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PRACA ORYGINALNA / ORIGINAL PAPER

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Potencjał zeta preparatów hemostatycznych TachoComb i Surgicel-Fibrillar krwi i kości zębodołu

The zeta potential of TachoComb and Surgicel-Fibrillar hemostatic agents in blood and alveolar bone

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STRESZCZENIE

Wstęp. Zainteresowania autorów, zjawiskami elektrycznymi i elektrostatycznymi zachodzącymi w tkankach, narządach i płynach ustrojowych żywych organizmów oraz człowieka, od dekady pojawiają się w piśmiennictwie medycznym. Dotyczy to nie tylko wyjaśnieniu patogenezy chorób, ale również stosowanych procedur i sposobów leczenia.

Cel. Celem pracy było określenie potencjału zeta, kolagenowo-fibrynowego opatrunku chirurgicznego TachoComb i preparatu utlenionej, regenerowanej celulozy Surgicel – Fibrillar, krwi i kości zębodołu.

Materiał i metoda. Badania przeprowadzono w Zakładzie Ceramiki i Biomateriałów Instytutu Elektrotechniki we Wrocławiu, wykorzystując do pomiaru potencjału zeta urządzenia Zetasizer 2000 – firmy Malvern Instruments (UK).

ABSTRACT

Introduction. The electrical and electrostatic phenomena that take place in tissues, organs and body fluids of living organisms and man have been the issue of numerous publications in the medical literature for a decade. This applies not only to clarification of the pathogenesis of diseases but also the procedures and treatments.

Aim. The aim of this study was to determine the zeta potential of collagen-fibrin TachoComb surgical dressing and oxidized regenerated cellulose Surgicel–Fibrillar preparation in blood and alveolar bone.

Materials and methods. The study was conducted in Department of Ceramics and Biomaterials, Institute of Electrical Engineering in Wroclaw. The measurements of zeta potential were conducted by means of Zetasizer 2000 device

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Udział współautorów / Participation of co-authors: A – przygotowanie projektu badawczego/ preparation of a research project; B – zbieranie danych / collection of data; C – analiza statystyczna / statistical analysis; D – interpretacja danych / interpretation of data; E – przygotowanie manuskryptu / preparation of a manuscript; F – opracowanie piśmiennictwa / working out the literature; G – pozyskanie funduszy / obtaining funds

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Wnioski. potencjał elektrokinetyczny w funkcji pH i funkcji temperatury obu badanych hemostatyków posiada ujemny ładunek. Potencjał zeta w funkcji pH dla TachoComb waha się od -15, 2 (+ -12)mV do -9, 8 (+ -0,9)mV, dla Surgicel-Fibrillar przyjmuje średnie wartości 17,5mV. Wartości potencjału zeta w funkcji temperatury dla TachoComb były nieco wyższe, średnio 15,9 mV, zaś dla Surgicel-Fibrillar wynosily od -16, 2 (+ - 1, 3)mV do -22, 0 (+ -1, 2)mV.

Słowa kluczowe: potencjał zeta, preparaty memostatyczne, TachoComb; Surgicel-Fibrillar

produced by Malvern Instruments (UK).
Conclusions. The electrokinetic zeta potential of pH and temperature function in both tested haemostatic agents has negative charge. The zeta potential of pH function for TachoComb ranges from -15.2 (+ -12)mV to -9.8 (+ -0.9) mV, for Surgicel-Fibrillar the average values are 17.5mV. The values of zeta potential of temperature function for TachoComb were slightly higher, on average 15.9 mV, while for the Surgicel-Fibrillar ranged from -16.2 (+ - 1.3) to -22 mV, 0 (+ -1.2) mV.

Key words: zeta potential, hemostatic agents, TachoComb, Surgicel-Fibrillar

Introduction

The issue on electrical and electrostatic phenomena occurring in the tissues, organs and body fluids of living organisms and in humans and research on these phenomena has occurred for a decade in the medical literature [1–7].

