Electrical properties of rat muscle after sciatic nerve injury: impact on surface impedance measurements assessed via finite element analysis

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Abstract. Tetrapolar surface electrical impedance methods are sensitive to changes in muscle status and can therefore provide a means for studying neuromuscular disease noninvasively. In order to better understand the relationship between surface impedance measurements and the actual muscle electrical properties, we performed measurements on 20 adult Wistar rats, 8 of which underwent sciatic nerve crush. Surface impedance measurements were performed on the left hind limb both before injury and out to 2 weeks after injury. In addition, both normal and sciatic crush animals were sacrificed and the dielectric properties of the extracted gastrocnemius muscle measured. We found that 50 kHz conductivities were greater in the animals that underwent crush than in the animals that did not. The permittivities in both directions, however, showed non-significant differences. In order to analyze the effect of these changes as well as the accompanying reduction in muscle volume, a finite element model of the hind limb was developed based on computerized tomographic imaging. The model successfully predicted the surface impedance values in the animals after crush injury and, by its inverse application, may be used to help determine the underlying electrical properties of muscle in various neuromuscular diseases based on surface impedance data.

1. Introduction
Electrical Impedance Myography (EIM) is a non-invasive electro-physiologic technique to assess muscle status in healthy as well as neuromuscular diseases. In EIM a low-intensity, high-frequency current is passed between two surface electrodes and the voltage pattern is observed by another pair of electrodes residing between the two current electrodes [1], [2]. Although under refinement, our initial work in humans [3], [4] has already shown the potential of this approach for the evaluation of a variety of neuromuscular diseases. In order to improve our understanding of this impedance data and its relationship to the muscle’s inherent electrical and pathological properties, over the past several years, we have been pursuing studies in rodents. We have already shown that surface EIM parameters such as resistance, reactance and phase change significantly after nerve injury in the rat [5].

The finite element method is a useful tool to study EIM data since it provides a means for determining how muscle geometry and the inherent electrical properties of the muscle itself impact our surface measurements both in health and in disease states. In this study, we measured the electrical properties such as conductivity and permittivity of rat gastrocnemius muscle for both normal rats and...
rats with sciatic nerve injury. A finite element model of rat leg was then developed both for normal and sciatic crush rats by incorporating the measured electrical values of the rat muscle and altering the shape of the leg based on computerized tomographic (CT) imaging.

2. Methods

2.1. EIM measurement
A total of twenty Wister rats weighing 425-475 grams were obtained from Charles River, Wilmington, MA. Of these twenty rats, 8 underwent sciatic nerve crush. All studies were approved by the Institutional Animal Care and Use Committee at Beth Israel Deaconess Medical Center. Detailed experimental procedures can be found in [5].

Ambu Neuroline 700 surface adhesive Ag-AgCl electrodes (Product # 70010-K/C/12, AMBU Inc., Bethesda, Maryland) were used for EIM measurement. These electrodes are Ag-AgCl with a conductive adhesive gel. To facilitate our measurements on rats, these electrodes were resized to 2cm by 3.5mm by using a razor press customized for this purpose, which consistently produced electrodes of these exact dimensions. The backs of 4 such electrodes were affixed to a piece of adhesive tape, maintaining precisely an inter-electrode distance of 0.75 cm (center to center) between electrodes, thus producing a tetrapolar electrode array. The outer two electrodes served as the current electrodes and the inner two electrodes served as the voltage electrodes. The entire array of electrodes was then placed on the shaved, depilated leg with the electrode axes perpendicular to the long-axis of the limb, keeping the center of the electrode set aligned with the previously applied tattoo [5].

A multi-frequency lock-in amplifier, Model 7280 of Signal Recovery, Oak Ridge, TN coupled with a very low capacitance active probe (Model 1103 of Tektronix, Beaverton, OR) was used to measure the impedance of the muscle [5]. The system was calibrated with resistor-capacitor circuits of known character, designed to mimic to the electrical properties of muscle; in addition, the system was also tested using different concentration of saline solutions to confirm data consistency.

2.1.1. Sciatic crush
A 1 mm length segment of sciatic nerve was crushed using a jeweler’s forceps by applying 3–4 MPa pressure for approximately 30 s. The incisions were then sutured closed, and the animals were allowed to recover until they were killed 1–2 weeks later. Baseline EIM measurements were performed on all the rats including the ones that were to undergo sciatic crush. EIM was also measured for all the rats for two successive weeks. Four rats with sciatic crush were killed a week after crush and the remaining four rats with crush were killed two weeks after crush.

