European Journal of Ophthalmology / Vol. 19 no. 1, 2009 / pp. 28-36

Scanning electron microscopy and energy dispersive spectroscopy findings in explanted PMMA and hydrophilic acrylic intraocular lenses

H. GUNGEL

Beyoglu Eye Education and Research Hospital, Istanbul - Turkey

PURPOSE. *To investigate the presence of calcium (Ca) aggregates influencing biocompatibility and the factors that affect calcium accumulation in explanted intraocular lenses (IOL) and to analyze the Ca distribution in an opacified hydrophilic acrylic lens.*

METHODS. *Surface irregularities and aggregates of 13 IOLs were studied with scanning electron microscopy, and their relative concentrations with energy dispersive spectroscopy (EDS). Relationships of distribution between Ca and silicone (Si) and nitrogen (N) and between N and Si and Na, and the influence of Si on Ca accumulation and the effect of differences in lens material on the distribution of N, and the effect of endophthalmitis on the distribution of Ca were evaluated statistically. EDS analyses were performed on the surface and cross-section of the opacified lens.*

RESULTS. *The statistically significant relationships between the distribution of Ca and N, and between the distribution of N and Na, the significant effect of Si on the Ca accumulation, significant relationship between endophthalmitis and the Ca accumulation in the aggregates were shown. The EDS analysis of the opacified IOL, Ca and P peaks were shown from the whole surface, Ca, O peaks were determined from cross-sections over a 70–80 µm distance.*

CONCLUSIONS. *In the aggregates influencing IOL biocompatibility, presence of proteins was determined to be more important than the presence of Si regarding the distribution of Ca, while the presence of Si affected the accumulation of Ca. Opacification, caused by the Ca accumulation within the lens, was found to result from Ca penetrating from lens pores. (Eur J Ophthalmol 2009; 19: 28-36)*

KEY WORDS. *Biocompatibility, Calcification, Calcium, Electron microscopy, Intraocular lens, Polymethylmethacrylate*

Accepted: July 1, 2008

INTRODUCTION

Advances that have taken place in the basic medical sciences and materials sciences during the last three decades have influenced medical sciences, particularly leading to rapid changes in methods and materials used in ophthalmology, dealing with the eye that is open to external factors. This is most evident in the devices and methods used in cataract surgery. The purpose of these studies is to improve biocompatibility, to maintain the achieved improvement, and to accelerate and maintain patient rehabilitation.

Increased numbers of lens epithelial cells (LECs) following cataract surgery leads to ECM formation on the lens, and these cells are transformed into mesenchymal cells with enhanced TGF-B effect. Strong evidence of this is the presence of TGF-B and ECM components in the protein deposits (1). Although the abovementioned is a natural process, it was shown that, in conditions including the presence of diabetes mellitus and proliferative vitreo-

1120-6721/028-09$25.00/0

This study was presented at the 25th Congress of the ESCRS, Stockholm, Sweden, September 8–12, 2007

retinopathy, these molecules have increased even further in the intraocular fluid (2, 3). Although the effect of the collagen existing in the structure of protein secreted by the cells that adhere to the temporary surfaces of plasma proteins such as fibronectin and vitronectin is not completely understood, it is asserted that it may affect intraocular lens (IOL) biocompatibility (4, 5). IOL calcifications may occur on the surface of or within the lens. There are many studies in the literature that aimed to explain the opacification during the postoperative period, which presented data concerning light microscopy, electron microscopy, histopathology, spectroscopy, and immunohistochemical characteristics of IOLs that were extracted due to opacification (5-14).

This study aimed to elucidate the chemical structure of surface deposits and the significance of the presence of protein and silicone (Si) on the accumulation of calcium (Ca) and Na, the effectiveness of polymer dystrophy on Ca accumulation that may potentially affect the biocompatibility and also the material significance of IOLs (PMMA and hydrophilic acrylic lenses) on N presentation. For this purpose, IOLs explanted from 13 patients whose initial intraocular surgeries were performed in different hospitals but who were directed to our clinic for various reasons were studied. The surfaces of IOLs, regardless of their chemical structures, were studied and deposits were detected with scanning electron microscopy (SEM), after which chemical structures of the deposits and relative concentrations of the elements were assessed via energy dispersive spectroscopy (EDS). The presence of nitrogen (N), as an indicator of protein deposits, was investigated in EDS studies. These EDS analyses were carried out on a total of 62 locations, with a varying number of analyses on each lens. Relationships among Si, N, and Ca were examined by statistical means. The effect of polymer dystrophy on Ca accumulation was examined by comparing the Ca distribution between the lenses removed from patients with endophthalmitis and anterior ischemic syndrome and the other lenses. The relationships between protein accumulation on IOLs made of PMMA and hydrophilic acrylic were also analyzed and the chemical structure of lenses were examined statistically. Distribution of calcification in lenses was investigated by performing EDS on the cross-section of an opacified hydrophilic acrylic lens which remained in the eye for 4 years.

