Terahertz Time-Domain Spectroscopy for Studying Absorbable Hemostats

Marie Nedvedova, Vojtech Kresalek, Zdenek Adamik, and Ivo Provaznik

***Abstract*—This study deals with the kinetics of absorbable hemostats used for supporting the hemostasis and wound closure during surgical intervention. There are several types of hemostats of different composition therefore with different mechanism of action. Here, terahertz (THz) time-domain spectroscopy is used for studying the absorption kinetics of the reactions between the hemostatic and the physiological saline solution (PSS). The reactions correspond with the changes of the THz radiation response measured in time. These time dependences are analyzed to find time constants for mathematical description of the hemostatic kinetics and the significant differences among all types of hemostats are found. The physiological saline solution is replaced by human blood in the additional measurement. Based on the results, we can compare the reaction rate of different types of hemostats that could be beneficial in the surgery practice.**

***Index Terms*—Absorbable hemostat, kinetics, physiological saline solution (PSS), terahertz (THz) time-domain spectroscopy.**

I. Introduction

BLEEDING is a typical reaction practically to each injury when a blood vessel is damaged. A natural response of the body is the hemostasis, which is a sequence of processes that lead to stoppage of bleeding. There are three basic mechanisms that are essential for normal hemostasis [1]. A vasoconstriction is the first reaction reflecting the damage of the vessel. As a result, the blood flow through the affected area is minimized to prevent other blood loss. An exposed subendothelial extracellular matrix contains several adhesive proteins that serve as ligands for platelet surface receptors. The blood platelets adhere to the exposed substrates (especially collagen) from the broken site of the vessel in a highly coordinated process and chemicals promoting the other platelet aggregation are released [2], [3]. The following coagulation cascade consists of the enzymatic reactions including the thrombin activation and conversion of soluble fibrinogen into insoluble fibrin. The adherence and cross-linking of the fibrin proteins together causes a loose mesh, where platelets and red blood cells are caught and finally, the blood clot (thrombus) is formed [4], [5]. When the injured blood vessel is healed, the effete clot is degraded in controlled fibrinolysis [5].

When the wound is relatively small, the hemostasis is fully capable of stopping bleeding without significant blood losses or serious consequences. However, there are also situations when the hemostasis alone is not sufficient to ensure effective stoppage of bleeding. This includes not only the injuries but typically also each surgical intervention. During surgery, the hemostasis is supported by various methods such as compression, clipping, ligation, and electrocautery. However, there are cases when these conventional methods are not adequate. Hemostatic agents can be used in this application [6].

Generally, hemostatic agents form five major groups according to the mechanisms of action on which they are based: physical, synthetic, biologically active agents, absorbable agents, and hemostatic dressings. The effect of mechanical agents such as bone wax and ostene is based on the mechanical occlusion of bleeding channels in bone. Synthetic agent works as a tissue adhesive; the mechanism of action can be based on the mixing of specific substances forming the final glue for wound closure (polyethylene glycol hydrogels, glutaraldehyde cross-linked albumin) or on the natural curing reaction when in contact with tissue (cyanoacrylates). Biologically active agents are usually based on fibrin and thrombin; thereby participate in the hemocoagulation cascade directly. Absorbable agents participate in the coagulation indirectly providing physical matrices for clotting initiation (gelatin, oxidized cellulose) or promoting platelet adherence and activation (collagen). Hemostatic dressings are based on the chitin, chitosan, mineral zeolite, or combined with the absorbable and biologic agents [7], [8].

All of the above-mentioned hemostats are widely used in surgical practice. There are descriptions of applications in a wide medical field, especially dermatologic and plastic surgery [9], gynecologic surgery [10], and practically all types of surgical interventions [8]. Some authors compare the effectiveness of hemostats using animal models [11]–[13]. As the mechanism of the action is different for each hemostatic, its usage is not completely universal and it is necessary to respect some recommendations. Choosing the most appropriate hemostat for a given situation is usually based on the clinical experiences of the surgical

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team [14]. An important factor can be the rapidity of hemostasis. Because the hemostats are made of various materials, the kinetics (and probably also the rapidity) of reaction with blood could differ. Knowing the hemostatic kinetics should be helpful in any hemostatic selection but relatively little is known about it. Because each biological object is individual in many ways, the objective evaluation of the reaction rate can be a problem. This is an opportunity for using the methods investigating physical or chemical material properties. In addition to basic physical–chemical measuring procedures (measurement of pH, etc.), modern methods were also applied such as scanning electron microscopy [15]–[17], transmission electron microscopy [16], magnetic resonance imaging [18], and spectroscopic methods (plasma atomic emission spectroscopy in [19], UV-VIS spectroscopy in [20], and Fourier transform infrared spectroscopy in [21]).

