



# Microscopic and spectroscopic investigation of an explanted opacified intraocular lens



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## ABSTRACT

The investigated polymethylmethacrylate intraocular lens explanted an year after implantation presented a fine granularity consisting of ring-like grains of about 15  $\mu\text{m}$  in diameter. In order to evidence the changes occurred on intraocular lens relative to morphology, elemental composition and atomic environments, microscopic and spectroscopic analyses were carried out using scanning electron microscopy (SEM), Fourier transform infrared (FTIR), energy-dispersive X-ray (EDS), and X-ray photoelectron (XPS) spectroscopies. The results revealed that the grains contain hydroxyapatite mineral phase. A protein layer covers the lens both in opacified and transparent zones. The amide II band is like in basal epithelial cells. The shape and size of the grains, and the XPS depth profiling results indicate the possibility of a cell-mediated process involving lens epithelial cells which fagocitated apoptotic epithelial cells, and in which the debris derived from cell necrosis were calcified. To the best of our knowledge, this is the first investigation on explanted intraocular lenses using XPS depth profiling in order to examine the inside of the opacifying deposits.

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## 1. Introduction

The research in the field of ocular biomaterials led to development of a wide range of implants and biomedical devices to correct the functional deficiencies of disease, age, and ocular trauma [1]. The most common way of replacing the focusing lens of the eye is with an artificial lens implant called intraocular lens (IOL). The intraocular lenses are probably the most frequent implanted ocular biomaterials. A large number of intraocular lenses fabricated from polymethylmethacrylate (PMMA) are in widespread use due to PMMA's excellent biocompatibility with ocular tissues and transparency to visible light [2]. Nevertheless, opacifications that usually became evident clinically one or more years postoperatively are undesirable effects that have to be as much as possible prevented. The occurrence of "snowflakes" at the beginning causes later the opacification of intraocular lenses with associated vision troubles. It was reported [3] that opacification can occur without infection in injured tissue wherein extracellular deposits of devitalized cells, blood cells, and lipids may act as a nidus for calcification.

Werner and collaborators published the first clinicopathological analysis with histochemical correlations of this complication [4,5] and further devoted lot of work on investigation of calcium-containing deposits that appear on intraocular lenses as irregular, spherical-ovoid granules. It was assumed that different patterns of calcium precipitation could be related to differences in the water content of the hydrophilic acrylic materials [6].

Many papers reported that the calcification takes place on or within the lenses, or both [7], or just beneath the surface [8–10], and several mechanisms were proposed to explain the experimental observations on explanted intraocular lenses [10,11]. Notwithstanding the relatively high number of studies, the processes contributing to opacification are still under debate. The degradation of the intraocular lenses is largely accepted to be determined by three major types of calcification, i.e. primary calcification, secondary calcification, and false-positive calcification or pseudo-calcification [12]. According to this classification, the primary form refers to calcification based on possible inadequate properties of the polymer, or to faults in lens fabrication or packaging; this calcification presumably occurs in otherwise normal eyes and generally is not associated with preexisting diseases. The secondary form refers to deposition of calcium onto the surface of the lens most likely associated with preexisting or concurrent diseases, and in this case this calcification is not related to any problem with the

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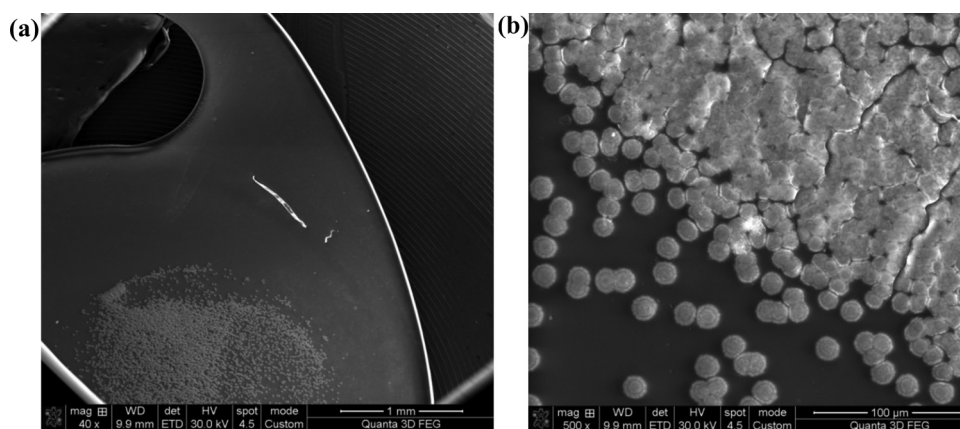


Fig. 1. SEM images of the explanted intraocular lens taken with different magnifications.

lens itself. The false-positive or pseudocalcification refers to those cases in which other pathology is mistaken for calcification or false-positive staining for calcium occurs. In a very recent study [13], the authors also considered the expiration dates of the lenses.