METHODS

This study was carried out in Beyoglu Ophthalmology Training and Research Hospital, 4th Ophthalmology Clinic, between March 2006 and January 2007. Thirteen lens materials explanted from 13 patients who were admitted to the hospital due to various reasons were used for the study. Of the cases, four were female and nine were male, with an average age of 64 (range 48–77). One of the cases had sarcoidosis, one had diabetes mellitus, and others had systemic hypertension. The explantation reasons were endophthalmitis, rhegmatogenous retina detachment accompanied by PVR, opacification, endothelium decompensation, and dropping into

TABLE I - CASE NUMBER, CHARACTERISTICS AND CAUSES OF EXPLANTATION OF INTRAOCULAR LENSES

Case	Interval	Lens localization	Material	Trademark	Cause of explantation
1	8 yr	ACIOL	PMMA	IOLtech	Endophthalmitis
2	1 wk	PCIOL	HA	HexaVision	Endophthalmitis
3	1 wk	PCIOL	HA	HexaVision	Endophthalmitis
4	2 mo	PCIOL	HA	Dr Schmidt	Endophthalmitis
5	4 yr	PCIOL	HA	Dr Schmidt	RRD (traumatic)
6	4 mo	ACIOL	PMMA	Dr Schmidt	RRD
7	2 yr	PCIOL	HA	Hanita	RRD (traumatic)
8	1 yr	PCIOL	HA	Ocuflex	RRD
9	2 yr	PCIOL	HA	Lenstech	RRD
10	1 yr	PCIOL	HA	Ocuflex	RRD
11	4 yr	PCIOL	HA	Ophthalmic Innovatives International	Opacification
12	2 y	ACIOL	PMMA	Dr Schmidt	Corneal decompensation
13	15 y	Iris claw lens	PMMA	Ophtech	Drop of IOL into AC

ACIOL = anterior chamber intraocular lens; PCIOL = posterior chamber intraocular lens; PMMA = polymethylmethacrylate acrylic; HA = hydrophilic acrylic; RRD = rhegmatogenous retinal detachment.

anterior chamber. Three of the 13 IOLs inspected were angle-supported anterior chamber lenses, 1 was a rigid iris claw lens, and 9 were posterior chamber lenses. Of the 13 lenses, 4 were PMMA and 9 were hydrophilic acrylic (Tab. I). All cases were treated with systemic and/or topical antibiotics and steroids. Lenses were penetrated by clear corneal incisions under local or general anesthesia. The anterior chamber was filled with viscoelastic material (methylcellulose). IOLs were taken out of the eye using forceps, taking care not to cause optic or haptic damage. IOLs were then washed with saline and were placed in separate containers filled with saline. They were stored at +4ºC until the date of analysis.

SEM and EDS methods

Lenses were mounted on aluminum blocks and coated with a gold-palladium alloy after applying bidirectional carbon tape. EDS analyses were then performed on the lenses using scanning electron microscopy (Oxford Instruments-INCA, Model No.7274, EDS Instrument) at 20 Kv and low vacuum (5–15 Pa). EDS studies were carried out on each IOL and the number of analyses varied according to the surface irregularity observed in respective IOLs. The following assays were performed according to the data obtained during EDS analyses:

1) Consistency of Ca with Si, N, and of Na with N, chi-square McNemar test.
2) Consistency of Si with Ca and Na with chi-square McNemar test.
3) Influence of Ca accumulation in the presence and absence of Si on the IOL surface, statistical differences between relative Ca concentrations at Si(+) and Si(–) points with Mann-Whitney *U* test. Variables in the statistical analyses were arranged as 2 x 2 tables. Statistical significance was accepted at the 0.05 level (two-tailed).
4) Hydrophilic acrylic and PMMA lenses were studied with regard to the presence and absence of N on the surface, and the difference between materials regarding the presence of N were analyzed statistically by Fisher exact test.
5) Statistical comparison of the difference between the Ca distribution in IOLs explanted from four cases with endophthalmitis and one case with endothelium deficiency accompanied with anterior segment ischemia, and Ca distribution in IOLs explanted from seven cases without endophthalmitis with Fisher exact test.
6) Also, an EDS analysis on the cross-section of the lens explanted due to opacification to examine the distribution of calcification within a hydrophilic acrylic lens.

