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Determination of electrophysical and structural properties of human cancellous bone and synthetic bone substitute material using impedance spectroscopy and X-ray powder diffraction

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Electrophysical stimulation is used to support fracture healing and bone regeneration. For design optimization of electrostimulative implants, in combination with applied human donor bone or synthetic bone scaffolds, the knowledge of electrophysical properties is fundamental. Hence further investigations of the structural properties of native and synthetic bone is of high interest to improve biofunctionality of bone scaffolds and subsequent healing of the bone defect. The investigation of these properties was taken as an objective of this study. Therefore, surgically extracted fresh cylindrical and consecutively ashed cancellous bone samples from human osteoarthritic femoral heads were characterized and compared to synthetic bone substitute material. Thereby, impedance spectroscopy is used to determine the electrophysical properties and X-ray powder diffraction (XRD) for analysis of structural information of the bone samples. Conductivity and permittivity of fresh and ashed cancellous bone amounted to $1.7 \cdot 10^{-2}$ S/m and $7.5 \cdot 10^{6}$ and $2 \cdot 10^{-5}$ S/m and $7.2 \cdot 10^{3}$, respectively. Electrical conductivity and dielectric permittivity of bone scaffold resulted in $1.7 \cdot 10^{-7}$ S/m and 49. Analysis of the structural properties showed that the synthetic bone scaffolds made of Brushite exhibited some reflections which correspond to the native bone samples.

The information in present study of the bone material (synthetic and autologous) could be used for later patient individual application of electrostimulative implants.

Key words: *human cancellous bone, synthetic bone, properties, impedance spectroscopy, X-ray powder diffraction*

1. Introduction

Electrophysical stimulation is used to support fracture healing and bone regeneration, e.g., in the case of neck fracture of the femur, non-union or avascular necrosis of the femoral head [16], [22]. Thereby, a bipolar induction screw system (BISS) can be used based on the approach of Kraus–Lechner [16], [22]. A frequency of 20 Hz, an electrical field between 5 and 70 V/m and a voltage of 0.7 V are commonly applied [7], [16], [22], [23]. This stimulation treatment can be combined intraoperatively with insertion of bone cylinders into the affected region [7], [16], [22], [23] of the femoral head, i.e., to repair the defected bone after avascular head necrosis, autologous bone or synthetic bone scaffolds as bone substitute material can be additionally used since surgical treatment [6], [15].

Synthetic bone scaffolds consist mostly of calcium phosphate, i.e., $Ca_5(PO_4, CO_3)_3OH$, (HA) [29]. Human bone consists of 50–60% of hydroxyapatite carbonate (HA), 30–40% of collagen, and 10% of water [6], and shows different mechanical and structural properties. In the long-term design of electrostimulative implants in combination with application of fresh autologous

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bone or synthetic bone scaffolds and knowledge of their biophysical properties is fundamental for patient's individual electrostimulating therapy of bone defects. The electrophysical properties of different materials are mainly described by electrical conductivity and dielectric permittivity [1], [8]–[10].

In a previous study we characterized the electrophysical properties of osteoarthritic bone [13]. However, the electrical and dielectric properties of synthetic bone scaffolds were not included. Furthermore, the mechanical and structural data of bone scaffolds can be involved in computational modeling of electrostimulating implants and treatments. Compositional and structural analyses have been performed using different spectroscopy techniques on various bone types, i.e., Fourier transform infrared microspectroscopy (FTIRM) and neutron-scattering [18], [24]. In previous works we analyzed electric field distribution in combination with various design parameters of electrostimulative implants for avascular femoral head necrosis [27]. Moreover, the investigation of bone substitute materials using high-energy X-ray diffraction (XRD) and impedance spectroscopy is of interest of research to improve biofunctionality of scaffolds and subsequent healing of the bone defect [25]. XRD is used to get structural information and data about the crystalline composition of bone tissue [26], [28]. Measurements were also done on synthetic bone materials and scaffolds [25]. Synthetic bone scaffolds are often made using calcium phosphate to approach the hydroxyapatite constitution of the human bone [31].

Following the results of XRD measurements of native bone, it might be possible to modify and improve structural properties of synthetic bone scaffolds. Hence, explicit information of structural properties of the bone materials is useful for application of patient-specific scaffolds. In this context, the electrophysical properties of native femoral bone, compared to synthetic bone substitute materials, were not measured so far. Moreover, the biophysical and structural properties of native as well as synthetic bone samples have not been investigated simultaneously.

