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Particle Induced X-ray Emission (PIXE) for elemental tissue imaging in hip modular prosthesis fracture case

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ABSTRACT

The increased modularity in the total hip arthroplasty (THA) allows adapting the prosthesis to the specific anatomical characteristics of each patient. However, the advantages of the modular THA are shadowed by the increased number of prosthesis failures observed in patients. The presence of junction in modular THA may increase its risk of mechanical failure. Moreover, the micro movements between neck and stem could lead to the production of metallic debris which may cause tissue inflammation and unsealing of prosthesis due to osseous dissolution. It is necessary to understand the mechanism of dispersion of the metal particles from the prosthesis into the tissue. Techniques currently applied in hospitals, such as X-ray scans or optical tissue microscopies, are able to distinguish metal particles, but unable to identify their specific metallic origin. In this work, within the TissueMaps project, we have proved that Proton Induced X-ray Emission (PIXE) is able to provide the distribution and elemental composition of particles from the prosthesis into the pseudo capsular tissue samples (near the femoral head) and identify the features observed under optical microscopy, in a case of broken neck prosthesis.

1. Introduction

Increasing modularity in total hip arthroplasty (THA) has been a clear trend in the last years. Modular Morse taper used to attach the femoral head to the femoral stem has become a well-established and commonly used design. During last years, the use of femoral stems with an additional modular junction between the neck and the body of the stem (component introduced into the femur) has gained popularity as method used for the primary THA (substitution of hip joint).

Modular stems for THA offer the possibility of selecting intraoperatively the components for a better adaption to the anatomy of each patient, by choosing neck and stem lengths and angles in the junction of both parts (degree of modular neck anteversion or retroversion and the varus/valgus orientation of the neck). However, higher revision rates of bi-modular junctions than monoblock stems (with modularity limited to the head-neck junction) have been reported [1]. Moreover, the presence of the junction increases the risk of mechanical failure and consequential fracture. Additional failures linked to the use of a modular prosthesis have been referred [2–5]. In particular, a nationwide study from Slovenia has proven that the risks of using modular prosthesis outweigh their benefits [1].

Fretting caused by micro movements between neck and stem, galvanic corrosion and crevice in the neck-stem interface may lead to the production of metallic debris, which are released to the surrounding tissue, possibly causing bioreaction and induction to prosthesis failure.

Metallic particles can get trapped between two articulating surfaces on the hip joint resulting in a process of third-body wear [6]. In addition, Titanium particles are associated with the cytokines release that may derive in periprosthetic bone loss [7]. The degradation and future break of the modular prosthesis takes place mostly in the junction of the neck and the stem, as previously studied [8]. In that study it was showed that the highest stress concentration occurred in very narrow region of the neck where tensile stress and fatigue crack appeared.

Imaging techniques currently applied in Hospitals, such as X-rays, magnetic resonance imaging (MRI) or optical tissue microscopies, do not optimally support the study of the effects of implants on the body, as they are not able to provide the chemical image of the periprosthetic tissue. The research needed to reveal important details of the body response on the implant is lacking of the incorporation of novel analytical methodologies and techniques, including a broad spectrum of elemental and molecular imaging techniques, which have recently become available for biomedical research. Modern tissue microscopy

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techniques have potential for providing elemental and chemical distribution of a tissue, with high sensitivity and lateral resolution. Among these techniques, Energy Dispersive X-ray analysis (EDX) and Electron Energy-Loss Spectroscopy (EELS) provide high lateral resolution (< 10 nm), however, they are unable to detect elements present at concentrations lower than 1 wt%. Proton-Induced X-ray Emission with focused high-energy proton beam (micro-PIXE) technique combines both high elemental sensitivity with detection limit down to 0.1 μg/g, as well as lateral resolution down to 600 nm. In particular, micro-PIXE allows determining the quantitative elemental mapping of a tissue combining high elemental sensitivity and high lateral resolution with the quantification ability [9]. Due to its features micro-PIXE has been previously proved to suit well, among others, for brain elemental distributions associated to Alzheimer's disease [10], trace element imbalances in bone and endometrium associated to osteoporosis and hormone replacement therapies [11], identification of inhaled particles in the human respiratory system [12], distribution and permeation in skin of nanoparticles of Ti used as physical filter or UV radiation in sunscreens [13] and study of size and distribution of gold nanoparticles used as agents in cancer fighting strategies [14]. Micro-PIXE is particularly suited to screen the tissue for the effects of prosthesis degradation [8,15].

