Published in final edited form as:

Physiol Meas. 2011 August ; 32(8): 1275–1283. doi:10.1088/0967-3334/32/8/017.

CONDUCTIVITY OF NEONATAL PIGLET SKULL

Shilpa Pant*, Tang Te§, Aaron Tucker, and Rosalind J. Sadleir†[Member, IEEE]

*Singapore Bioimaging Consortium, Biomedical Sciences Institutes, Singapore 138667

§Department of Psychology, Florida State University, Tallahassee, FL, 32306-1270, USA

J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, Gainesville
FL 32611-6131, USA

†Department of Biomedical Engineering, Kyung Hee University, Yongin-si, Gyeonggi-do, 446-701,
Korea

Abstract

We report the first measured values of conductivities for neonatal mammalian skull samples. We measured the average radial (normal to the skull surface) conductivity of fresh neonatal piglet skull samples at 1 kHz, and found it to be around 30 mS/m at ambient room temperatures of about 23° C. Measurements were made on samples of either frontal or parietal cranial bone, using a saline-filled cell technique. The conductivity value we observed was approximately twice values reported for adult skull (Oostendorp et al. 2000) using a similar technique, but at a frequency of around 5 Hz. Further, we found that the conductivity of skull fragments increased linearly with thickness. We found evidence that this was related to differences in composition between the frontal and parietal bone samples tested, which we believe is because frontal bones contained a larger fraction of higher conductivity cancellous bone material.

Keywords

biological tissues; skull; neonatal; electrical conductivity

I. Introduction

Our research is directed towards development of electrical impedance tomography (EIT) methods that can be used to detect intraventricular hemorrhage in the premature human neonatal brain. EIT involves reconstruction of three-dimensional images of the electrical properties of a subject via multiple impedance measurements made on an array of surface electrodes. The adult skull's characteristically low conductivity is believed to obstruct the ability of EIT to faithfully reconstruct images of the head (Holder 2005). However, the geometry and mechanical properties of the developing neonatal cranial vault are significantly different from adult skull, with infant skull consisting of a large number of small bones connected by thin sutures and large skull openings at the anterior and posterior fontanelles (Kiesler 2003, Henderson et al. 2004, Margulies et al 2000). The neonatal cranial bones grow together and fuse over a period of approximately 24 months. However, after skull formation is complete there may still be openings in the skull along suture lines due to incomplete fusing and the natural presence of foraminae such as those for optic nerves or blood vessels (Law 1993).

(Corresponding Author, phone: 352-273-9326; fax: 352-273-9221; sadleir@ufl.edu)..

Skull bones have a characteristic three-layered structure (Sadleir and Argibay 2007) with two outer surfaces composed of cortical (hard) bone and the inner layer being cancellous or spongiform bone. Relative thicknesses of these layers vary throughout the cranial vault and the inner layer is sometimes not present. Measurements of isolated samples of live skull layers Akhtari et al. (2002) found average conductivities of 6.17 and 4.87 mS/m in compact bone, and 21.4 mS/m in spongiform bone.

Imaging of conductivity changes within the adult brain using scalp electrodes is made difficult by the low sensitivity to conductivity related changes occurring within the highly resistive skull and highly conductive cerebrospinal fluid encapsulating the brain (Gibson et al. 2000, Sadleir and Tang 2009). The presence of openings in the neonatal skull may make detection of phenomena such as intracranial bleeding or ischemia via EIT easier (Sadleir and Tang 2009). The absolute conductivity of neonatal skull tissue should also affect EIT's sensitivity to intracranial conductivity changes. The conductivity of adult human skull has been measured in several studies, with recently cited values for live adult skull of around 15 mS/m (Oostendorp et al. 2000) and about 10 mS/m (Akhtari et al. 2002). However, to date there have been no published measurements of neonatal mammalian skull conductivities. Because our model for detection of neonatal intraventricular hemorrhage is the neonatal piglet, we were able to make measurements of radial cranial conductivities in numerous samples dissected from postmortem piglet specimens. We obtained piglet skulls immediately after the animal had been sacrificed and measured radial conductivities within (using a homogeneous field applied normal to the skull surface) using a saline-filled cell. Measured radial conductivity values were compared with average thicknesses in both term neonatal and preterm piglet skulls.