RESULTS

According to the surface opacities observed with SEM in 13 IOLs:

1) Consistency between Ca and Si on the lens surface was investigated and the following findings were obtained: silicone was detected in 31/62 aggregates analyzed by EDS. The consistency between Ca and Si was 18/31. Calcium was observed in 16/31 aggregates without silicone. The consistency between Ca and Si was found to be statistically insignificant (χ^2mn=0.13, p=0.71). When the consistency between Ca and N was examined, Ca was present in 21/23 aggregates with N, but was not present in 26/39 aggregates without N; the relationship between Ca and N was found to be highly significant (χ^2mn=6.66, p=0.009). Analysis of the consistency of N and Na showed that Na was present in 14/23 aggregates with N but not in 9. Na was present in 23/39 aggregates without N but it was not present in 16. The consistency of N and Na was statistically significant (χ^2mn=5.28, p=0.02).
2) The rate of the presence of N was 23/62 aggregates analyzed by EDS. Si was present in 14 /23 aggregates with N but not in 9. Si was present in 17/39 aggregates without N, but was not present in 22. The consistency between N and Si was not statistically significant (χ^2mn=1.88, p=0.16). Analysis of consistency between Si and Na gave the following findings: Na was present in 14/31 aggregates with Si but not present in 17. Si was present in 23/31 aggregates with Na, but not in 8. Consistency between Si and Na was statistically insignificant (χ^2mn=0.62, p=0.42).
3) The relative concentrations of Ca in the presence or absence of Si on the lens surface was 7.5% and 2.5%, respectively. The difference between the relative concentrations of Ca was highly significant (p=0.001) statistically.
4) Difference in N accumulation on acrylic and PMMA surfaces: analyses performed on 26 aggregates for PMMA lenses showed that N was present in 7 aggregates while absent in 19 aggregates. On the other

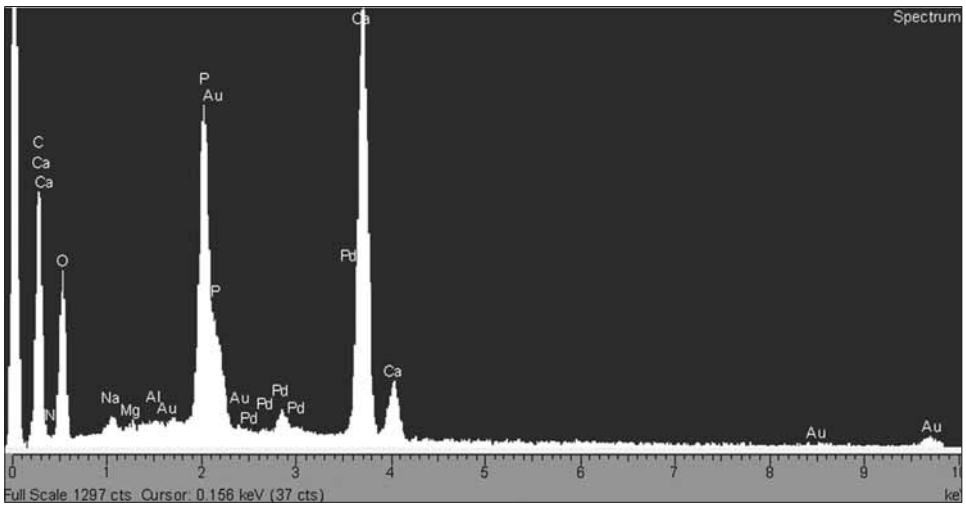

Fig. 1 - *Energy dispersive spectroscopy analysis of the opacified lens matrix.*

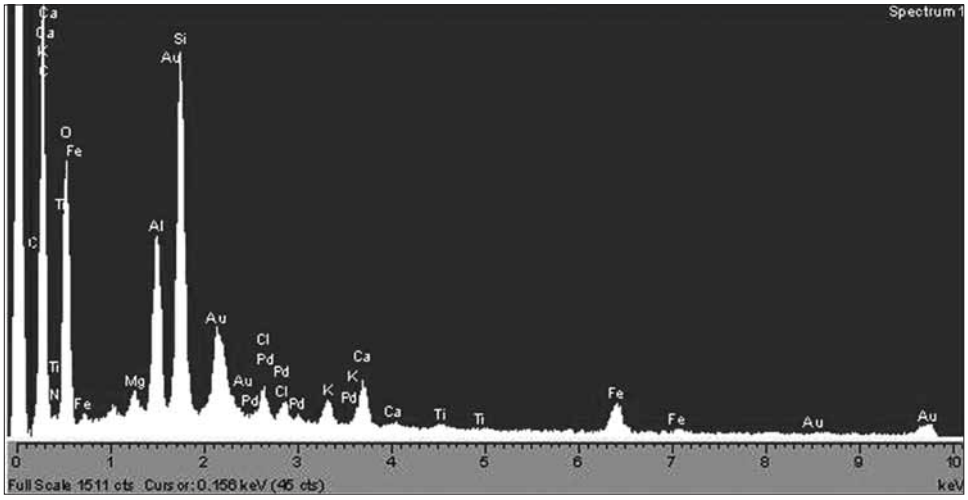

Fig. 2 - *Energy dispersive spectroscopy findings of a granular opacity on the surface of the opacified lens.*