The main goal of this study was to investigate to what extent the qualitative representation of the apatite phase of substitute materials may be a relevant factor in regeneration of bone defects in the case of electrostimulative therapy and whether parts of the apatite phase do correspond between the native and synthetic bone. Therefore, XRD measurements on samples of osteoarthritic human cancellous as well as synthetic bone scaffold were carried out in this study. By means of XRD, the qualitative pattern of the apatite structure of the synthetic bone scaffolds as well as ashed human bone samples were determined. Furthermore, electrical and dielectric properties of a synthetic bone scaffold material (Brushite) in comparison to fresh human cancellous bone samples as well as consecutively after ashing were investigated by impedance spectroscopy.

2. Materials and methods

2.1. Test samples

Human femoral heads of osteoarthritic patients were obtained during the procedure of implantation of hip prosthesis. Cylindrical samples were cut from each femoral head with the use of a diamond hollow drill (Günther Diamantwerkzeuge, Idar-Oberstein, Germany). Six fresh cancellous bone cylinders from different patients, 12 mm in diameter and 4.4 ± 0.3 mm in length were frozen at –20 °C. Before processing and testing, the bone samples were stored in temperature in the range of 6 to 8 °C for 12 hours. The fresh-frozen bone samples and their ashed bone samples (800 °C, 5 h [3]) from six different patients, after the ashing process, resulted to be 10 mm in diameter and 3.8 ± 0.4 mm in length. This study was approved by the local Ethics Committee of the University of Rostock (registration number: A 2009 38). The sample processing is described in detail in the study by Haba et al. [11], [14]. Three different bone scaffold samples (provided by DOT GmbH, Rostock, Germany) 4.4 ± 0.3 mm in length and 12 mm in diameter were investigated. The synthetic bone scaffolds were composed of Brushite, a calcium phosphate mineral ($CaHPO_4{\cdot}2H_2O$) with monoclinic prismatic crystals [30].

2.2. Impedance spectroscopy

For measurement of electrical and dielectric properties of the samples, an impedance spectrometer system (Broadband Dielectric Spectrometer System BDS 4000 – Alpha high-resolution dielectric analyzer, Novocontrol Technologies GmbH & Co. KG., Montabaur, Germany), equipped with a Quatro Cryosystem for temperature control and WinDETA software version 4.1, was used. The calibrating process was prepared before starting the sample measurement using the AIS 100 Ω calibration normal.

According to Maxwell's equations [20], [21], the current density $\vec{J} = \kappa^* \vec{E}$ and dielectric displacement

$\frac{d\vec{D}}{dt} = i\omega\varepsilon^{*}\varepsilon_0\vec{E}$ are equivalent to $\kappa^{*}(\omega) = \kappa'(\omega) + i\kappa''(\omega)$ $= i\omega\varepsilon_0\varepsilon^{*}(\omega)$. The real and imaginary part of electrical conductivity $\kappa^{*}(\omega)$ can be estimated by $\kappa'(\omega) = \omega\varepsilon_0\varepsilon''(\omega)$, $\kappa''(\omega) = \omega\varepsilon_0\varepsilon'(\omega)$.

The bone samples and Brushite scaffolds (provided by DOT, Rostock, Germany) had a mean length of about 4.4 mm, between 4 mm to 4.6 mm, and diameter of 12 mm. The thickness of the fresh bone samples measuring between 4.1 mm and 4.9 mm after the ashing process (800 °C, 5 h) resulted in a mean length of 4.4 mm and with a diameter of 10 mm. According to [3], the bone samples were combusted in a tube furnace (Nabertherm, Lilienthal, Germany) at 800 °C for 5 h. To obtain a better view of a parallel surface the bone samples were plated with gold leaf (Noris Blattgold GmbH, Schwabach, Germany) as shown in Fig. 1.

