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Electrical and dielectric properties of bovine trabecular bone—relationships with mechanical properties and mineral density

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Abstract

Interrelationships of trabecular bone electrical and dielectric properties with mechanical characteristics and density are poorly known. While electrical stimulation is used for healing fractures, better understanding of these relations has clinical importance. Furthermore, earlier studies have suggested that bone electrical and dielectric properties depend on the bone density and could, therefore, be used to predict bone strength. To clarify these issues, volumetric bone mineral density (BMD_{vol}), electrical and dielectric as well as mechanical properties were determined from 40 cylindrical plugs of bovine trabecular bone. Phase angle, relative permittivity, loss factor and conductivity of wet bovine trabecular bone were correlated with Young's modulus, yield stress, ultimate strength, resilience and BMD_{vol}. The reproducibility of *in vitro* electrical and dielectric measurements was excellent (standardized coefficient of variation less than 1%, for all parameters), especially at frequencies higher than 1 kHz. Correlations of electrical and dielectric parameters with the bone mechanical properties or density were frequency-dependent. The relative permittivity showed the strongest linear correlations with mechanical parameters ($r > 0.547$, $p < 0.01$, $n = 40$, at 50 kHz) and with BMD_{vol} ($r = 0.866$, $p < 0.01$, $n = 40$, at 50 kHz). In general, linear correlations between relative permittivity and mechanical properties or BMD_{vol} were highest at frequencies over 6 kHz. In addition, a significant site-dependent variation of electrical and dielectric characteristics, mechanical properties and BMD_{vol} was revealed in bovine femur ($p < 0.05$, Kruskall–Wallis H-test). Based on the present results, we conclude that the measurement of electrical and dielectric properties provides quantitative information that is related to bone quantity and quality.

1. Introduction

Osteoporotic fractures and joint diseases are major public health problems inducing vast human and economic burdens on society (Yelin and Callahan 1995). Several diagnostic techniques applying dual energy x-ray absorptiometry (DXA) or ultrasound measurements have been introduced for the evaluation of bone quality (Blake *et al* 1997). Various medical, physiological and nutritional treatments, including electrical stimulation, are used for the treatment of osteoporosis and fractures (Evans *et al* 2001). Although, a previous study suggests that the measurement of bone electrical and dielectric properties may provide a tool for the diagnosis of bone quality (Saha and Williams 1989), little is known about the relations between electrical, mechanical and structural properties of trabecular bone. In fact, even the current definition of osteoporosis is based on one parameter only, i.e. the bone mineral density (BMD) value only (Kanis 1997). Since electrical and dielectric properties of tissue determine the pathways of current flow, a deeper understanding of these properties is crucial for the measurement and analysis of parameters obtained by impedance techniques (Foster and Schwan 1989). In therapeutic applications, detailed knowledge of the interrelationships between the bone density and electrical and dielectric properties is necessary, e.g. for the calculation of electrical field and current distribution during electrical stimulation of bone fracture (Williams and Saha 1996). Obviously, poorly known interrelationships between these properties may diminish the effectiveness of the treatment.

Since the trabecular bone is an inhomogeneous composite material, containing porous bony matrix filled with fluid, and being structurally and mechanically anisotropic, its electrical characteristics are complex (Chakkalakal and Johnson 1981). Several *in vitro* studies have been conducted to clarify the electrical and dielectric properties of either compact, trabecular or whole bone (Freeman 1967, Cochran *et al* 1968, Behari *et al* 1974, Chakkalakal and Johnson 1981, Singh and Saha 1987, De Mercato and Garcia-Sanchez 1988, Saha and Williams 1989). In order to separate the effect of the fluid phase from that of the solid matrix, the studies on dry bone (Garcia-Sanchez and De Mercato 1995) and fluid-saturated tissue (Kosterich *et al* 1983) have been conducted. Those studies have shown that the high-frequency limit of relative permittivity depends on the water content of the tissue, and that the collagen fibres are the main source of dielectric dispersion in a long bone. Conflicting results exist on the interrelationships between bone density and electrical or dielectric properties. In contrast to the findings of De Mercato and Garcia-Sanchez (1988), Williams and Saha (1996) found that there is a statistically significant and positive, linear correlation between the bone density and specific capacitance, which is strongly and positively related to relative permittivity. Besides the composition, the bone structure affects the electrical and dielectric properties. Earlier studies investigating properties of compact and trabecular bone in three orthogonal directions revealed strong electrical and dielectric anisotropy of compact bone (Reddy and Saha 1984) and transversely isotropic nature of cancellous bone (Saha and Williams 1989). In addition to *in vitro* studies, only a few *in vivo* studies exist (Tzukert *et al* 1983, Rubinacci and Brigatti 1984, Skinner *et al* 2001). In a recent *in vivo* study, the bone healing after corticotomy was successfully followed by measuring electrical impedance (Skinner *et al* 2001).

