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●*Original Contribution*

CORRELATION OF TISSUE CONSTITUENTS WITH THE ACOUSTIC PROPERTIES OF SKIN AND WOUND

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Abstract—The purpose of this study was to compare measurements of ultrasound properties of skin and wound tissue with measurements of material properties such as total collagen concentration, acetic acid soluble collagen concentration, water concentration, and morphologic properties. Using a scanning laser acoustic microscope (SLAM), both ultrasonic speed and attenuation coefficient values were obtained for control skin (2–3 cm from the wound), for skin immediately adjacent to wounds (within 0.3 mm), as well as for wound tissue itself. The attenuation coefficient and speed measurements were lowest for wound tissue followed by adjacent skin and then control skin. As the wounds healed there appeared to be an increase in both speed and attenuation coefficient although the wound age at which these increases started and the length of time for which they continued varied from one dog to the next. The precision of duplicate sample measurement of wave speed was ±1.7% for control skin, whereas that for attenuation coefficient it was ±16%. Both ultrasonic speed and attenuation coefficient were directly correlated with tissue collagen concentration and inversely correlated with tissue water concentration ($p < 0.001$). Attenuation coefficient correlated best ($r = 0.73$) with acetic acid soluble collagen concentration which reflects the changes in collagen taking place during the repair process. These attenuation measurements made at 100 MHz using the SLAM were compared for control skin and wound samples with measurements made at 10–40 MHz using backscatter acoustic techniques (BAT). The tissue samples analyzed by each ultrasound technique were from adjacent locations on the animals.

Key Words: **Ultrasound, Ultrasonic tissue characterization, Scanning laser acoustic microscope, Biochemistry, Morphology, Skin, Wounds.**

INTRODUCTION

Ultrasound is an important clinical modality for non-invasively imaging internal organs and, further, it may prove to be a valuable tool in the evaluation of skin and healing wounds. The feasibility of using ultrasound for the evaluation of wounds depends on whether ultrasound measurements correlate with tensile strength as well as morphological and biochemical properties of wounds at various stages of repair. In our previous study of 3 dogs (Olerud et al. 1987), ultrasonic propagation properties in wound tissue and adjacent skin were shown to be correlated with the material properties of collagen concentration, water concentration, and tensile strength. The results suggested that ultrasonic speed and attenuation coefficient measurements made with the scanning laser acoustic microscope (SLAM) reflected the wound healing process.

The present study was undertaken to extend our data base and verify the results of that previous investigation as well as to study other aspects of the wound model. Matched pairs of wounds from individual animals were analyzed in the present study in order to evaluate the degree of reproducibility of the acoustic, biochemical, and morphological measure-

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ments. In this study we analyzed acoustically control skin remote from the wound because in our prior study (Olerud et al. 1987) the acoustical properties of the skin immediately adjacent to the wound appeared to be influenced by the wound healing process. We correlated tissue water concentration, total collagen concentration and acetic acid soluble collagen concentration with both acoustic speed and attenuation coefficient for the four animals studied in these experiments. In addition, similar plots and equations were derived for all seven animals studied to date including the three animals from our earlier study. Finally, we compared our attenuation measurements made in a through transmission mode at 100 MHz using SLAM with measurements made at 10–40 MHz using BAT (Forster et al. 1990, companion paper).

METHODOLOGY

The animal model, biochemical, morphological, and ultrasound methods employed in this study are described in detail elsewhere (Olerud et al. 1987; Steiger et al. 1988; Riederer-Henderson et al. 1988; Forster et al. 1990) and are only summarized here. The SLAM was used in this study to analyze samples of both wounds and control skin from locations on the animals immediately adjacent to those samples measured in the companion paper by Forster et al. using BAT (see Fig. 1).

Animal model

Standard paravertebral skin incisions 6 cm in length were created on four mongrel dogs (21–25 kg). Pairs of wounds were created in a humane manner under sterile surgical conditions using intravenous sodium thiamyl and Halothane for anaesthesia at time points 49, 34, 20, and 9 days prior to euthanizing the animals. At the time the animals were euthanized, tissue was removed and allocated in the manner depicted in Fig. 1. In every case the samples were either placed in the appropriate fixative or frozen in liquid nitrogen within 5 minutes of being excised from the animal.

Morphology

Samples for morphology were placed in half strength Karnovsky's fixative (1965). Samples were subsequently either imbedded in Epon 812 (Luft 1961) for light microscopy (LM) and transmission electron microscopy (TEM) studies or further prepared for assessment of three dimensional architecture using the scanning electron microscope (SEM).

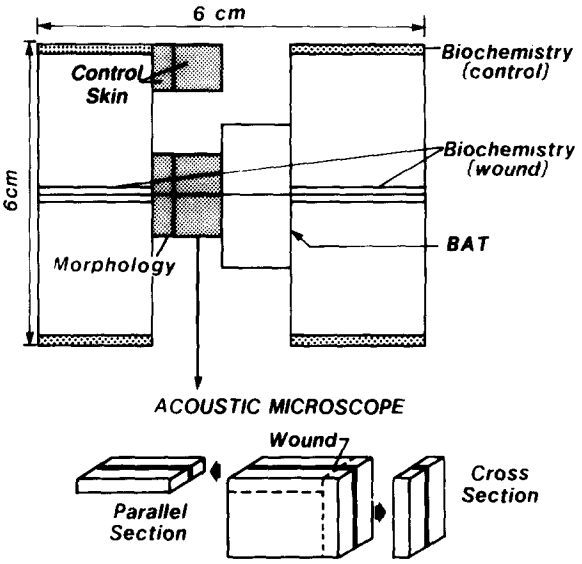

Fig. 1. Skin and wound sample allocation. For acoustic microscopy samples were oriented both parallel and perpendicular (cross-section) to the epidermal surface. The sample designated BAT was studied using backscattered acoustic techniques and is described in a separate report (Forster et al. 1990).

