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Atomic layer deposition to prevent metal transfer from implants: An X-ray fluorescence study

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ABSTRACT

We show that Atomic Layer Deposition is a suitable coating technique to prevent metal diffusion from medical implants. The metal distribution in animal bone tissue with inserted bare and coated Co–Cr alloys was evaluated by means of micro X-ray fluorescence mapping. In the uncoated implant, the migration of Co and Cr particles from the bare alloy in the biological tissues is observed just after one month and the number of particles significantly increases after two months. In contrast, no metal diffusion was detected in the implant coated with TiO_2. Instead, a gradient distribution of the metals was found, from the alloy surface going into the tissue. No significant change was detected after two months of aging. As expected, the thicker is the TiO_2 layer, the lower is the metal migration.

1. Introduction

Biomedical and clinical applications of implants have increased. The materials used in implants must be inert and resistant to any kind of deterioration by biological or chemical agents. Today, titanium or Co–Cr alloys are widely used for load-bearing applications [1]. Often the devices suffer from adverse reactions, including inflammation, fibrosis, thrombosis, infection of surrounding tissues and corrosion of implant material. Thus in depth investigation about their biocompatibility, in terms of not causing adverse effects [2], is mandatory.

For example, the diffusion of some metals in the biological tissue determines negative effects on human health [3], including cytotoxicity and carcinogenesis, Ni produces genomic instability [4], while Co–Cr alloys can cause chromosome aberrations or DNA damages [5,6]. The metal distribution in bones is also relevant in several diseases such as osteoporosis, hyperthyroidism and hyperparathyroidism [7] and bone turnover in general [8]. It is therefore crucial to evaluate the metal diffusion in the biological tissues and to develop new materials and/or coatings as a physical barrier to diffusion.

Several techniques may be used for surface modification of implants, such as physical vapor deposition [9], chemical vapor deposition [10], thermo-mechanical processing [11], anodic oxidation [12], sol–gel coating [13] and hydrothermal deposition of hydroxyapatite [14]. All these studies report some advantages and drawbacks mainly related to cost and effectiveness of surface covering. One of the main issue to select a coating technique is the reliable and strong coating adhesion to the substrate.

Atomic Layer Deposition (ALD) allows the conformal deposition of ultrathin layers with high adhesion and well-defined thickness [15]. There are already reported the benefits of ALD compared to other above mentioned coated techniques: the deposition temperature did not affect the microstructure or surface properties of the film, the residual stress is very low due to molecular self-assembly, suitable process for sensitive substrates, even on Teflon. Moreover, the films are continuous and pinhole-free. Finally, ALD offers the possibility of parallel processing of multiple substrates [16]. Among all, TiO_2 is one of the most attractive functional oxides because of its properties such as high durability, good resistance to

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corrosion, and well-tested biocompatibility. Indeed, TiO_2 is already used to coat biomedical alloys and implants to improve the short- and long-term body responses [17].

Micro-X-ray fluorescence analysis (μ-XRF) is a unique technique to determine the distribution of major, minor, and trace elements present in the sample surface. μ-XRF can also be combined with Synchrotron Radiation (SR), leading to detection limits in the pg range [18,19]. μ-XRF offers the possibility to compare the elemental distribution in different areas of the sample and to perform the semi-quantitative analysis as well. μ-XRF was successfully used to investigate different kinds of biological samples such as tissue [20], cells [21], and bioindicators [22,23].

The aim of this study is to evaluate by μ-XRF the metal diffusion from the alloy into the bone tissue and the barrier effect of ALD TiO_2 layers using μ-XRF. For this purpose, Co–Cr alloy, bare and coated by ALD with TiO_2 coatings of different thickness using ALD, were embedded in pig bone tissues and the distributions of Co, Cr, Fe, Mn, and Mo were compared.

2. Materials and methods

2.1. Elemental analysis of the implant alloy

Co–Cr alloy powder with a nominal composition of Co 86/Cr 14 (Goodfellow Cambridge Limited, England) was molded into solid discs with a 25 mm diameter and a 1.5 mm of thickness. The discs were cut into 2 mm thick slices and implanted in the pig bone.

The composition of the implant material was determined by a LEO EVO 40XVP scanning electron microscope, equipped with a Link Analytical probe for energy dispersive X-ray spectroscopy. Total reflection X-ray fluorescence (TXRF) was used to determine the presence of trace elements. The powder was directly deposited on the reflector, where a thin layer of silicone grease was added by a cotton wool bud in order to stick the powder. Measurements were performed using a S2 Picofox (Bruker) TXRF instrument equipped with a Mo tube operating at 50 keV and 750 μA.

