PAPER

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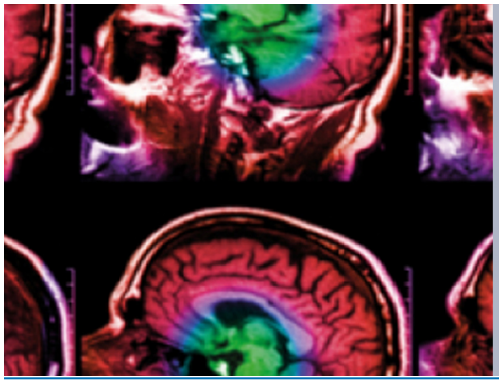

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PAPER

Gelatin: a skin phantom for bioimpedance spectroscopy

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Abstract

Phantoms mimic the properties of tissues, like skin. The electrical response of a skin phantom should be similar to the electrical response of an *in vivo* skin tissue. In this work the electrical characterization of gelatin based skin phantoms and of different skin sites are presented. The bioimpedance measurements of the gelatin based phantoms are compared with *in vivo* skin measurements, demonstrating that gelatin has potential as the basis material for the fabrication of low-cost skin phantoms for bioimpedance spectroscopy.

1. Introduction

The electrical properties of a material are a measure of its ability to interact with electromagnetic energy. This interaction is a consequence of the presence of constituents on the material, which can be affected by the electric and magnetic forces generated by electromagnetic fields. As so, a material's electrical properties are a direct consequence of its composition and structure [1]. In the analysis of a tissue's response to electric stimulation, the microscopic description depends on the variety of cell shapes and their distribution inside the tissue as well as the different properties of the extracellular media. While the macroscopic response depends on the tissue orientation relative to the applied field (directional anisotropy), the frequency of the applied field (the tissue is neither a perfect dielectric nor a perfect conductor), and time- and space-dependency (e.g., changes in tissue conductivity during electro-permeabilization) [2].

The human tissues are considered to be dielectric materials, meaning they can be polarized by an applied electric field. Even more, each tissue is thought to be composed by various dielectric components, their constitution being responsible for their electric properties [3]. The skin is the largest tissue of the human body. It is most important functions are to form a physical barrier to the exterior environment, to allow and limit the inward and outward passage of water, electrolytes and various substances while providing protection against micro-organisms, ultraviolet radiation, toxic agents and mechanical stresses [4]. The epidermis is the layer of skin in direct contact with the environment, acting as the physical and chemical barrier between the body and external environment. The epidermis outermost structure is the stratum corneum, composed of dead epidermal cells, presenting important dielectric characteristics. The rest of the epidermis structure characteristics cannot be separated dielectrically. The dielectric properties of this structure are determined by the membranes of the epidermal cells as well as intra- and extracellular content. The dermis is the deeper layer providing structural support to the skin. Its dielectric properties depend mostly on the extracellular liquid [3]. Below the dermis there is a loose connective tissue layer, the hypodermis, which is a depot of fat. The skin is a dynamic organ that, although structurally consistent throughout the all body, varies in thickness according to the anatomical site on the individual, among other factors [5].

Phantoms are objects that mimic or imitate human tissues, like skin. Phantoms are used to evaluate, analyze and tune the performance of medical devices. A phantom is more readily available than living tissues and provides consistent results. Phantoms can be found in the commercial market, however due to the high demand and application related specificity, finding less expensive solutions would be of interest. The development of low cost phantoms has been extensively studied in fields such as optical spectroscopy, imaging and dosimetry [6]. A phantom should respond in a similar manner as do human tissues for a

specific device or application. A potential candidate for this task is gelatin, which has been used in several applications such as ultrasound imaging [7], ultra wide band microwave applications [8], optical spectroscopy and dosimetry [6], electrography [9], and even in the study of ballistic behavior [10]. Gelatin is an irreversibly hydrolyzed form of collagen obtained from various animal by-products. It is formed by natural macromolecules and present characteristics such as biodegradability and low antigenicity, acting as a good basis for biomaterials in biomedical, pharmaceutical and cosmetic applications [11]. Gelatin is easy available, nontoxic, low cost, and allows easy modeling of the phantom's shape.

