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Investigation of the inverse piezoelectric effect of trabecular bone on a micrometer length scale using synchrotron radiation

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ABSTRACT

In the present paper we have investigated the impact of electro stimulation on microstructural parameters of the major constituents of bone, hydroxyapatite and collagen. Therapeutic approaches exhibit an improved healing rate under electric fields. However, the underlying mechanism is not fully understood so far. In this context one possible effect which could be responsible is the inverse piezo electric effect at bone structures. Therefore, we have carried out scanning X-ray microdiffraction experiments, i.e. we recorded X-ray diffraction data with micrometer resolution using synchrotron radiation from trabecular bone samples in order to investigate how the bone matrix reacts to an applied electric field. Different samples were investigated, where the orientation of the collagen matrix differed with respect to the applied electric field. Our experiments aimed to determine whether the inverse piezo electric effect could have a significant impact on the improved bone regeneration owing to electrostimulative therapy. Our data suggest that strain is in fact induced in bone by the collagen matrix via the inverse piezo electric effect which occurs in the presence of an adequately oriented electric field. The magnitude of the underlying strain is in a range where bone cells are able to detect it.

Statement of Significance

In our study we report on the piezoelectric effect in bone which was already discovered and explored on a macro scale in the 1950. Clinical approaches utilize successfully electro stimulation to enhance bone healing but the exact mechanisms taking place are still a matter of debate. We have measured the stress distribution with micron resolution in trabecular bone to determine the piezo electric induced stress. Our results show that the magnitude of the induced stress is big enough to be sensed by cells and therefore, could be a trigger for bone remodeling and growth.

1. Introduction

Bone defects are critical when the ability of the bone to regenerate itself is disturbed or limited. Clinical diagnoses of bone defects with the necessity to promote bone regeneration are e.g. avascular necrosis of the femoral head, delayed fracture healing or bone tumors.

An effective adjuvant for enhanced bone regeneration is electrical stimulation therapy. Thereby, three different ways to induce an electric stimulation in bone are applied; by direct current (DC), conductive coupling (CC) and inductive coupling (IC) [1–3] DC is an invasive technology that requires the placement of electrodes directly to the bone. The electrodes are placed under the skin and connected to the power supply. CC is a non-invasive technology that uses extracorporeal electrodes on the skin above the bone lesion. IC is often referred to pulsed electromagnetic fields (PEMF), which induce electric fields in the bone lesion through a magnetic field created by an extracorporeal coil.

Kraus and Lechner designed an inductively coupled system which works at low frequencies of 12 and 20 s^{-1} [4]. This system is based on the interplay of two coils and two electrodes. A primary coil is placed outside of the human body while the secondary coil is an implanted transducer coil. The latter receives an induced voltage from an external alternating magnetic field and connects to two electrodes of an implant. One electrode is placed within the bone to be healed, and another is placed in the immediate proximity. Based on this approach, Mittelmeier et al. [5] proposed a bipolar induction screw system, which is clinically applied as the

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so-called Asnis III s-series screw (Stryker Trauma, Kiel, Germany). In this system, the transducer coil is embedded in the screw, and thus there are no extra wires to be implanted. After implantation, the patient is asked to wear an extracorporeal coil around the body three times a day for 45 min. Thereby, an external sinusoidal oscillating magnetic field induces voltage in the embedded secondary coil. In this activated area around the implant electrodes, the electric field can stimulate bone regeneration [6].

In vitro studies showed that human osteoblasts cultured under electromagnetic stimulation exhibit higher proliferation, calcium deposition, and greater expression of decorin, osteocalcin, osteopontin, type I collagen, and type III collagen [7,8]. On the other hand, an in vitro study using rat osteoblasts under sinusoidal magnetic fields with different intensities between 0.9 and 4.8 mT indicated that osteoblast proliferation was inhibited while differentiation and mineralization potentials were significantly promoted [9]. Our own research towards different parameters in electrostimulation using the bipolar induction screw system in combination with cell culture is ongoing [10]. In clinical practice, the bipolar induction screw system shows promising results [11], and exact placement of the implant needs to be taken care of due to the its influence on the electric field distribution [12]. An extensive literature review into clinical studies of electrical stimulation indicates an enhancement of bone healing [13]. However, with regard to levels of evidence of the studies and limited comparability of the different methodologies an optimization of electrical stimulation for clinical practice could not be drawn. The authors conclude that the exact mechanism by which electrostimulation enhances bone regeneration is still not fully understood and needs more investigation. It is assumed that a reciprocal piezoelectric effect in combination with piezoelectric properties of bone tissue [14,15] is the underlying mechanism which in turn results in mechanical stimulation of bone. Nevertheless, the response of the bone to the electromagnetic field is unclear and adjustment of stimulation parameters has yet been performed only empirically. The question remains which is the optimal electrostimulation method in combination with which electric parameters. Therefore, a deeper insight into the inverse piezoelectric behavior of bone is required.