Most earlier studies have focused on the compact bone and, therefore, knowledge on the electrical and dielectric properties of trabecular bone is limited (Saha and Williams 1989). Since most bones are composed of compact and trabecular tissue, knowledge on the electrical and dielectric properties of both components is desirable. The objective of the present study was to investigate relationships between bovine trabecular bone electrical and dielectric properties, mechanical characteristics and mineral density. The electrical measurements were conducted in a wide frequency range, as motivated by the fact that electro-magnetic pulses used in the stimulation of bone healing contain a wide range of frequencies as well. As BMD can be measured with non-invasive techniques such as dual energy x-ray absorptiometry (DXA), information on the interrelationship between electrical and dielectric properties with BMD would enable the estimation of field distribution during electrical stimulation (Williams and Saha 1996). Although, stress-generated electric potentials and piezoeffects have been described earlier (Cochran *et al* 1968, Maeda and Fukada 1982), the electrical and dielectric properties of trabecular bone have not been, to our knowledge, previously compared with the mechanical properties.

2. Materials and methods

2.1. Sample preparation

Cylindrical trabecular bone plugs ($n = 40$, $d = 25.4$ mm, $h = 14.2$ mm) were drilled from the bovine femoral lateral condyle (FLC; $n = 9$), femoral medial condyle (FMC; $n = 11$), femoral head (femoral caput: FC; $n = 10$) and femoral greater trochanter (femoral trochanter major: FTM; $n = 10$) within a few hours post-mortem. An Isomet low-speed diamond saw (Buehler, Lake Bluff, IL, USA) was used for cutting parallel sample faces in the sagittal plane. During the cutting process, the samples were sprayed with phosphate buffered saline (PBS) in order to prevent loss of moisture. Sample orientation, irrespective of the anatomical position, was always medio-lateral. After preparation, the samples were immersed in phosphate buffered saline (PBS), frozen (-20 °C) and finally thawed just prior to measurements. The freeze–thaw cycle has only a minor effect on the trabecular structure (Linde and Sorensen 1993). Similar to other preserving methods, however, it can significantly affect the electrical properties (Saha and Williams 1988). In order to minimize these effects and to improve the comparison between samples, their treatment was carefully standardized. The times for freezing, thawing and measurements were constant for all samples. The humidity during experiment was kept constant.

2.2. Bone density measurements

The areal BMD (g cm^{-2}) was measured with a clinical DXA instrument (Lunar Expert, Lunar Co. Madison, WI, USA). The volumetric BMD (g cm^{-3}) (BMD_{vol}) was calculated by dividing the areal BMD by the sample thickness measured with a micrometer (Mitutoyo, Kawasaki, Japan).

2.3. Electrical measurements

The electrical and dielectric parameters were measured by applying electrical current on samples through two round ($d = 30$ mm) stainless-steel (AISI316L) electrodes using an LCR meter (HIOKI 3531 Z HiTester, Koizumi, Japan) (figure 1). To minimize measurement artefacts, the electrical examination was conducted in a Faraday cage. In order to provide a good contact between the electrodes and the sample, a thin layer of conducting gel was applied to the sample surface. To avoid drying of the sample, known to significantly affect electrical properties (Saha and Williams 1995), the time between the removal of a sample from the PBS and the end of the measurements was minimized and kept constant (2 min). In addition, the samples were moistened with PBS just before measurements. In order to minimize the effects of stray capacitance, inductance of the leads or leakage conductance, short- and open-circuit corrections were determined (Kosterich *et al* 1983). To investigate the reproducibility of electrical measurements, one sample was repositioned and measured nine times with all frequencies.