hand, EDS analyses performed on acrylic lenses at 36 aggregates revealed the presence of N at 16 aggregates and its absence at 20 aggregates. The difference, however, was not significant (p=0.16).

5) Ca distribution was 17:22 (72.3%) in IOLs explanted from four cases with endophthalmitis and one case with anterior segment ischemia and was 17:40 (42.5%) for others. The difference was statistically significant (p=0.03).
6) Surface irregularity was not detected for opacified lens electron microscopy. C, O, N, Ca and P were observed

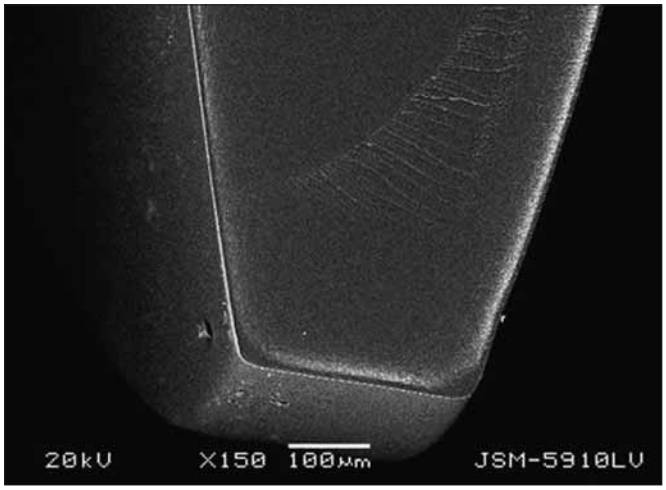

Fig. 3 - *The cross-section of the opacified lens.*

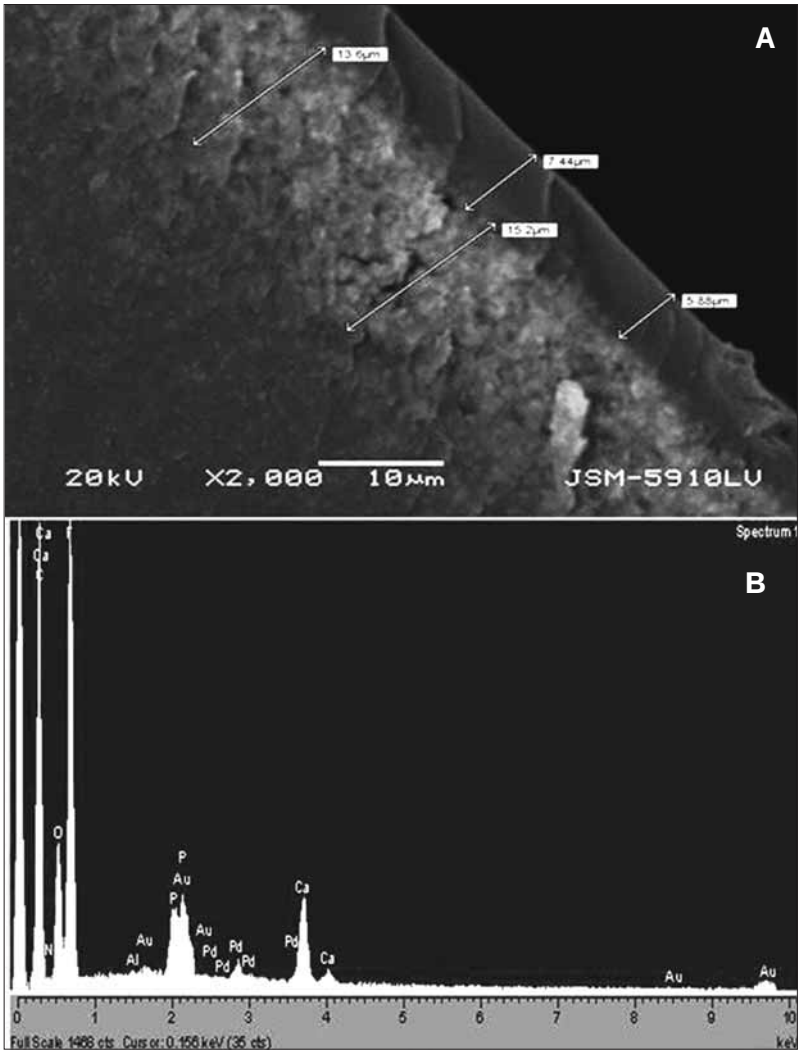

Fig. 4 - (A) *Photograph of the cross-section of the opacified lens that shows the distances of the opacities.* **(B)** *Energy dispersive spectroscopy findings performed on the opacified area parallel to the surface.*

