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SCANNING ELECTRON MICROSCOPY AS AN ANALYTICAL TOOL FOR THE STUDY OF CALCIFIED INTRAUTERINE CONTRACEPTIVE DEVICES

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Abstract

Within the endometrial cavity intrauterine contraceptive devices (IUDs) become encrusted with cellular, acellular, and fibrillar substances. Scanning electron microscopy was used to study the crust. Cellular material consisted mainly of blood cells and various types of bacteria. The fibrillar material appeared to be fibrin which was omnipresent in the crust and formed a thin layer immediately over the IUD surface. X-ray microanalysis of the acellular component of the crust revealed the presence of calcium. No other major peaks were identified. Near the IUD surface characteristic calcium phosphate crystals were present. Their microanalysis showed peaks for calcium and phosphorus. X-ray diffraction of the crust however, showed it to contain only calcite. It is through the use of scanning electron microscopy that calcium phosphate has been detected in the IUD crust and a fibrillar layer has been visualized on the IUD surface. This study further demonstrates the effectiveness of SEM analytical techniques in the area of biomedical research.

KEY WORDS: Calcification, Biomineralization, Calcium Phosphate, Calcium Carbonate, X-ray Microanalysis, Copper IUDs, Plastic IUDs, Intrauterine Contraceptive Devices, Scanning Electron Microscopy, Calcific deposits.

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Introduction

Intrauterine contraceptive devices (IUDs) are one of the most popular means of reproduction control in the world today. They are effective, economical and generally safe. A number of clinical studies have however, shown that use of IUDs leads to an increased incidence of pelvic inflammation and that the duration of the use of an IUD is the single most important risk factor in the development of the inflammation [3, 12, 13, 14]. Within the endometrial cavity, IUDs are capable of inducing changes that can lead to inflammation. In the process the IUD surface becomes encrusted with a light brownish-yellow material [3, 4, 5, 9] which, in cases of heavy deposition, can be seen with unaided eyes. Our interest in biomineralization led us to study this phenomenon. Changes that affect IUDs are surface related, permitting their study using scanning electron microscopic techniques.

Materials and Methods

The IUDs used in this study were extracted for various reasons. Two Lippes loops and one copper IUD were removed because of the finding of actinomycosis-like bacterial groups in their cervical cytology specimens. One Saf-T-Coil and one copper IUD were removed because of the diagnosis of pelvic inflammation disease. One Saf-T-Coil was extracted because the patient presented with vaginal bleeding and was found to have stage IIB carcinoma of the cervix. One copper IUD was removed as a routine care procedure. All intrauterine devices were removed at the clinics of Shands Hospital. They were within the endometrial cavities of women of reproductive age for approximately one to eight years.

Immediately after extraction the IUDs were placed in 10% buffered formalin. Within 48 hours they were transferred to a half strength Karnovsky's fixative [7]. After several hours in this fixative they were processed for scanning electron microscopy (SEM): washed in deionized distilled water, then dehydrated through a graded series of alcohols and critical point dried. The dried specimens were then coated with silver or gold-palladium using a sputter coater and examined with a Hitachi S-450 scanning electron

microscope. Following surface examination, the crust was removed from the IUD and was crushed and teased apart to observe its interior.

Uncoated or silver or gold-palladium coated IUD crust was analyzed using a Kevex 7000 energy dispersive x-ray microanalyser, attached to the scanning electron microscope. The crust was also analyzed by x-ray diffraction using a Philips automated powder diffraction unit APD 3520.

Results

The IUD surface was covered with a flaky or cracked (Fig. 1) crust which consisted of cellular, acellular and fibrillar substances. The cracked or flaky appearance of the crust may be artifactual and a result of the dehydration process. The amount of crust deposited appeared to depend upon the duration the devices were in utero. Based on morphology some of the cells were identified as erythrocytes (Fig. 2) and leukocytes while others, because of the characteristic ridges on their exposed surfaces, were recognized as exfoliated epithelial cells. Rod shaped bacteria and spermatozoa (Fig. 3) were also seen on the surfaces of some IUDs. Embedded in the crust were filamentous microorganisms, most probably bacteria (Fig. 4).

Fibrillar material appeared as a reticulum of fine threads and was present both on the surface of (Fig. 2), as well as within the crust and was also present under the crust, coating the IUD surface. The morphological appearance of the fibrillar material and its intimate association with blood cells would strongly suggest that the fibrillar material was fibrin. Sheppard and Bonnar [15] have also reported the presence of fibrin in IUD crust. Some of the fibrillar material could be pre-ovulatory mucoproteins derived from the endometrial fluid.