Recently, more and more attention is paid to the use of electrostatic and electrokinetic phenomena to determine the biocompatibility and biofunctionality between human tissues and organs, implant materials and all kinds of biomaterials that are widely used in medical practice and associated disciplines, i.e. surgery, biotechnology and dentistry [8–11].

Examples of materials commonly used in surgical practice and emergency medicine are hemostatic agents administered in the treatment and prevention of bleeding. Most of the available hemostatic agents used in general surgery, trauma surgery, maxillofacial surgery and otolaryngology have been examined for their structural, chemical, microbiological properties and their influence on tissues of living organisms both in vitro and in vivo studies. No studies have so far undertaken the subject of electrokinetic phenomena occurring at the border of the hemostatic material and body fluids as well as the impact of these phenomena on the hemostatic properties and clinical utility [12–15].

No studies on the biocompatibility of hemostatic agents based on electrostatic and electrokinetic phenomena have been conducted. The use of hemostatic agents on wounds in oral surgery after tooth extraction in order to stop bleeding creates a system of double layer in an aqueous phase in contact of the following materials: haemostatic agent, blood, morphotic elements of blood, alveolar bone, saliva. Their electrostatic properties depend on theelectrokinetic phenomena such as electrophoresis, electroosmosis, sedimentation potential Each particle in the colloidal system has an electric charge on their surface, which causes and streaming potential mutual interaction between colloidal particles [16–18]. These interactions occur between the particles, regardless of whether they are in contact or are at a distance from each other and constitute the electrical properties of the system [3–6, 10, 11]. Based on theoretical assumptions, confirmed by practical models it was proved that the electrical force plays an important role in biological processes. Considering the structure of colloidal systems it was noted that the characteristic feature of the colloidal particles is surface charge adsorbed in the form of ions electrostatically compensated by the ions contained in the dispersion medium. The colloidal particles in the middle are surrounded by dispersive film of electrically charged atoms or a group atoms. In this way double electrical layer is created and a layer of counterions, a micelle is formed. Surface charge of the particle present in the adsorption layer and the charge contained in the layer of counterions in the dispersion medium form double electrical layer (del).

Aim

The aim of the study was to determine the zeta potential of the next generation of hemostatic agents, i.e. collagen-fibrin TachoComb surgical dressing and oxidized regenerated cellulose Surgicel–Fibrillar preparation in blood and alveolar bone.

Material and methods

The study used blood, alveolar bone obtained with the consent of patients during diagnostic-treatment procedures performed in these patients during the hospitalization at the Clinical Department of Maxillo-Facial Surgery of Provincial Specialist Hospital in Rzeszów.

The study of electrokinetic potential was conducted at the Department of Ceramics and Biomaterials, Institute of Electrical Engineering in Wrocław. The measurements of zeta potential were conducted by means of Zetasizer 2000 device produced by Malvern Instruments (UK).

Laboratory has ISO certificates and approvals of the EU, among others, in the respect of electrokinetic potential measurements. Device parameters: range of particle diameter from 5 nm to 30 μm, the conductivity of the medium of 0 to 30 mS / cm, pH from 2 to 12, the angle of rays intersection 12 °, temperature of measurement from 10 ° to 70 ° C.

Material for the examination, i.e. collagen-fibrin dressing, oxidized regenerated cellulose, alveolar bone and blood were prepared for the physicochemical assessment by pre-fragmentation to colloidal size. These samples were dispersed in aqueous solutions at various pH, constant ionic strength, determined on the basis of ten times diluted physiological saline.

The measurement of electrokinetic potential was performed by collecting the results of every 30s at 25° C – 42° C. The results were read as the average of 30 consecutive measurements.

Results

The results of the study of the zeta potential are presented in the tables and figures in the form of line charts with their descriptive interpretation, taking into account the patient's natural physiological reaction. A reference for the human pH was accepted at 7, 3 to 7, 35 [2, 7, 9].