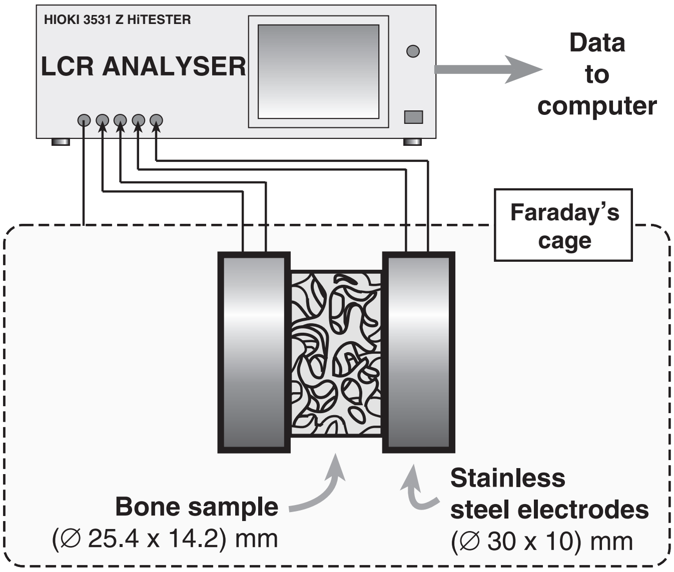

Figure 1. Measurement set-up for the determination of bone electrical and dielectric properties. The electrical and dielectric properties were investigated by applying electrical current to samples through two round ($d = 30$ mm) stainless steel (AISI316L) electrodes using an LCR meter (HIOKI 3531 Z HiTester, Koizumi, Japan). Electrical measurements were conducted in a Faraday cage.

The modulus of impedance, parallel resistance (R), parallel capacitance (C) and phase angle (θ) were measured as a function of frequency (52 frequencies, from 20 Hz to 5 MHz). From these measurements, the relative permittivity (ε_r'), the loss factor (ε_r'') and the conductivity (σ') were determined by using the following formulae (3)–(6), respectively (MacDonald 1987, Grimnes and Martinsen 2000). Admittance ($\bar{Y}$) is related to parallel capacitance and parallel resistance as follows:

$$\bar{Y} = G + \mathrm{j}B = \frac{1}{R} + \mathrm{j}\omega C \tag{1}$$

where G is the conductance, B is the susceptance and $\omega = 2\pi f$ is the angular frequency. Complex relative permittivity ($\bar{\varepsilon}_r$) can be determined as follows:

$$\bar{\varepsilon}_r = \varepsilon_r' - \mathrm{j}\varepsilon_r'' = \frac{\bar{Y}}{\mathrm{j}\omega C_e} \tag{2}$$

thus

$$\varepsilon_r' = \frac{C}{C_e} \tag{3}$$

and

$$\varepsilon_r'' = \frac{1}{R\omega C_e} \tag{4}$$

where $C_e = \varepsilon_0 A_C/l$ is the capacitance of an empty measuring cell, i.e. when the sample was removed (A_C is the electrode area, l is the electrode separation length and ε_0 is the permittivity of free space). The complex conductivity can be determined as follows:

$$\bar{\sigma} = \sigma' + \mathrm{j}\sigma'' = \mathrm{j}\omega\bar{\varepsilon}_r\varepsilon_0 \tag{5}$$

thus

$$\sigma' = \frac{\varepsilon_0}{RC_e}. \tag{6}$$

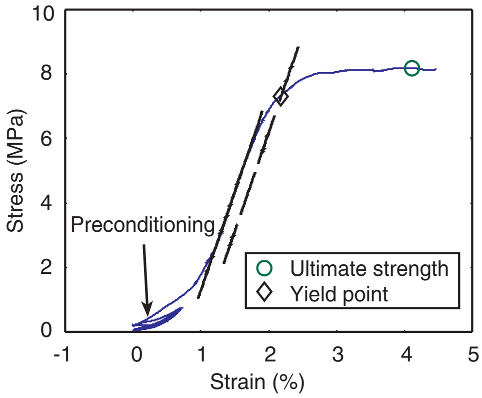

Figure 2. At the beginning of the destructive test, a small preload (0.13 MPa) was applied to a sample, which was then preconditioned by loading and unloading the sample five times to 0.7% strain. Subsequently, a 4.5% strain (destructive) was applied to the sample with the strain rate of 0.35×10^{-3} s^{-1}. Young's modulus was determined as a slope of the fit (——) to the linear part of the stress–strain curve (i.e. to data between 40 and 65% of ultimate strength). The line (– – –) parallel to the linear fit was calculated with an offset strain of 0.2%. The yield point (◇) was determined as the intersection between the calculated line and the stress–strain curve. The ultimate stress (○) was determined as the maximum stress recorded during the experiment.

