OsteoArthritis and Cartilage (2006) **14,** 906–913

doi:10.1016/j.joca.2006.03.001

Osteoarthritis and Cartilage

International Cartilage Repair Society

OARSI OSTEOARTHRITIS RESEARCH SOCIETY INTERNATIONAL

Lead accumulation in tidemark of articular cartilage

N. Zoeger Ph.D.†*, P. Roschger Ph.D.‡, J. G. Hofstaetter M.D.‡§, C. Jokubonis†, G. Pepponi Ph.D.‖, G. Falkenberg Ph.D.¶, P. Fratzl Ph.D.††, A. Berzlanovich M.D.‡‡§§, W. Osterode M.D., Ph.D.‖‖, C. Streli Ph.D.† and P. Wobrauschek Ph.D.†

† *Vienna University of Technology, Atominstitut, Stadionallee 2, 1020 Vienna, Austria*

‡ *Ludwig Boltzmann Institute of Osteology, Hanusch Hospital of WGKK and AUVA Trauma Centre Meidling, 4th Medical Department, Hanusch Hospital, Vienna, Austria*

§ *Department of Orthopaedics, Vienna General Hospital, Medical University of Vienna, A-1090 Vienna, Austria*

‖ *ITC-irst, Centro per la Ricerca Scientifica e Tecnologica, via Sommarive 18, 38050 Povo, Trento, Italy*

¶ *Hamburger Synchrotronstrahlungslabor HASYLAB am Deutschen Elektronen-Synchrotron, Notkestrasse 85, 22603 Hamburg, Germany*

†† *Max-Planck Institute of Colloids and Interfaces, Department of Biomaterials, Am Mühlenberg, D-14476 Potsdam-Golm, Germany*

‡‡ *Department of Forensic Medicine, Medical University of Vienna, A-1090 Vienna, Austria*

§§ *Department of Forensic Medicine, University of Munich, D-80377 Munich, Germany*

‖‖ *Universitätsklinik für Innere Medizin IV, Vienna General Hospital, Medical University of Vienna, A-1090 Vienna, Austria*

Summary

Objective: Determination of the spatial distribution of the toxic element lead (Pb) and other trace elements in normal articular cartilage and subchondral bone from adult humans with no history of work-related exposure to Pb.

Methods: Four macroscopically normal femoral heads and three patellas were harvested from randomly selected forensic autopsies. All subjects died of acute illnesses, had no history of work-related exposure to Pb and had no metabolic bone disease. The elemental distribution of lead (Pb) together with zinc (Zn), strontium (Sr) and calcium (Ca) in the chondral and subchondral region was detected using high resolution synchrotron radiation induced micro X-ray fluorescence (SR μ-XRF) analysis. SR μ-XRF line scans in conventional and SR μ-XRF area scans in confocal geometry were correlated to backscattered electron (BE) images visualizing the mineralized tissue.

Results: In all samples, we found a highly specific accumulation of Pb in the tidemark, the transition zone between calcified and non-calcified articular cartilage. Pb fluorescence intensities in the tidemark, which is thought to be a metabolically active mineralization front, were 13-fold higher when compared to subchondral bone. Pb intensities in the subchondral region were strongly correlated with Zn, but were distinctly different from Ca and Sr.

Conclusions: The finding of the highly specific accumulation of lead in the tidemark of human articular cartilage is novel. However at this point, the exact mechanisms of the local Pb accumulation as well as its clinical implications are unknown.

Key words: Lead accumulation, Tidemark, Synchrotron micro XRF, Calcified cartilage.

Introduction

One of the main threats to human health from heavy metals is associated with exposure to Pb. Exposure to Pb is associated with chronic diseases in the nervous, hematopoietic, skeletal, renal and endocrine systems[1]. Although much progress has been made to limit lead exposure in industrialized countries, primarily through the elimination of leaded gasoline, workplace exposures and leaded pipes, most adults have already accumulated a substantial body burden of Pb[2,3]. The half-life of Pb in the blood is 1 month, but it accumulates in the skeleton, where approximately 95% of the total body burden of Pb is present[4] with an estimated half-life up to 20 years[5]. Diseases or states with increased bone turnover, such as osteoporosis, pregnancy, hyperthyroidism and hyperparathyroidism are associated with increased mobilization of Pb from the skeleton[6–8]. Aging-associated release of bone lead into the circulation is a potentially important source of soft-tissue lead exposure and toxicity[9]. Moreover, reports in humans and animals support a role of Pb in osteopenia[9,10] and Carmouche *et al.* have recently shown in an experimental mouse model that exposure to lead inhibits fracture healing[11]. They were able to

*Address correspondence and reprint requests to: Dr Norbert Zoeger, Vienna University of Technology, Atominstitut, Stadionallee 2, A-1020 Vienna, Austria. Tel: 43-1-58801-14133; Fax: 43-1-58801-14199; E-mail: zoeger@ati.ac.at

Received 17 August 2005; revision accepted 5 March 2006.

show that Pb exposure leads to an increased cartilage formation with delayed maturation and calcification and increased formation of fibrous tissue during bone repair.