The acellular material on the crust surface was amorphous to spheroidal in appearance (Fig. 3). Elemental analysis of this substance revealed that calcium was its main component (Fig. 5a, 5c). Inside the crust, and near the IUD surface, there were round crystals (Fig. 6a) which

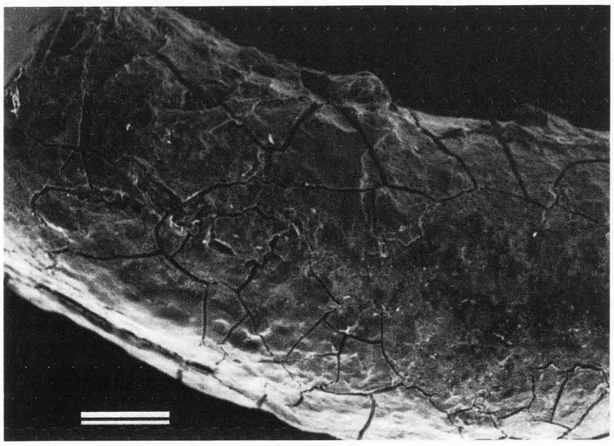


Fig. 1. A plastic IUD covered with a cracked crust. Bar = 500 μm.

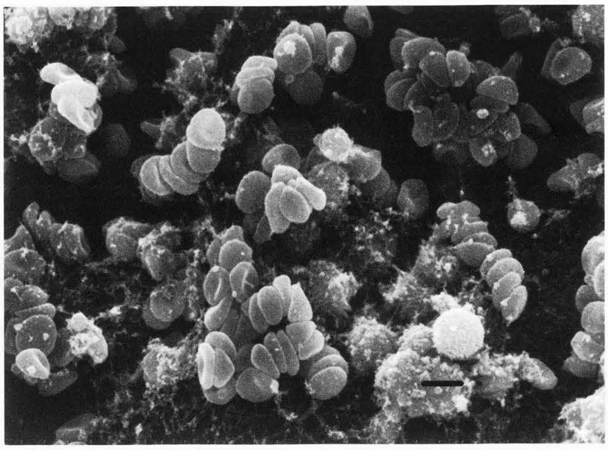


Fig. 2. Surface of the IUD crust showing erythrocytes and fibrillar material along with amorphous substances whose microanalysis showed a presence of only calcium. Bar = 5 μm.

Fig. 3. Surface of an IUD crust showing acellular, cellular and fibrillar substances. A spermatozoan can be identified at the top right. Microanalysis of large spheroidal structures showed the presence of only calcium. Bar = 50 μm.

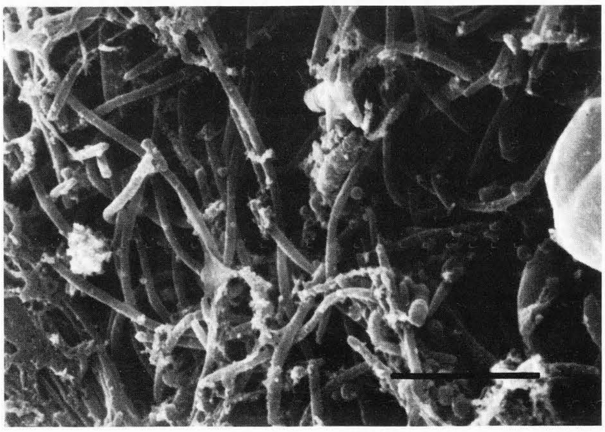
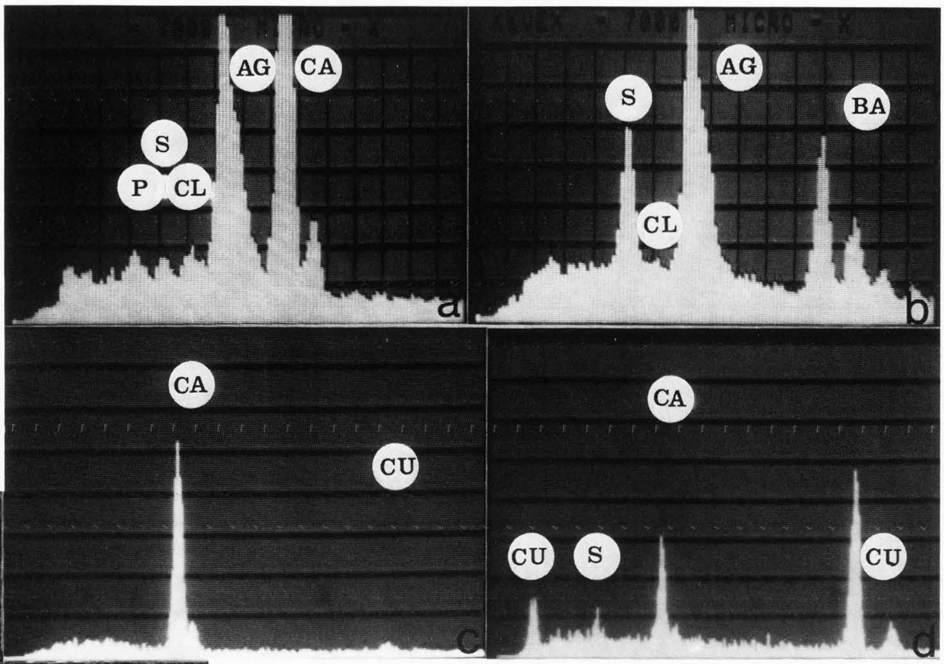