2.4. Biomechanical methods

A destructive compressive test was used to determine the mechanical properties of the samples (figure 2). Prior to the examination, a smaller plug ($n = 40$, $d = 19$ mm, $h = 14.2$ mm) was drilled from the centre of the original sample in order to ensure the capability of our 12.5 kN servo-hydraulic material testing device (Matertest Oy, Espoo, Finland) to conduct the tests. The samples were immersed in PBS during tests. To begin with, 0.13 MPa prestress was applied for 2 min to samples. Next, the samples were preconditioned non-destructively with five consecutive cycles to 0.7% strain. Subsequently, the samples were destructively compressed to 4.5% strain with the strain rate of 0.35×10^{-3} s^{-1}. Young's modulus, yield stress, ultimate strength and resilience were determined. Young's modulus was determined as a slope of linear fit to data between 40 and 65% of maximum stress of the stress–strain curve. The yield stress was determined using the offset method by calculating a line parallel to the tangent of the stress–strain curve (between 40 and 65% of maximum stress) with a strain offset of 0.2% and determining the intersection between the calculated line and the stress–strain curve (Turner and Burr 1993). The ultimate compressive strength was determined as the maximum stress recorded during the test. The resilience was calculated as the area under the stress–strain curve to the point of yielding. For the data analysis, a customized analysis function was programmed using Matlab 5.3 (The Mathworks Inc, Natick, MA, USA).

2.5. Statistical analysis

The Kruskall–Wallis H-test was used to investigate the significance of site-dependent variation of parameters. The reproducibility of all electrical and dielectric properties was determined in terms of standardized coefficient of variation (sCV%) obtained by dividing the coefficient of variation (CV) by 4SD/mean of all samples (Njeh *et al* 2000). The linear correlation coefficients were determined by using the Pearson correlation analysis. SPSS 8.0 software (SPSS Inc., Chicago, IL, USA) was used in the statistical analyses.

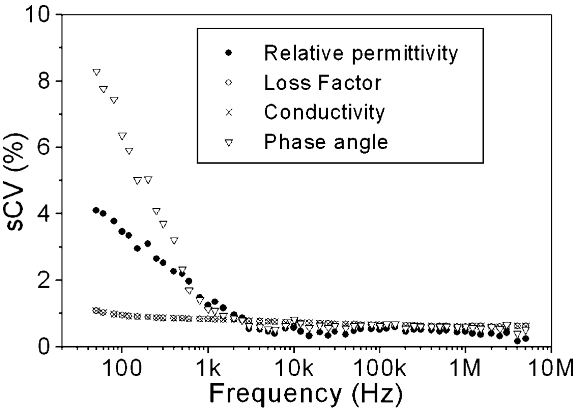

Figure 3. Standardized CV (sCV (%)) of measured electrical and dielectric parameters as a function of frequency. At frequencies over 1 kHz, sCV was less than 1% for all electrical and dielectric parameters.