This study is focused on the studying kinetics of the absorbable hemostats using THz time-domain spectroscopy that could provide a better insight of their mechanism of action. The goal is to determine parameters characterizing the investigated system (such as time constants) and to compare reaction rates of the hemostats each to other.

II. Materials and Methods

A. Instrumentation

THz time-domain spectroscopy is a modern method that has found many useful applications in technical areas. Especially because of its advantages, it has taken its place also in biological and medical sciences. An important aspect in studying the biological objects is the noninvasiveness and nondestructiveness of the investigating method; both are fulfilled. Other advantages beneficial to our experiment include no special requirements for sample preparation, water sensitivity and optional system settings. Water occur strong absorption of the terahertz radiation that could make some measurements impossible, consequently it is usually considered as something unwanted. In our case, we can this fact take as an advantage because it characterizes the absorption capability of a hemostatic sample. THz time-domain spectroscopy system TPS Spectra 3000 by TeraView Ltd. was used as an analyzing device in this experiment. The spectrometer has an ability to set parameters of scanning (number of scans, resolution, scanning frequency) according to our requirements. For our purpose, we were focused on accurate sampling and scanning time reduction that made the measurement sufficiently fast.

TPS Spectra 3000 is capable of measuring in several modes. As the most appropriate method, the attenuated total reflection (ATR) mode was used. Generally, an investigated sample is placed on ATR crystal which has a significantly higher refractive index. In this case, the ATR crystal made of silicon with refractive index 3.42 is used. A THz radiation enters the crystal at the angle of 35°. At the crystal–sample interface, the terahertz wave is totally reflected. An evanescent wave forms and penetrates into the sample down to a specified depth depending on the wavelength, refractive index of the sample and crystal, and angle of incidence [22]. The penetration depth is about 100 μm, thus there must be achieved a good contact of the sample with the ATR crystal.

B. Samples

Six types of absorbable hemostats were used in this study: Surgicel Nu-Knit, Surgicel SNoW, Surgicel Fibrillar, Gelita-Spon, Hypro-Sorb, and TachoSil. The first three Surgicel hemostats by Ethicon, Johnson and Johnson, are forms of oxidized regenerated cellulose that could have various formats (woven/nonwoven), fiber density, and basis mass that influence the hemostasis process. This type of hemostatic agent is used to control bleeding in open and endoscopic procedures. When absorbing water, the material swells and entraps blood proteins and platelets forming a gel-like barrier to blood flow [12]. Other mechanisms of the action lies in the pH decreasing which has an antimicrobial effect [7]. Gelita-Spon is an absorbable gelatin sponge hemostat, a type of gelatin foam. Gelatin is a pure collagen structural element of human tissue [23], [14]. Gelatin foam provides a matrix for clotting and helps to stop capillary, venous, and arteriolar bleeding. Gelatin hemostat swells more than cellulose [7], [8]and can absorb more than 40 times its own weight [23], [14]. Because of neutral pH, it can be used in conjunction with other pH neutral biologic agents to enhance the hemostasis [7]. Hypro-Sorb R is a bioabsorbable atelocollagen hemostatic felt manufactured from bovine atelocollagen. It has specific activity to thrombocytes and releases clotting factors [24]. TachoSil is an absorbable fibrin sealant patch that combines advantages of biologic agents with a classical absorbable hemostat. A layer of human fibrinogen and thrombin is coated onto an equine collagen sponge [25]. The strength of this combined adhesive is significantly higher than fibrin glue alone [25]. The mechanism of action is based on an interaction between active biological substances and following fibrin clot formation. All mentioned materials are biodegradable usually in several weeks [7], [8]. A cellulose swab was added to measurement as a reference absorption material with no other special expected properties.

