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Determination of the water content of an ex vivo porcine liver

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Abstract: When modeling High-Frequency (HF) surgical applications the choice of the right dielectric tissue parameters is a key factor for success. The water content of biological tissue is of particular importance since the dielectric tissue properties highly depend on it. Therefore, we investigate a simple and fast method for the determination of relative tissue water content using small samples of an ex vivo porcine liver.

To identify the water content, we utilized a desiccation and weighing experiment. It is assumed that the weight loss can be directly attributed to the loss of water in the case of desiccation. Based on the ratio between the initial and dry weight of a sample, the relative water content can be determined. The drying procedure lasted 28 hours, with 16 small samples taken from an ex vivo porcine liver.

At the end of the drying procedure, a relative tissue water content of $69.6\ \% \pm 1.1\ \%$ was observed. Using a curve fit to the measured data, it can be assumed that approximately 99.3 % of the tissue water content had evaporated by the end of the drying process.

With the drying and weighing method used, we were able to determine the water content of the ex vivo porcine liver relatively easily. However, our results also suggest that optimizations of the applied method should be considered for future measurements. Especially, if a faster determination of the water content is required, which in a broader sense should afterward serve for the validation of a simulation model.

Keywords: tissue water content, porcine liver, desiccation

1 Introduction

High-Frequency (HF) alternating current (AC) is commonly used in a variety of different surgical procedures to achieve hemostasis. Here, hemostasis is achieved by Joule heating. In this process, the tissue is selectively heated at the cellular level using the HF current, causing tissue water to evaporate and the tissue to shrink. However, despite the known advantages and decades of use of HF medical devices, evidence of their effectiveness and safety for regulatory purposes is still lacking, even for products already on the market.

Therefore, computational modeling and simulation are becoming increasingly important not only for development purposes but also for medical approvals [1]. This is because it can provide evidence to support medical approval processes in terms of product safety and functionality. This can save money and time. Nevertheless, computational modeling and simulation can only be helpful if they adequately represent reality. To achieve this, it is essential to understand the process to be modeled in question and be able to describe it in sufficient detail. In the end, a validated simulation model is required if a medical approval process has to be supported. This means that whenever an experiment of an HF application has to be performed to validate a corresponding simulation model, it is important to know as many boundary conditions and parameters of the setup as possible in order to consider them in the simulation accordingly.

Consequently, accurate knowledge of the different tissue parameters is required when modeling and simulating tissue heating induced by HF currents. As shown in Busch et al. [2] even small changes in a dielectric tissue parameter can lead to significant changes in the simulation. However, the tissue water content is also an important factor to be considered in modeling HF-induced heating since the properties highly depend on it [3]. This is because tissue water is a central prerequisite for the flow of current and consequently for the heating process in the tissue. Therefore, the knowledge of the water content in the tissue to be used for tests to validate a simulation model is essential.

This importance has also been identified by other research groups. Yang et al. [4] reported a measured water content of 73 % for ex vivo bovine liver tissue determined by a wet-dry method and also measured the water content of ablated tissue, among others. Others, such as Chen et al. [5], Karaki et al. [6], and Yang et al. [7] have considered temperature-dependent water content in different modeling approaches but have not measured it themselves. In those cases, an initial water content of 70 % was used.

Nevertheless, in this contribution, we present a simple and relatively fast desiccation and weighting method for measuring the tissue water content of an ex vivo porcine liver tissue. This experiment is anticipated to be performed with only small

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pieces of tissue. This will allow the remaining ex vivo tissue to be used for further testing to validate a modeling approach of an HF application. In addition, existing limitations and optimizations of the used method shall be identified. If it proves to be practical, the water content of other tissue samples that shall be used for future model validation can be determined similarly and considered adequately in a simulation model.