To this end, we have investigated the effect of electro stimulation on femoral bone by scanning X-ray diffraction (XRD) experiments to investigate changes in the crystal structure of hydroxyapatite (HA) with respect to the orientation to electric field. In these experiments we simultaneously recorded small and wide angle scattering data from the HA crystals. By this we were able to determine the local orientation of the HA crystals and their lattice constant with high spatial resolution. The evaluation of both scattering signals was necessary as the textured two dimensional WAXS pattern was not completely recorded due to experimental constraints. To our knowledge, the presented study is the first to investigate the inverse piezoelectric behavior with a spatial resolution across the bone on a micrometer length scale using scanning X-ray diffraction.

2. Materials and methods

The scanning X-ray diffraction and scattering data with in situ application of an electrostatic field were recorded at the Nanofocus Endstation of P03 beamline [16,17] of PETRA III synchrotron radiation source (DESY, Hamburg, Germany) and at beamline X9 [18] of the NSLS synchrotron radiation source (Brookhaven National Laboratory, Upton NY, USA). The experiments were performed in transmission mode and data were recorded in both, wide and small angle regimes (WAXS and SAXS) using a monochromatic beam with a photon energy of 13.0 keV (P03) and 13.5 keV (X9), i.e. at wavelengths of 0.954 and 0.918 Å, respectively. The beam sizes (horizontal × vertical) at the sample position were 1.5 × 1.5 μm^2 (P03) and 20 × 60 μm^2 (X9), focused using elliptically shaped mirrors in crossed geometry (KB-mirrors). The photon flux was 10^{10} photons/s and the acquisition time was 60 s for one image at the P03. At the X9 photon flux was 10^{10} photons/s and the acquisition time was 20 s for one image. A schematic representation of the experimental setups is shown in Fig. 1. Bone sample acquisition was performed by extracting a 10 mm diameter cylinder of trabecular bone from the femoral head of a fresh frozen bovine femur. From the extracted cylinder, slices comprising the trabecular network were cut to 150 μm thickness and stored in the freezer at −20 °C. The slices were thawed and placed in clamps immediately before examination. The samples were mounted on top of a micropositioner (X9) or a piezo-driven nanopositioner, each allowing for sufficiently precise movements of the sample with respect to the (fixed) beam. Using these setups, micro- and nanodiffraction data were recorded in a 2D scanning fashion, with step sizes (horizontal × vertical) of 1.5 × 4 μm (P03) and 40 × 100 μm (X9).

Different sample regions were chosen using the in-line video microscopes installed at the beamlines and the scanned areas ranged from 45 × 80 μm^2 (P03) to 100 × 400 μm^2 (X9), generating in this way several hundreds of diffraction images. In order to record diffraction data from one region at different electrostatic fields (at voltages from 0 to 100 V), each subsequent scan was shifted vertically by 1.5 μm (P03) and 20 μm (X9) (see Fig. 3). Using this interlined scan pattern, no spot was irradiated twice, allowing for each recorded data set to remain unharmed by the radiation damage from the previous scan.

In order to enable application of the electrostatic field onto the samples in situ, i.e. while diffraction data was recorded, special sample holders were used. At P03 beamline, the sample was placed onto a 200 μm Kapton foil while in wet condition where it remained stuck to once dried. Next to the sample, two metal-foil electrodes were attached to the Kapton foil, with the sample sitting in between the electrodes but without any direct contact to them. This way, the Kapton foil acted as both, a support of the brittle sample as well as a support for the electrodes (made of aluminum foil glued to the Kapton foil). Using thin gold wires, a DC source was connected to the electrodes without inducing mechanical strain onto the sample holder and data were recorded at 0, 5 and 10 V voltage and an electrode spacing of 4 mm, yielding a maximum electrostatic field of 2500 V/m. For the experiment at X9, on the other hand, a plastic sample holder was manufactured using

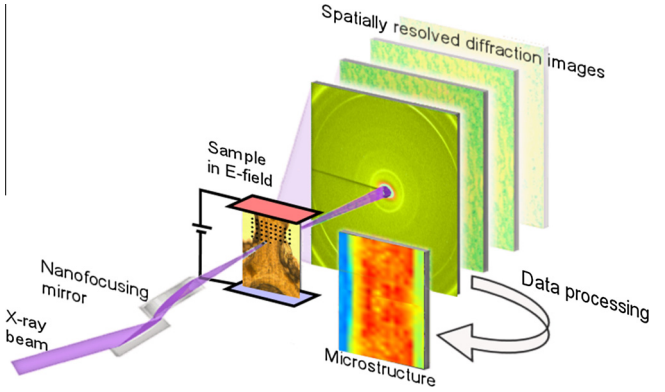

Fig. 1. Schematic representation of the scanning X-ray microdiffraction experiment performed at P03 beamline. The monochromatic beam, as delivered by the beamline, was focused using a pair of elliptical mirrors. The sample was positioned at the focal plane and inside a homogenous electrostatic field. While the sample was scanned across the beam in small steps, diffraction images were recorded at each individual position. A subsequent automated processing of the recorded data evaluated it's the SAXS and WAXS portions and delivered a spatially resolved map of hydroxyapatite orientation and lattice spacing.