Studies have shown that the zeta potentials of the tested biological materials (blood, bone) and hemostatic agents demonstrate significant changes in pH function of the systems, temperature, and even the time of measurement which indicates a dynamic physicochemical nature of the measured parameter. The values of electrokinetic potential may determine the course of a number of physiological and physicochemical processes in living organisms and it is believed that they are significant for clinical purposes. Number of cases observed in this study cannot form the basis for a very general conclusions about the specific influence of the zeta potential on the processes of bleeding. However, it is a guideline to further research of noninvasive laser diffraction method used while measuring the electrokinetic potential. The results presented below may form the basis of modeling a chosen clinical process in terms of mass transfer, movement of colloidal particles at the surgical site. The proposed model – a representation of a real system with haemostatic agent is to define, as was assumed in the present study, the importance of the double electrical layer around the colloidal particle measured quantitatively in the form of the zeta potential and to identify possible interactions of cellular and tissue properties as a function of pH and temperature.

Data on zeta potential of whole blood as pH function are shown in Table 1 and in Fig. 1 and in Fig. 2 and Tab. 2 show its function of temperature. The presented zeta potential decreases significantly from the value of minus several mV to -30mV in the physiological range of pH, what confirms the possible changes in colloidal stability of poli-dispersive structure of the contained in an aqueous medium. The increase in acidity of the environment below 6 pH may promote settling of the individual particles making up blood cells on other negatively charged particles or other surfaces, which is course dependent on the charges of the phases. The zeta potential as a function of temperature changes in the range from -23 mV to -16mV for the range from 37 to 42 degrees C.

Multiple correlation coefficients confirm that these changes in a relatively narrow range of changes of the independent variables are linear. Further research is justified to determine the correlation of other materials that may affect the inhibition of bleeding during or after surgery.

The values of the zeta potential of the alveolar bone as a function of pH and temperature are presented in Figures 3 and 4. The value of the zeta potential as pH function ranges from -8mV to -15mV for similar pH as in case of blood. The graph shows that in the physiological range of pH, the zeta potential of this tissue is approximately -13mV, which enables reaching of the individual particles of whole blood. The zeta potential as a function of temperature for bone, which is illustrated in Fig. 7, increases linearly to about -12mV for the temperature of 40 C, and thus less and less negativevalues. Therefore, it becomes easier for the elements of blood to reach the site with elevated temperature.

Table 5 and Fig. 5 and Table 6 and Fig. 6 show the electrokinetic potential of the colloidal particles obtained from collagen-fibrin agent (TachoComb) and oxidized regenerated cellulose (Surgicel-Fibrillar) as a function of acidity of the environment and temperature. The zeta potential increases with increasing pH, both for the collagen-fibrin agent and for oxidized regenerated cellulose. The values of the zeta potential in a pH range close to the physiological range fluctuate for TachoComb -11.6 (+ -1.1) mV and about 16.6 (+ - 1.3) mV for Surgicel-Fibrillar. The value of the zeta potential for both TahoComb and Surgicel Fibrillar decreases, ie. it becomes more negative with increasing temperature reaching the values of -21.1 (+/- 1.9) for the collagen-fibrin agent and -22 mV (+/- 1.2) mV for the oxidized regenerated cellulose at the temperature of 42.0 C. In the temperature of about 37.0 the values of zeta potential for each agent were -10.7 (+ -1.0) mV and about – 16.0 (+ -1.6) mV, respectively.

Fig. shows the values of the zeta potentials for blood and blood – collagen-fibrin dressing (blood-TC) systems and blood – cellulose agent (blood-SF) as a function of pH while Fig. 13 as a function of temperature. The values of the zeta potentials for the collagen-fibrin dressing – blood system and blood – cellulose agent system are becoming increasingly negative with increasing pH. Both graphs of the zeta potential for haemostatic agents are shifted in the direction of less negative values in comparison to the zeta potential values for blood.

The zeta potential of the collagen-fibrin dressing ranges from -11.8 (+ -1. 2) mV for acidic pH to -19.0 (+ -1.8) mV in terms of more alkaline pH. The zeta potential of oxidized regenerated cellulose assumes values -12.2 (+ -1.1) mV at pH<5 and at pH> 8.3 – 26.2 mV.