Even though gelatin has been used as a phantom in several application fields, there are not many studies on the dielectric properties of gelatin, and there are even less studies on using gelatin as a phantom for electrical analysis. The properties of the conductivity and permittivity of different gelatins were studied at 10, 27, 50 MHz [12]. The characterization of the permittivity, dielectric loss and dielectric module of gelatin in the frequency range of 10 mHz and 1 MHz was also performed [13]. The resistivity of a 25% concentrated gelatin was also measured in frequencies in the range of 10 kHz and 50 MHz [14]. And recently, a preliminary study on gelatin's electrical characteristics and its similarity to skin was presented [15].

In this manuscript, the development of a methodology to design new phantoms for electrical analysis is presented. Gelatin is evaluated as a skin phantom for bioimpedance spectroscopy (BIS). The electrical characteristics of different gelatin based phantoms are presented. *In vivo* electrical measurements performed on different skin sites of the author are also provided. The comparison of the *in vivo* skin spectra and the phantom's characteristics advocates that gelatin is able to emulate the impedance spectrum of skin and, as so, can be used as the basis material for low-cost skin phantoms.

2. Experimental method

In order to test gelatin as a skin phantom for BIS, different gelatins were prepared. Additionally, different skin sites were prepared for BIS measurements, aiming to compare gelatin-skin spectra. Finally, BIS measurements were taken in both gelatins and skin sites. The procedures for each part are described below.

2.1. Gelatin preparation for BIS measurement

Different gelatins were prepared. The gelatin preparation was performed using the powder of edible gelatin (*Gelco Gelatinas do Brasil-GGB*). The gelatin's physical and chemical characteristics are presented in table 1.

The gelatins preparation consisted on dissolving the powder in 100 ml of deionized water at 80 °C. The gelatin powder was dissolved on the water at controlled temperature under constant stirring in a beaker. All gelatin based phantoms were prepared at the same time to insure the same conditions on their preparation. Six phantoms were prepared using different combinations of gelatin and salt. Table 2 presents the composition of each phantom.

Table 1. Gelatin's physical and chemical characteristics.

Parameters	Results
Bloom[a]	228 g/Bl
Viscosity	36, 7 mP
Humidity	9, 05%
pH	5, 45
Color	Light yellow

[a] Bloom—strength of the gel.

In order to increase the phantoms melting temperature and, consequently, their stability over time, formaldehyde (37% Quimidrol) was added to each gelatin liquid solution. Formaldehyde is an organic compound that increases the cross-linking of the gelatin matrix fibers and allows it to be used at room temperature without the need for refrigeration [6]. After all components were mixed together and all liquid solutions were fully prepared, the phantoms were left to solidify at room temperature. After their solidification, all phantoms were placed in the refrigerator. Twenty four hours prior to the BIS measurements, all phantoms were removed from the refrigerator and left at room temperature, insuring this way that the measurements were carried out at room temperature.

2.2. Skin preparation for BIS measurement

To prepare the skin for the BIS measurements, all the skin sites to be assessed were stripped of its stratum corneum with a cellulose-tape. After stripping, the skin was cleaned with ethanol. A saline solution (0, 9%) was moistened in the skin site in order to improve the contact between probe and tissue.

2.3. BIS measurements

The BIS measurements were taken using a tetra polar probe coupled to an impedance analyzer (Agilent, model 4294S). The tetra-polar probe BIATRON I was built in the Electronic and Biomedical Engineering Laboratory at Santa Catarina State University in Brazil [16]. Figure 1 illustrates the set up used to take the measurements. The Agilent analyzer applies auto-balancing bridge technique, allowing high precision measurements on a high range of frequencies. The measurements were recorded in the frequency range of 100 kHz–10 MHz, using 500 frequency points.

The BIS measurements were first made putting the BIATRON I probe in contact with each gelatin (figure 1(a)) and subsequently applying the same set up to skin sites (figure 1(b)), insuring no pressure was

Table 2. Composition of the phantoms prepared.