in the EDS analysis of the lens matrix (Fig. 1). In the EDS of a granular opacity on the surface of the same lens, Na, Ca, Si, K, Mg, Fe and Ti were detected (Fig. 2). In the cross-section of the lens, opacity was identified below and parallel to surfaces (Fig. 3). This opacity began from 6–7 µm and was 13–14 µm thick parallel to the back-surface, and was 6–9.5 µm distant from the front-surface and was 15–20.5 µm thick. N, F, P, and Ca was detected in the EDS performed on this location (Fig. 4). Electron microscopy performed on the surface of the same cross-section at 100 µm showing needle-shaped deposits (Fig. 5) and EDS analyses carried out at a diameter of 60–70 µm (Fig. 6) revealed the presence of only Ca, besides C and O. In the EDS analysis of the central part of the cross-section of the lens, C and O were detected only (Fig. 7).

DISCUSSION

It has been well documented that Si is a nidus for Ca deposition that affects the biocompatibility. Also, proteins on the surfaces are known to cause Ca deposition. It is well known that amine groups of proteins and Ca tend to bind acrylic acid which is an important material in hydrophilic lenses. The presence of N may be due to ECM proteins on the lens surfaces, degenerated cells, or proteins in the anterior chamber fluid. Histologic, immunologic, and spectroscopic studies were performed on explanted IOLs to determine the structure of proteins and cells that cover the surfaces. Fibronectin and complement components had been detected by the electrophoresis of proteins from extracted IOLs (14). Also, it is inevitable that cell and protein accumulations on IOL surfaces for months and years

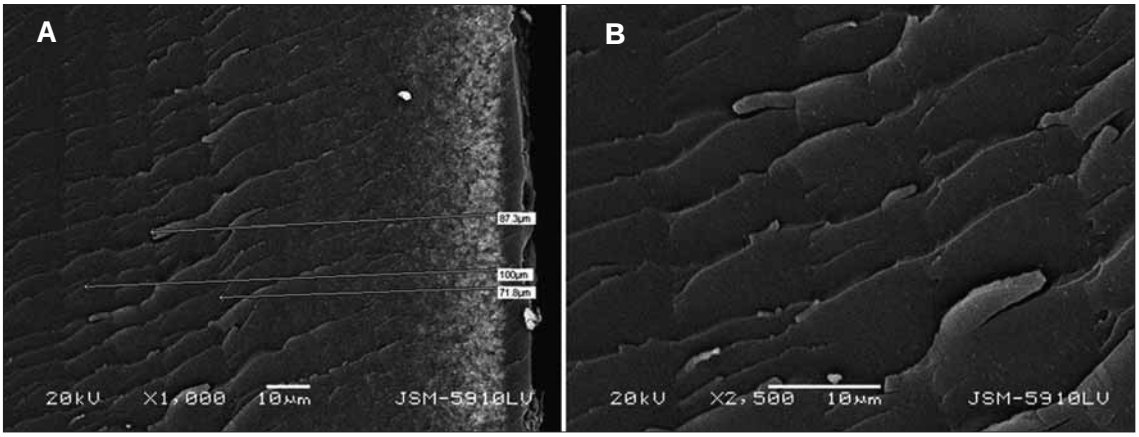

Fig. 5 - A) *Photograph of the cross-section of the opacified lens showing the distances of the needle-shaped crystals.* **B)** *Photograph showing the needle-shaped crystals.*