C. Experimental Design

Samples of dry hemostats were prepared for ATR measurement. The size of each sample was fit to the sampling window of ATR crystal. The thickness of each dry hemostatic sample varies in the range 400–800 μm, however, the volume each of them was kept on 27 ± 3 mm^3. The parameters of the spectrometer were set up for fast measurements; each scan lasted 1 s. The measurement was carried out at the laboratory temperature of 30 °C and relative humidity of approximately 25%. Every act during the measurement was timed precisely. First, the measurement of the spectrometer started when the exact amount (15 μl) of liquid medium was placed on the silicon crystal, and the hemostatic sample was applied on and fixed with the screw. The whole procedure is shown in Fig. 1. Two liquid media were used for the hemostat activation there—physiological saline solution (PSS, 0.9% solution of sodium chloride and water) and human blood. The measurement of the reaction with PSS was repeated five times for each type of investigated material. Blood was drawn from a fingertip of a volunteer (56-year-old healthy man) using a device with a tiny needle (part of a glucose tester)

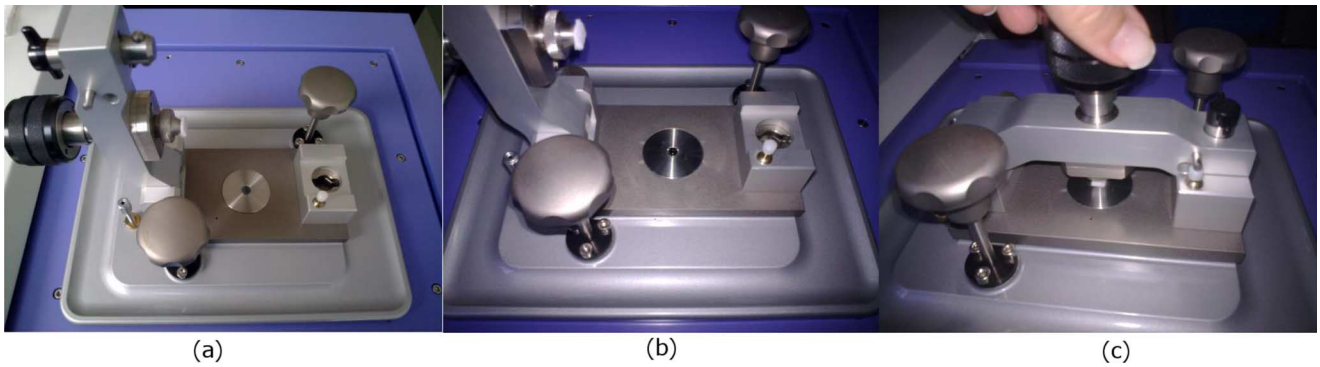

Fig. 1. Measurement procedure. (a) Clean ATR silicon crystal. (b) Liquid medium application (here PSS). (c) Hemostatic sample application and fixing with the screw.