	Gelatin		Salt	
Name	Weight (g)	Concentration	Weight	Concentration
Phantom 1	10	10%	—	—
Phantom 2	10	10%	0.2	0.2%
Phantom 3	10	10%	0.5	0.5%
Phantom 4	10	10%	1	1%
Phantom 5	20	20%	—	—
Phantom 6	30	30%	—	—

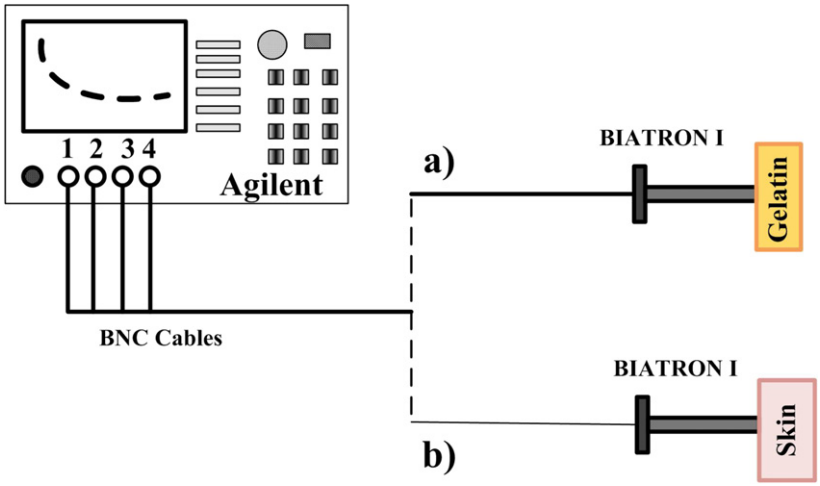

Figure 1. Schematic diagram of the set up used for measurements: (a) of gelatin, (b) of *in vivo* skin. Legend: 1, 2, 3 and 4 are the BNC terminals for current injection and potential retrieval.

applied. The BIS *in vivo* measurements were made on the author's skin on different body sites: wrist, pectoral, heel and inflammation site on the foot (from now on called allergy spot). For each gelatin and skin site, ten consecutive measurements were realized. The results are the arithmetic mean values to each gelatin and skin site. The measurements were repeated in three different moments, along three months, using gelatin samples prepared at different moments.

3. Results and discussion

In this section, the phantom's measurements will be presented and discussed. Also, the measurements performed on skin sites will be shown and debated. Finally, the feasibility of gelatin as a material for skin phantom's development will be featured.

3.1. Phantoms measurements

The measurements of the electrical impedance modulus for the different phantoms with no salt on their composition are presented in figure 2. The phantoms with higher concentration of gelatin present higher values of impedance modulus for the same frequencies. All through the measurement frequency range phantom 6 (30% gelatin) has higher impedance modulus than phantom 5 (20% gelatin) and phantom 1 (10% gelatin). In the same way, phantom 5 has higher impedance modulus than phantom 1 in all the frequency range.

The tendency observed in figure 2 for phantoms with different concentrations of gelatin but no salt is quite different from the variation of the impedance modulus observed in phantoms with different salt concentrations. Figure 3 presents the results obtained from measurements done in phantoms with 10% gelatin concentration and different concentration of salt.

The addition of salt to gelatins induces profound changes in the impedance modulus profile, as can be seen in figure 3. This may be due to the fact that gelatin gels have both cationic and anionic groups. Gelatin is formed by the cross-linking of polymer chains forming an interconnected three-dimensional network arranged into triple helices by intermolecular hydrogen bonds. The gelatin's chain has both positive and negative charged sites, which makes the electrostatic interactions of great importance in the gel stabilization process. The presence of salt in the gelatin mix shields the electrostatic interactions between the charged sites. This happens in such a manner that as the salt concentration rises the extent of shielding also increases, leading to a reduction of the electrostatic interactions, which allows the gel's triple helix to freely reorganize in the medium [12, 17]. Additionally, adding