The width of each wound was estimated by LM using a calibrated eyepiece micrometer.

Biochemistry

Biochemistry of wound and control skin samples are described in detail elsewhere (Forster et al. 1990).

Ultrasound

A scanning laser acoustic microscope (SLAM) (Sonomicroscope 100, Sonoscan Inc., Bensenville, IL), operating at 100 MHz, was used to determine the ultrasonic attenuation coefficient and wave speed in the tissue specimens. The basic operating principles of the SLAM as they related to this study have been described in detail elsewhere (Steiger et al. 1988; Olerud et al. 1987; Embree et al. 1985; Tervola et al. 1985; Tervola and O'Brien 1985). The method of sample preparation to allow analysis of frozen sections both parallel and perpendicular to the epidermal surface is described previously (Olerud et al. 1987). The tissue areas studied with acoustic techniques included: *wound, adjacent skin* (within 0.3 mm of the wound edge), and *control skin* (2–3 cm medial to the wound). Samples sectioned parallel to the epidermal surface were typically taken at depths ranging 100 to 500 μm from the surface. Hence, the parallel samples included speed and attenuation coefficient measurements for both papillary and reticular dermis. Speed measurements for the cross-sections

typically were taken 300–700 μm from the epidermal surface (*i.e.*, in the reticular dermis) whereas for attenuation measurement sites were more randomly selected in both papillary and reticular dermis. Hair shafts were avoided because we observed very high attenuation and speed of these structures.

Statistical analysis

A standard statistical software package (SPSS) was used to perform statistical tests on the data. Linear least square fits analyses were performed to describe functional relationships between two variables such as speed vs. water and attenuation coefficient vs. collagen. In situations where relationships appeared to be nonlinear, other fits such as exponential, log, and power (to the fourth order) were also examined. The correlation coefficient, r, was used in all cases as an indicator of goodness of fit. The 2-tailed Student's t test was used to determine the significance of r. The F test for significance of the multivariant analysis of variance (ANOVA) was utilized. A probability of $p < 0.05$ was considered significant for all tests.

Precision of analyses of duplicate samples

In order to assess the precision (reproducibility) of the biochemical and ultrasonic measurements, duplicate (matched-pairs) wound and control samples from opposite sides of the back of the four animals were evaluated for each wound age. To quantify this precision, the absolute percent difference for duplicate samples was determined for the following factors: water concentration, total collagen concentration (as a percent of DDF weight), acid soluble collagen (as a percent of total collagen), speed, and attenuation coefficient.

RESULTS

Clinical observations

The dogs ranged in weight from 21 to 25 kg (22.6 kg $\pm$1.8, mean $\pm$ SD) at the beginning of the study. Dog A gained 2.4 kg during the study period and all wounds appeared normal and well healed. Dog B had a nasal discharge and cough for 2 weeks during the study period and lost 1 kg. Both 9 day and both 20 day wounds had to be resutured because the animal chewed sutures out. The 9 day wounds appeared slightly separated at the time the sutures were removed, and as noted below contained histological evidence of wound infection in one of the 9 day wounds. One of the 20 day wounds showed an elevation of one of the margins and a 34 day wound was noted to be wider than normal where the wound had spread apart early in the repair process, then healed by secondary intension. Dog C had a nasal discharge and cough for 2 weeks, but weight remained constant. Wounds appeared well healed. Dog D remained in good health with constant weight. Both 20 day wounds were noted to be widened and thickened.

Morphology

LM and SEM studies were done on both wound and control skin for each animal. Wounds ranged in width from approximately 0.3 to 2 mm with the older wounds generally being wider than the more recent wounds. Normal repair sequences were documented in all four animals with the exception of one of the 9 day wounds for dog B which showed diffuse tissue infiltration with neutrophiles consistent with a wound infection. TEM studies document the normal repair process as illustrated in Fig. 2. In a normal 9 day wound there are only scattered collagen fibrils and no collagen fiber bundles. A progressive increase in both the amount of collagen and the fiber bundle size is shown in the 20, 34, and 49 day wound samples. They are contrasted with control skin.

Biochemistry

Biochemistry data are detailed elsewhere (see Table 1, Forster et al. 1990). Only values for wounds and control skin are reported in Table 1 because of the technical difficulty of separating the wound tissue from adjacent skin (within 0.3 mm of the wound).