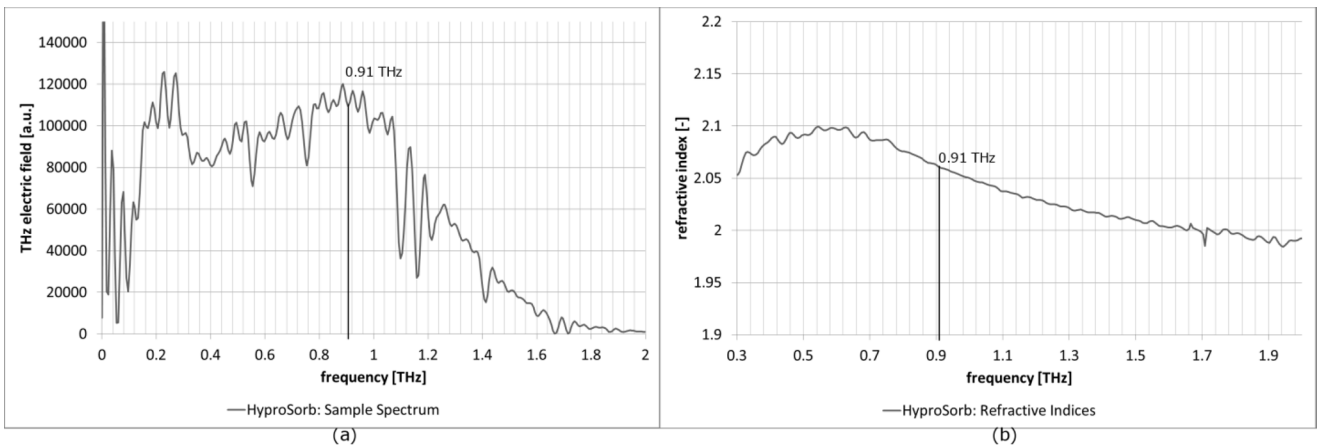

Fig. 2. (a) Typical reflection spectrum and (b) measured refractive index one of the investigated sample (HyproSorb) in contact with PSS.

after obtaining the informed consent. The whole procedure was conducted in accordance with the Helsinki Declaration. Blood was used once for each investigated material in a preliminary experiment.

III. Data Analysis and Results

Using the TPS Spectra 3000 spectrometer, the THz waveforms in the time domain were recorded, and optical parameters were calculated. As an investigated parameter, the refractive index of the sample was calculated using the original Teraview software [27].

For a detailed analysis, the frequency of 0.91 THz was chosen as the frequency with a good signal-to-noise ratio. The typical spectrum and corresponding refractive index are shown in Fig. 2 for the sample of HyproSorb. Fig. 3 shows the time course of the whole ATR measurement as the time dependence of refractive index. The reactions of the hemostat with PSS and blood can be compared. Also shown are the time dependences for the PSS and blood without application of hemostat to verify the influence of other processes on the crystal. As PSS had practically constant refractive index during the whole measurement, the refractive index for blood was slowly decreasing. Therefore, the curve of PSS without hemostat was not further processed.

There were extracted and further processed only the sequences from the point when the hemostatic sample was applied and fixed, or when only blood was applied on the crystal for the measurement without hemostat. These signals were filtered by moving average (window length 30) to reduce random noise. As we are focused on the description of a dynamic system, the exact value of refractive index is not as important as its change corresponding with kinetics of the hemostatic reaction with liquid medium. Consequently, the measured signals were normalized by scaling between 0 and 1. The normalized value (normalized $n(t)$) for refractive index n in the time t was calculated using

$$\text{normalized } n(t) = \frac{n(t) - n_{\min}}{n_{\max} - n_{\min}} \tag{1}$$

where the values $n_{\min}$ and $n_{\max}$ are the minimum and maximum of the variable n, respectively. The normalized signals of all hemostats are shown in Fig. 4 for reaction with PSS and Fig. 5 for reaction with blood.

As we can see, a refractive index of all the materials was changing during the measurements; an exponential decreasing trend is obvious at whole the data set. Consequently, the mathematical model was derived from the decreasing form of exponential decay. The mathematical approximation model $n(t)$ of the normalized data in time t has simple exponential form given by