The aim of our study was to investigate microscopically and spectroscopically the aggregates occurred on an explanted opacified hydrophilic acrylic lens and to think about a scenario for the mechanism of intraocular lens calcification.

## 2. Experimental

The investigated hydrophilic intraocular lens of polymethylmethacrylate (PhysIOL, +4.00 D) was explanted from a clinically healthy 28 years old male patient, a year after implantation with silicone oil, due to significant visual disturbances. An opacification could be slightly observed with the naked eye in the middle of the inner surface.

Scanning electron microscopy (SEM) and chemical analysis of local area by energy-dispersive X-ray spectroscopy (EDS) were carried out with an FEI Quanta 3D FEG dual beam microscope.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were recorded at a resolution of  $2\text{ cm}^{-1}$  with a Bruker Equinox 55 spectrometer, at room temperature.

X-ray photoelectron spectroscopy (XPS) analysis was performed using a SPECS PHOIBOS 150 MCD system equipped with monochromatic Al  $K\alpha$  source (250 W,  $h\nu = 1486.6\text{ eV}$ ). The vacuum in the analysis chamber during the measurements was in the range of  $10^{-9}$ – $10^{-10}$  mbar. Charge neutralization was used for all measurements. The binding energy scale was charge referenced to the C 1s photoelectron peak at 284.6 eV. The elemental composition on the outermost layer of samples (about 5 nm deep from surface) was estimated from the areas of the characteristic photoelectron lines in the survey spectra assuming a Shirley-type background. High-resolution spectra were obtained using analyzer pass energy of 30 eV. The spectra deconvolution was accomplished with Casa XPS (Casa Software Ltd., UK). In order to acquire XPS information from deeper layer, the depth profile was examined after sample ablation by the  $\text{Ar}^+$  ion beam sputtering (at a sputter angle of  $45^\circ$ , exposure time 8 min, acceleration voltage 1.5 kV, emission current 10 mA).

All examinations were carried out both on transparent and opacified areas of the explanted lens, as well as on a completely new reference lens of same origin.

## 3. Results and discussion

Scanning electron microscopy images (Fig. 1) evidence the granular deposition in the middle of the lens, and at higher

magnification one remarks a characteristic rounded shape. Surprisingly similar SEM images were published [14] for a hydrophilic intraocular lens explanted from a patient with injection of intraocular gas. In earlier studies, the patterns of the opacifying deposits are described as characteristic ring-like crystalline structure [14], but also as “cerebriform” pattern [15–18]. The shape of the grains (Fig. 1b) appears very similar to a cell image and further we pay a special attention to this aspect. Moreover, it might be tempting to consider even a cell division. Recently, it was reported [19] the presence of foreign body giant cells on the lens surface in the early postoperative phase. Otherwise, long time ago was settled that neovascularization, if present, tends to occur accompanied by giant cells and macrophages [20–23].

The elements identified from X-ray EDS analysis in the reference lens and in the transparent area of the explanted lens are only carbon and oxygen (Fig. 2), excepting a very small amount of nitrogen, 0.7 at.%, detected on the transparent zone of the explanted lens. The C/O ratio of  $69.8/30.2 = 2.31$  for the reference lens, and of  $69.2/30.1 = 2.3$  for the explanted lens in the transparent area (Table 1) are close to the theoretical value of 2.5 in PMMA-( $\text{C}_5\text{O}_2\text{H}_8$ ) $_n$ . Neither EDS nor XPS analyses can detect the hydrogen atoms. Thereupon, it has to be mentioned that the elemental concentrations delivered by EDS reflect the composition of a deeper layer than that explored by XPS on the outermost atomic layer of the sample.

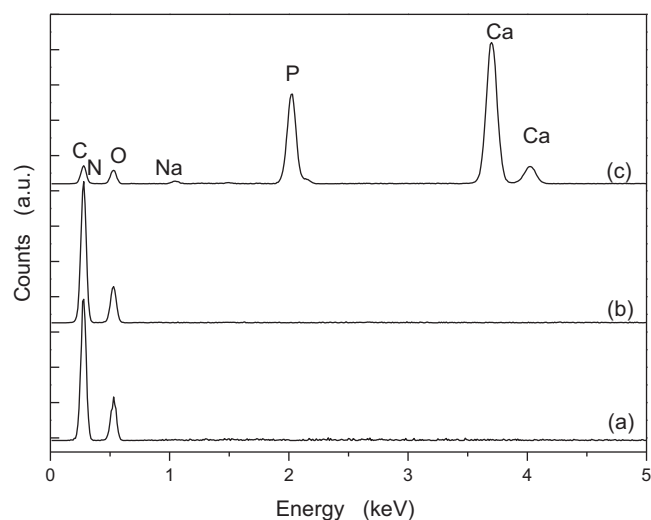


Fig. 2. EDS spectra recorded from reference lens (a), and explanted lens in transparent zone (b) and on deposited grains (c).

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