2 Materials and methods

A desiccation experiment was performed using an ex vivo porcine liver to determine the intracellular and extracellular water bound in the liver tissue. It was assumed that the intracellular and extracellular water can be removed from the tissue by slow and gentle air drying using a slightly elevated temperature. Measuring the weight loss of tissue over time then gives a direct measurement of the loss of tissue water. The percentage weight loss of a tissue sample at a time t_m can be determined by relating its weight at t_m to its initial weight at t_0. Therefore the percentage loss of tissue water at time point t_m of the n-th tissue sample can be expressed by

$$W_{\mathrm{n,loss}}(t_\mathrm{m}) = \left(1 - \frac{w(t_\mathrm{m})}{w(t_0)}\right) \cdot 100\%, \tag{1}$$

where $w(t_0)$ and $w(t_\mathrm{m})$ are the initial weight of the tissue and the tissue weight measured at t_m respectively.

Since it is unknown when the tissue samples can be considered as dry, a criterion was defined for the end of desiccation. For each tissue sample, the relative percentage difference between measured weight at t_m and $t_{\mathrm{m}-1}$ was calculated by $\Delta W_\mathrm{n}(t_\mathrm{m}) = W_{\mathrm{n,loss}}(t_\mathrm{m}) - W_{\mathrm{n,loss}}(t_{\mathrm{m}-1})$. Which becomes reformulated to

$$\Delta W_\mathrm{n}(t_\mathrm{m}) = \left(\frac{w(t_{\mathrm{m}-1}) - w(t_\mathrm{m})}{w(t_0)}\right) \cdot 100\%. \tag{2}$$

The arithmetic mean $\overline{\Delta W}(t_\mathrm{m})$ of (2) for all tissue samples was then used to determine a dried tissue condition and as the criterion to stop the drying process. We defined the threshold for dry tissue as $\overline{\Delta W}(t_\mathrm{m}) \leq 0.2\ \%$. A subsequent curve fit to the measured data should provide information about the remaining water content in the samples. This is because the water loss is assumed to take an exponential progression. This, in turn, means that a very long drying time is required for a completely dry sample.

A freshly slaughtered porcine liver was obtained from a local butcher shop and 16 small liver samples were collected from it. The samples were cut into different sizes with the largest possible surface area (for fast drying). The size of the samples varied from about $5 \times 5 \times 10$ mm to about $30 \times 40 \times 2.5$ mm. When preparing the tissue samples, care was taken to ensure that they were not crossed by visible vessels. Thus the water content of the liver tissue can be determined without being influenced by other types of tissue. The samples were initially weighed using the XA503S precision balance from Mettler-Toledo GmbH. Subsequently, the samples were placed in a heating oven for drying. The UNE 200 universal oven from Memmert GmbH & Co. KG was used.

For gentle tissue drying, a temperature of 55 °C was selected for the heating oven. Every two hours, the 16 samples were individually removed from the heating oven and weighed using the XA503S precision balance. Weighing of the tissue samples was performed as quickly as possible to avoid interruption of the drying process. Based on the weight measurements of the samples, the relative weight loss in percent was determined by (1). Because the criterion of dried tissue samples was not reached after ten hours of drying, the samples were stored overnight (for 14 hours) at room temperature (21 °C). The next day, drying was continued at 55 °C for additional four hours until the criterion of $\overline{\Delta W}(t_\mathrm{m}) \leq 0.2\ \%$ was reached.

3 Results

In Figure 1, the mean value of the percentage weight loss of the 16 tissue samples during the drying time of 28 hours is shown. Also shown are the three phases of the drying process. The first phase lasts 10 hours, during which tissue drying was performed at 55 °C in the heating oven. The second phase lasted 14 hours, with samples stored at 21 °C. In the third phase, the tissue was dried again at 55 °C.

The measured weight loss of the samples was equated to the loss of tissue water. The measurements indicate that after the first phase, the tissue samples have already lost an average of 68 % of their total weight. During the second phase, the weight decreased on average only slightly by a further 0.9 %. The last four hours, the third phase, resulted in a further 0.7 % weight loss, so the samples lost an average of 69.6 % of their weight at the end of the 28 hours. The mean and standard deviation of the measured relative water loss of the tissue samples are also given in Table 1. Furthermore, the relative mean change in water loss (with min. and max. values) is listed in Table 1, which was used as a criterion for identifying dry samples.