$$\text{model } n(t) = \exp^{-t/\tau} \tag{2}$$

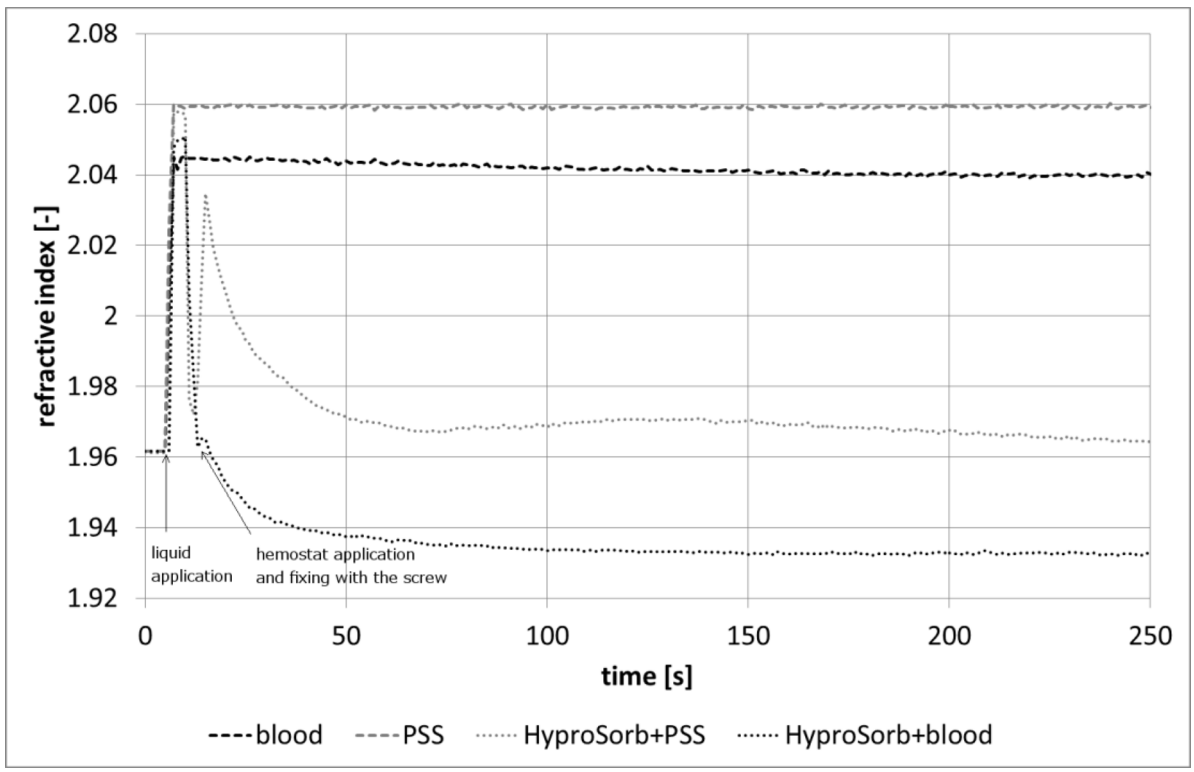

Fig. 3. Time dependencies of the refractive indices for the PSS and blood without and with application of hemostat (HyproSorb).