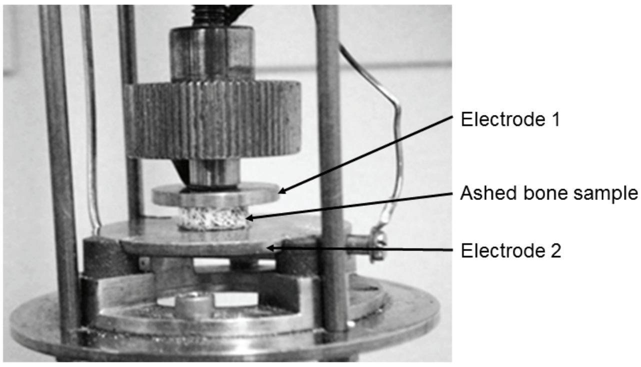

Fig. 1. Impedance spectroscopy setup used for measurement of ashed cylindrical cancellous bone sample, which consisted of two gold plated brass plates, electrode 1 and electrode 2 (without demonstration of Quatro Cryosystem)

The frequency sweeps were performed at the frequency in the range between 0.01 Hz and 0.1 MHz. The synthetic bone scaffolds and the fresh and ashed bone samples were measured at room temperature (20 °C).

2.3. X-ray powder diffraction

High-resolution X-ray powder diffraction patterns of microcrystalline samples were measured with Cu-Kα1 radiation (λ = 1.5406 Å) using a STOE Stadi-P diffractometer in transmission mode with curved Ge monochromator and PSD detector. According to Bragg's law [4] (1) d is the spacing between diffracting planes and θ is the angle between diffracted and incident beam:

$$2\,d\sin\theta = n\,\lambda \qquad (1)$$

λ is the wavelength of the beam and n is an integer for the diffraction order.

The fine-grain powdered, ashed bone samples (Fig. 2), treated at 800 °C for 5 h [3], were placed between two plastic tapes and rotated during the measurements. Additionally, measurements were also done on synthetic bone Brushite samples (fine-grain powders). Measurements were conducted at room temperature (20 °C) with settings of 40 kV and 40 mA for the X-ray generator. Scans were done with steps of 0.01° and 30 sec. measuring time in a 2θ region from 8 to 80°. Data treatment was done with the WinX^{Pow} software (STOE&Cie GmbH, Darmstadt, Germany).

3. Results

3.1. Electrical conductivity and permittivity of fresh and ashed cancellous bone compared to synthetic bone scaffold

The measured electrical (κ' and κ'') and dielectric (ε' and ε''), bone properties were determined. Electri-

Fig. 2. Sample preparation for X-ray powder diffraction; fine powdered ashed bone respective Brushite (left); the placed samples between two plastic tapes in the sample holder (middle and right)

cal and dielectric parameters of the fresh, ashed bone and the bone scaffold samples revealed high standard deviations (Table 1). The calculated real and imaginary part of the electrical and dielectric parameters of $n = 6$ fresh and their ashed cancellous bone samples are shown in Figs. 3 and 4. The mean value and the standard deviation for the electrical conductivity κ' amounted to $1.7 \cdot 10^{-2} \pm 1.5 \cdot 10^{-2}$ S/m (fresh samples) and $2 \cdot 10^{-5} \pm 1.9 \cdot 10^{-5}$ S/m (ashed samples) at the frequency of 20 Hz. Additionally, the relative permittivity ε' at 20 Hz was equal to $7.5 \cdot 10^{6} \pm 7.2 \cdot 10^{6}$ (fresh samples) and $7.2 \cdot 10^{3} \pm 7.3 \cdot 10^{3}$ (ashed samples), respectively (Table 1). Furthermore, the real and imaginary part of the electrical and dielectric parameters of three scaffold bone samples were investigated at a temperature of 20 °C (Fig. 5). The mean value and standard derivation at 20 Hz of the electrical conductivity κ' was found at $1.7 \cdot 10^{-7} \pm 1.9 \cdot 10^{-8}$ S/m and relative permittivity ε' of $4.9 \cdot 10 \pm 6.3$ was determined (Table 1).

Comparison between the electrical conductivity of the fresh and ashed cancellous bone and the bone substitute material, Brushite, showed a difference by about two orders of magnitude. On average, the dielectric parameters of ashed cancellous bone were twenty times higher than those of Brushite.