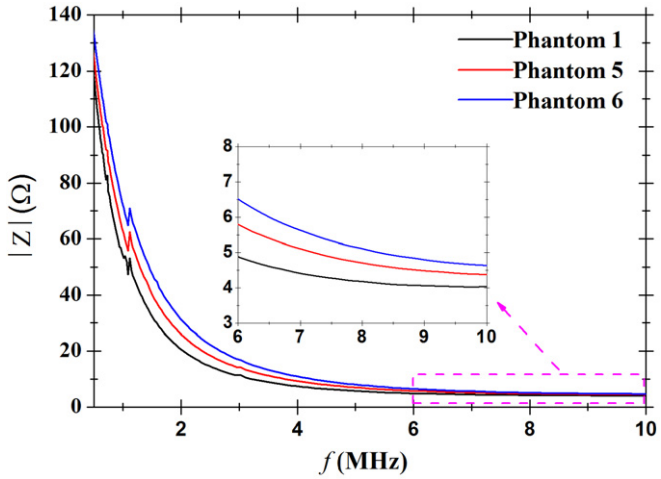

Figure 2. Impedance modulus as a function of the frequency, for phantoms with different gelatin contents and no salt.

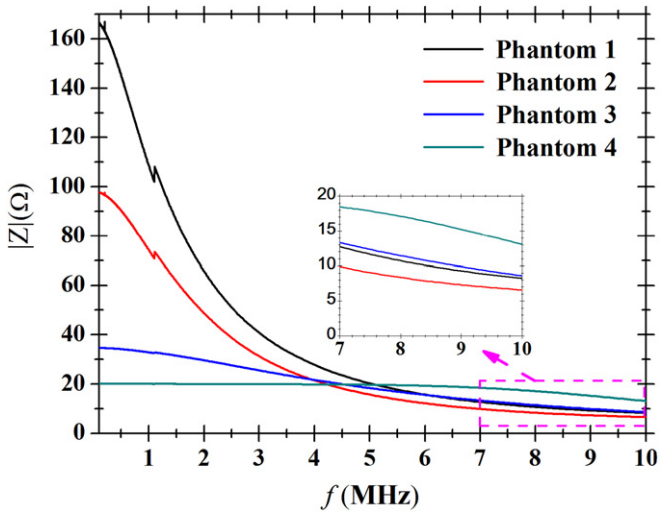

Figure 3. Impedance modulus as a function of the frequency, for phantoms with different salt content and 10% gelatin concentration.

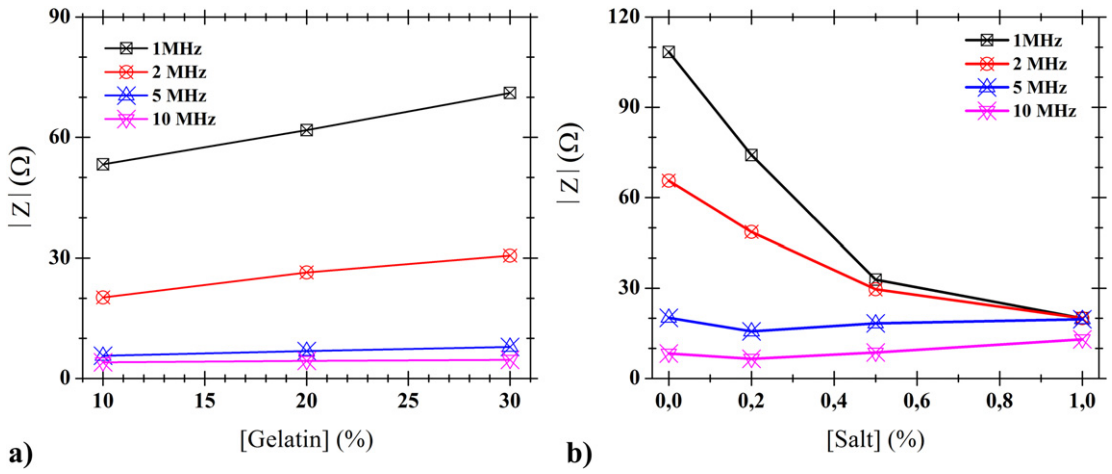

Figure 4. Impedance modulus as a function of (a) gelatin concentration and (b) salt concentration.