II. Methods

A. Sample Preparation

Samples were extracted from the skulls of piglets euthanized less than 4 hours prior to measurement. Figure 1(a) shows a piglet skull with scalp removed prior to sample dissection. Left and right frontal and parietal skull bones, a total of four samples per skull, were easily separated along suture lines, as shown in Figure 1(b). Samples were scraped on both surfaces to remove the dura and any other membranes. Sample thicknesses were irregular, but were between 1 and 2 mm. Thicknesses were measured at a minimum of three locations over the surface using a vernier caliper, or at six locations by using the caliper for three, and a vernier micrometer for the remainder. The conductivity measurement procedures described below were performed over a period of less than 4 hours after animals were sacrificed to avoid degradation of the samples. A total of 35 samples from 9 term piglets were measured in these experiments, with a total of four samples (left and right frontal, left and right parietal) harvested from each animal. In one animal, the left parietal section of the skull was broken during preparation and measurements were not possible from this sample. Samples remained in air until conductivity measurement commenced, as soon as possible after segments were dissected (approximately 30 min). Visual inspection of frontal bones revealed that they were more opaque (under laboratory light) than parietal bones, and more often contained a layer of cancellous bone at the sample margins (Figure 1(c)).

The typical swine gestation period is about 115 days (Straw 1999). In addition to 35 samples derived from term piglets one or two days after birth, we obtained 12 samples from three preterm piglets that had been delivered by Caesarian section at around 99 days' gestation. This period represents 85% of full term gestation. Therefore this gestational age was estimated to be equivalent to a neonatal human at about 35 weeks' gestation (assuming a human gestational period of 40 weeks). We both measured radial conductivities and

obtained transmission micrographs of these samples. Data from term and preterm piglets were analyzed separately.

B. Measurement Apparatus

1) Measurement cell—The cell used to obtain radial conductivity measurements is shown in Figure 2. This cell was similar to that described by Oostendorp et al (2000) and one used in our other study of skull impedance (Sadleir and Argibay 2007). The cell had an internal diameter of 18 mm, and an effective length of 60 mm. The two saline-filled halves of the cell were sealed with O-rings, and connected by screws passing through the two flanges. Current was passed between electrodes $E1$ and $E2$, which also formed the ends of each tube, and voltage differences were measured at two electrodes ($V1$ and $V2$) positioned in the tube wall. Electrodes $V1$ and $V2$ were positioned about 10 mm from the flange on each side. The complex impedance of the cell (in Ω) was measured before (Z_s) and after (Z_{B+S}) inclusion of the bone sample between the flanges, and the difference was converted to radial skull impedivities (in Ωm) by multiplying the impedance difference by a cell factor that was calculated from the cross sectional area of the cell (A , about 81 mm^2) divided by the measured bone sample thickness (t).

$$\rho_B = (Z_{B+S} - Z_s) \frac{A}{t} \quad (1)$$

Here, ' $B + S$ ' refers to a measurement with both bone and saline, and ' S ' refers to the impedance measured with no sample between the two halves of the cell. The complex impedance Z (in Ω), was measured as

$$Z = R + jX \quad (2)$$

where R is resistance and X is the reactive component.

Samples were placed in the saline-filled measurement cell and were therefore in contact with saline, for a period of 60–90 seconds during the final stage of the differential measurement, and therefore we did not expect that samples would have been affected by the conductivity of the fluid (Kosterich et al.).

2) Radial Skull Conductivity measurements—The Kyung Hee Bioimpedance Spectrometer (Oh et al. 2008) was used to collect radial impedance measurements. This device applies a constant current of approximately 1 mA to samples and simultaneously measures the voltage difference between two other points of the sample, thus measuring a transimpedance. Digital demodulations of voltage signals may be used to extract both real and imaginary parts of the impedance. Impedances may be measured sequentially at 10 frequencies logarithmically distributed between 500 Hz and 500 MHz. We chose only to record values gathered at 1 kHz. Noise in resistance and reactance measurements was reduced by averaging measurements over 64 cycles at the measurement frequency.

3) Digital micrography—The twelve preterm piglet skull samples were micrographed on the stage of a Leica DM IL optical microscope (Leica Microsystems GmbH, Wetzlar, Germany). Each sample was illuminated similarly, and the segment of the sample in the (fixed) focal plane of the instrument in each case. This strategy assisted with qualitative examination of the sample thickness and compositions.

C. Data Collection Strategy

Impedivities were then transformed to complex conductivities in S/m by complex inversion.

$$\sigma_B = \frac{A}{t Z_B} \quad (3)$$

In equation (3), A represents the tissue area (in m^2) and t represents the skull thickness (approximately 1×10^{-3} m). Magnitudes of the complex conductivity were used as an approximation to the conductivity at 1 kHz. Reactive impedances were found to be less than 0.1% at the measurement frequency.