2. Materials and methods

This work presents the case of a patient who suffered from a modular hip prosthesis failure due to a fracture in the prosthesis neck, which connects head and stem. The patient is a male with body mass index of 31 kg/m^2. The broken prosthesis was Profemur® Z (Wright Medical Technology, now MicroPort Orthopedics, Arlington, TN, USA) with straight short neck. Both, neck and stem were made of Ti alloy: Ti, 6Al, 4 V (wt%).

The sample collection for the serum and tissue was carried out at the Department of Orthopedics at University Medical Centre Maribor. Institutional review board approval was obtained for the study. All the information regarding the medical history of patients is confidentially kept at the Hospital files. All the samples and files used are codified to avoid the identification of the patient.

Blood samples were taken before revision surgery and analyzed for Ti, Al and V content.

Human peri-prosthetic tissue was collected during the revision surgery and sent for cultures and histological examination. The biopsy tissue was stained to delimit the surgical biopsy borders with The Davidson Marking System ® (DMS) in blue color (#3408-5), from Bradley Products, Inc. After that, samples were fixed in Formalin (10% neutral buffered Formalin) and placed in paraffin blocks with the standard process using a Excelsior^{TM} AS Tissue Processor (Thermo Fisher Scientific) and Tissue Embedding System TES99 (MEDITE Cancer Diagnostics).

Thin slices, from 2 to 40 μm thickness were cut from the paraffin block using a microtome. The 2 μm thick tissue slices layered on the object glass were analyzed at the Department of Pathology at University Medical Centre Maribor using the Hematoxylin and Eosin standard staining protocol [16].

The 10–40 μm thick slices were sandwiched between two aluminium frames with 1 μm thick mylar windows for the micro-PIXE analysis. Micro-PIXE measurements were done in vacuum at the high-energy focused ion beam facility at Jožef Stefan Institute (JSI) [17]. Micro-PIXE analysis was carried out with 3 MeV proton beam of size 1X1 μm^2 and analytical ion current up to 300 pA.

The beam line is coupled with 2 MV tandem accelerator available at JSI and can produce bright ion beam of 14 A m^{-2} rad^{-2} eV^{-1} [18], at that time, the highest proton beam brightness reported at any tandem accelerator. The high brightness of the proton microprobe allows the reduction of object slit aperture and the reduction of acceptance angle at the nuclear microprobe, resulting in a reduced beam size, which makes it especially suited for this type of experiments.

The micro-PIXE measurements run simultaneously with the Elastic Backscattering Spectroscopy (EBS) to detect the backscattered protons from the sample, and on–off axis Scanning Transmission Ion Microscopy (STIM) to determine the proton exit energy after passing through the sample. These two methods give information about the areal density and composition of the tissue matrix, allowing the elemental quantification [19].

3. Results and discussion

The metal content in serum of the patient before the surgery was measured. The resulting concentrations were 60.5 μg/L for Ti, 6.1 μg/L for Al and 5.8 μg/L for V. The normal values for the body concentration of these elements are < 6 μg/L, < 10 μg/L and < 0.14 μg/L respectively (values determined by Institute of Clinical Chemistry and Biochemistry – UMC Ljubljana, Slovenia), meaning that the patient had 10 and 40 times the normal values for Ti and V, respectively, while the Al concentration exhibits the normal value. High amounts of metal elements (Ti, V or Al) in blood may serve as a rough estimation of the degree of prosthesis degradation.