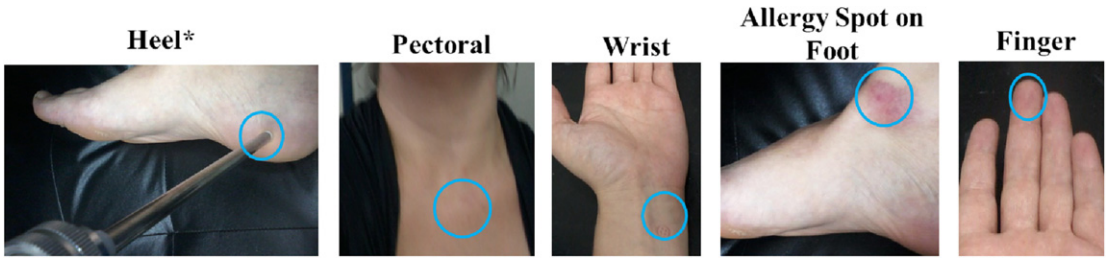

Figure 5. Photographs of the skin sites were the measurements were taken. Blue circle—spot of measurement. ∗ The silver instrument on this picture is the BIS probe.

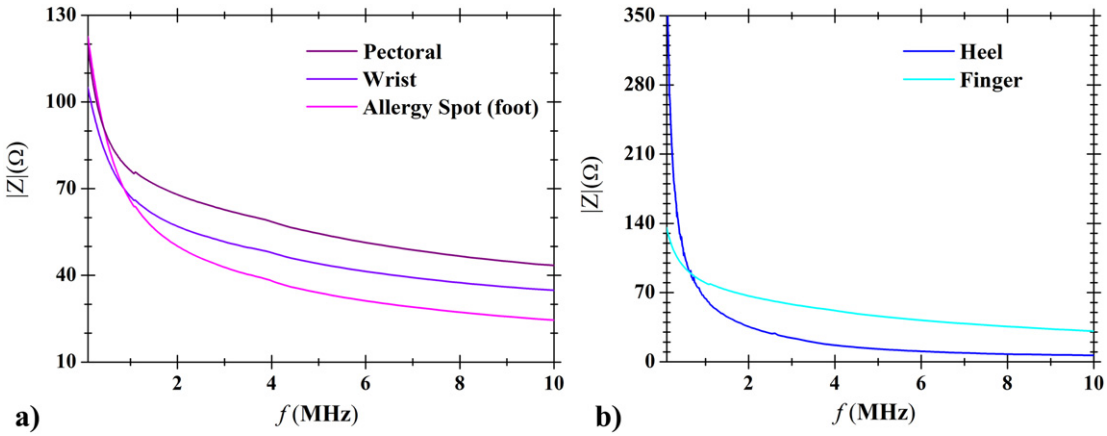

Figure 6. Impedance modulus versus frequency, for different skin sites: (a) pectoral, wrist and allergy spot, and (b) heel and finger.

salt to the gelatin leads to an ionization increase due to the formation of complex ions, which in turn entails a conductivity raise [12, 18], thus lowering the value of the impedance.

When depicting the impedance modulus as a function of gelatin and salt concentration, as shown in figure 4, it can be seen that different concentrations of this components imply different impedance modulus. When analyzing the impedance modulus variation with gelatin concentration (see figure 4(a)), it can be observed that the impedance increases with the concentration of gelatin, being that this augment is less noticeable for higher frequencies. This might be due to the fact that different frequency regions provide information about different constituents of the material. Low frequency current only allows the analysis of the biological material that is in extracellular fluids, since the reactance of the cell membrane does not allow the low frequency current to penetrate it. However, high frequency current penetrates cell membranes and passes through the cell (membranes and intracellular fluids), allowing to analyze intracellular biological material such as proteins, nucleic acids and membranes [19]. By observing figure 4(b), it can be seen that the influence of salt in the impedance modulus value is not linear, seeming to present a saturation value near 1% salt concentration. This is probably related to the polarization dependence of the dielectric properties of gelatin [20], which are highly influenced by the shielding of the electrostatic interactions in the presence of salt, as explained above.

It can be seen by the spectra shown above that the phantoms have quite different properties and characteristics. Even more, from figure 4 it is quite clear that by changing the constituents in the gelatin solution the impedance spectra can be easily tailored.