In comparison, the zeta potential of physiological blood ranges from -16.9 mV for low pH to -24.3 mV for pH> 7.5. It decreases with increasing pH.

In the physiological range of pH adopted at 7.3 – 7.35 the values of the potential for both haemostatic agents and blood differ slightly and assume the values for the blood of

Tab. 1. The zeta potential of the blood as a function of pH

pH	zeta potential [mV]	Standard deviation
6.0	-14.3	1.4
6.5	-15.3	1.6
7.3	-26.7	2.5
7.6	-29.7	2.9

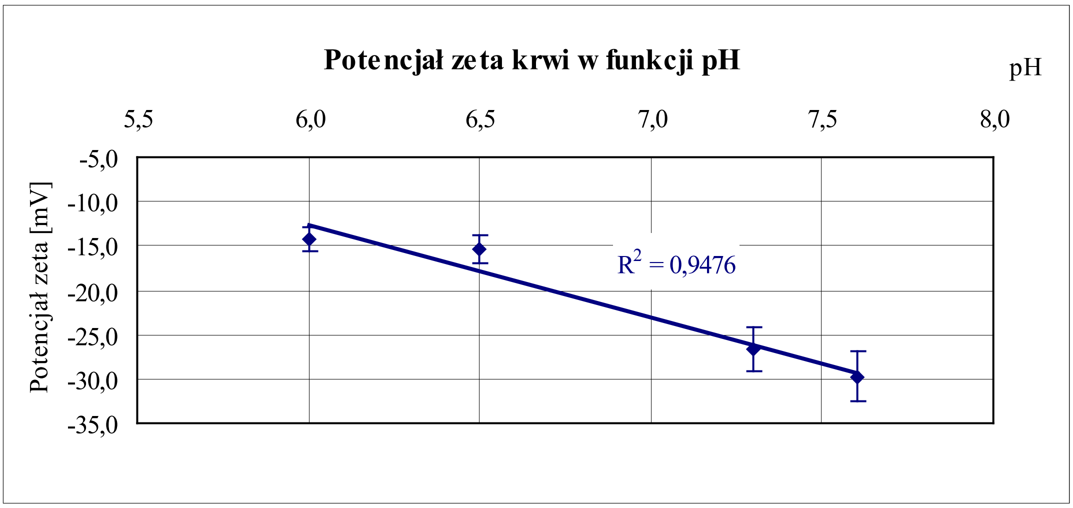


Fig. 1. The zeta potential of the blood as a function of pH

Tab. 2. The zeta potential of the blood as a function of temperature

Temperature	Zeta Potential [mV]	Standard deviation
36.9	-22.7	2.1
38.0	-22.5	2.4
39.2	-20.7	2.0
40.1	-17.6	1.5
41.9	-16.1	1.6