The normalized water loss of the total 16 samples within the first 10 hours of drying (phase 1) is shown in Figure 2. There, the dry tissue weight of each sample was subtracted from the measured sample weight and then normalized to account for the different sample sizes. In addition, an exponential curve fit was performed based on these data, plot-

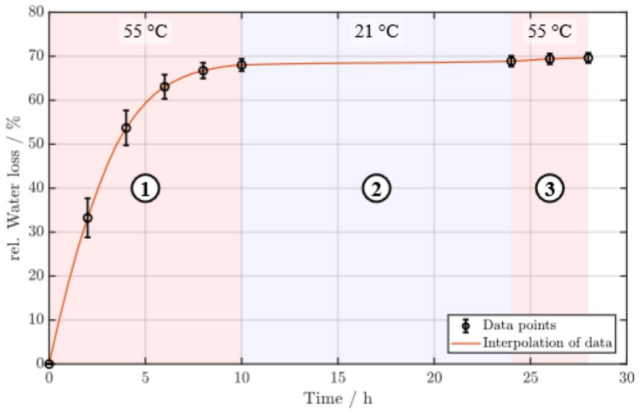

Fig. 1: Relative water loss of a porcine liver during a drying time of 28 hours with the three phases of the drying process. Here, phases 1 and 3 represent drying at 55 °C. Phase 2 shows the time the samples were stored overnight at room temperature. The black circular data points, including error bars, show the mean values of relative water loss of the 16 tissue samples and their standard deviation. A shape-preserving piecewise cubic interpolation of the mean value of relative tissue water loss is plotted in red.

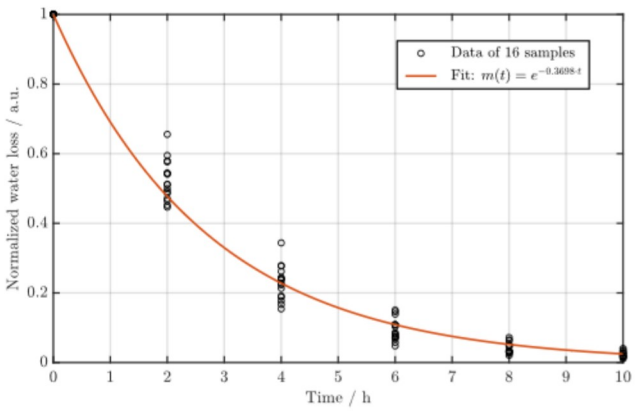

Fig. 2: Normalized water loss during the first 10 hours of drying. The normalized water loss of the samples is plotted in the black circular data points. Whereby the data only shows the normalized water amount of the samples without the pure dry tissue weight. An exponential curve fit of the data is shown in the red line.

ted in the red line. The curve fit thereby led to the function $m(t) = e^{-0.3698 \cdot t}$, which describes the exponential water loss behavior of our used samples during drying at 55 °C.

Tab. 1: Relative water loss of liver samples during drying

Time t_m	Rel. mean water loss $\overline{W_{loss}}$	Rel. mean change $\overline{\Delta W}$
0 h	0 %	–
2 h	33.3 ± 4.5 %	33.3 % (23.6; 39.3)
4 h	53.7 ± 4.0 %	20.5 % (16.4; 23.9)
6 h	63.1 ± 2.7 %	9.4 % (6.8; 13.2)
8 h	66.7 ± 1.8 %	3.7 % (1.9; 6.0)
10 h	68.0 ± 1.4 %	1.3 % (0.7; 2.2)
24 h	68.9 ± 1.2 %	0.9 % (0.3; 1.8)
26 h	69.4 ± 1.2 %	0.5 % (0.3; 0.8)
28 h	69.6 ± 1.1 %	0.2 % (0.1; 0.4)

4 Discussion

The results presented in this contribution show that the tissue water content of our used porcine liver tissue can be estimated with a relatively simple experiment. It was assumed that tissue water could be determined by desiccating small biological tissue samples in a heating oven and measuring the resulting change in weight. The loss in weight is a direct measure of the loss in tissue water. Therefore, the relative water content of our porcine liver tissue could be determined.