D. Error Analysis

Conductivity errors were calculated taking into account contributions from errors from resistance measurement and thickness measurement errors. The cell constant used to convert impedance to impedivity was the result of two different measurements (for A and l), rather than from a reference measurement. Therefore the errors were estimated as

$$\delta\sigma = \sqrt{\left(\frac{\partial\sigma}{\partial A}\delta A\right)^2 + \left(\frac{\partial\sigma}{\partial t}\delta t\right)^2 + (\delta\sigma_{\text{sig}})^2} \quad (4)$$

where $\delta\sigma_{\text{sig}}$ represents the factor contributed by uncertainty in measured impedances.

We found that the principal contribution to conductivity estimations was uncertainty in thickness measurements, because of the sample irregularity. This factor contributed about 10% error to conductivity estimations. The KHU BIS device contributed the smallest error to conductivity values, less than 0.7%. We included a fixed error contribution because of uncertainty in measurement of the cell diameter and therefore in cell area. While there was an extra contribution to area uncertainty caused by variations in sealing O-ring diameter caused when the cell was clamped onto samples, we elected to neglect contributions from this factor. Including all error contributions, typical total relative errors were of the order of 11–15%.

III. Results

A. Sample Thicknesses

A summary of measured frontal and parietal sample thicknesses of term samples is shown in Table 1. Average samples thickness was about 1.3 mm. Comparison of frontal and parietal thicknesses using a two-tailed paired t-test found no significant differences between the two sample sets ($p < 0.05$).

B. Conductivities at 1 kHz

Results of radial piglet skull conductivity measurements taken less than 4 hours post-mortem are shown in Table 1 and Figure 3. The mean conductivity value found at 1 kHz for frontal samples was 35.8 ± 8.7 mS/m, and for parietal samples 25.5 ± 8.2 mS/m. A t-test comparison of conductivity values at 1 kHz found a significant difference in mean values ($p < 0.05$), with frontal bone conductivities being larger than parietal.

C. Preterm Bone Samples

The preterm bone sample thicknesses were similar but more uniform than term samples, with measured thicknesses of 1.32 ± 0.33 mm and a thickness range of only 0.40 mm. Conversely, conductivities of preterm bone samples were very variable. The mean conductivity of all samples was 31 ± 9.7 mS/m, similar to those found in term samples. No significant difference was found in either thicknesses or measured conductivities of the two tissue types. Figure 4 shows micrographs of the 12 preterm samples. While there were differences between the opacity of tissues varying from sample to sample, but no overall qualitative difference between the sample types.

IV. Discussion

A. Comparison of values with adult mammalian skull, and other tissue values

Oostendorp et al. (2000) and Akhtari et al. (2002) measured values for radial conductivities in fresh or live adult skull. Oostendorp found a conductivity of 15 ± 3 mS/m (measured at 37°C) in fresh and live skull using a similar apparatus to that shown in Figure 2(a). Akhtari et al. found a radial conductivity of 9.53 ± 0.29 mS/m in live adult skull samples. Because of the incomplete development of neonatal bone, the higher conductivity we find for neonatal piglet skull was likely a consequence of the larger water fraction in the tissue and its reduced level of mineralization. We also note that because our measurements were performed at 23°C , we would expect radial bone conductivity measured at body temperature to be higher, possibly up to 30% (assuming a conductivity temperature coefficient of around $+2\%/^\circ\text{C}$) or about 40 mS/m (Grimnes and Martinsen, 2000).

B. Dependence of conductivity on sample thickness

While there were no significant differences observed in measured sample thicknesses of the two bone types, frontal bone conductivities were significantly higher than those for parietal bones. We believe this is a consequence of the larger fraction of higher conductivity cancellous bone in frontal bone samples, but this has not been systematically evaluated. Certainly, inspection of frontal bone margins of term and preterm samples showed that most contained bone marrow (cancellous bone), whereas many parietal bone samples were translucent under laboratory lighting, with edges that showed no evidence of a layered inner structure.

C. Properties of premature bone

We found no clear dependence between tissue type (frontal or parietal) and measured conductivities in premature piglet skull tissue. Two parietal samples had large conductivity values (> 50 mS/m) while the remainder had conductivities below 25 mS/m. Several frontal samples had conductivities above 40 mS/m. Broad examination of Figure 5 showed a greater qualitative opacity in frontal bones (left) than in parietal samples. However, the parietal sample with the largest conductivity (Piglet 2, right parietal) did not appear more opaque than other parietal samples. Similarly, the frontal sample having the largest conductivity (Piglet 3, right frontal) did not have an appreciably darker micrograph than other frontal samples. We concluded that the micrograph brightness was more likely related to local illumination of features in the inhomogeneous bone and not to the average sample thickness. Another approach, for example tissue staining, would likely constitute a better measure of relative bone compositions.