Ultrasound studies

To examine changes during the wound healing process the ultrasonic speed (Fig. 3) and attenuation coefficient measurements (Fig. 4) in the wound, adjacent skin, and control skin were plotted as a function of wound age. For the speed measurements, all thicknesses (50, 100, and 150 μ), for both section types (parallel vs. perpendicular) and wound age duplicates were combined, yielding an average of 10 to 28 values per dog for each wound age. For the attenuation coefficient, four values for each wound age were averaged. In all cases, the average values were plotted. The range of standard deviations (SD) for the mean values are also indicated on the figures (1% to 4% for speed and 15% to 20% for attenuation coefficient).

In dog B, for the histologically normal 9 day wound and the 9 day wound with evidence of infection the difference in mean values was 1% for speed and 16% for attenuation coefficient. Hence, the difference in the mean values for those samples was similar to the difference in mean values seen for other pairs of samples.

For all dogs and all wound ages, both the speed and attenuation coefficient were lower in the wound

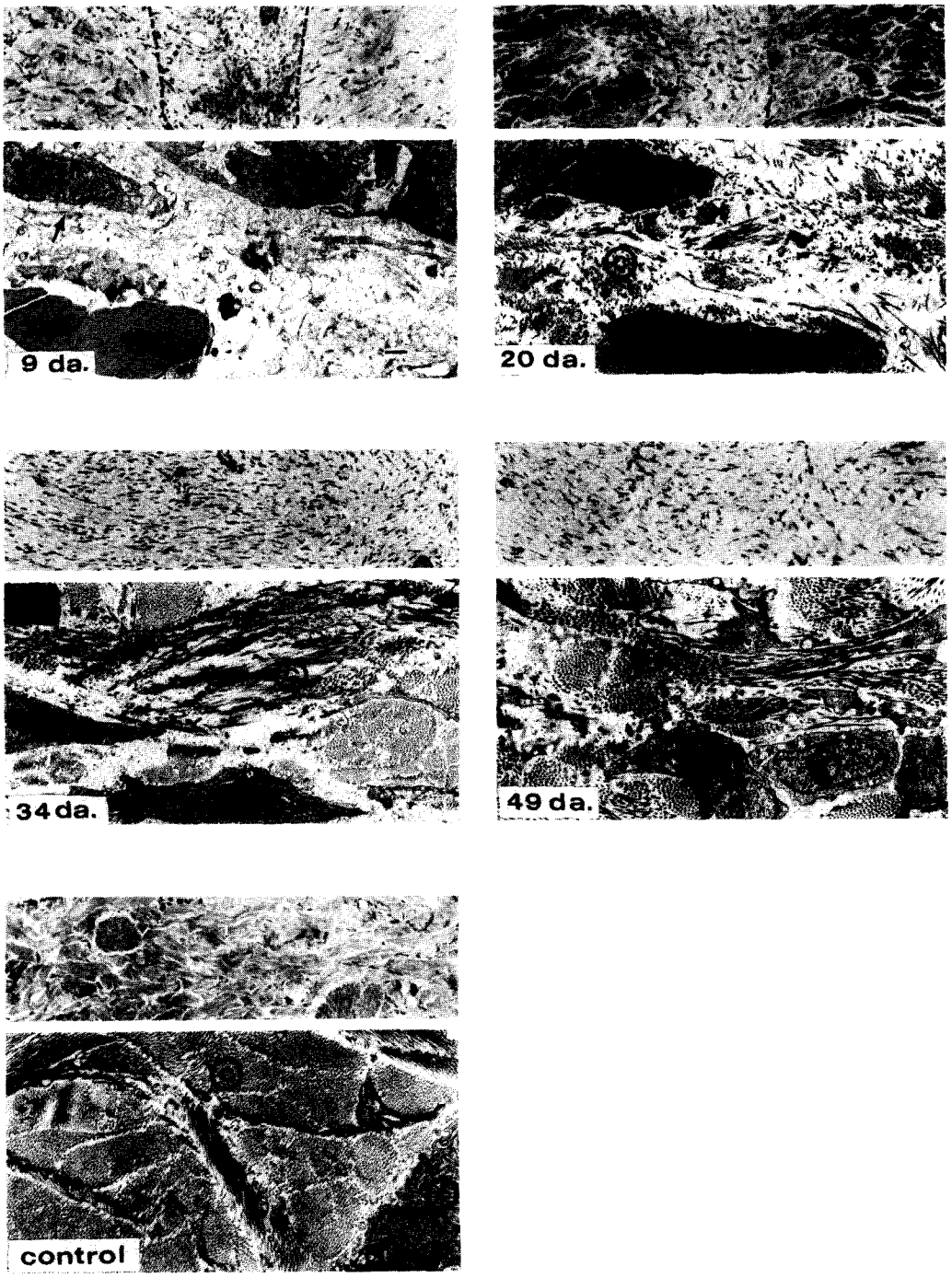

Fig. 2. Light and transmission electron micrographs (LM and TEM) of wounds and control skin from dog A depict tissue from skin wounds of 9, 20, 34, and 49 days of age and normal control skin. Each of the 5 panels illustrates an LM at magnification 63× (upper) and a TEM at magnification 4050× (lower). The scale bar on the 9 day TEM = 1 μm. In the photomicrographs of the 9 and 20 day specimens the broken vertical lines define the lateral limits of the wound whereas in the 34 and 49 day wounds the dermal repair response occupies the entire width of the sample. Note the scanty distribution of isolated collagen fibrils in the TEM of a 9 day wound and compare the progressive increments in collagen fibrils and the increasing size of bundles of collagen fibrils (*i.e.*, collagen fiber bundles) with advancing age of the wounds. Observe also the changes in cellularity of wounds with age and the progressive decrease of interfibrillar space with advancing repair. Red blood cells (rbc), fibroblast (f) and collagen (c) are labeled.