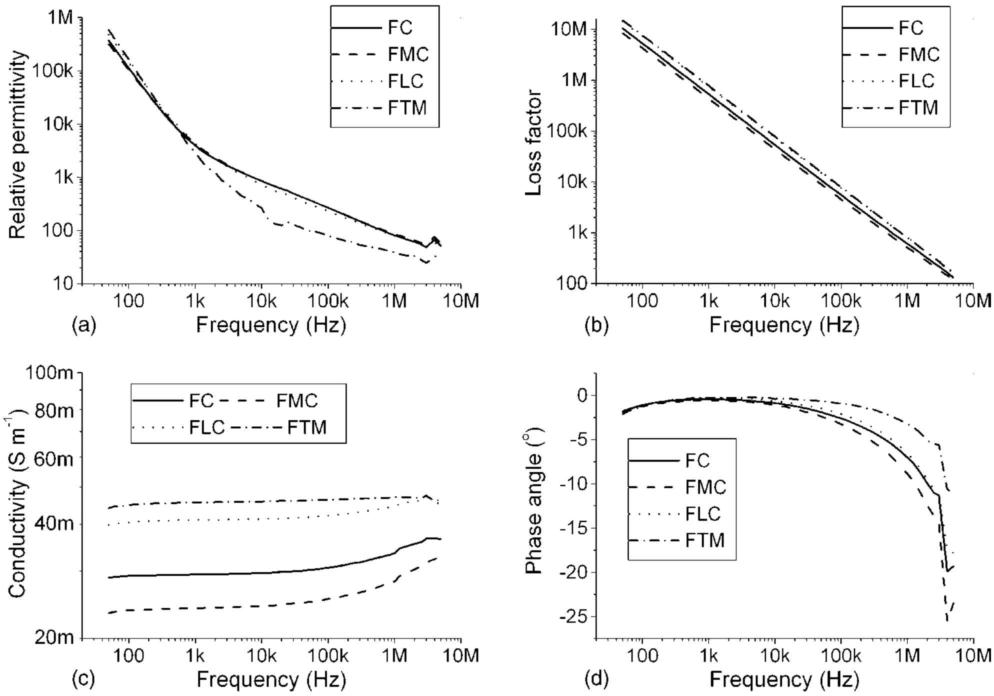

Figure 4. The electrical and dielectric properties of bovine trabecular bone at different anatomical locations as a function of frequency: relative permittivity (a), loss factor (b), conductivity (c) and phase angle (d). For measurement sites, see section 2 .

3. Results

Measurements of trabecular bone electrical and dielectric properties were highly reproducible over a wide range of frequencies (figure 3). At frequencies over 1 kHz, sCV was less than

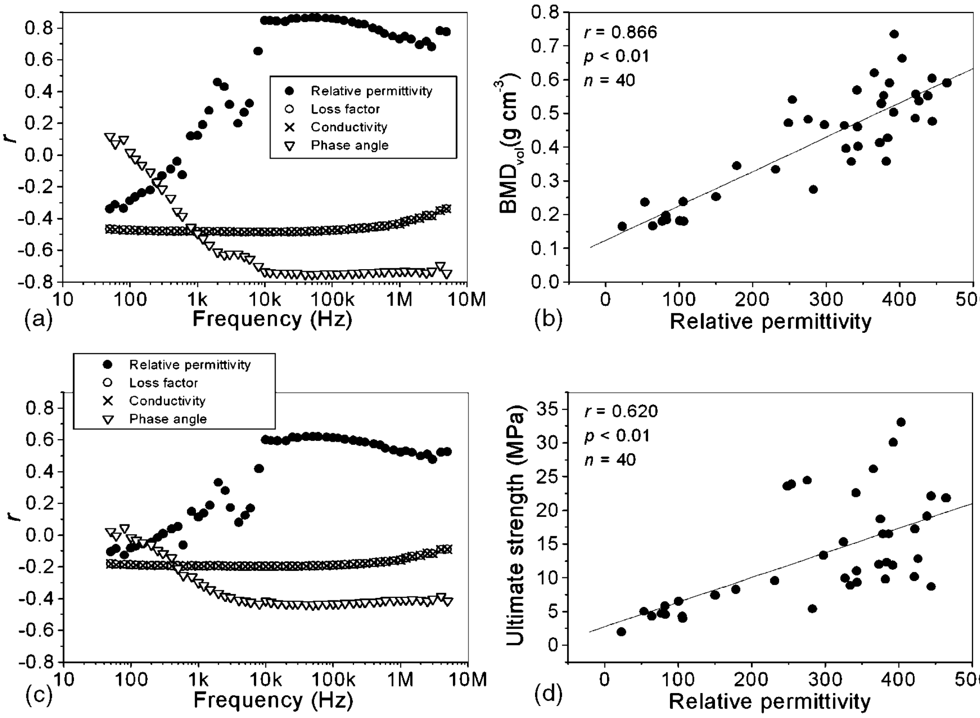

Figure 5. The linear correlation coefficients (r) between BMD_{vol} and the electrical and dielectric parameters (a) and between ultimate strength and electrical and dielectric properties (c) as a function of frequency. The highest correlations ((b), (d)) were revealed with the relative permittivity at the frequency of 50 kHz.