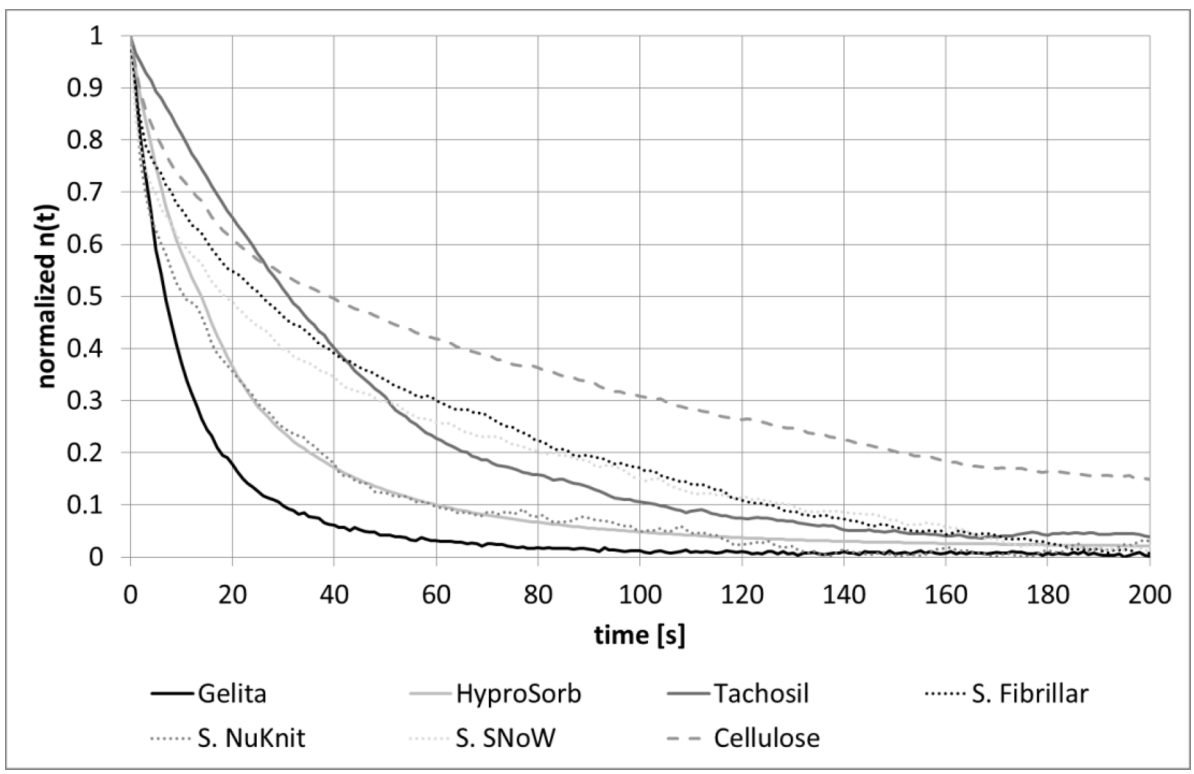

Fig. 4. Time dependence of the normalized refractive index monitoring the reactions of the hemostat with PSS.

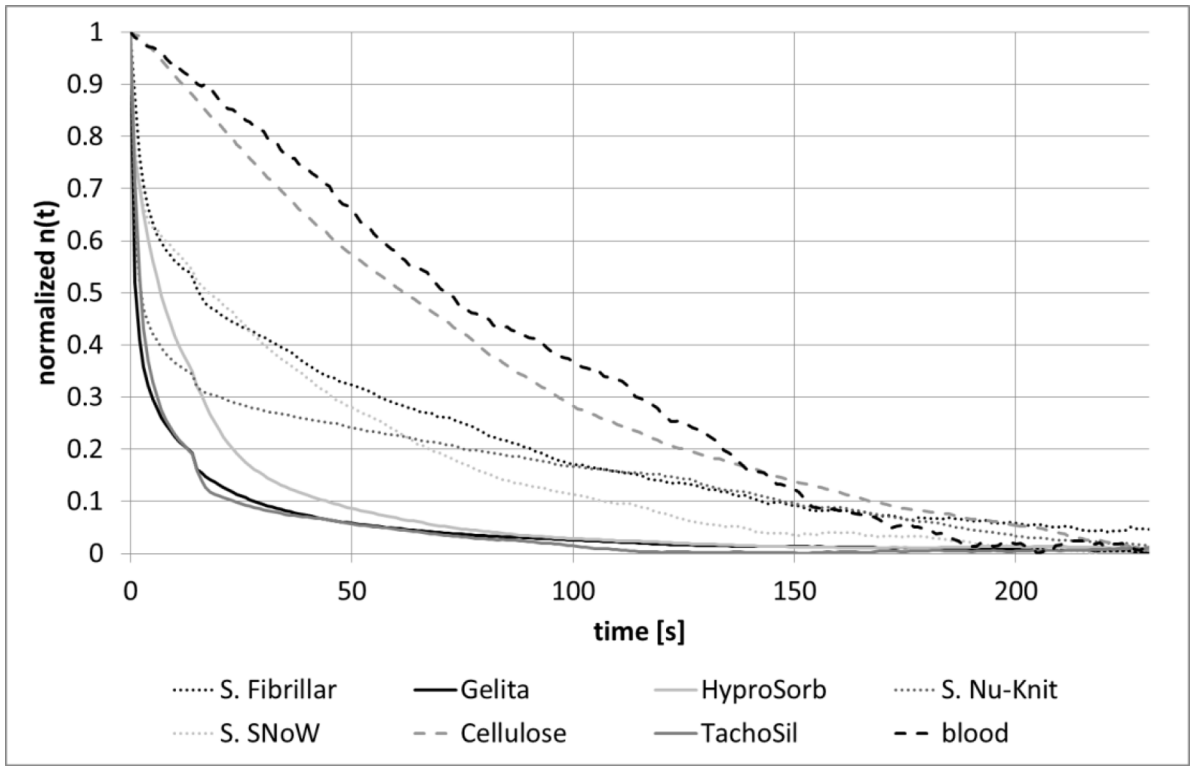

Fig. 5. Time dependence of the normalized refractive index monitoring the reactions of the hemostat with blood.