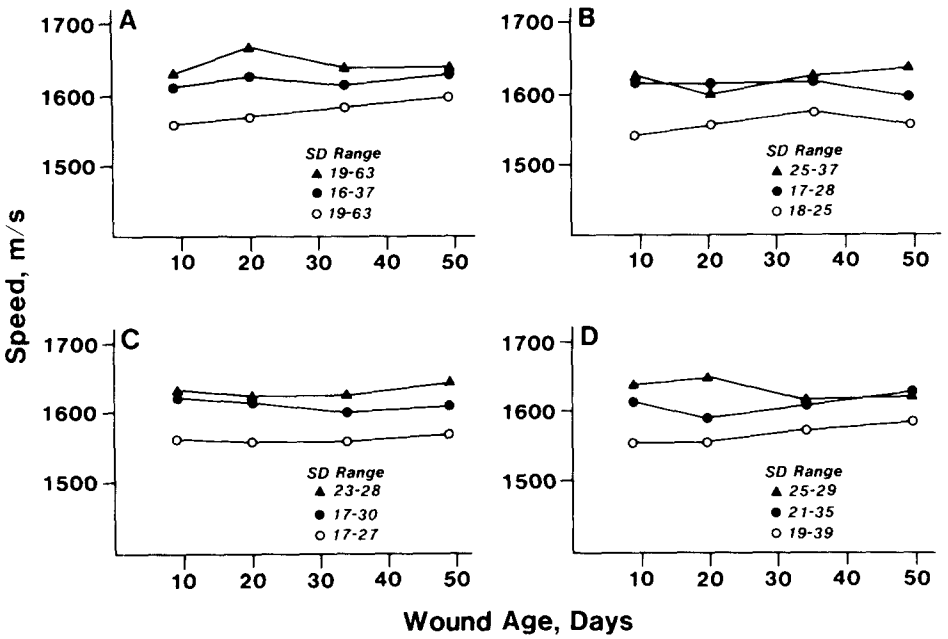

Fig. 3. Plots of ultrasonic speed for dogs A, B, C, and D as a function of wound age for wound tissue (open circles), adjacent skin, within 0.3 mm of wounds (closed circles), and control skin, 2–3 cm from wounds (closed triangles). Although the curves appear relatively flat across the time points examined the mean wave speed in control skin is generally greater than adjacent skin, which in turn is greater than wound tissue. SD denotes standard deviation.

than in the adjacent skin. In almost all cases, the speed and attenuation coefficient in the adjacent skin were slightly lower than in the control skin. The changes with increasing wound age varied from dog to dog, as was suggested by an ANOVA.

The ultrasonic speed and attenuation data were

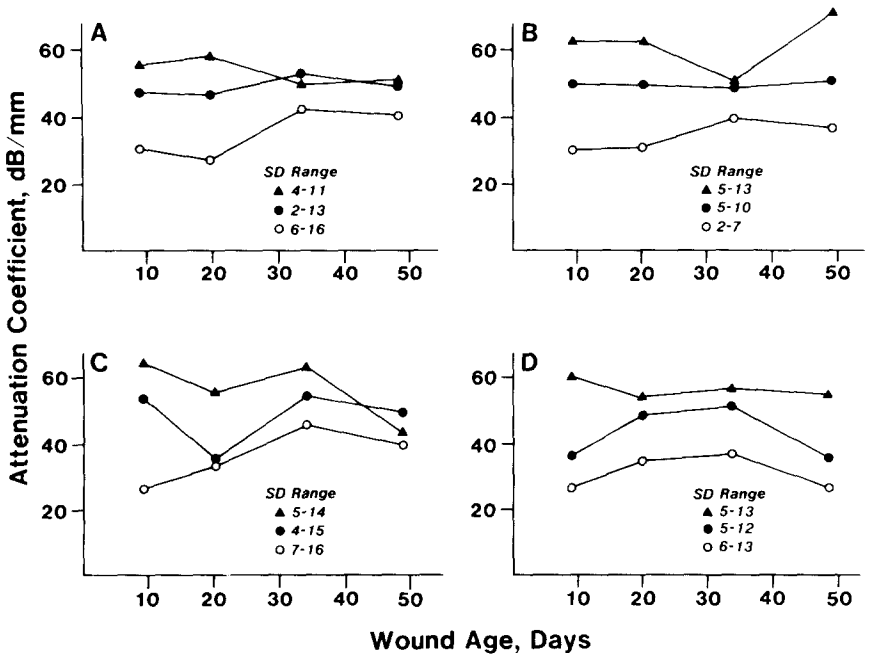

Fig. 4. Plots of attenuation coefficient for dogs A, B, C, and D as a function of wound age for wound tissue (open circles), adjacent skin, within 0.3 mm of the wounds (closed circles), and control skin, 2–3 cm from wounds (closed triangles). Attenuation coefficient values are generally lowest in wound tissue at day 9 and highest at day 34 with a suggestion of a decrease in attenuation coefficient at day 49. The mean control values are consistently higher than the adjacent skin values which in turn are higher than wound values. SD denotes standard deviation.

statistically analyzed using ANOVA to estimate the percent of variance in the data explained by each of the following factors: dog (A, B, C, and D), wound age (9, 20, 34, and 49 days), section type (parallel vs. perpendicular), tissue type (wound and adjacent skin), and specimen thickness (50, 100, and 150 μm).