2.2. FEM model
Two distinct current types, the conductive current and displacement current, contribute to the total current that flows through the muscle. The total current in frequency domain is thus given by:

\[ J_{\text{total}} = \sigma E + j \omega \varepsilon_0 \varepsilon_r E \]  

(1)

where \( \sigma \) is the conductivity of the tissue and \( E \) is the electric field, \( \varepsilon \) is the permittivity and \( \varepsilon = \varepsilon_0 \varepsilon_r \) where \( \varepsilon_0 \) is permittivity of air (8.854 \times 10^{-12}) and \( \varepsilon_r \) is the relative permittivity. Since we are assessing the potential on the surface of the muscle, we must calculate the potential \( \varphi \), which is directly related to electric field as:

\[ E = -\nabla \varphi \]  

(2)

In this simulation, the magnetic induction effects are negligible and the field is considered quasi static. Conservation of charge requires that:

\[ \nabla \cdot J = 0 \]  

(3)
Thus the equation that needs to be solved for the potential measurement is:

\[ \nabla \cdot J_{\text{total}} = \nabla \cdot (\sigma E + j \omega \varepsilon_0 \varepsilon_r E) = 0 \]

\[ = \nabla \left[ (\sigma + j \omega \varepsilon_0 \varepsilon_r) \nabla \varphi \right] = 0 \quad (4) \]

A 1mA test current was supplied between the two current electrodes. The normal component of the electric current is assumed to be continuous between any two media. Also it is assumed that there is no current flow out of the boundary at the two ends of the model. This assumption is reasonable, as one side of the model ends at ankle and other side ends at knee, both consisting of mostly poorly conductive bone.

The rat finite element model that we have developed, consists of leg from ankle to knee joint which has a skin layer, a subcutaneous fat layer, the superficial biceps femoris muscle, the gastrocnemius-soleus complex beneath, and two bones (the fibula and the tibia). The shape of the leg was adopted from a computerized tomographic (CT) scan of the rat leg that we had been obtained while maintaining the rat in an identical position as when performing EIM. Angular anisotropy [6] was incorporated for gastrocnemious and bicep-femoris muscle layer. Electrode size and thickness were also modelled according to the actual size of the measuring electrodes used in the experiment.

2.3. Measurement of dielectric properties

A superficial portion of gastrocnemius muscle, approximately 1 cm × 1 cm square was excised with a scalpel, and its height precisely measured. The tissue was kept moist and warm by its being covered with 0.9% saline-saturated gauze under a heating lamp at 36–37°C. Dielectric properties were measured with a four electrode measurement technique as previously described [7].

3. Results

Table 1 shows the measured longitudinal (\( \sigma_l \)) and transverse (\( \sigma_t \)) conductivities and longitudinal (\( \varepsilon_l \)) and transverse (\( \varepsilon_t \)) permittivities for normal and sciatic crush rats. These values are in good conformity with reported values in [8], [9]. For the purposes of employing the finite element method, isotropic conductivities and permittivities of skin, fat and bone were adopted from [8] and [10]. A complete finite element model of a rat with sciatic crush is shown in Figure 1 with all layers labelled. Adult normal rats showed 50 kHz surface EIM resistance (R) of 73±4 ohms (mean +/- SD) and reactance (X) of 23±2 ohms. Because of the degree of crush, the surface EIM parameters and muscle shape varied considerably between the crushed rats. We incorporated the geometry of one of the sciatic crushed rats. The measured and modelled R and X values for both the normal and sciatic-crushed animals are presented in Table 2.

Table 1. Measured conductivities and permittivities at 50 kHz and level of significance.

|         | \( \sigma_l \) (S/m) | \( \sigma_t \) (S/m) | \( \varepsilon_l \) | \( \varepsilon_t \) |
|---------|----------------------|----------------------|--------------------|--------------------|
| Normal mature rats (N=12) | 0.38 ± 0.02          | 0.15 ± 0.01          | 47400 ± 3100       | 47600 ± 1900       |
| Sciatic crushed rats (N=8)  | 0.55 ± 0.03          | 0.21 ± 0.01          | 45800 ± 2700       | 48500 ± 2000       |
| Significance                  | 0.0004               | 0.0002               | ns                 | ns                 |

Table 2. Comparison of experimental EIM data and FEM data at 50 kHz (in ohms).

|         | Measured R | Modelled R | Measured X | Modelled X |
|---------|------------|------------|------------|------------|
| Normal rats          | 73 ± 4     | 69         | 23 ± 2     | 22         |
| Sciatic rat           | 64         | 55         | 15         | 12         |
4. Discussion
By employing finite element model based on the actual muscle electrical constants and limb geometry, we have obtained results that are in reasonable agreement with the experimental surface EIM data. There are several factors that impact the outcome of the model results presented here. First, variability in the severity of the experimental crush lesion contributes to some of the variability in the observed measurements of the electrical constants. Second, the conductivities and the permittivities measured depend critically upon accurately measuring the muscle sample size, maintaining a consistent temperature, and performing the measurements quickly after sacrifice, so as to avoid degradation of the tissue. Third, the geometry of the leg incorporated in the model needs to be as accurate as possible. We attempted to model the geometry as by obtaining CT measurements with the leg in the same position as we perform EIM, but inaccuracies are inevitable. Fourth, the actual internal anatomy of the leg, including the orientation of the bones, the skin and fat thickness and the angles of different layers of muscle need to be precise [6]. Finally, the electrical values for fat, skin and bone will impact results.

The dielectric values that we reported here are average values and crush severity plays a role in measured dielectric values. Moreover while developing a finite element model we adopted a particular rat with crush and its own shape derived by CT scan. The shape of crushed leg is more irregular than that of a normal rat leg and thus likely contributed to the observed differences between the experimental and the modelled data. A MRI-based finite element modelling is thus anticipated to be more accurate in predicting the EIM data, with incorporation of each crushed animal’s unique geometries. Nevertheless, the results found here provide a basis for this basic analytic approach in neuromuscular diseases, including muscular dystrophy and amyotrophic lateral sclerosis.

5. References
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