3.2. Skin measurements

In vivo BIS measurements were made in different sites of the author's skin: heel, pectoral, wrist, inflammation site on the foot side, and finger. The inflammation site in the foot side (red area) was the result of an insect bite, presenting swelling. Figure 5 shows the photographs of the skin sites were the BIS measurements were taken (indicated by the blue circle). The silver instrument shown on the heel photograph of figure 5 is the BIS probe.

Figure 6 shows the measured impedance modulus versus the frequency for different skin sites As can be seen in figure 6(a), the impedance modulus profiles of the three skin sites (pectoral, wrist and allergy spot) present different descents whereas presenting similar overall values, at low frequencies. Nevertheless, they present similar decline for high frequencies, although having different values. The heel and finger impedance modulus spectra are quite different in shape from

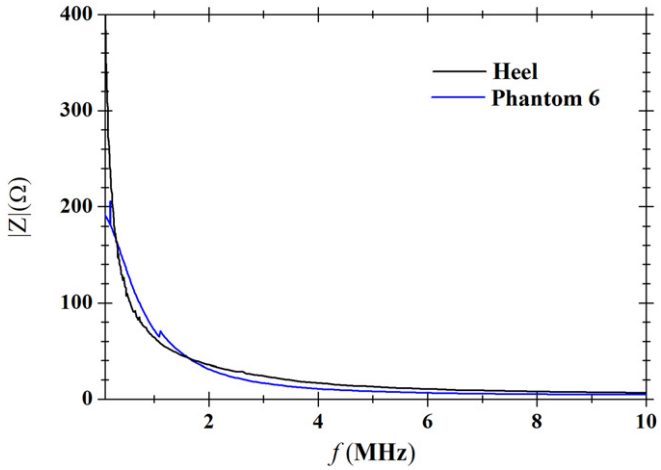

Figure 7. Impedance modulus as a function of the frequency, for phantom 6 and *in vivo* heel skin measurements.

other skin sites, as shown in figure 6(b). The heel spectrum presents high values for low frequencies followed by a high descent leading to low values at high frequencies. The finger spectrum is similar in values to other skin sites spectra like the wrist, the pectoral and the allergy spot (figure 6(a)), but its descent is slightly different leading to a different shaped spectrum.

At lower frequencies, all skin spectra present high impedance modulus. This may be due to a poor stratum corneum stripping. The stratum corneum is the exterior sublayer of the epidermis. Its thickness ranges from 8 to 15 μm. This sublayer is composed of several layers of hexagonal-shaped flat and hard cells named horny cells. These are dry dead cells without organelles and filled with keratin fibers. This sublayer prevents excessive dehydration of the skin tissue. Despite being very thin, this sublayer presents highly resistive properties at low frequencies, dominating the skin impedance spectra [2]. In the case of the heel spectra the high impedance modulus at low frequencies is even more pronounced, probably because the epidermis of the heel skin is much thicker than that of other skin sites, as well as it has a bigger layer of stratum corneum. A more thorough stripping process should be done, as the one presented in [21].

At high frequencies, the differences in spectra might be explained by the dissimilarity of skin's structure in different sites of the body. Skin has three layers: epidermis, dermis and hypodermis. Even though skin is structurally consistent all through the body, it varies in thickness according to the anatomical site, and other factors (age, gender, pigmentation, among others) [2]. All skin layers present different thickness at different body sites. The epidermis thickness varies from 0.05 mm for eyelids to 0.8–1.5 mm in soles of the feet and palms of the hands. Furthermore, the thickness also varies according to the volume of water that the epidermis holds. The dermis fluctuates in thickness from 0.6 mm on the eyelids to 3 mm on the back, palms and soles. The hypodermis, being an important depot of fat, can reach up to 3 cm thick on the abdomen [4]. Furthermore, the spectrum of the allergy spot on the skin is due to several changes triggered by the damage inflicted by the insect bite to the skin. These changes are characterized by variation of the blood flow, increase in permeability of blood vessels, and migration of fluid, proteins, and white blood cells from the circulation to the site of skin damage. These might be the cause for the decreased of the impedance modulus at higher frequencies. The slight different shape of the finger's spectrum on the other hand, might be due to the pressure applied during measurement. The pressure applied to the skin by the probe can influence the BIS output signal [21].