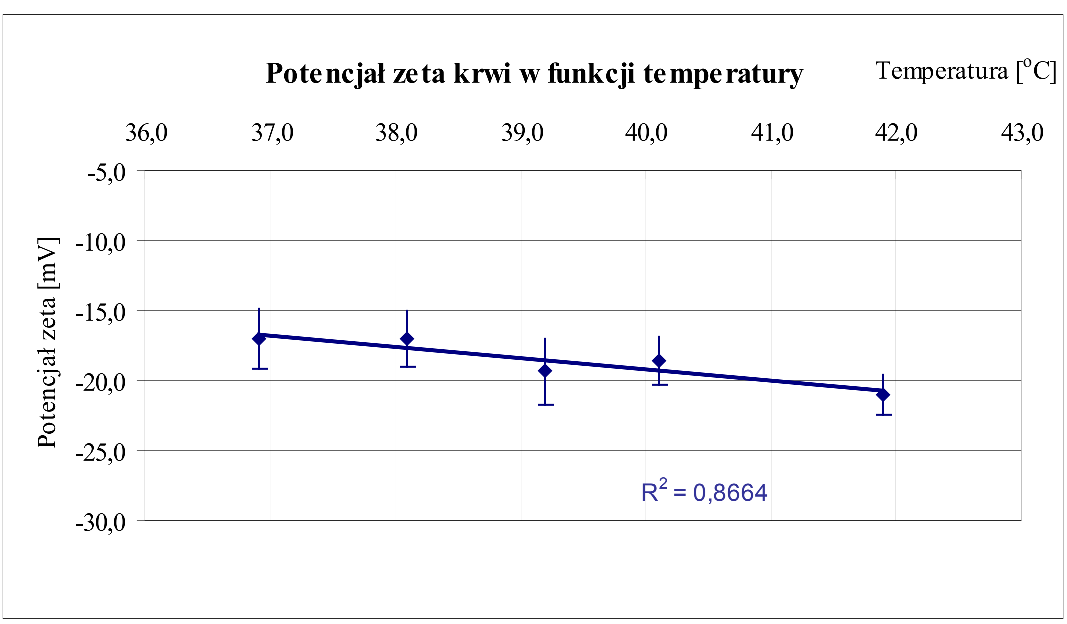


Fig. 2. The zeta potential of the blood as a function of temperature

Tab. 3. The zeta potential of the alveolar bone as a function of pH

pH	zeta potential [mV]	Standard deviation
6.3	-8.5	0.7
7.0	-13.1	1.1
8.2	-15.1	1.2

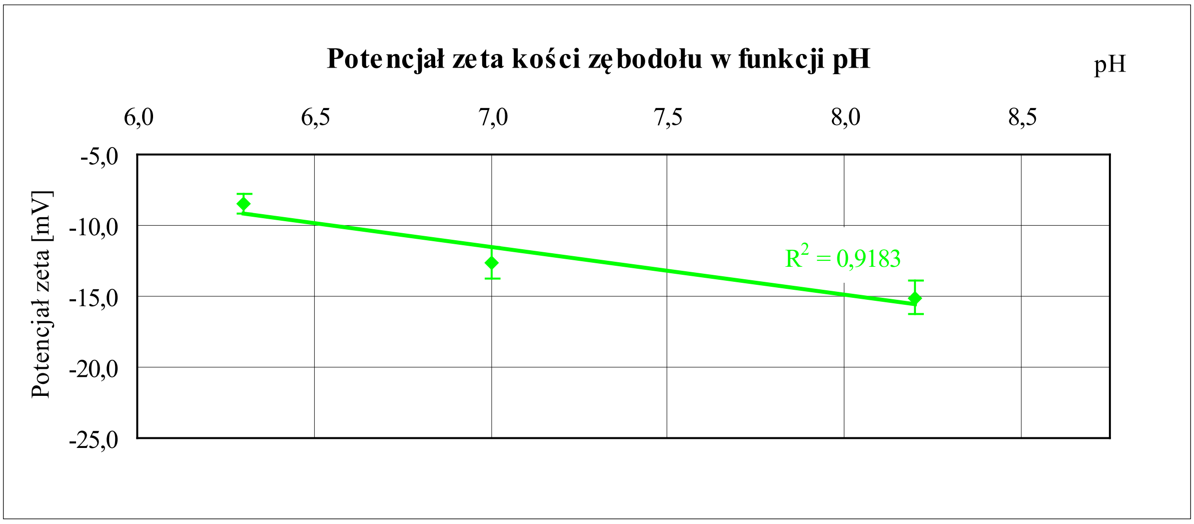


Fig. 3. The zeta potential of the alveolar bone as a function of temperature

Tab. 4. The zeta potential of the alveolar bone as a function of temperature

Temperature	Zeta potential [mV]	Standard deviation
36.0	-15.5	1.5
38.0	-13.4	1.1
40.0	-12.2	1.2