Table 1. Mean values (±SD) of the electrical and dielectric properties at 50 kHz, the mechanical properties and BMD_{vol} of bovine femur trabecular bone at different anatomical sites (for measurement sites, see section 2).

	All ($n = 40$)	FC ($n = 10$)	FMC ($n = 11$)	FLC ($n = 9$)	FTM ($n = 10$)
Relative permittivity	290 ± 130	381 ± 60	380 ± 50	300 ± 70	85 ± 35
Loss factor (10^3)	13 ± 5.2	11 ± 2.4	9 ± 2.7	15 ± 6.5	17 ± 4.5
Conductivity (10^{-2} S m^{-1})	3.6 ± 1.4	3.0 ± 0.7	2.5 ± 0.8	4.2 ± 1.8	0.6 ± 1.3
Phase angle (deg)	−1.6 ± 1.1	−2.1 ± 0.5	−2.6 ± 0.7	1.4 ± 0.8	−0.3 ± 0.1
Young's modulus (MPa)	1200 ± 800	2100 ± 500	1000 ± 300	1200 ± 800	420 ± 110
Yield stress (MPa)	12.4 ± 7.3	21.7 ± 4.7	11.3 ± 2.7	12.1 ± 6.5	4.7 ± 1.4
Ultimate strength (MPa)	13.3 ± 7.9	23.3 ± 5.3	12.4 ± 3.2	12.8 ± 6.7	4.8 ± 1.5
Resilience (10^4 J m^{-3})	9.0 ± 5.5	15.7 ± 3.6	8.3 ± 2.5	8.9 ± 4.7	3.2 ± 1.5
BMD_{vol} (g cm^{-3})	0.418 ± 0.158	0.586 ± 0.075	0.489 ± 0.069	0.389 ± 0.068	0.198 ± 0.032

1% for all electrical and dielectric parameters. Below 1 kHz, the reproducibilities for the measurements of phase angle and relative permittivity deteriorated significantly.

The electrical and dielectric properties were strongly dependent on the frequency (figure 4). The relative permittivity showed the strongest linear correlations with the mechanical properties ($r > 0.547$, $p < 0.01$, $n = 40$, at 50 kHz) and with BMD_{vol} ($r = 0.866$, $p < 0.01$, $n = 40$, at 50 kHz). In general, at frequencies over 6 kHz, the linear