Fig. 1 corresponds to a microphotograph taken at 40X magnification from a 2 μm thick slice stained for pathological examination. Under the light microscope, candidates for metallic particles (marked with red circles) are black, grey-light violet or yellow-green color [20]. Some of them have a crystalloid appearance and others are amorphous. The metal particles in this biopsy material are not birefringent. The bigger particles are located in the extracellular substance made out of collagen produced by the fibroblasts. The fine dusty material, with crystalloid appearance, is placed in the cytoplasm of the macrophages, the so-called arthroplasty effect. It is not possible to differentiate the metallic origin of the particles under the light microscope. Therefore, from the pathologist analysis, it is not possible to conclude if there are Ti, V or Al particles coming from the broken prosthesis.

The entire tissue sample, sandwiched between two 1-μm thick mylar foils, is showed in Fig. 2. The tissue slice is 20 μm thick and has been obtained from the same tissue block as the 2 μm thick slice in Fig. 1. The blue borders in the tissue are due to the ink used to delimit the cross-sectional surgical biopsy borders. This ink is rich in Fe and is used to mark the borders of the tissue obtained in the biopsy. The area analyzed has been marked with a black square. 3 a) and 3b) represent the x-ray

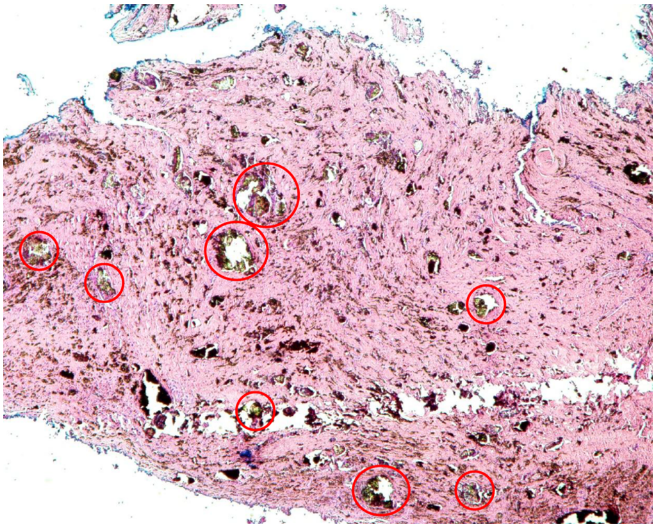

Fig. 1. Corresponds to a microphotograph taken with at 40X from a 2 μm thick slice stained for pathological examination using Hematoxylin and Eosin. The candidates to metallic particle (in yellow-green color) are marked with red circles.

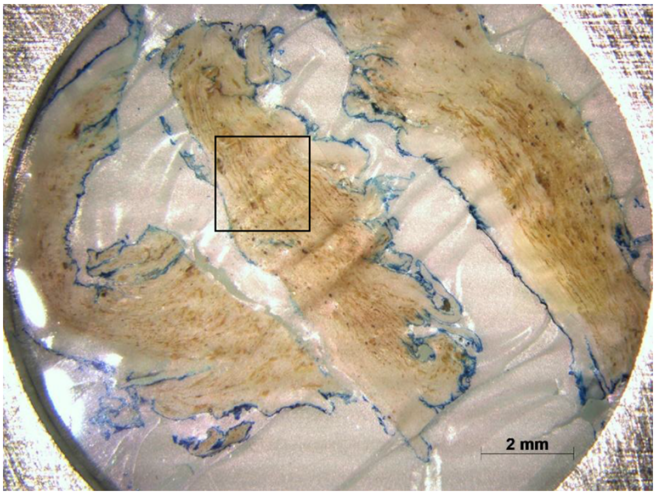

Fig. 2. Image obtained with the stereoscopic microscope of the entire tissue sample sandwiched between two mylar windows of 1 μm thick. The area measured with micro-PIXE (2000 μm × 2000 μm) is delimited with a black square.

spectra obtained from that area with the silicon drift detector (SDD) and the intrinsic Germanium (iGe) detector, respectively (Fig. 3).