For this experiment, 16 small samples were intentionally prepared with different sizes/weights. The reason for this was that the water content, and thus the relative weight loss due to evaporation of the tissue water, has to be almost the same between samples, even if their size and weight vary. Nevertheless, a slight variation in the drying process is expected since the specimens had a different surface area for the evaporation of tissue water. It is assumed that the larger standard deviation on the relative mean water loss in the first four desiccation hours (see Table 1) is due to the different surface areas of the prepared tissue samples. To investigate this assumption, more comprehensive tests and analyses must be carried out. There, the focus then has to be on the drying rate of different sample sizes with a larger sample quantity.

Furthermore, it is assumed that the varying thickness of the samples also influences the desiccation process. For thicker ones, it could take longer for the water to evaporate from the center because it needs to diffuse through a thicker dry outer tissue layer. This assumption is hardly reflected in the data. Therefore, to show the significance of the sample thickness on the desiccation processes, further investigations with a larger sample quantity are required.

The results in Figure 1 indicate that the weight loss due to a desiccation process carried out by heat leads to exponential weight loss and, consequently, water loss. Nevertheless, a break in the data curve through the overnight storing phase (phase 2 in Figure 1) can be identified. The second phase illustrates that even when the tissue samples have already lost 68.0 ±1.4 % of their weight, storing at room temperature leads

to further desiccation. This is, of course, due to the fact that the tissue water had not yet completely evaporated at this point. Furthermore, the humidity in the storage room also influenced the evaporation process during storage. However, since the humidity was unknown, it is difficult to make any conclusions other than that the tissue lost an additional 0.9 % weight during these 14 hours.

After the last four hours of desiccation (third phase), a further weight loss of 1.6 % was seen. Even though the established criterion for dry tissue was reached in the last two drying hours, the curve progression suggests that there is still a small amount of water. The min. and max. values of the relative water change given in Table 1 column $\overline{\Delta W}$ show that the samples can sometimes differ considerably. This implies that the dry tissue criterion must be applied individually to each specimen, causing them to take different lengths of time to dry, or that the size of all tissue samples should be uniform for future measurements.

The 14-hour storage of the tissue samples (second phase) leads to an interruption of the drying process that was continuous until then. This caused a deviation in the recorded weight loss curve, as described earlier and also evident from the data plotted in Figure 1. For this reason, a curve fit describing the normalized water loss of our samples during air drying at 55 $^{\circ}$C was performed using only the data from the first 10 hours. The measured data and the fitted curve shown in Figure 2 indicate that the water loss of the samples is exponential. By using this function to identify the water loss during continuous 55 $^{\circ}$C drying, it can be derived that after 12 hours and 30 minutes, 99 % of the tissue water has evaporated. If drying continues, 99.9 % of the water is expected to be evaporated after a total of 18 hours and 42 minutes. Since our tissue samples were exposed to a temperature of 55 $^{\circ}$C for a total of 14 hours and neglecting the water loss during the storage phase, at least 99.4 % of the tissue water should have evaporated.

Comparing our results with the values used in other publications ([4–7]), they are in good agreement. Thus, a water content of about 70 % could also be determined in our tissue samples.

5 Conclusion

In this contribution, we investigated a simple and relatively fast method to determine the water content of an ex vivo porcine liver. 16 small samples of the liver were dried and the weight change was measured. Our measurements showed that about 70 % of the weight of our tissue samples was related to tissue water. Nevertheless, analysis of our results showed that improvements for even faster identification of the tissue water should be considered for further measurements. These improvements include increasing the temperature for drying, uniform and thin thickness of the tissue samples, and not interrupting the desiccation process by storing overnight to obtain continuously acquired data.

It should be noted that we used only one porcine liver for our experiment and were not interested in conducting a study to determine the water content of porcine liver tissue in general. For our purpose, we need a quick and easy method to measure water content using only small tissue samples. The remaining tissue will then be used for further HF application testing needed to validate a simulation model. Tissue water content is therefore only a small subset of our model, but even this needs to be adequately adjusted to the conditions and tissue parameters prevailing in the real test setup.

In conclusion, we were able to measure the tissue water content of one porcine liver tissue, but still need to improve the method for an even faster determination.

Author Statement

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