A release test of the bare alloy in Milli-Q (MQ) water was performed. 50 ml MQ water was transferred into a 100 ml beaker and stirred for 168 h. 10 μl of the test solution was pipetted at the following times: 8, 24, 36, 48, 56, 72, 80, 96, 104 and 168 h, respectively. The sample was deposited on a siliconized quartz sample carrier and dried on a hot plate at 50 °C. Dried samples were spiked with 10 μl of 1 mg/L Ga, used as internal standard to perform quantitative analysis. Samples were dried and measured by TXRF spectrometer for 600 s.

2.2. Surface modification of the implants

Surface modification of the implants was performed by ALD in an Ultratech/Cambridge Nanotech Inc Savannah 100 system. The reactor is a stainless steel cylinder (18.1 cm diameter and 3.6 cm height) with a bottom-heated plate with an area of 179 cm^2. Titanium oxide was deposited starting from tetrakis(dimethylamido)titanium(IV) (TDMAT) as the titanium source and Milli-Q (MQ) water as the oxygen source. TDMAT (99,999%) (Sigma-Aldrich Chemical Co., Germany) was used without any further purification. MQ water was produced in the laboratory with a Millipore DirectQ-5 purification system starting from tap water. The precursors were injected into the reactor directly from 25 g stainless steel reservoirs, held, respectively at 363 K for the TDMAT and at room temperature for the water, to develop enough vapor pressure. Nitrogen gas with 99,999% purity was used as the carrier gas to feed the precursor vapors alternatively to the reaction chamber. The deposition temperature was 363 K and the base pressure of the reactor was 66.66 Pa [15].

Table 1
Samples and experimental conditions.

Type of sample	Sample number	TiO_2 thickness (nm)
Bone	0	–*
	1	0
	2	20
	3	200
	4	300

* Reference sample.

Samples were prepared for coating by washing with MQ water and acetone, dried with nitrogen gas and then inserted in the UV cleaner immediately before the deposition. The following thicknesses of TiO_2 were deposited: 20, 200, and 300 nm.

2.3. Implanted bone samples

Fresh marrow animal bone samples were cut into 1 cm thick slices by using a low-speed diamond saw from ISOMET 1000. The entire embedding surface was prepared using a diamond cutter. Bare and coated implants were embedded in bones samples, then sandwiched between two polymeric foils in order to avoid contamination and to prevent the natural degradation processes [24]. One bone sample without implant was also prepared as reference. The sample list is reported in Table 1.

2.4. μ-XRF measurements

μ-XRF was used to map the area at the interface of the implant and the bone. The instrument is located at the Atominstitut, Vienna and is equipped with a Rh anode X-ray tube "Apogee" from Oxford XTG, which operates at 50 kV and 0.4 mA. It has a small focal spot of 35 μm and a thin 125 μm Be window. The fluorescence radiation from the sample is detected by means of a Si (Li) detector (Oxford Premiumgrade B35) with an active area of 30 mm^2. It features an ultrathin 300 nm polymer window (Moxtek AP3.3) that allows low energy photons to be detected. A collimator with an electron trap is placed at the end of the detector snout. After the right determination of XYZ positions, an area of interest was chosen on the sample and it was scanned with a measurement time of 100 s per pixel. The resolution was $50 \times 50\ \mu m^2$ per pixel. The sample was monitored using an optical microscope with a charge coupled device (CCD) camera. The measurement process was controlled by a specialized computer software developed specifically for this spectrometer at the Atominstitut [20]. It controls the sample stage, the spectrum acquisition process and the X-ray generator. Automatic region of interest (ROI) images were generated during the scan and the spectral deconvolution was performed using the QXAS AXIL software package (IAEA, Vienna, 1995), leading to more accurate elemental maps compared to those obtained by automatic evaluation. The sample was scanned from top (implant) to bottom (bone) with a beam diameter of about 50 μm. Due to the absence of the suitable reference standards for this experimental set up, which would have allowed calculation of Co, Cr, Mo, Fe, Mn and Ti concentrations, results are given in absolute XRF count rates. All the samples were scanned one month after the sample preparation. Sample 1 and 2 were measured again after two months. Fluorescence maps of elements detected in bone tissue such as Ca, P, Sr, Zn and those detected in implant alloy like Ti, Co, Cr, Fe, Mn, Mo, were obtained.

3. Results and discussions

The composition of the alloy used for implants was evaluated by scanning electron microscopy (EDX-SEM): Co 64.7%, Cr 28.5%