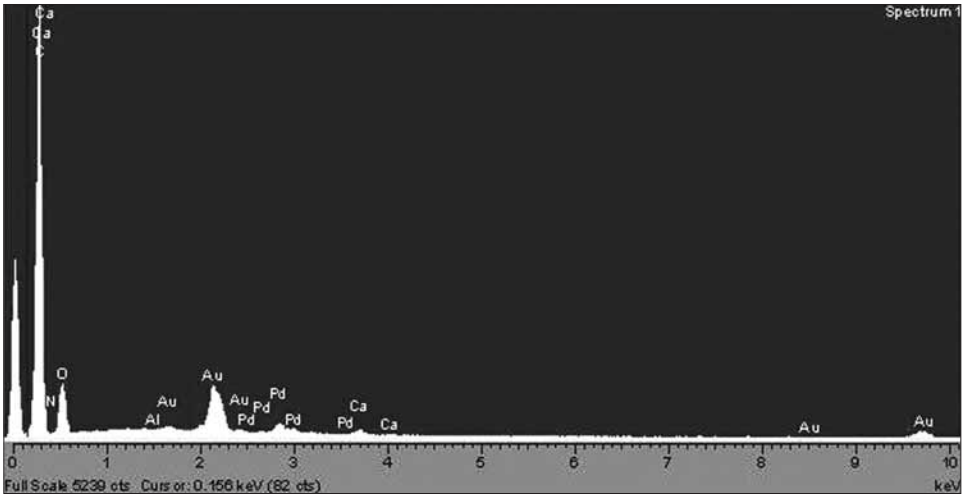

Fig. 6 - *Energy dispersive spectroscopy findings of the opacified lens at 6-7 µm.*

result in the deposition of Ca and Na. In this study, the effect of Si on Ca distribution was found statistically insignificant whereas N was statistically highly significant. Therefore, the presence of proteins on the lens surfaces affects the Ca and Na distribution in the aggregates more significantly than Si.

The chemical binding of Ca and Na to the Si on the lens surface is not an expected relationship. However, surface energy of Ca, due to its ionic characteristics, is higher than that of Si. Ca may blanket Si. The significant effect of the presence of Si on the Ca accumulation has been demonstrated statistically in this study. However, surface energy of Na is lower than Si and cannot blanket Si. In this study, the consistency of Na and Si was not significant, which supports this argument.

Significant differences in the types of proteins covering the surfaces between soft acrylate, PMMA, and silicone lenses have been reported in human autopsy studies. Fibronectin was shown to bind mostly to hydrophobic acrylate. It has been shown that all surfaces of acrylate lenses were covered with vitronectin (13). Although the type of the protein could not be determined in this study, the incidence of the presence of N, as an indicator of protein involvement in surface opacities, was shown to differ insignificantly between PMMA and acrylic lenses. No difference could be detected between the two lens groups regarding protein distribution, most importantly because both may contain acrylic acid and blood–eye barrier was disrupted in all of the 13 cases; the eyes were inflamed, and pathologies leading to the breakdown of the eye–blood barrier could be due to the accumulation of both cellular and noncellular proteins.

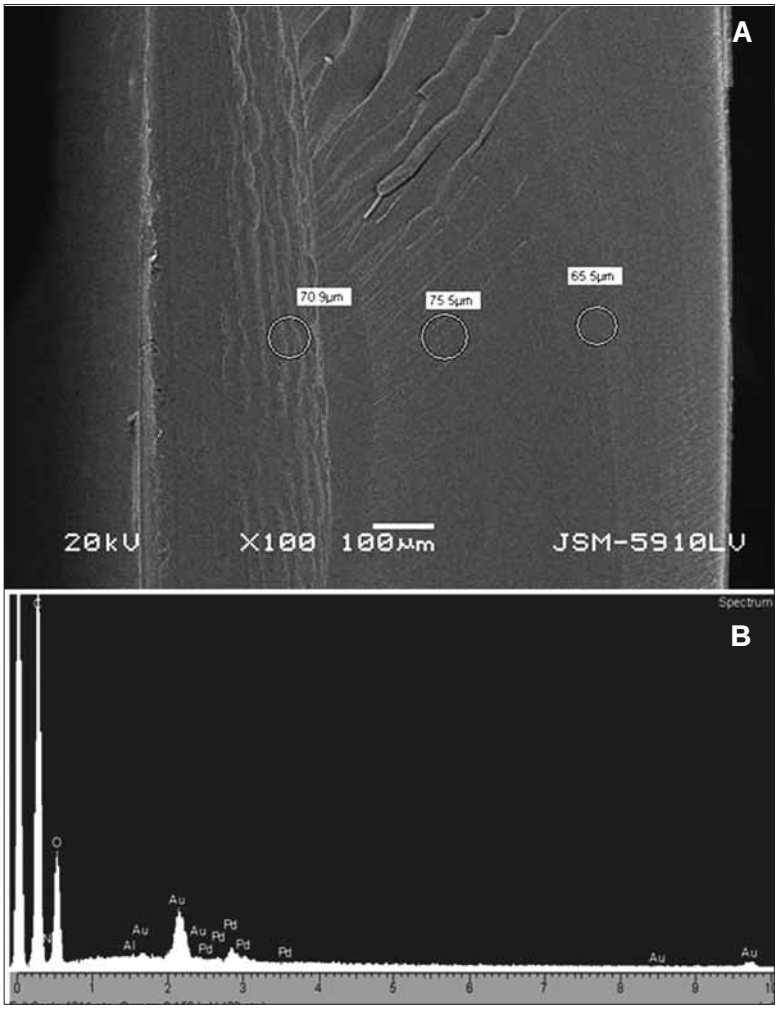

Fig. 7 - (A) *Photograph of the center of the opacified lens.* **(B)** *Energy dispersive spectroscopy findings performed on the center of the opacified lens.*