Speed

The ANOVA of the ultrasonic speed data indicated that all of the factors except section type had a significant effect on the variance of the speed results. The specimen thickness, however, explained only 0.6% of the speed variance, so its effect was considered to be negligible. Tissue type (wound and adjacent skin), on the other hand, explained most of the variance (45%) in the speed results. Although still significant, wound age explained only 1.2% of the variance in speed. Contrary to our earlier study (Olerud et al. 1987), the variation among dogs was considered significant. This indicated that it was not statistically appropriate in the present study to pool ultrasound results from all of the dogs.

Based on the first ANOVA all sections (parallel and perpendicular) and all thicknesses (50, 100, and 150 μ) were combined and a second ANOVA was done to assess the effect of age on the ultrasonic speed in wound and adjacent skin for each dog. The results for the second ANOVA showed that in dogs A, B, and D, age explained a significant portion (about 20%) of the variance in speed. For dog C, the ultrasonic speed in the wound did not appear to depend on wound age. For the adjacent skin, wound age had a significant effect on the speed variance in dogs B, C, and D.

Attenuation coefficient

To determine if the attenuation coefficients were different for the perpendicular and parallel sections, a dependent Student's t test was performed. The difference in attenuation coefficient between perpendicular and parallel sections was small (about 1 Np/cm) and was not significant in either the wound or adjacent skin. Thus, these data were combined and an ANOVA was done to determine the percent of variance in the data explained by the different dogs and by wound ages. For the wound, age explained a large (23%) and significant portion of the variance in attenuation coefficient. The effect of variation among dogs was small and nonsignificant. In the adjacent skin, wound age did not explain a significant portion of variance (7%) in attenuation coefficient. The effect of variation among dogs, however, was quite large (12%) and was considered significant.

In general, there appears to be an increase in both speed and attenuation coefficient with increasing wound age. The age at which these increases start and the length of time for which they continue vary from one dog to the next. To determine how well these variations in speed and attenuation coefficient reflect variations in the wound healing process requires a direct comparison between the ultrasonic and biochemical measurements.

Correlation between ultrasonic and biochemical results

Ultrasonic speed and attenuation coefficient for each specimen were correlated with tissue water, total collagen, and acetic acid soluble collagen concentration of the same specimens. The significant ($p < 0.05$) linear correlations determined for the *wound tissue alone* are listed in Table 1. Ultrasonic speed in the wound was significantly correlated with all three measures of collagen concentration. The regression equation with collagen as a percent of DDF weight explained the greatest amount (20%) of the variance in speed, but it was only slightly (1 to 3%) better than the equation using total collagen (as a percent of wet weight) or acid soluble collagen.

The data in Table 1 also show that the attenuation coefficient in the *wound tissue alone* was significantly correlated with collagen concentration when expressed as a percent of DDF weight and with acid soluble collagen concentration. The correlation between attenuation coefficient and collagen concentration (percent wet weight) was not very strong, and the equation explained only 15% of the variance in the attenuation coefficient. The relationship between attenuation coefficient and acid soluble collagen concentration in the wound was stronger ($r = 0.73$).

The significant correlations between the ultrasonic and biochemical measurements when *both wound and control skin* were included in the analysis are given in Table 2. Speed and attenuation coefficient were all negatively correlated with tissue water concentration. This is clearly shown in Fig. 5. The best fit relationship between speed or attenuation coefficient and water concentration was linear. Speed and attenuation coefficient were all significantly correlated with the collagen concentration (expressed either as a percent of wet or DDF weight) in the control skin and wound as clearly shown in Fig. 6. The relationship between both ultrasound measurement and collagen as a percent of DDF weight was nonlinear.

Precision of analysis of duplicate samples

The precision of the biochemical analyses based on duplicate control skin values was ±2.8% for water,

Table 1. Wound tissue only: correlations between ultrasonic speed (c), or attenuation coefficient (A) and biochemical measurements.

Variables	Regression Equation	r	Significance
Speed, m/s vs.:			
Collagen, % wet weight (CW)	$c = 2.53CW + 1539$	0.41	0.009
Collagen, % DDF weight (CD)	$c = 0.90CD + 1521$	0.45	0.005
Collagen, % acid soluble (CS)	$c = 1.43CS + 1554$	0.43	0.017
Attenuation coefficient, dB/mm vs.:			
Collagen, % DDF weight (CD)	$A = 0.29CD + 19.21$	0.37	0.036
Collagen, % acid soluble (CS)	$A = 0.94CS + 27.10$	0.73	<0.001

±3.5% for total collagen, and ±12.6% for acid soluble collagen determinations (Riederer-Henderson et al. 1988). If wound tissue values were included in the statistical analysis, a measure of the reproducibility of duplicate wounds as well as control tissue on the same dog was obtained. These were ±3.9% for water, ±8.5% for total collagen, and ±26.4% for acid soluble collagen determinations. The large variation in acid soluble collagen related in part to the relatively small values from which the absolute percent differences were calculated (Table 1 in Forster et al. 1990), and in part to the differences in the rate of repair between the duplicate wounds.