3.3. Gelatin as a skin phantom for BIS

To observe gelatin's feasibility as a skin phantom, the electrical impedance spectra of the different phantoms and the different skin sites were compared. To each skin site impedance spectrum the most similar gelatin's spectrum was attributed and compared.

Figure 7 presents the spectra of the impedance modulus for the heel skin and phantom 6, as a function of the frequency. From all the phantoms developed, phantom 6 (30% gelatin and 0% salt) was the one with the similar impedance spectrum. As can be seen in this figure, phantom 6 and the heel have very similar impedance spectra in the whole frequency range.

In figure 8, the impedance modulus spectra of different skin sites and phantoms are presented. For all the three skin sites (pectoral, wrist and allergy spot), phantom 3 presented the most similar shaped spectrum. For each skin site, the phantom's spectrum mean level was shifted in order to compare it directly with the spectrum of the skin site. As so, for each skin site the phantom's mean level was shifted by a different value. In figure 8(a) the phantom's mean line was shifted 36 Ω, in figure 8(b) the phantom's mean level was shifted by 26.3 Ω, and in figure 8(c) it was shifted by

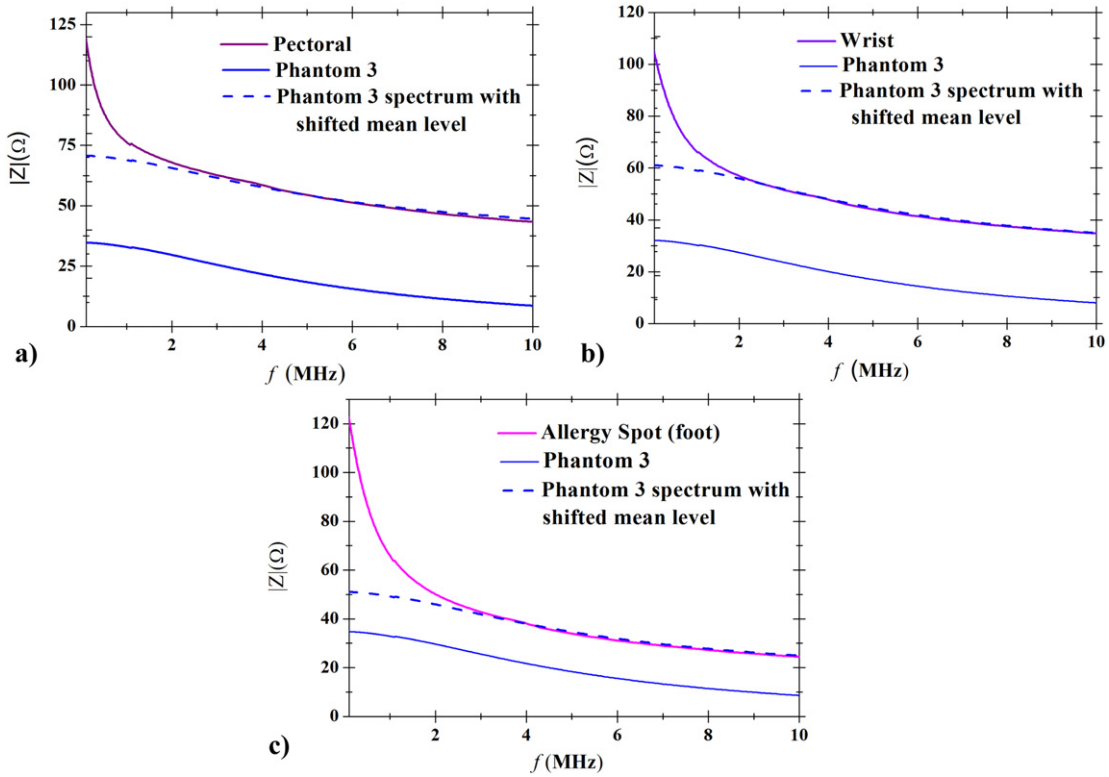

Figure 8. Impedance modulus versus frequency for *in vivo* skin sites, phantom 3 and phantom 3's shifted spectrum. (a) Pectoral, (b) wrist and (c) allergy spot.