Endophthalmitis and anterior segment ischemia increase the PH, consequently speeding up the hydrolysis of lens material. As a result, dystrophy of the polymer may occur and Ca binding is facilitated. Similarly, in this study, Ca distribution in IOLs explanted from patients with endophthalmitis and anterior segment ischemia were statistically different than those observed in IOLs of nonendophthalmitis patients, supporting the above argument (15-17). The presence of needle-shaped crystals comprised of Ca, P, and oxygen was demonstrated in EDS studies of IOLs explanted due to opacification. Opacification is known to be caused by intense Ca and P accumulations in the surface on or within the lens. The presence of Si, together with Ca and P, was shown in EDS studies. A silicone–gasket packaging system (B&L Surgical, Rochester, NY) is suggested as the cause of opacification of Hydroview foldable hydrogel lenses, which has been frequently reported in the literature (18, 19). Silicone adhering to the optical surface, which occurs in the presence of fatty acids and all other metabolic diseases, is alleged to form a nidus for Ca storage (11, 20). This study has also shown that Si indeed formed a nidus for Ca accumulation. That delayed opacification in hydrophilic acrylics is caused by the presence of Ca, P, and Si on lens surfaces has been demonstrated by findings of EDS studies (11, 18, 21, 22). The presence of Ca and P in the substance of the optic in Hydroview IOLs, Memory lenses, SC60B-O4V IOLs (MDR), and Aquasense IOLs has been reported in the studied literature. Dystrophic calcifications develop in a multifactorial manner (23, 24). In this study, EDS studies revealed the presence of Ca and P on the whole surface of the IOL explanted due to opacification. Besides, Ca, Cl,

Fe, K, Mg, Si, and Ti were detected in individual granular opacities on the surface. The presence of Ti, however, could not be explained. Ca, P accumulations were observed in cross-sections, parallel to the surface. On the other hand, Ca aggregates at 80 µm distance from the surface, demonstrating the existence of Ca; O via EDS was noted, as well. This study yielded findings which suggest that calcium accumulation may occur during transportation through the lens pores, rather than the dystrophy of lens polymer. It was shown that elements accompanying calcium differed at different depths of the opacified lens.

Cell and protein accumulation to some degree is expected to occur on the IOL surface for biocompatibility. With this study, it was demonstrated through SEM and EDS findings of explanted hydrophilic IOLs that the presence of protein was more important than the presence of Si in the distribution of Ca, and that sites containing Si formed a nidus for Ca accumulation. The study findings suggested that both acrylic acid on the surface and protein storage resulting from biocompatibility might lead to the storage of salts such as Ca and Na, and that these might take place in a natural manner. Dystrophic alterations seem to be inevitable by time and/or due to diseases of the eye that disrupt the blood–eye barrier. Endophthalmitis is a factor that facilitates calcification. Ca ions may also find passage into lenses through lens pores, resulting in Ca accumulation within the lens material and opacification of the lens and elements accompanying calcium differed at different depths of the opacified lens.

In conclusion, in order to identify the ideal lens material, clinically supported further studies are indicated. These studies should aim to define surgical equipment and skills that help minimize inflammation and cell proliferation on the lens surfaces.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Atilla Güngör, Marmara University Art and Science Faculty, for guidance and Prof. Dr. Halim Issever, Istanbul University, Public Health, for statistical analysis.

The authors report no financial support or conflicts of interest.

Reprint requests to:
Hulya Gungel, MD
4. Gazeteciler Sitesi C1 Blok D: 9, 34330
Levent, Istanbul, Turkey
hulyagungel@msn.com