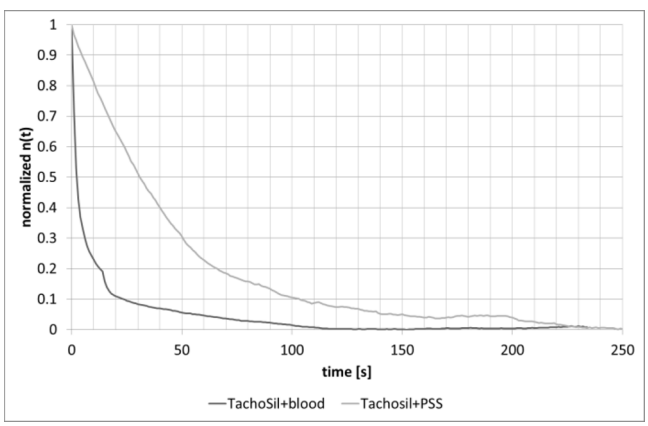

Fig. 6. The comparison between the reactions of the Tachosil hemostat with the PSS (light line) and blood (dark line).

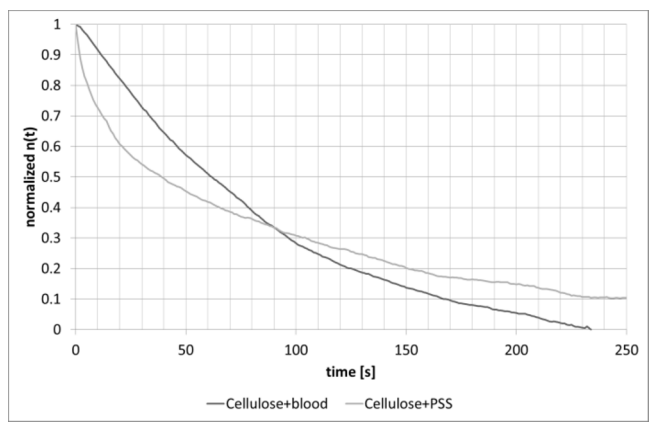

Fig. 7. Comparison between the reactions of the cellulose with the PSS (light line) and blood (dark line).

where τ is a time constant of the system.

After the mathematical model formulation, a goal is to adjust the parameter τ of a model function to the best fit with the data set. A generalized reduced gradient method was used to find the optimal solution. An optimization criterion for estimated parameters' selection was based on the least squares method. Data set consisted of m data pairs sampled in time t $(\text{model } n_i, \text{normalized } n_i)$, i $= 1$ up to m, where the normalized n_i is the independent variable and model n_i is a variable dependent on the input parameters. When the sum of squared residuals is minimal, the optimal value of τ is found. A residual is defined as a difference between the actual normalized n_i and model n_i values predicted by the mathematical model.

For the reaction with blood, the situation seems to be more complex as the significant changes in the kinetics of hemostats are obvious. This change is evident e. g. in the sample of the hemostatic TachoSil, see Fig. 6. Conversely, practically no significant variations are present in the reference sample of cellulose without any known active hemostatic effect, see Fig. 7.

IV. Discussion

The presented method allows a monitoring of the hemostatic reactions with liquid media. The specifications given by a manufacturer describe the principle of material hemostatic action and total time of achieving hemostasis based on the *in vivo* studies. However, there is no detailed information about the hemostatic kinetics to validate this physical–chemical process. Usage of the

TABLE I
TIME CONSTANTS OF REACTIONS FOR HEMOSTATS WITH PSS (τ_{PSS})

Hemostatic type	τ_{PSS} [s]
Cellulose	80 ± 14
TachoSil®	45 ± 9
S. Fibrillar®	44 ± 4
S. SNoW™	37 ±15
S. Nu-Knit™	28 ± 18
HyproSorb®	24 ± 11
Gelita®	12 ± 3

appropriate hemostatic depends on the experience of the surgeon and an orientation in an amount of hemostats could be problematic. Therefore, we focused on the description of hemostatic kinetics in an objective way, based on the physical parameter measurement. THz time-domain spectrometer was used for ATR measurement of refractive index changes that correspond to the reactions of hemostat with used liquid media.

