Δ Impedance Measurements Correlate to Callus Maturation of Mice Tibia Fractures

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PAPER ABSTRACTS

Background/Purpose: Approximately 15 million fracture injuries occur in the United States each year. Accurate monitoring of fracture healing can determine timing of return to function or the need for early intervention in case of a fracture nonunion. Fracture healing is currently monitored by radiographic methods, which rely on mineralization of tissue that only occurs in the later stages of fracture healing, and other monitoring techniques are either subjective or inaccurate. Electrical impedance spectroscopy (EIS) provides a measure of the dielectric properties of a medium and has been used to differentiate between different tissue types. We hypothesized that EIS can be used to monitor fracture healing by tracking the changing tissue composition of a fracture callus as it progresses through the various stages of healing.

Methods: Standardized, closed fractures were created in the middiaphyses of mice tibia according to an established murine model of endochondral repair. Mice were euthanized and their fracture callus tissues dissected out at days 8, 14, and 21 postfracture for measurement (N = 11). Each intact callus was pressed onto custom-made sensors with a 590-g weight, and 2-point impedance measurements were taken across two gold electrodes (150-μm diameter) over a range of frequencies (20 Hz to 1 MHz). Samples were also fixed in 4% paraformaldehyde overnight, decalcified in 19% EDTA (pH 7.4) for 14 days at 4°C, and embedded in paraffin. Serial 10-μm longitudinal sections throughout the entire callus tissue were collected and stained with modified Milligan's Trichrome. To quantify tissue volume fractions, histomorphometric analyses of total callus, cartilage, trabecular bone, cortical bone, muscle, fibrous tissue, and bone marrow space volumes were performed using an Olympus CAST system and Visiopharm software. The total tissue volumes were calculated in cubic millimeters (mm3) using the equation for a conical frustum and Cavalieri's principle. Univariate linear regression analysis was performed to assess correlative relationships between impedance measurements and volume fraction percentages of the various tissues present in the fracture calluses, and two-tailed t tests were used to determine whether regression slopes were significantly different than 0. Significance was set at $P < 0.05$ and trends were defined as $0.05 < P < 0.1$.

Results: Linear regression analyses indicated negative relationships between impedance magnitude (|Z|) and % trabecular bone as well as % marrow space, and positive relationships between |Z| and % cartilage as well as % fibrous tissue. The opposite trends were found when comparing phase angle (θ) to these same volume fractions of tissues. These correlations were as expected; as healing time increases, % cartilage decreases and % trabecular bone increases as the spongy bone replaces the early soft callus. As a result, |Z| rises over the course of healing as more conductive tissue (cartilage) is remodeled into more resistive tissue (bone). % fibrous tissue decreases with healing time as it is replaced by trabecular

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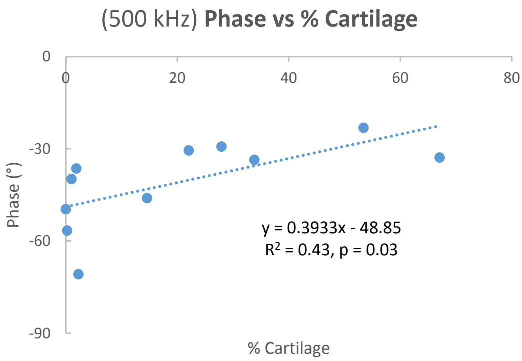
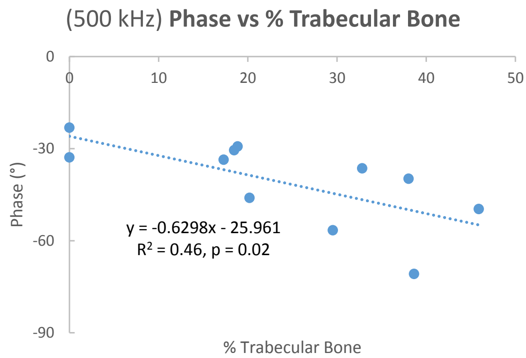
See pages 49 - 106 for financial disclosure information.

bone or marrow space, and consequently % marrow space increases. Specifically at 500 kHz, |Z| and phase both showed significant correlation with % cartilage and % trabecular bone (R2 >0.40, P <0.05). At 1 MHz, phase became less negative with greater % cartilage and % fibrous tissue (R2 >0.54, P <0.01) and more negative with greater % trabecular bone (R2 = 0.58, P = 0.007). In addition, phase became less negative significantly with % trabecular bone at 5 kHz (R2 = 0.39, P = 0.04), and this trend was maintained for frequencies less than 5 kHz (P <0.1).





Regression analysis of phase angle (θ) correlated to % volume fractions of cartilage and of trabecular bone for fracture calluses. Significant relationships are shown here for measurements at 500 kHz.

Conclusion: Impedance magnitude and phase angle have significant correlations with volume fractions of cartilage, trabecular bone, fibrous tissue, and marrow space at multiple frequencies, particularly below 5 kHz and above 500 kHz. These findings support use of electrical impedance spectroscopy for monitoring fracture healing.