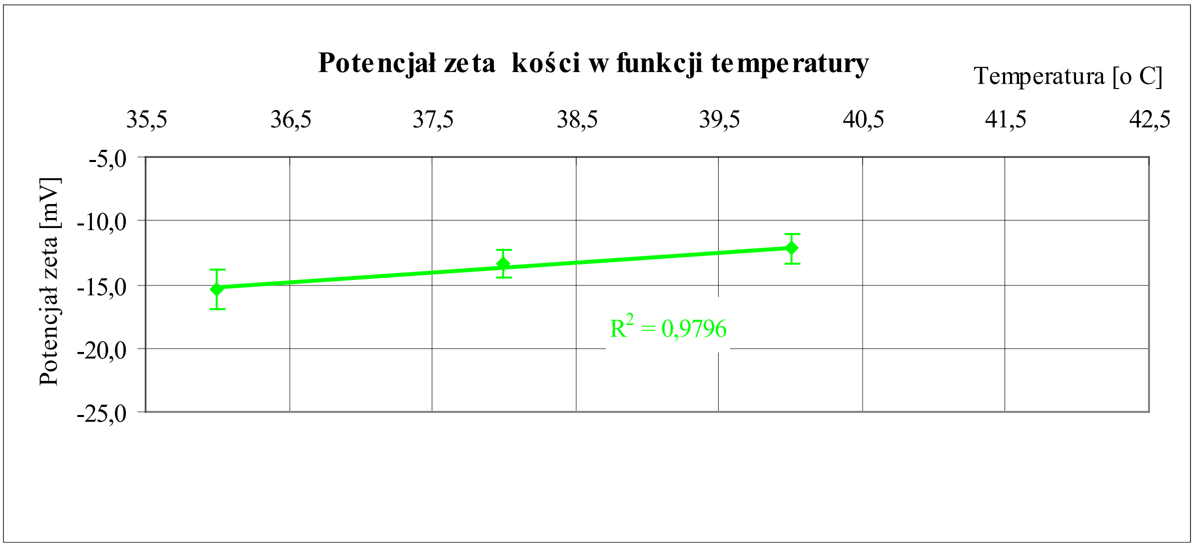


Fig. 4. The zeta potential of the alveolar bone as a function of temperature

Tab. 5. The zeta potential of TachoComb and Surgicel-Fibrillar as a function of pH

TachoComb		**Surgicel -Fibrillar**	
pH	**zeta potential**	**pH**	**zeta potential**
5.6	-15.2 (1.2)a	**4.7**	-21.2 (+ - 1.7)a
7.1	-11.6 (1.1)	**6.2**	-17.9 (+ - 1.4)
7.8	-10.5 (1.0)	**6.9**	-16.6 (+ - 1.3)
8.5	-9.8 (0.9)	**8.3**	-13.9 (+ - 1.1)