V. Conclusions

Measurements of neonatal piglet skull conductivity at 1 kHz showed that its approximate radial conductivity was around 30 mS/m at 23°C . This conductivity was around twice

recently quoted values for adult human skull bone (Oostendorp et al 2000). Frontal bones had significantly higher conductivities, but non-significantly different thicknesses than parietal bones. It is speculated that this is because frontal bones contained a larger fraction of spongiform bone. Measurements of preterm skull conductivities obtained similar quantitative results at 1 kHz.

Acknowledgments

RJS would like to acknowledge the kind cooperation of Professor Peggy Borum, of the University of Florida Department of Food Science & Human Nutrition, for providing the preterm piglet skull samples used in this work, and Dr Peter McFetridge, J. Crayton Pruitt Family Department of Biomedical Engineering, University of Florida, for assisting us in micrographing preterm piglet skull samples.

This work was supported in part by NIH grant RO1EB-002389 to RJS.

References

- [1]. Agilent Technology Company Ltd. The Impedance Measurement Handbook. Santa Clara, CA, USA: 2003.
- [2]. Akhtari M, Bryant HC, Mamelak AN, Flynn ER, Heller L, Shih JJ, Mandelkern M, Matlachov A, Ranken DM, DiMauro MA, Lee RR, Sutherland WW. Conductivities of three-layer live human skull. *Brain Topography*. 2002; 14:151–167. [PubMed: 12002346]
- [3]. Grimnes, S.; Martinsen, OG. *Bioimpedance & Bioelectricity Basics*. 1st ed.. Academic Press; London, San Diego: 2000.
- [4]. Holder, DS. Electrical Impedance Tomography. Methods, history and applications. In: Orton, CG.; Nagel, JH.; Webster, JG., editors. *Series in Medical Physics and Biomedical Engineering*. 1st ed.. Bristol and Philadelphia: Institute of Physics Publishing; 2005. p. 4562000
- [5]. Kosterich JD, Foster KR, Pollack SR. Dielectric properties of fluid-saturated bone - the effect of variation in conductivity of immersion fluid. *IEEE Transactions on Biomedical Engineering*. 1984; BME-31:369–374. [PubMed: 6745971]
- [6]. Kosterich JR, Foster KR, Pollack SR. Dielectric permittivity and electrical conductivity of fluid saturated bone. *IEEE Transactions on Biomedical Engineering*. 1983; BME-30:81–86. [PubMed: 6832795]
- [7]. Law SK. Thickness and resistivity variations over the upper surface of the human skull. *Brain Topography*. 1993; 6:99–109. [PubMed: 8123431]
- [8]. Oh TI, Koo H, Lee KH, Kim SM, Lee J, Kim SW, Seo JK, Woo EJ. Validation of a multi-frequency electrical impedance tomography (mfeit) system klu mark1: Impedance spectroscopy and time-difference imaging. *Physiological Measurement*. 2008; 29:295–307. [PubMed: 18367806]
- [9]. Oostendorp TF, Delbeke J, Stegeman DF. The conductivity of the human skull: results of in vivo and in vitro measurements. *IEEE Transactions on Biomedical Engineering*. 2000; 47:1487–1492. [PubMed: 11077742]
- [10]. Sadleir RJ, Argibay A. Modeling skull electrical anisotropy. *Annals of Biomedical Engineering*. 2007
- [11]. Straw, BE. *Diseases of swine*. Iowa State University Press; Ames, IA: 1999.

Figure 1.

Preparation and presentation of piglet cranial bone samples. (a) Term piglet skull prior to dissection, (b) four separated skull samples, comprising left and right frontal and left and right parietal samples, and (c) side views of frontal and parietal sample from preterm piglet, showing cancellous bone on frontal bone margin (arrowed, top) and uniform composition at margin of parietal bone (bottom).

Figure 2. Radial conductivity cell, showing current application points (E1, E2), voltage monitoring points (V1, V2), and a schematic of measurements.

Figure 3. Plot of sample thickness against measured radial skull conductivities of term piglet samples at 1 kHz.

Figure 4. Optical Micrographs of preterm piglet bone tissues showing (left) Frontal and (right) Parietal fragments.

Table 1

Measurements of frontal and parietal skull fragment thicknesses and conductivities in term piglets

	Frontal	Parietal
Number of samples	18	17
<i>Thickness (mm)</i>		
Mean	1.40	1.25
Standard Deviation	0.30	0.36
Minimum	0.76	0.58
Maximum	1.78	1.78
<i>Conductivity (1 kHz, mS/m)</i>		
Mean	35.8	25.5
Standard Deviation	8.7	8.2
Minimum	18.1	12.4
Maximum	46.7	39.4