The precision (worst case) of speed and attenuation coefficient measurements for duplicate samples of control skin was ±1.7% and ±16%, respectively. Precision (worst case) for measurements of duplicate wounds was ±1.6% for speed and ±24% for attenuation coefficient. The details of these measurements which deal with the uncertainty of SLAM measurements are presented elsewhere (Steiger et al. 1988). The control skin is more heterogeneous overall than wound tissue; however, the precision of the measurement in control skin is not affected by differences in rate of wound healing.

DISCUSSION

One important observation in the present study is the variation of acoustic propagation properties as a function of distance from the wounds. A consistent feature of the acoustic data is the distinction between control skin (2–3 cm from wound) vs. adjacent skin (0.3 mm from the wound) vs. wound tissue (Figs. 3 and 4). Control skin values for speed and attenuation coefficient are consistently greater than adjacent skin values which are in turn consistently greater than wound values regardless of wound age. One explanation for this phenomenon is that adjacent skin samples were influenced by tissue edema, collagenase, or other aspects of the repair and remodeling process whereas control skin samples were far enough from the wound that they were not affected.

Another observation is that reliability measurements for duplicate samples show speed measurements to be considerably more precise than attenuation coefficient measurements. The precision of measurement for speed in duplicate samples of the control skin (worst case assumption for errors) is ±1.7%, whereas that for the attenuation coefficient is ±16%. This can be seen graphically when biochemical data from all seven animals used in past and present studies are plotted as a function of the acoustic propagation properties (Figs. 5 and 6). One sees substantially more scatter around best fit lines for attenuation coefficient than the lines for speed.

Attenuation coefficient was more strongly correlated with acetic acid soluble collagen concentration ($r = 0.73$) than was speed. Acetic acid soluble collagen has relatively little intermolecular cross-linking

Table 2. Wound and control skin: significant best fit correlations between ultrasonic speed (c), attenuation coefficient (A), and biochemical measurements.

Variables	Regression Equation	r	Significance
Speed, m/s vs.:			
% Water (W)	$c = -4.43W + 1893$	−0.75	<0.001
Collagen, % wet weight (CW)	$c = 5.34CW + 1516$	0.68	<0.001
Collagen, % DDF weight (CD)	$c = 0.023(CD)^2 + 1516$	0.67	<0.001
Attenuation coefficient, dB/mm vs.:			
% Water (W)	$A = -1.40W + 138.2$	−0.68	<0.001
Collagen, % wet weight (CW)	$A = 2.00CW + 14.1$	0.73	<0.001
Collagen, % DDF weight (CD)	$A = 1.00 \times 10^{-4}(CD)^3 + 22$	0.73	<0.001

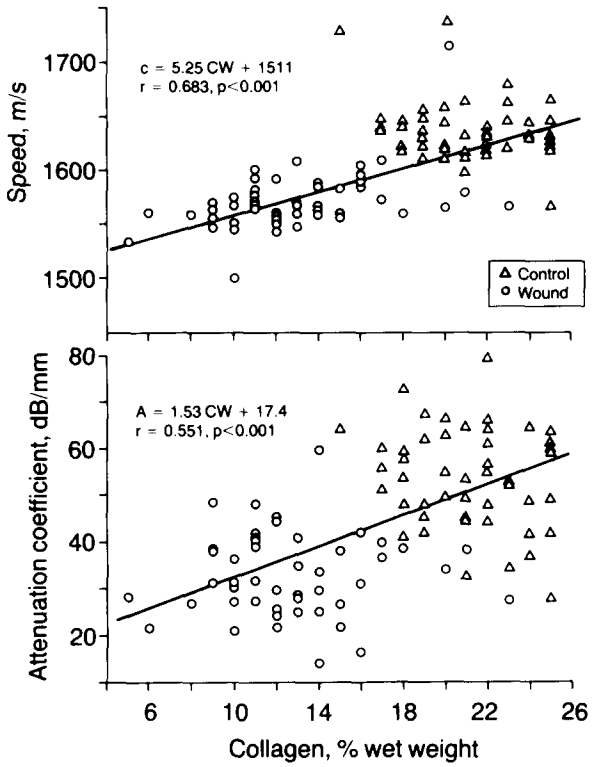

Fig. 5. Scatter plots and best fit curves for wave speed and attenuation coefficient as a function of total collagen concentration (percent wet weight) for wound tissue (circles) and control skin (triangles). The equations which describe the best fit line and the correlation coefficient of the fit are shown as well. There appears to be less scatter around the line for wave speed vs. total collagen.

compared to more mature collagen. We have also observed that breaking intermolecular cross-links using a protease (Starkey et al. 1977; Burleigh 1977) in a well characterized cartilage model (Chun et al. 1986; Schmidt et al. 1987) results in a dramatic change in attenuation coefficient (a large increase) (Agemura et al. 1987). Hence, even though the precision of our method for measuring attenuation coefficient may be relatively low, attenuation coefficient may still be the most sensitive acoustic modality for detecting important changes in collagen configuration in wounds.