Table 1. Measured electrical and dielectric parameters in terms of mean ± standard deviation (SD) of the real part of the dielectric permittivity (ε') and conductivity (κ') of $n = 6$ human fresh and their ashed cancellous bone samples and $n = 3$ bone scaffold samples at 20 °C, 20 Hz and 1 V

Electrical and dielectric parameters	Mean ± SD	Range
κ' [S/m] $n = 6$ (fresh cancellous bone)	$1.7 \cdot 10^{-2} \pm 1.5 \cdot 10^{-2}$	$2.2 \cdot 10^{-3}$–$4.2 \cdot 10^{-2}$
κ' [S/m] $n = 6$ (ashed cancellous bone)	$2 \cdot 10^{-5} \pm 1.9 \cdot 10^{-5}$	$2.9 \cdot 10^{-6}$–$5.5 \cdot 10^{-5}$
κ' [S/m] $n = 3$ (bone scaffold: Brushite)	$1.7 \cdot 10^{-7} \pm 0.2 \cdot 10^{-7}$	$1.6 \cdot 10^{-7}$–$2 \cdot 10^{-7}$
ε' $n = 6$ (fresh cancellous bone)	$7.5 \cdot 10^{6} \pm 7.2 \cdot 10^{6}$	$8.9 \cdot 10^{5}$–$2.2 \cdot 10^{7}$
ε' $n = 6$ (ashed cancellous bone)	$7.2 \cdot 10^{3} \pm 7.3 \cdot 10^{3}$	$8.4 \cdot 10^{2}$–$2.1 \cdot 10^{4}$
ε' $n = 3$ (bone scaffold: Brushite)	49 ± 6.3	42–5.4·10

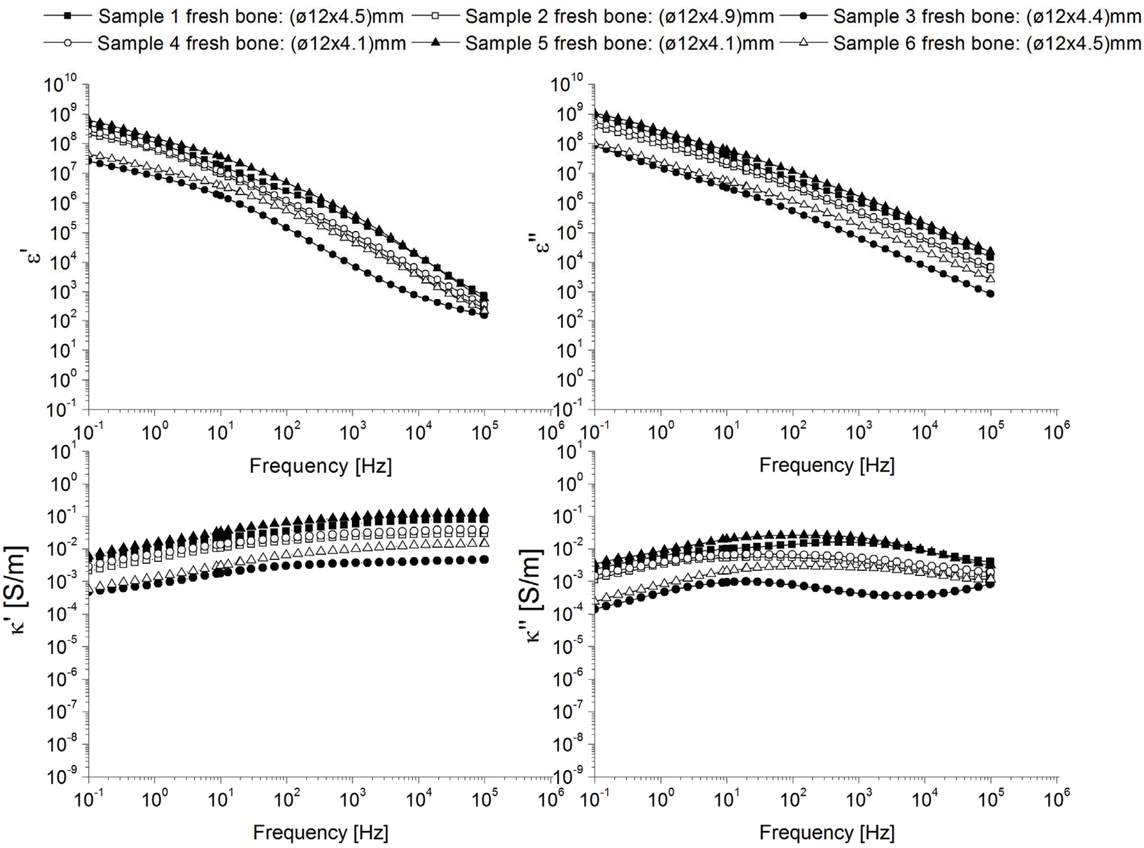

Fig. 3. Real and imaginary part of dielectric permittivity (ε' and ε'', top figures) and conductivity (κ' and κ'', bottom figures) of $n = 6$ fresh cancellous bone samples measured at 20 °C and 1 V (between 0.01 Hz and 0.1 MHz)