REFERENCES

1. Gonzalez-Avila G, Lozano D, Manjarrez ME, et al. Influence on collagen metabolism of vitreous from eyes with proliferative vitreoretinopathy. Ophthalmology 1995; 102: 1400-5.
2. Yang CM, Cousins SW. Quantitative assessment of growth stimulating activity of growth stimulating activity of the vitreous during PVR. Invest Ophthalmol Vis Sci 1992; 33: 2436-42.
3. Miyake K, Asakura M, Kobayashi H. Effect of intraocular lens implantation on the blood–aqueous barrier. Am J Ophthalmol 1984; 98: 588-90.
4. Sanders DR, Kraff MC, Lieberman HL, Payman GA, Tarabishy S. Breakdown and reestablishment of blood–aqueous barrier with implant surgery. Arch Ophthalmol 1982; 100: 588-90.
5. Saika S, Miyamoto T, Yamanaka A, et al. Immunohistochemical evaluation of cellular deposits on posterior chamber intraocular lenses. Graefes Arch Clin Exp Ophthalmol 1998; 236: 758-65.
6. McMahon MS, Weiss JS, Reidel KG, Albert DM. Clinically unsuspected phacoanaphylaxis after extracapsular cataract extraction with intraocular lens implantation. Br J Ophthalmol 1985; 69: 836-40.
7. Wolter JR. Foreign body giant cells implants. Graefes Arch Clin Exp Ophthalmol 1982; 219: 103-11.
8. Wolter JR. Fusion of macrophages on lens implants resulting in the formation of giant cells. Graefes Arch Clin Exp Ophthalmol 1983; 221: 1-7.
9. Gayton JL, Apple DJ, Peng Q, et al. Interlenticular opacifica-

tion: clinicopathological correlation of a complication of posterior chamber piggyback intraocular lenses. J Cataract Refract Surg 2000; 26: 330-6.
10. Lai JY, Chen KH, Hsu WM, Lee TH, Lin SY. Multiple elements in the deposits of opacified Hydroview intraocular lens. Am J Ophthalmol 2005; 139: 1123-5.
11. Dorey MW, Brownstein S, Hill VE, et al. Proposed pathogenesis for the delayed postoperative opacification of the Hydroview hydrogel intraocular lens. Am J Ophthalmol 2003; 135: 591-8.
12. Saika S, Tamaka S, Ohmi S, et al. Deposition of extracellular matrix on intraocular lenses in rabbits: an immunohistochemical and transmission electron microscopic study. Graefes Arch Clin Exp Ophthalmol 1997; 235: 241-7.
13. Linnola RJ, Werner L, Pandey SK, Escobar-Gomez M, Znoiko SL, Apple DJ. Adhesion of fibronectin, vitronectin, laminin, and collagen type IV to intraocular lens materials in pseudophakic human autopsy eyes Part 2: explanted intraocular lenses. J Cataract Refract Surg 2000; 26: 1807-18.
14. Kochounian HH, Kovacs SA, Sy J, Grubbs DE, Maxwell WA. Identification of intraocular lens adsorbed proteins in mammalian in vitro and in vivo systems. Arch Ophthalmol 1994; 112: 395-401.
15. Werner L, Apple DJ, Kaskaloglu M, Pandey SK. Dense opacification of the optical component of a hydrophilic acrylic intraocular lens: a clinicopathological analysis of 9 explanted lenses. J Cataract Refract Surg 2001; 27: 1485-92.
16. Macky TA, Werner L, Soliman MM, Pandey SK, Izak AM, Apple DJ. Opacification of two hydrophilic acrylic intraocular lenses 3 months after implantation. Ophthalmic Surg Lasers Imaging 2003; 34: 197-202.
17. Izak AM, Werner L, Pandey SK, Macky TA, Trivedi RH, Apple DJ. Calcification on the surface of the Bausch & Lomb Hydroview intraocular lens. Int Ophthalmol Clin 2001; 41: 63-77.
18. Shek TW, Wong A, Yau B, Yu AK. Opacification of artificial intraocular lens: an electron microscopic study. Ultrastructural Pathol 2001; 25: 281-3.
19. Moesen I., Maudgal PC, Foets B. Late opacification of Hydroview intraocular lenses: report of 11 cases. Bull Soc Belge Ophthalmol 2006; 299: 13-8.
20. Trivedi RH, Werner L, Apple DJ, Pandey SK, Izak AM. Post-cataract intraocular lens (IOL) surgery opacification. Eye 2002; 16: 217-41.
21. Werner L, Apple DJ, Pandey SK, et al. Analysis of elements of interlenticular opacification. Am J Ophthalmol 2002; 133: 320-6.
22. Yu AK, Shek TW. Hydroxyapatite formation on implanted hydrogel intraocular lenses. Arch Ophthalmol 2001; 119: 611-4.
23. Neuhann IM, Stodulka P, Werner L, et al. Two opacification patterns of the same hydrophilic acrylic polymer: case reports and clinicopathological correlation. J Cataract Refract Surg 2006; 32: 879-86.
24. Izak AM, Werner L, Pandey SK, Apple DJ. Calcification of modern foldable hydrogel intraocular lens designs. Eye 2003; 17: 393-406.