The above observations have important implications for making clinical measurements of material properties *in vivo*. While prototype instruments are already available for measurements of attenuation coefficient from backscattered acoustic signals (Forster et al. 1983, 1990), such technology is not yet available for making speed measurements. Curves relating wound age to ultrasonic propagation properties are somewhat flat in the relatively mature wounds studied to date (9 to 50 days), perhaps because of changes in wound architecture which have competing effects on ultrasound properties (*e.g.*, increasing total collagen, which increases attenuation coefficient and increasing cross-links which decrease attenuation coefficient). Hence, it may be difficult to detect changes in normally healing wounds within the time period of the present study. However, such measurements may be clinically useful over earlier time points where tissue water concentration is very high relative to the amount of collagen present. Wounds between 1 and 21 days may show a greater change in attenuation because a large increase in total wound collagen occurs during that time period, but the collagen still has relatively little intermolecular cross-linking.

Both attenuation coefficient and speed consistently demonstrate that their values for wound specimens are less than that for control specimens. This is true not only for the ultrasonic values derived from the SLAM at 100 MHz but also for the integrated attenuation coefficient values derived from the BAT technique (Forster et al. 1990), over the frequency

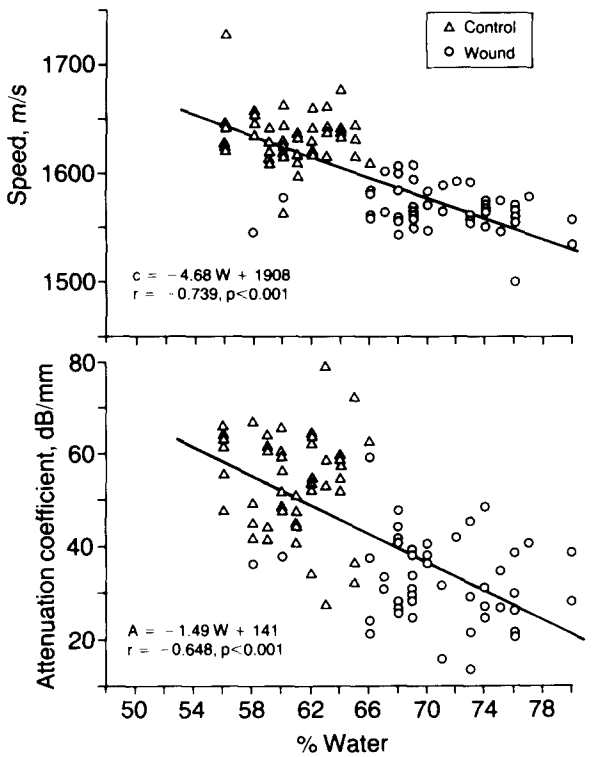

Fig. 6. Scatter plots and best fit curves for wave speed and attenuation coefficient as a function of water concentration for wound tissue (circles) and control skin (triangles). The equations which describe the best fit line and the correlation coefficient of the fit are shown as well. There appears to be less scatter around the line for wave speed vs. total collagen.

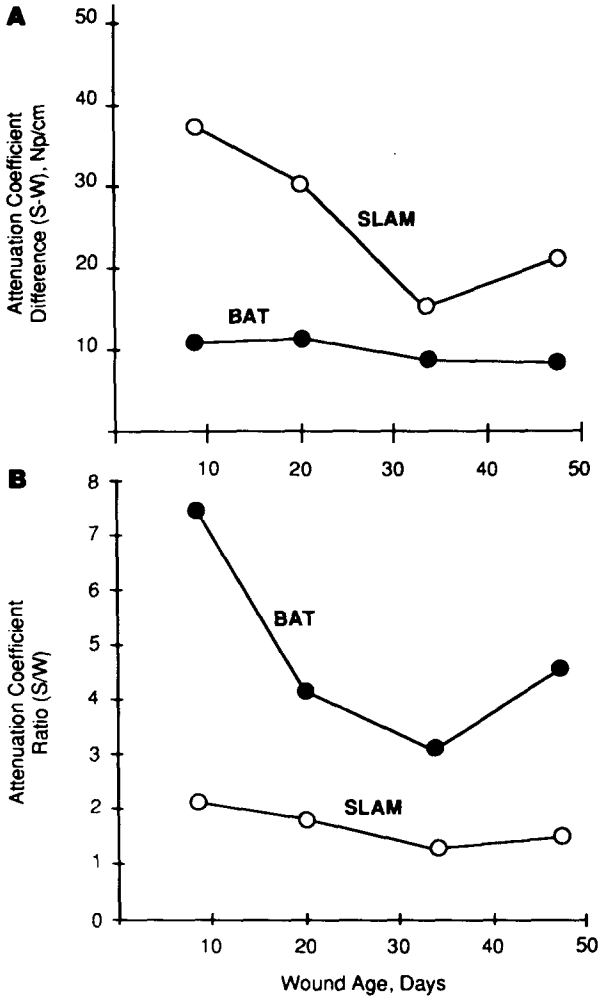

Fig. 7. (A) Differences in attenuation coefficient between control skin and wound tissue for SLAM and BAT plotted as a function of wound age. The general trend appears to be that values for the wound specimens are approaching those for control skin as wound maturation occurs. (B) Ratios for attenuation coefficient values measured by SLAM and BAT [control skin (*s*) divided by wound (*w*), *i.e.*, *S/W*] plotted as a function of wound age. The BAT attenuation coefficient for control skin is 7.4 times greater than wound at day 9 and decreases to 3.1 at day 34, while the SLAM attenuation coefficient for control skin is 2.1 times greater than wounds at day 9 and decreases to 1.3 at 34 days.

range of 10–40 MHz (Fig. 7A). The integrated attenuation coefficient values reported by Forster et al. (1990) were measured for wound and control skin samples obtained at the same time from the same animal evaluated in our study, thus permitting direct comparisons between the two studies.