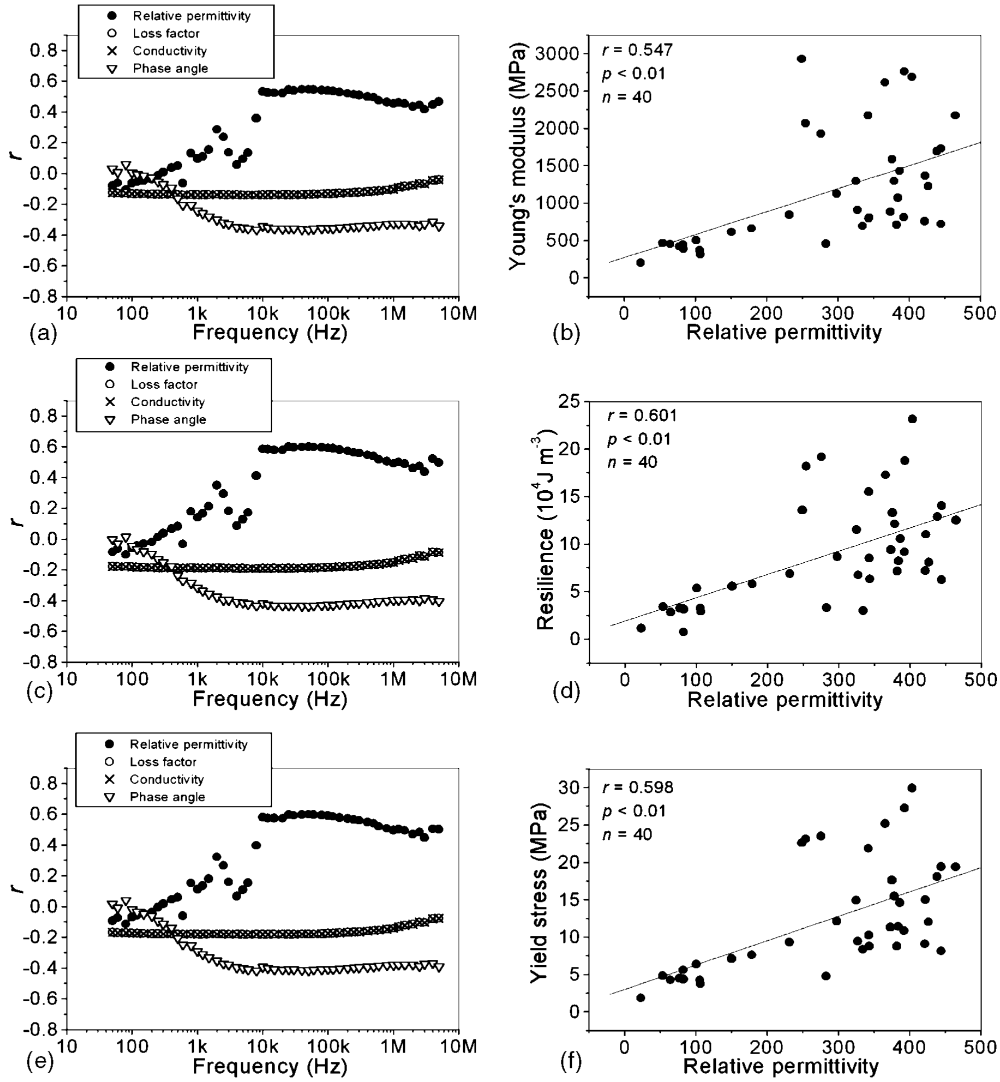

Figure 6. The linear correlation coefficients (r) between the electrical and dielectric parameters and Young's modulus (a), the resilience (c) and the yield stress (e) as a function of frequency. The highest linear correlations ((b), (d) and (f)) were revealed with the relative permittivity at the frequency of 50 kHz.

correlations between relative permittivity and BMD_{vol} or mechanical properties were highest, even though still dependent on frequency (figures 5 and 6).

BMD_{vol}, mechanical properties and electrical and dielectric characteristics showed significant site-dependent variation ($p < 0.05$, Kruskall–Wallis H-test, table 1, figure 4). At a frequency of 50 kHz, the relative permittivity reached the highest value (mean $\pm$ SD, 381 $\pm$ 60) in the femoral head and the lowest (85 $\pm$ 35) in the greater trochanter. The highest BMD_{vol} (0.586 $\pm$ 0.075 g cm^{-3}) was also found in the femoral head and the lowest (0.198 $\pm$ 0.032 g cm^{-3}) in the greater trochanter. Similarly, all mechanical properties reached the highest and the lowest values in the femoral head and greater trochanter, respectively.

4. Discussion and conclusions

In the present study, electrical, mechanical and density measurements were conducted for the same bovine trabecular bone samples. The electrical measurements showed an excellent reproducibility at frequencies higher than 1 kHz, whereas at frequencies below 1 kHz, the reproducibility of the measurements declined. This was probably due to the shortcomings of the technique arising especially from the polarization of electrode contacts (Gabriel *et al* 1996a, b).

New samples were drilled from the original plugs in order to ensure mechanical testing within the loading limits of the device. This was done carefully, e.g. paying special attention to heating, contamination and mechanical damage. Using the present technique, we have earlier shown high linear correlations between the mechanical properties and BMD (Töyräs *et al* 1999, 2002). These results suggest that the specimens for mechanical testing were not damaged during processing.

In the present study, parallel with an earlier investigation (Williams and Saha 1996), both relative permittivity and phase angle showed a strong linear correlation with BMD_{vol}. However, our study suggests an even stronger relation between the relative permittivity and density ($r = 0.866$, at 50 kHz) than that revealed in the previous study ($r = 0.617$, at 100 kHz). Possibly, this small difference may be related to the use of bovine femoral bone, instead of human tibial bone, studied by Williams and Saha (1996). Bovine trabecular bone is known to be denser and stiffer than human bone (Jiang *et al* 1998, Töyräs *et al* 2002). Initially, De Mercato and Garcia-Sanchez (1988) found that the relative permittivity decreased with increasing compactness of bovine bone, i.e. they proposed a negative correlation between the bone mineral density and relative permittivity. That result contradicts our present findings, as well as those of Williams and Saha (1996), on a positive density–permittivity relation.