a) Standard deviation

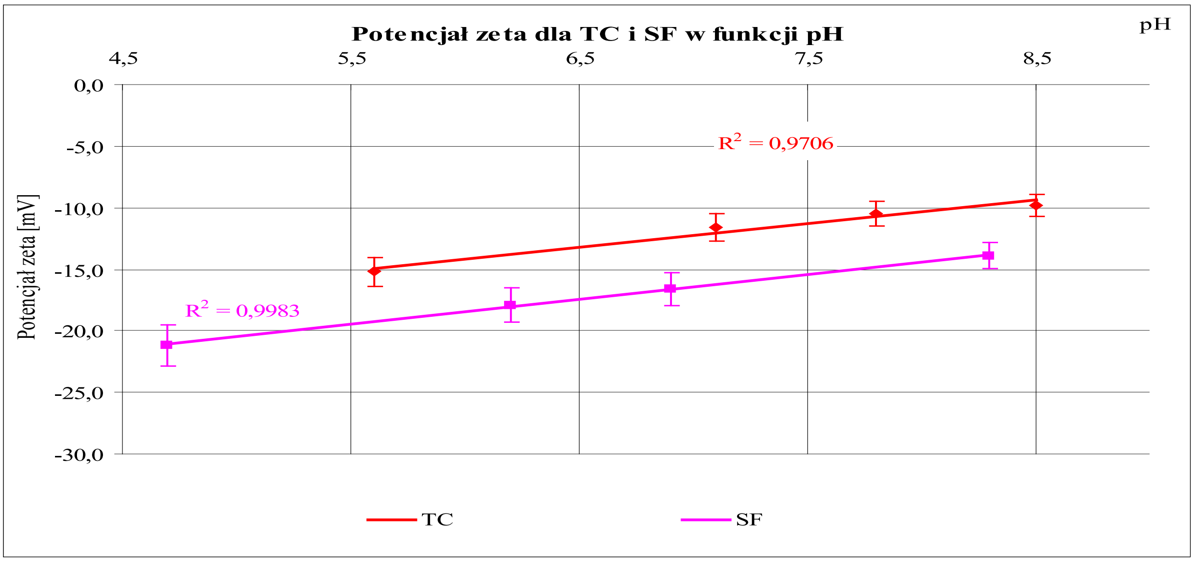


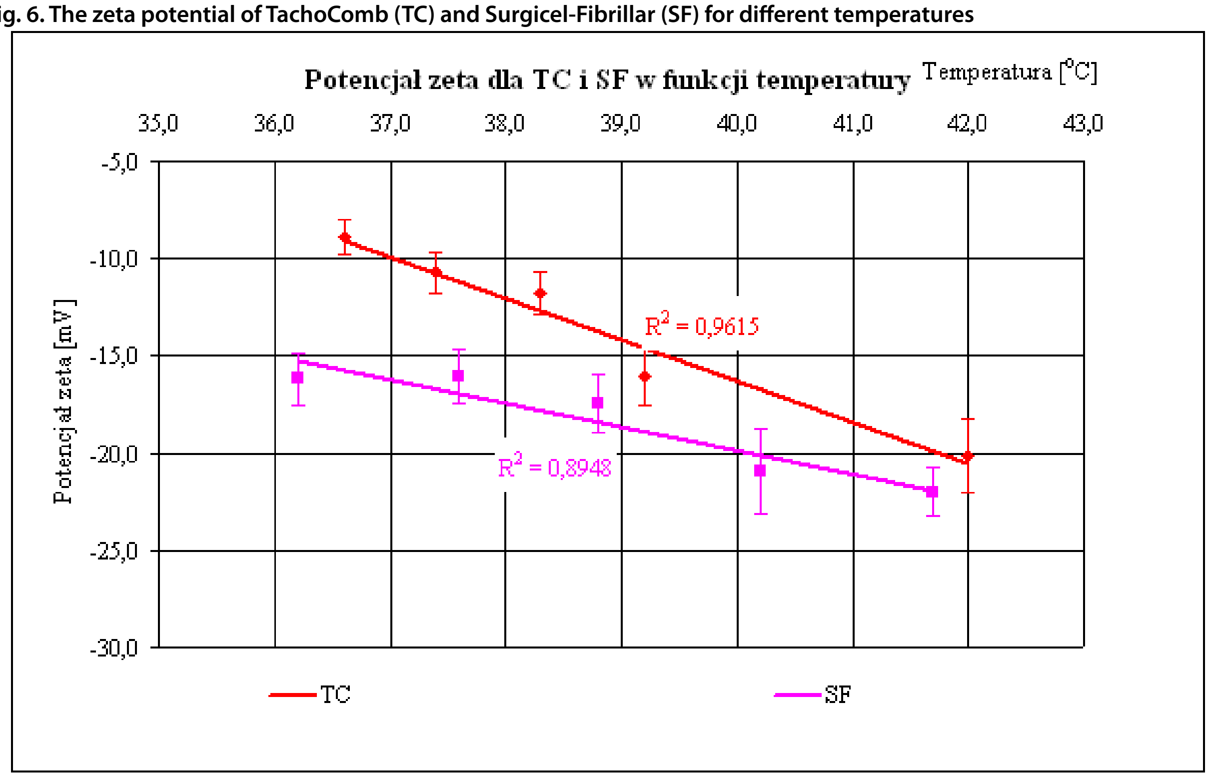
Fig. 5. The zeta potential of TachoComb and SF as a function of pH