Moreover, intra-articular lead has shown to lead to osteoarthritic changes in the knee joint in humans[12] as well as in experimental animal models[13,14]. Osteoblasts and chondrocytes seem to be important target cells for the toxic effects of Pb[15], however, surprisingly little is known about how Pb is distributed in bone and cartilage at the microscopic level. Pb becomes incorporated into bone during mineralization[16] and remains there until bone is resorbed by osteoclasts[4]. Previous studies on the distribution of Pb in bone differentiated only between compact and trabecular bone and lacked any further spatial resolution[4,17,18]. To date, there are no data available on Pb distribution in the osteochondral unit of articular joints.

The osteochondral unit has a highly complex structure designed to enable friction free movements in articulating joints, to resist static and dynamic loads and to transfer loads from articular cartilage to the underlying bone. It is composed basically of articular cartilage forming superficial, transitional, deep and calcified zones with different fibril features and subchondral bone tissue[19]. The zone of calcified cartilage is about 100 μm thick and forms a tight bonding of cartilage to bone, two materials very different in stiffness[20]. Interestingly, the mineral particles impregnating the organic matrix in calcified articular cartilage are similar with that in bone[21], though its chemical composition is very different[19]. In general the mineralization density in calcified articular cartilage is higher than in bone, while surprisingly the stiffness of both calcified materials is comparable[20]. However, little is known about mechanisms and dynamics of the mineralization process in this region. The transition zone between calcified and non-calcified cartilaginous matrix, the so called tidemark, seems to be the metabolically active front of calcification[22].

The purpose of this study was to establish a spatially resolved distribution of Pb in the chondral and subchondral region from normal humans using synchrotron radiation induced micro X-ray fluorescence (SR μ-XRF) analysis. This technique enables non-destructive detection of Pb and other trace elements in the femtogram range at the micrometer level, which cannot be achieved by other micro analytical techniques (e.g., Electron Probe Micro Analysis, EPMA). Moreover, an inherent advantage of this method is its multielement detection capability, which enables to detect different elements simultaneously[23].

Methods

SAMPLES AND SAMPLE PREPARATION

Four femoral heads and three patellas were harvested from five (three females, two males) randomly selected forensic autopsies. All individuals died of illnesses of the heart and the great vessels or accidents and had no history of metabolic bone disease or Pb exposure. Furthermore, samples were selected on basis of having no macroscopical signs of osteoarthritis. In two cases, one femoral head and one patella were taken from the same individual. In two cases, one single femoral head and in one case, a single patella was harvested. The average age of the subjects was 57 years (ranging from 48 to 65). The study was approved by the Institutional Ethical Review Board of the Department of Forensic Medicine of the Medical University of Vienna.

Five-millimeter-thick sections were cut perpendicular to the articular surface from the central region of patella (sagittal plane) as well as from the superior, weight bearing region of the femoral head (frontal plane). Samples were fixed in 70% ethanol, dehydrated through a series of alcohol, and embedded in polymethylmethacrylate (PMMA)[24]. After trimming, surfaces of the PMMA-blocks were polished using diamond suspension and carbon coated for backscattered electron (BE) imaging. Afterwards, 200-μm-thick slices containing the bone area analyzed by BE imaging were cut using a low speed diamond saw (Buehler Isomed, Lake Pluff, USA) for SR μ-XRF analysis.

BE IMAGING

BE imaging is a validated technique to visualize and quantify calcium concentration distribution in bone and mineralized cartilage[20,21,25] and is based on the backscattering of electrons from the sample surface in a scanning electron microscope. A digital scanning electron microscope (DSM 962, Zeiss, Oberkochen, Germany) equipped with a four quadrant semiconductor BE-detector was employed. The microscope was operated at an acceleration voltage of 20 kV, the working distance kept at 15 mm, and the probe current was maintained at 110 pA. Images at a series of magnifications 12−500× were acquired. The intensity of the BE signal is proportional to the average atomic number of the target material. In case of bone the BE signal is dominated by its Ca content − the element with the highest atomic number ($Z = 20$). Thus, areas in the BE image with bright gray levels reflect mineralized matrix with high Ca contents, whereas areas with dark gray levels indicate low mineral density.

The BE images provide the spatial distribution of mineralization density at the sectioned tissue surface with an information depth about 1 μm. Areas of mineralized cartilage have a more homogenous intrinsic structure and have a higher degree of mineralization than bone. Cement-lines are transition zones between different bone packets and/or mineralized cartilage with a generally high mineral content and a homogenous intrinsic structure.

The tidemark is a typical narrow seam of gradually increasing mineral content at the border between non-calcified and calcified articular cartilage[20,21]. Younger bone packets have a characteristic lower mineral content (lower gray level in BE images) than older more mature bone packets[26].

SR μ-XRF ANALYSIS (LINE SCANS)

SR μ-XRF is a powerful analytical tool for qualitative and quantitative analysis of the chemical elements present in the sample[23], based on the detection of characteristic X-rays induced by high energy primary photons.

An inherent advantage of this method is its multielement detection capability, which enables the detection of different elements simultaneously[23].

The unique properties of synchrotron radiation, notably high photon flux, natural collimation, polarization and tunability of the energy of the primary photons, resulting in absolute detection limits on the femtogram level for medium Z elements, allow the analysis of trace elements in bone. The outstanding features of synchrotron radiation in combination with novel X-ray optics were exploited to investigate the elemental distribution in bone on a microscopic scale. SR μ-XRF measurements have been carried out at the micro focus end-station at HASYLAB, beamline L, Hamburg, Germany, by scanning the sample along a line in steps of