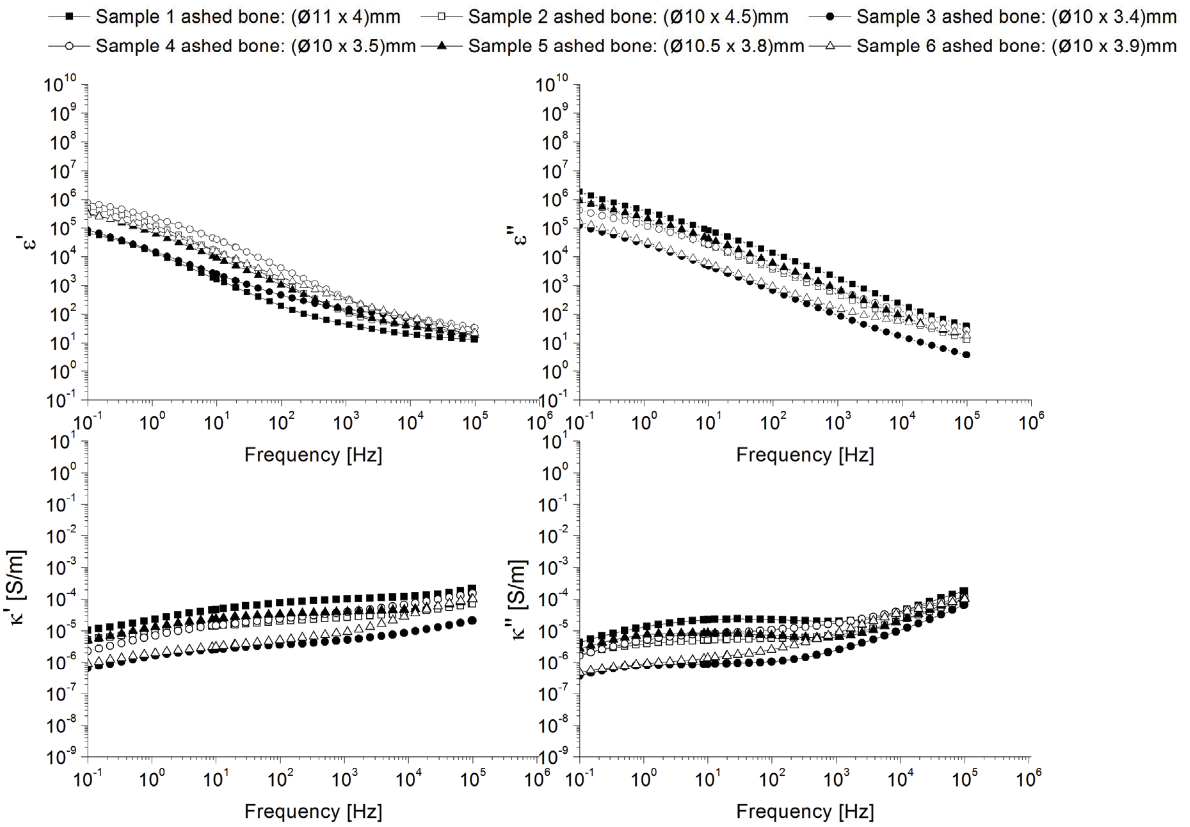

Fig. 4. Real and imaginary part of dielectric permittivity (ε' and ε'', top figures) and conductivity (κ' and κ'', bottom figures) of $n = 6$ ashed cancellous bone samples measured at 20 °C and 1 V (between 0.01 Hz and 0.1 MHz)