Tab. 6. The zeta potential of TachoComb and Surgicel –Fibrillar as a function of temperature

TachoComb		Surgicel-Fibrillar	
Temp.	**zeta potential**	**Temp.**	**zeta potential**
36.6	- 8.9 (0.9)a	**36.2**	-16.2 (1.3)a
37.4	-10.7 (1.0)	**37.6**	-16.1 (1.4)
38.3	-11.8 (1.1)	**39.8**	-17.5 (1.5)
39.2	-16.1 (1.5)	**40.2**	-20.9 (2.2)
42.0	-20.1 (1.9)	**41.7**	-22.0 (1.2)

a) Standard deviation

Fig. 6. The zeta potential of TachoComb (TC) and Surgicel-Fibrillar (SF) for different temperatures



about -24,3 mV, and for cellulose (Surgicel-Fibrillar) -23.2 mV and for collagen-fibrin dressing (TachoComb) -19.0 mV. The combination of particles of blood with hemostatic agents and simultaneous measurement resulted in reduction of the potential of the system due to the presence of particles of blood. A comparison of the graphs in Fig. 10 and 12 shows that the blood "imposes" type of potential changes in TC and SF on pH, which is consistent with that observed for the blood. The values of the potential for the systems: TachoComb – blood, Surgicel – blood is conducive to clinical activity of both agents slightly raising the potential for blood and enabling the inhibition of bleeding. The graph in Fig. 12 shows also that the directional attribute of a straight line changes the potential for SF – blood and becomes more negative than in case of TC–blood and from this point of view it indicates a possible advantage of TC in surgical practice.

Discussion

The available literature data on the properties of electrostatic biomaterials applied in surgery and implantology, among other things, as vascular prostheses and various types of implants indicate that, in the process of clot formation an electrostatic charge of the material upon which the clot is formed plays an important role. It has been found that the positive charge facilitates the clot formation and the negative one hinders it [9, 12, 14, 15].

It seems interesting to know, inter alia, that electrokinetic potentials occurring at the Interface between the hemostatic preparations for the treatment and prevention of bleeding in the surgery of oral cavity and forming a clot [6, 16, 17].

In order to achieve it, some measurements of zeta potentials of hemostatics; agents of collagen-fibrin and oxidized cellulose, have been carried out. Hemostatics used to obtain a stable clot in a post-extraction wound interact directly with blood and its components e.g. platelets, red blood cells and an alveolar bone. The double electrical layer forming a clot may determine the impact forces of individual components on the surface of a bone and hemostatics [10, 11, 14].

The measurements of the zeta potential values indicate that it is a further convenient and simple criterion of the biocompatibility of a collagen-fibrin sealant preparation and regenerated oxidized cellulose applied in practice, the topical treatment of bleeding. It has been shown that the zeta potential values in the pH range about 7.0 are negative for both the collagen-fibrin dressing, which is approximately 11.6 (± 1.1) mV, and the regenerated oxidized cellulose, taking a higher zeta potential about -16.6 (± 1. preparation of 3) mV. According to Ney's interpretation (acc.17) concerning the stability of various colloidal solutions depending on the size of the zeta potential, regenerated oxidized cellulose, with a lower value of the zeta potential, has a higher stability of the system whereas a collagen-fibrin dressing according to this classification is less stable.

Conclusions

1. Studies have shown that the zeta potential as a function of pH and temperature for both tested haemostatic agents has negative charge.
2. The zeta potential as a function of pH for TachoComb ranges from -15.2 (+ -12) to -9.8 (+ -0.9) mV and for Surgicel Fibrillar the values are between -21.2 (+ -1.7) mV to -13.9 (+ -1.1) mV.
3. The values of the zeta potential as a function of the temperature for the TachoComb collagen-fibrin agent range from -8.9 (+ -0 9) mV to -20.1 (+ - 1.9) mV and from -16.2 (+ - 1.3) to -22.0 mV (+ -1.2) mV for Surgicel Fibrillar agent.

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