The contribution of Fe can be seen in the spectrum obtained with iGe detector b). The spectrum obtained with the SDD detector shows that the Al concentrations are on the detection limit of micro-PIXE method. The Al signal is not discernible from the background in the maps obtained with GEOPIXE [21], in which almost no Al features are shown (Fig. 4f). In fact, we have observed that, when Al is a component of the Ti alloy (Ti6Al4V), the aluminium signal is low and not correlated with its concentration in the alloy. This fact is in concordance with the serum analysis content, where the concentration of Al in blood is within the normal limits, even when the Ti and V contents in blood were highly elevated. This fact has been observed by our group in a previous work where a broken prosthesis of the same type was analyzed in detail [8]. Low amount of Al in PIXE maps was also found in periprosthestic tissue around Ti6Al4V knee prosthesis [22], attributed to the absorption by C filter for light element X-rays. Conversely, high amount of Al was found, by our group with the same detector configuration, in the periprosthetic tissue of a patient with failure of acetabular inlay made from zirconia toughened alumina (not published). This may indicate a selective Al leaching from the debris into the physiological fluid, specific for the Ti-Al-V alloys.

The Fig. 4 shows the picture a), obtained with the stereoscopic microscope, from the analyzed area, and the elemental micro-PIXE maps. The scanned area is 2000 × 2000 μm^2. The micro-PIXE maps have been extracted by software GEOPIXE and show the quantified elemental distribution of particles in the tissue, allowing the univocal identification of the features observed with the optical microscopy.

The Ti and V signals, are both co-localized, as expected for the two of the components of the Ti-Al-V alloy. Al map shows almost no features, apart from the small one in the upper-left corner. The observed co-localization of Ca and P with Ti and V is in correlation with other works where it has been observed that Ca and P are incorporated in the surface of titanium implants into human jaw bone. Oxide formed on titanium implants grows and takes minerals after implantation [23,24]. Calcium phosphate is naturally formed on native titanium oxide. The result is the formation of apatite layer on titanium implant surfaces when in contact with body fluids [25]. We have observed the same ratio Ca/P (5/3) as apatite in some of the areas where Ca, P and Ti are co-localized. Further work will be necessary to confirm these preliminary results.

The elemental maps indicate that during the degradation processes of the prosthesis, the periprosthetic tissue was loaded with excessive amount of solid metal debris, with particulate sizes ranging from the submicron to 300 μm. In the case of studied patient, the serum concentrations of Ti and V were very high and correlate with very high concentration of metallic particles in periprosthetic tissue.

4. Conclusion

The aim of TissueMaps project is to assist doctors in the analysis of the biopsy tissues and develop a more complete diagnostic method for cases of prosthesis failure.

Micro-PIXE technique allows to identify the different features observed with the optical microscopies and clarify the potential presence of the metallic particles. In addition, it allows the determination of the metallic origin, giving us information of the size, the distribution into the tissue and the concentration of the metal particles. With micro-PIXE technique we have been able to identify the yellow-green particles observed in the optical microscopy as metallic particles, mainly consisting of Ti and V. In the PIXE maps, large amounts of metallic particles (from sub-micron to 300 μm size) have been found incorporated in the periprosthetic tissue, indicating strong mechano-chemical degradation of the prosthesis before the failure.

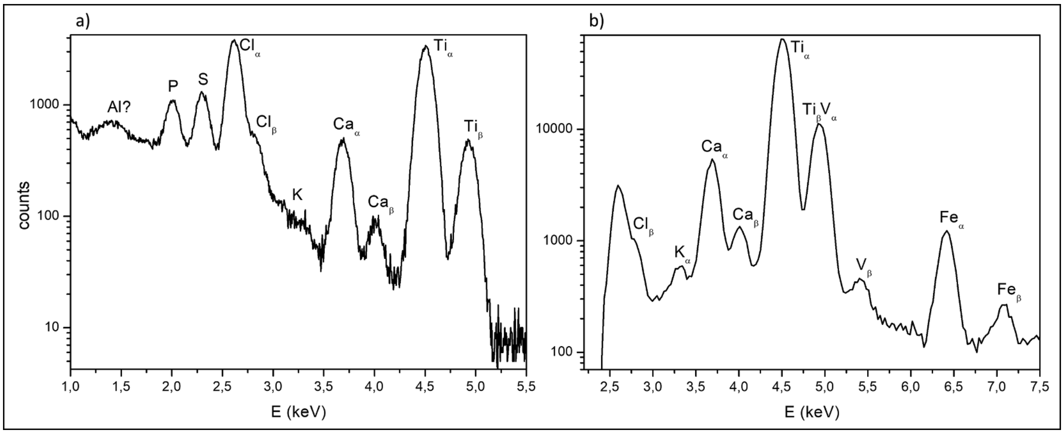

Fig. 3. The plots show the spectra collected with the silicon drift a) and the intrinsic Germanium b) detectors during the PIXE measurements in the area selected in Fig. 2.