The temporal trends in the differences between the skin and wound ultrasonic attenuation coefficient for both the SLAM and BAT studies are clearly demonstrated in Fig. 7A. The general trend appears to be that the values for the wound specimens are approaching those for the control skin specimens with wound maturation. The SLAM attenuation coefficient difference decreases from 39 Np/cm to 21 Np/cm and the BAT integrated attenuation coefficient difference decreases from 11 Np/cm to 9 Np/cm. Further, by evaluating the attenuation coefficient ratios of skin to wound values (Fig. 7B), the BAT attenuation coefficient for skin is 7.4 times greater than wound at day 9 and decreases to 3.1 at day 34 while the SLAM attenuation coefficient for control skin is 2.1 times greater than wounds at day 9 and decreases to 1.3 at 34 days.

One would expect to minimize the frequency dependence on attenuation between SLAM and BAT by using their skin/wound ratio (Fig. 7B). However, the differences in attenuation coefficient ratio between the two methods are still considerable. As suggested by Forster et al. (1990), this difference may be related in part to the fact that the acoustic effect of hair was eliminated from the SLAM measurements but not from the BAT.

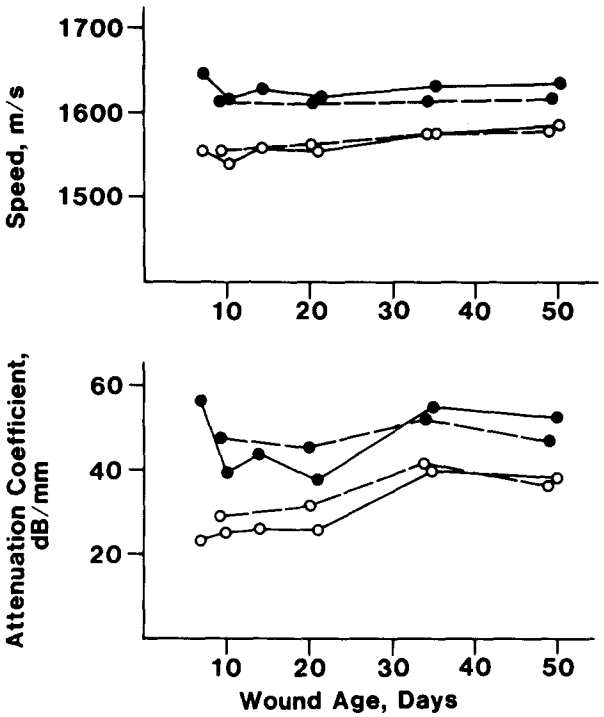

Fig. 8. Plots of mean values for prior study (Olerud et al. 1987) (solid lines) and present study (dashed lines) of ultrasonic wave speed (upper panel) and ultrasonic attenuation coefficient (lower panel) as a function of wound age for wound tissue (open circles) and adjacent skin, within 0.3 mm of wounds (closed circles). The measurements are quite consistent from one study to the next and show that both wave speed and attenuation coefficient appear greater in adjacent skin than in wound tissue.

The results from this study confirm our previous work (Olerud et al. 1987) in a number of important ways. They substantiate our conclusions that both ultrasonic speed and attenuation coefficient are directly correlated with tissue collagen concentration and inversely correlated with tissue water concentration ($p < 0.001$). Good agreement exists between past (Olerud et al. 1987) and present studies for speed and attenuation coefficient values of wounds and adjacent skin at the various time points studied. A plot of the mean values for the two studies of attenuation coefficient and wave speed as a function of wound age demonstrates this point (Fig. 8). Differences in behavior of acoustic propagation properties in skin cannot be distinguished between sections parallel to the epidermis and those oriented perpendicular to the epidermis (cross-section), thus showing the isotropic behavior of ultrasound in skin. Reproducible biochemical measurements of total collagen and acetic acid soluble collagen can be made using this wound model as demonstrated by the reproducibility of matched pairs of wounds from the same animal, wounds of the same age on different animals, and wounds from one series of experiments to the next. Additionally, comparison of the attenuation coefficient made using through transmission measurements at 100 MHz with SLAM and backscattered measurements made at 10–40 MHz with BAT show consistently greater attenuation in skin than wounds and the skin/wound ratio values are greater in earlier than later wounds with both methods.

A long-term goal of this work is to develop acoustic methods capable of making objective noninvasive clinical measurements of material properties for surgical wounds. We believe careful definition of biochemical, morphological, mechanical, and acoustic properties of connective tissues by a collaborative effort of investigators with expertise in each of these disciplines holds the greatest promise of achieving that goal.

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