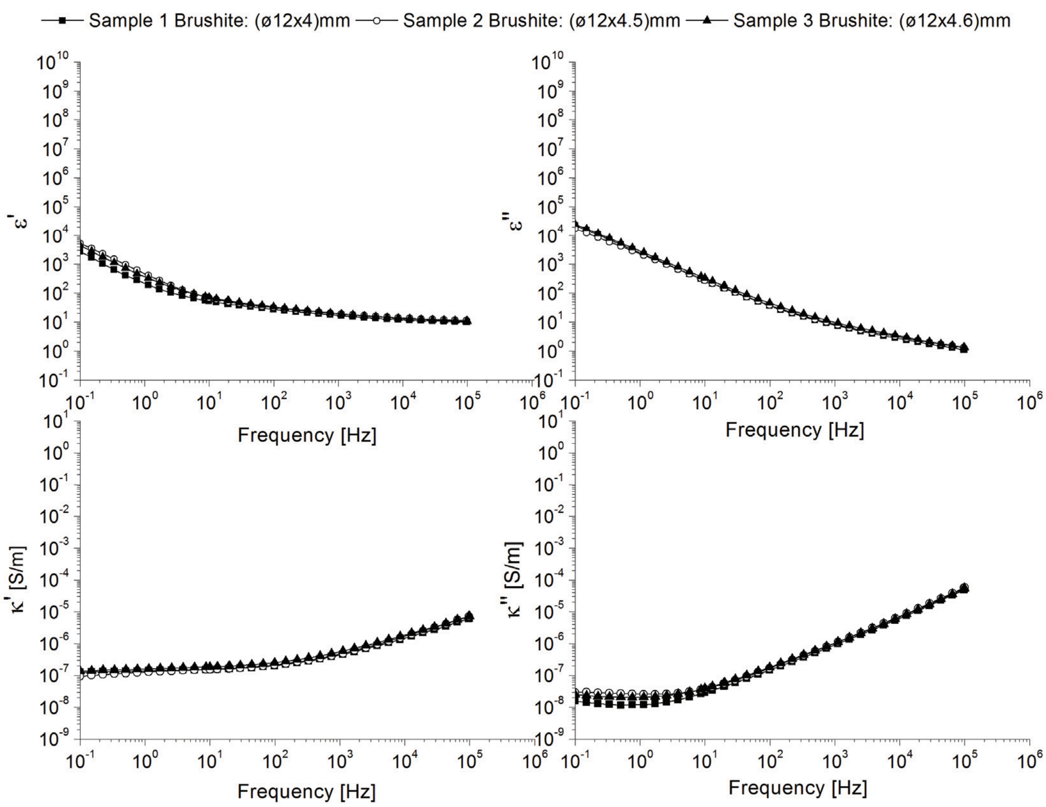

Fig. 5. Real and imaginary part of dielectric permittivity (ε' and ε'', top figures) and conductivity (κ' and κ'', bottom figures) of $n = 3$ cylindrical synthetic bone scaffold Brushite samples measured at 20 °C and 1 V (between 0.01 Hz and 0.1 MHz)

3.2. X-ray powder diffractometry

The X-ray powder diffraction pattern of the ashed cancellous bone sample treated for 5 h at 800 °C is shown in Fig. 6. Most of the peaks of the pattern correspond to those of apatite.

After indexing and refining the reflex positions, the hexagonal cell parameters (space group $P6_3/m$) amounted to values of a = 9.4450(5) and c = 6.8975(4) Å. Few reflexions detected could not be accounted for the hexagonal apatite structure pattern. The strongest ones were at 2θ = 20.83° and 26.61°, but they did not correspond to reflections of the Brushite, Calcium or Magnesium carbonate or oxide structure. Comparison of the pattern of the ashed cancellous bone sample with that of Brushite is shown in Fig. 7. The synthetic bone

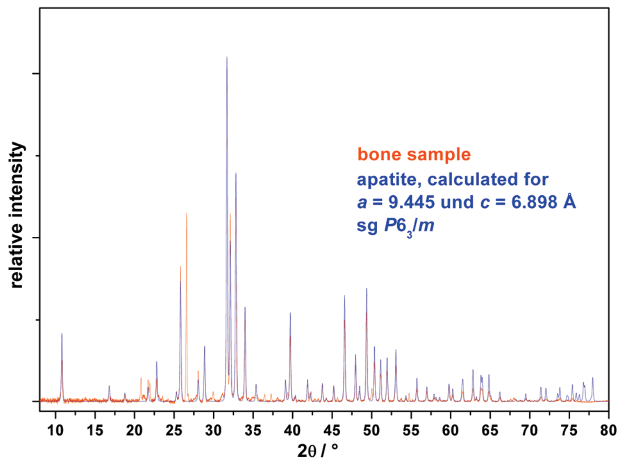

Fig. 6. X-ray powder diffraction pattern of an ashed cancellous bone sample from osteoarthritic human femoral head compared to the calculated pattern of apatite