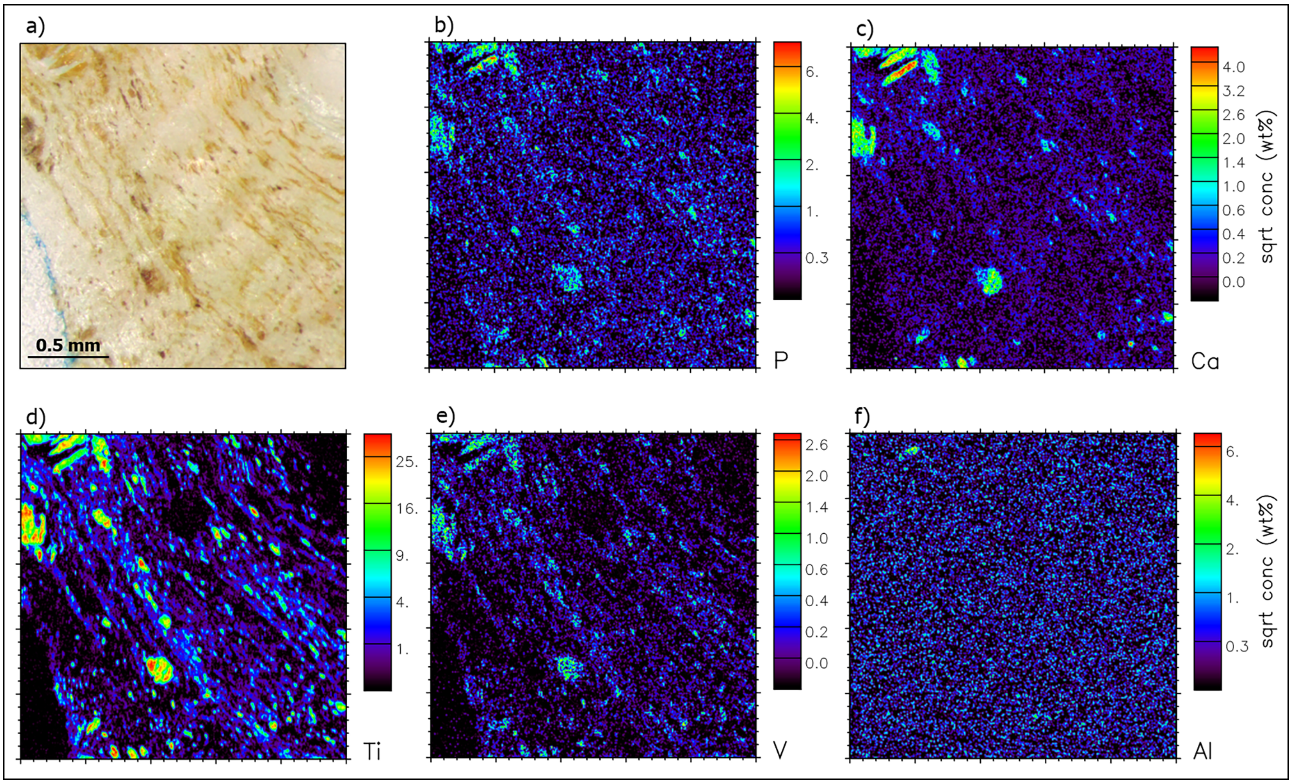

Fig. 4. Fig. 4a) corresponds to microphotograph taken in the area measured with the microprobe. b-f) represent the PIXE maps for Phosphorous, Calcium, Titanium, Vanadium and Aluminium, produced with GEOPIXE software, showing the distribution and concentration of the different elements into the same area. The PIXE map allows the univocal identification of the different features observed with the optical microscope. Scanned area 2000 × 2000 μm^2.

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