Our data revealed that in two samples from different anatomical sites and with similar BMD_{vol} value, permittivity is also highly similar. The values of conductivity, however, are different. Obviously, the conductivity is affected by different factors from permittivity, and different properties of bone are reflected by these two electrical parameters. The values of electrical properties for wet trabecular bovine bone, as determined in our experiments, were between the values reported earlier for compact bone and those for bone marrow (Smith and Foster 1985, Gabriel *et al* 1996a). Actually, the value of conductivity in bone with high BMD approaches the value of compact bone.

Based on the discussion above, we assume that both phases (mineralized tissue and marrow) contribute to the results. From the data obtained in the present study, however, it is not possible to determine the quantitative contribution of each phase, especially in a wide frequency range. The simple rule of mixture for trabecular bone matrix with physiological solution or bone marrow as an inclusion cannot be applied successfully either for conductivity or for permittivity. The effect of the wet phase in the composite structure of bone is smaller than the estimate based on the amount and the values of the separated wet phase (Kosterich *et al* 1984). For example, the conductivity of the bone is only 1% of the value of the immersion solution, although the content of the wet phase is 15–20% in volume. In the frequency range used by us, other factors, such as microstructure or polarization by diffusional processes in double layers surrounding charged interfaces, may play a major role as discussed by Kosterich *et al* (1983). Measurements of the electric properties of wet bone with and without marrow could provide more evidence about the role of the wet phase in the electrical properties of trabecular bone.

Phase angle, loss factor and especially conductivity were poorer estimates of bone density or mechanical properties than the relative permittivity. The conductivity is mainly a consequence of ion movements through the fluid phase (Kosterich *et al* 1983) and may change without any change in mineral density (Williams and Saha 1996).

The absolute values of electric and dielectric parameters determined in this study were in agreement with earlier studies (Saha and Williams 1989, Gabriel *et al* 1996a). Both absolute values of electrical and dielectric parameters as well as the correlations between the mechanical properties or BMD_{vol} with relative permittivity were significantly dependent on the frequency. At frequencies over 6 kHz, the relative permittivity predicted well both the mechanical properties and the BMD_{vol}. The site-dependent variation of electrical and dielectric parameters was revealed. This variation may be related to differences in tissue structure (e.g. size and orientation of trabeculae) as well as to differences in spatial distribution and density of the liquid phase (Kosterich *et al* 1983, De Mercato and Garcia-Sanchez 1988, 1992). The variation can also be affected by detected differences in the density of tested bone samples.

The electrical parameters account for about 30% of the variation in mechanical properties (ultimate strength, Young's modulus), as measured in the present study. Our mechanical analyses characterized mainly the elastic properties of trabecular bone. Possibly, the correlations might be even higher with some viscoelastic parameters of bone. Although the electrical parameters do not characterize mechanical properties of bone perfectly, they could still be used in some special cases, e.g. open surgery. Based on our data, we found that the electric parameters predicted mechanical characteristics of bovine bone better than the broadband ultrasound attenuation (BUA) measurements (Hakulinen *et al* 2002), which are known to be feasible in osteoporosis diagnostics, and predict risk of fracture for elderly people as effectively as BMD measurements (Kroger *et al* 1995, Schott *et al* 1995, Pfeifer *et al* 1997).

The significant linear correlations of trabecular bone density and mechanical properties with the relative permittivity suggest that measurement of electrical and dielectric properties may provide the means for quantitative diagnostics of bone status. Our results indicate that the measurement frequency of 50 kHz could be an optimal choice in diagnostic measurements. The correlations between the mechanical parameters and BMD_{vol} were higher than those for electrical parameters. Electrical parameters may not provide more information on the bone strength or stiffness than that obtained with DXA. They may offer some complementary data on bone quality and structure to supplement DXA measurements. In special cases, such as during open surgery, e.g. during bone grafting or total hip replacement, there is no method for direct measurement of bone quality. Electrical measurements utilizing open-ended probe technology (Whit Athey *et al* 1982) are potentially feasible in this kind of application. In addition, better understanding of the relationships between bone density, mechanical, electrical and dielectric properties may help us to improve techniques related to electrical stimulation and follow-up of healing processes in fractured bone (Williams and Saha 1996).

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