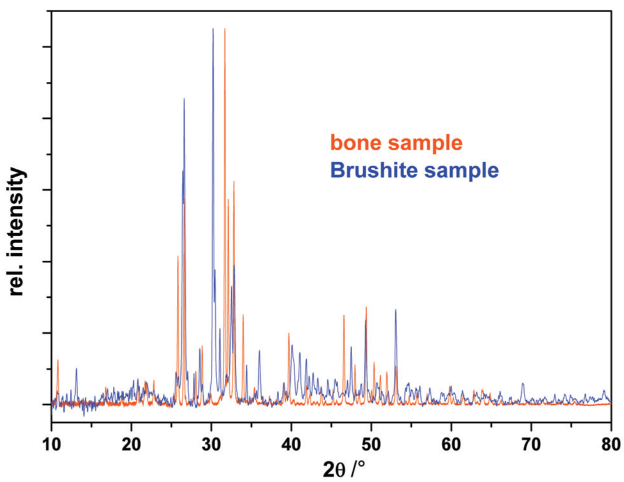

Fig. 7. X-ray powder diffraction pattern of the ashed cancellous bone sample of osteoarthritic human femoral head and that of Brushite phase of the synthetic bone scaffold

scaffold made of Brushite exhibited some reflections which correspond to the native bone sample.

4. Discussion

The goal of this experimental study was to determine the electrical and dielectric properties of synthetic bone material in comparison with fresh one and, consecutively, ashed samples obtained from human osteoarthritic cancellous bone, by means of impedance spectroscopy [13]. Biological structures like bone are heterogeneous systems [2]. Therefore, the bone scaffold and ashed bone tissue were also analyzed by X-ray powder diffraction (XRD) to derive the structural properties with respect to the three-dimensional space groups of inorganic crystals.

Bone is composed of hydroxyapatite (HA) and type I collagen [29]. Ashed bone samples have lost the collagen part during the combustion process at 800°C [19]. Gaseous pyrolysis products react with dioxygen in the combustion zone, resulting in formation of carbon dioxide and water under release of heat. Non-vaporized materials remain as coke [5]. This might result in differences in the electrical and dielectric ashed bone properties, compared to the synthetic bone material. The XRD pattern of our ashed bone samples gives no indication of the existence of graphite-like structures because only the apatite phase was found. However, the comparison with the bone substitute material Brushite shows no complete conformity.

The bone samples (fresh vs. ashed) from six different human donors and three synthetic bone scaffold samples made of Brushite showed high standard deviations within the impedance spectroscopy measurements. Impedance spectroscopy is used to determine electrical conductivity (κ' and κ'') and electrical permittivity (ε' and ε''). The dielectric spectra are consistent with Maxwell–Wagner polarization [17]. The range of frequency spectrum used was derived from our previous study [13]. However, regarding comparison of fresh bone [13] to ashed bone and bone substitute samples, no further numerical description of their electrical and dieletrical properties seems currently possible. The electrical properties, measured at 20 °C for the fresh and the ashed cancellous bone samples show differences at 20 Hz (Table 2). Comparison with the measurements reported by Gabriel et al. in the same range of frequencies [1], [8]–[10] shows good agreement of the electrical and dielectric properties (Table 2).

Table 2. Electrical and dielectric properties of bone samples at 20 Hz and 37 °C from the literature [1], [8]–[10] compared to our test data* at 20 Hz and 20 °C

		κ' in S/m	ε'
Fresh bone	Own n = 3, 20 °C	$1.7{\cdot}10^{-2} \pm 1.5{\cdot}10^{-2}$	$7.5{\cdot}10^{6} \pm 7.2{\cdot}10^{6}$
	Haba et al. [13] n = 20, 37 °C	$4.3{\cdot}10^{-2} \pm 2.4{\cdot}10^{-2}$	$8.1{\cdot}10^{6} \pm 5.2{\cdot}10^{6}$
	Gabriel et al. [1], [8]–10] 37°C	$7.8902{\cdot}10^{-2}$	$4.0202{\cdot}10^{6}$
Ashed bone n = 3, 20 °C		$2{\cdot}10^{-5} \pm 1.9{\cdot}10^{-5}$	$7.2{\cdot}10^{3} \pm 7.3{\cdot}10^{3}$
Synthetic bone (Brushite) n = 3, 20 °C		$1.7{\cdot}10^{-7} \pm 0.2{\cdot}10^{-7}$	49 ± 6.3