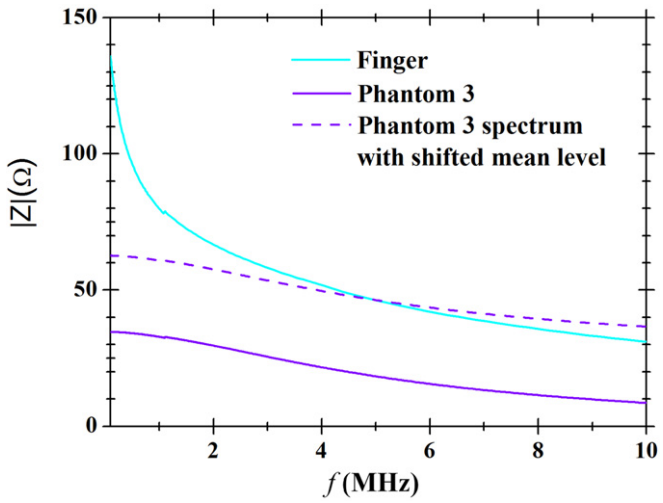

Figure 9. Impedance modulus versus frequency for an *in vivo* finger, phantom 3 and phantom 3's shifted spectrum.

16.3 Ω. As can be observed in figure 8, for frequencies higher than 2 MHz the phantom's spectrum fits perfectly the skin sites spectra, showing that gelatin based phantom are a good option to mimic theses skin sites electrical characteristics, especially at high frequencies.

Another skin site where the impedance spectroscopy measurements were done was the finger. Figure 9 shows finger and phantom 3 spectra, as well as the phantom's shifted spectrum. In this case, as can be seen in figure 9, the phantom spectrum is not such a good match. This may be due to different facts related with the structure of the finger's skin: its dermis connects to the epidermal layer through many papillae, which are tiny finger like projections of connective tissue, and additionally the skin in this site it is thicker than the skin in the pectoral, wrist, and allergy spot [5].

As can be seen from the figures presented above, for each skin site there was a gel phantom that was an optimal match. Table 3 summarizes the skin

Table 3. Phantoms recommended for each skin site.

Skin site	Phantom	Comments
Heel	Phantom 6	—
Pectoral	Phantom 3	Mean level must be shifted[a]
Wrist	Phantom 3	Mean level must be shifted[a]
Allergy spot	Phantom 3	Mean level must be shifted[a]
Finger	—	No phantom was a good fit

[a] The mean level shift is different for each skin site (see text above).

site/phantom's recommended equivalence. It should be noticed that for three skin sites (pectoral, wrist and allergy spot) phantom 3 is recommended, although for each skin site a different shift of the mean level is required (as explained above). In the finger case, no phantom was a good fit. Further studies must be done in order to obtain a gelatin based phantom to mimic this skin site.

The results presented above demonstrate that gelatin is a good material to use as the basis of a phantom for skin in BIS. Gelatin is easy to prepare, can be easily mixed with other materials, allowing fitting the spectrum shape. Even more, gelatin allows flexibility on the modeling phantom's shape. To improve our phantom's impedance characteristics, other materials can be added during gelatin preparation, such as bone powder, glass fiber, blood, paints and tofu, among others.

4. Conclusions

The work presented in this manuscript demonstrates that gelatin can be used as a skin phantom in BIS. Different gelatin based phantoms were analyzed and discussed. Finally, a direct comparison between impedance measurements made in *in vivo* skin and phantoms was presented. Similarities were found on the impedance modulus spectra of skin and gelatin based phantoms showing that gelatins are appropriated to be use as a skin phantom. It is our belief that, as for many other areas of application, also for BIS, gelatin is a good material to be used as the basis of phantoms. Nonetheless, additional research must be done in order to implement gelatin as a realistic skin phantom. It is important to emphasize that these kinds of skin phantoms would be useful not only for BIS, but they would also be of great interest in experiments with other biomedical instrumentation.

Acknowledgments

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