosphate-buffered saline (PBS). The increase of stationary potential in TNTs after annealing is also confirmed in the survey in Hank's solution [38]. Figure 3 also shows the evident change of OCP potential value after 8000 s for unmodified TNTs. The reason for this phenomenon may be settling ions on its surface, as confirmed by EDS test results shown in Table 2. The greatest stabilization of OCP waveforms is observed for samples annealed at 450 and 550 °C, which indicates that thermal treatment at higher temperatures has a protective effect on TNTs samples.

The EIS measurements results (Fig. 4) indicate that thermal modification causes the increase of impedance modulus and the phase angle of TNTs, and the highest |Z| value was observed for TNTs annealed at 450 °C. These results are in line with the work of Yu et al. [38] and confirm that annealed TNTs have better protective properties and surface homogeneity. The phase angle recorded at the highest frequencies characterizes the part of the electrode in contact with the electrolyte, while at the lowest frequency, it refers to the barrier layer under the nanotubes and above the titanium foil. Bode plots show the most significant differences in waveforms at lower frequencies. It proves that the thermal treatment did not damage the nanotubular structure and that the boundary (barrier) layer has the greatest influence on corrosion resistance [38].

The voltammograms recorded for the annealed TNTs (Fig. 6) show much stronger peaks related to Ti^{4+} reduction and the opposite reaction, which indicates that the heat treatment improves the oxidation-reduction properties of nanotube layers. These results are in agreement with the results of Xiao et al. [23] and Macak et al. [36]. The absence of other peaks indicates the stability of the TNTs substrates, i.e., no byproduct formation.

Based on the discussed results, TNTs annealed at 450 °C were selected for further measurements performed in dynamic conditions. The highest corrosion resistance dictated this choice confirmed in OCP and EIS tests. Moreover, in the OCP studies, this sample showed the most stable behavior during the four-hour measurement. These properties are essential for the dialysis membrane in hemodialysis.

4.3. Characterization of thermally modified TNTs in dialysis fluid and dynamic conditions

SEM micrographs of TNTs annealed at 450 °C (Fig. 6) before and after electrochemical measurement in dynamic mode do not show any significant morphological changes in nanotube layers due to dialysate flow, in contrast to unmodified TNTs (Fig. 7). These results also show that there was no adsorption of fluid components on the TNTs surfaces. It means that thermally modified TNTs can serve as dialysis membranes [33]. Referring to the EDS test results collected in Table 3, the non-annealed TNTs underflow conditions are more susceptible to adsorption of elements of body fluids than annealed TNTs. The research of Park et al. [28] suggests that low adsorption of solution components may be caused by too short reaction time of the samples with the solution, but they confirm that more calcium ions are deposited on non-annealed TNTs than on annealed samples. Additionally, the result of the test is influenced by the flow used in our study, which hinders the deposition of ions from the solution. There have been no studies on the influence of electrolyte flow on TNTs morphology and adsorption properties in the literature so far.

The OCP curves recorded under flow conditions show that the flow shifted the open circuit potential values towards positive values for both samples. It means that the TNTs surface shows a higher corrosion resistance during dynamic measurements, and the dialysis fluid elements do not deposit on it (Table 4). Adsorption of proteins on the TNTs surface can be used to capture proinflammatory compounds; however, this property can also cause adverse effects such as adsorb morphotic elements in blood on the surface of TNTs. Wu et al. [35] conducted studies on the adsorption of proteins to TNT, which showed that these properties could be controlled by changing the diameter of TNT, which is made possible by changing the anodizing parameters. The adsorption and activation of a platelet on TNTs have been examined by Zhang et al. [39]. In particular, it has been confirmed that the best platelet adhesion and activation are obtained with TNTs annealed in 450 °C compared to samples annealed at 350 and 550 °C, and unannealed. These studies confirmed the deposition of blood components on annealed TNTs, but they do not capture the blood flow during hemodialysis. Our blood tests (Subsection 4.4) do not confirm the deposition of platelets or proteins on TNTs using blood flow. The flow also induced oscillations of OCP potential values in time for annealed and non-annealed TNTs. This phenomenon can be observed when the applied flow rate is closed to critical speed for a sample. According to literature reports [38], the corrosion resistance reaches the maximum for a critical flow speed and can increase its value as a result of flow. The oscillations are distinctly stronger for annealed TNTs.

4.4. *Ex vivo* evaluation of the blood components deposition onto TNTs

SEM pictures (Fig. 8) and EDS analysis (Table 5) showed that TNTs before and after heat treatment owns adsorption properties for carbon and calcium ions included in swine blood. These ions create calcium carbonate on TNTs surface. The larger crystals were formed on non-annealed TNTs. In static conditions (i.e., without flow) of blood components, sedimentation on the surface of TNTs, revealed by Smith and Popat [30] and Smith et al. [31] confirmed that adsorbed elements are blood platelets. Our results performed in dynamic conditions (including blood flow) did not cause blood platelets deposition on the TNTs surface, therefore, using flow prevents the creation of blood clots on TNTs dialysis filter. The deposition of a small number of blood platelets and a prolonged clotting time of the TNTs membranes were also confirmed by research performed by Gao et al. [14].

For patients with kidney insufficiency, the primary role in the pathogenesis of bone changes is phosphate retention. In that case, it is recommended to limit phosphate intake and to take medications that reduce phosphate absorption from the digestive tract, as the most commonly used compound is calcium carbonate. The bonding of calcium carbonate by TNTs can help maintain the correct concentration of calcium for patients requiring high doses of calcium carbonate. TNTs dialysis filters can also reduce positive calcium balance and hypercalcemia occurring for one third of patients [32]. Calcium carbonate deposition on the surface of TNTs allows the application of $CaCO_3$ for patients with calcifications in the walls of blood vessels and soft tissues, with a tendency to increase serum calcium levels. For these patients, less effective sevelamer medicine is applied against phosphatemia [13].

5. Conclusion

The constantly increasing number of patients requiring renal replacement therapy is a reason to improve and develop dialysis techniques continuously. Therefore, in this work, the properties of unmodified titanium dioxide nanotubes and annealed at 350, 450, 550 °C in air atmosphere had been analyzed in dialysis fluid and evaluated in terms of the possibility of using them as a filter part in dialysis techniques. The results demonstrated that the TNTs annealed at 450 °C TNTs can be potentially applied for constructions dialysis membrane in the area of hemodialysis. Compared to currently used dialysis membranes, the TNTs layers have a smooth and flat surface, leading to the elimination of hemolysis in dialysis patients. Furthermore, the heat treatment process and surveys in the dialysis fluid with and without flow do not cause any damages to the passive TNTs layers, which could be adverse for the dialysis process. Our study also indicates that annealed process cause improvement of corrosion resistance and protective properties for TNTs in the dialysis fluid. So far, in the literature, the survey about titanium dioxide nanotubes characterized in dialysis fluid has substantially supplemented the existing literature reports.

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