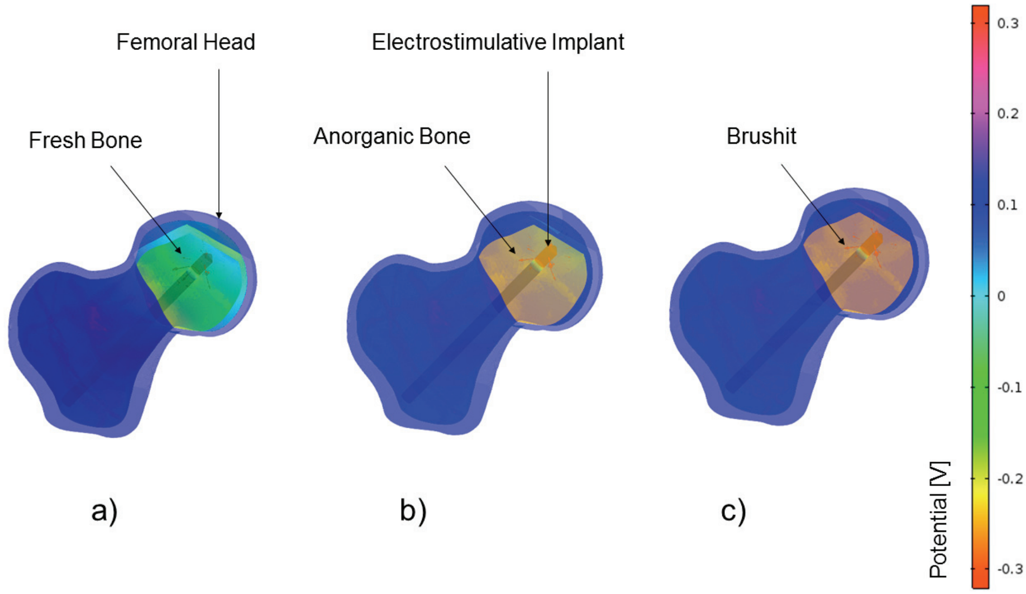

Fig. 8. Femoral head simulation model (potential field) of the implanted BISS screw system based on the Kraus–Lechner system used 20 Hz, 0.7 V and dielectric permittivity ε' and electrical conductivity κ' (Table 1): a) fresh bone, b) inorganic bone (ashed bone), and c) synthetic bone Brushite

The significance of electrical characteristics in computational simulations depends on different boundaries, i.e., the condition of bone stock and the bone substitute material (Fig. 8). Different electrical and dielectric bone parameters lead to various electric potentials in computational simulation. To generate an individual treatment for patients with electrostimulative implants, the determination of electrical and dielectric parameters of osteoarthritic femoral bone is necessary. Electrical and dielectric properties of bone scaffold can be measured non-invasively by impedance spectroscopy, using a bone scaffold sample and invasively, using an apparatus [12] in orthopaedic surgery.

Additionally, it seems possible to introduce the structural details from the XRD analysis in a statistical multiscale model in the future works. Further investigations, i.e., a comparison of normal and degenerated ashed bone, seem useful.

Limitation of the impedance determinations in this study was to determine a small sample size, not 37 °C, composite material collagen-calcium phosphate (Ca-P Collagen) and bone scaffold in blood. There are differences of material properties of the modified osteoarthritic bone comparable with the bone scaffolds (Table 2). It will be useful to compare normal and weak bone and synthetic collagen-calcium phosphate (Ca-P Collagen) scaffolds for treatment of bone defects in further works.

5. Conclusions

The electrical, dielectric, and structural bone parameters derived in the course of this work might be useful for an individual treatment of patients with electrostimulative implants, such as the electromagnetic screw implant adapted to the Kraus–Lechner approach [16], [22] in combination with bone scaffolds. Therefore, *in situ* measurement of electrical and dielectric parameters should be realized in the future, e.g., by means of electrical impedance spectroscopy analysis [12]. Structural parameters of further native bone samples after preparation of *ex vivo* as well as of other bone substitute materials should be analyzed and compared using XRD.

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Disclosure agreement

The authors state that there are no conflicts of interest for any of the authors. No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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