For the measurement of absorption properties of hemostats, the PSS was added. As we can see in Fig. 4, the process had the exponential course for all of the samples. Therefore, the measured data were approximated with the decreasing exponential function. The sum of squared residuals was considered as a criterion of optimality and it does not exceed the value 0.9 for any hemostatic. A slope of the individual curves is given by their time constants τ, see Table I. All the processes were approximated by the exponential decay for a simple first-order system, thus the time constant is defined as a time it takes for the $\mathrm{normalized} n(t)$ to reach 0.37 of its maximal value [28]. The time constant characterizes the rapidity of studied reaction. The hemostats based on the natural collagen show the shortest time constant (Gelita, HyproSorb). Then, the group of the cellulose hemostats (S. NuKnit, S. SNoW, S. Fibrillar) with the slower reaction rate follows. The curves reflecting the reactions of cellulose hemostats S. SNoW and S. Fibrillar are almost identical and this correlates with their similar time constants. The hemostat with fibrin sealant (TachoSil) seems to have a special position to others because of a different time course. The sample of the cellulose pad was used as a reference sample with no provable active hemostatic effect for comparison with hemostats. It is obvious that the reaction is the slowest one, and the time constant is almost double compared with the time constant of the slowest hemostat. We assume that the reference cellulose sample only absorbs the liquid and no further active mechanisms occur. These mechanisms should be the main cause of the reaction rate acceleration.

In the second measurement, the PSS was replaced by human blood. The results are shown in Fig. 5. Comparing them with the previous measurements, the differences are obvious. All the hemostats register the faster reaction with blood than with the PSS. The hemostats based on the biological active agent (TachoSil, Gelita, HyproSorb) show the most significant changes. Fig. 6 compares reactions of the TachoSil with PSS and blood. If the same simple mathematical model for a first-order system was used for the description of the reaction with blood, the time constant of the reaction of TachoSil with blood would be several times (up to eight times by estimation from Fig. 6) shorter than for reaction with PSS. Considering the group of cellulose hemostats, the reaction with both media is more moderate compared to the biological agents. Contrary to the progressive initial decline, the estimated time constants do not differ so much from those of the reaction with PSS. As it is obvious, a simple first-order mathematical model will not be sufficient for the data approximation bringing good results. The reaction seems to be more complex, therefore the mathematical approximation is not to be as simple as before. We suppose this relates to the blood composition in comparison to the PSS. The hemostatic reaction with blood will be further analyzed in following studies. In contrast with hemostats, the reactions of reference cellulose sample with both media stay almost unchanged, see Fig. 7. The cellulose has the same behavior whether blood or PSS is used. Its time constant for both reactions seems to be also the same. These results testify that all types of measured hemostats really react with the tested media in a specific way which reflects their hemostatic effect unlike the reference cellulose.

To summarize the results, the hemostats based on collagen (Gelita, HyproSorb) show the fastest reactions both with PSS and blood. Therefore, they should be the best for a rapid control of hemorrhage. However, TachoSil registers a significant change in reaction with blood causing the shortening of the time constant and the time course of the reaction is comparable with those of previous mentioned collagen hemostats. Probably, this relates to the fibrin sealant layer that is coated on the collagen sponge. As it was described in Introduction, fibrin is biologically active agent that participates in the hemocoagulation cascade directly. Therefore, the hemostatic effect of absorbable collagen matrix is multiplied by this fibrin layer.

V. CONCLUSION

The presented study shows a capability of THz time-domain spectroscopy to study kinetics of absorbable hemostats. The differences between the reactions of the hemostatic samples with two physiological media were described. The hemostatic reaction with blood seems to be more complex compared to the physiologic saline solution. Especially the nature of the hemostatic action of each type of hemostat with blood will be a subject of further studies. Using other methods (e.g., spectroscopic or microscopic) and comparing the experimental results, we could get more information to find out some other specifics for a detailed description of hemostatic mechanism.

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