Fig. 4. Filamentous microorganisms found embedded in the crust from a plastic IUD. Bar = 50 μm.

Fig. 5. Elemental analysis spectra of the IUD surfaces a. encrusted Lippes loop. b. decrusted Lippes loop. c. encrusted copper IUD. d. decrusted copper IUD. AG, silver, BA, barium; CA, calcium; CL, chloride; CU, copper; P, phosphorus; S. sulphur. Lippes loop were coated with silver, hence the silver peaks. Copper IUDs were analyzed without coating. Counting time 100 seconds, specimen tilt angle 30°, a and b spectra 0.64-5.76 keV and c and d spectra 0-10.24 keV.



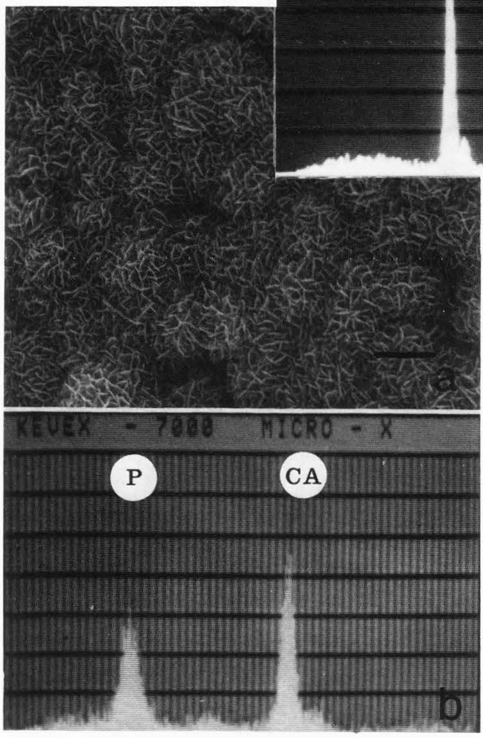


Fig. 6. a. Calcium phosphate crystals present on the crust's undersurface. Bar=5μm. b. Elemental analysis spectrum of calcium phosphate crystals shown in Fig. 6a. 0.72 keV-5.84 keV. For other legends see Fig. 5.

morphologically resembled urinary apatite crystals [16]. They were either smooth surfaced or had radially arranged plate-like crystallites on their periphery (Fig. 6a). The x-ray microanalysis spectrum of these crystals showed calcium and phosphorus peaks (Fig. 6b). Based on their habit and chemical composition these crystals were identified as apatite.

The x-ray microanalysis of encrusted copper, as well as inert plastic, IUD surfaces, showed major peaks for only calcium (Figs. 5a, 5c). When the crust was removed from an IUD surface, microanalysis of plastic intrauterine devices showed peaks for chloride, phosphorus, sulphur and sometimes barium and sulphur (Fig. 5b) in addition to smaller peaks for calcium. The microanalysis of copper IUDs, after crust removal, showed peaks for copper in addition to calcium (Fig. 5d). All of the calcified material did not come off the IUD surface when the crust was removed. The residual calcified material on the IUD surface may be responsible for the calcium and phosphorus peaks in the spectra of decrusted IUDs. Barium and sulphur peaks may have originated from barium sulphate which is used to make the plastic IUDs radiopaque. Biological material present in the crust may also have contributed to the sulphur peaks. Sulphur peak in the spectrum of encrusted IUD (Fig. 5a) was much smaller than in the spectrum of decrusted IUD (Fig. 5b) suggesting that sulphur responsible for the peaks was mostly confined to the IUD itself.

Disscussion

Used IUDs have been analyzed by various microscopic [2, 4, 5, 9, 10, 11, 15, 17, 18], chemical [1, 10, 18] and crystallographic techniques [10] to study the changes that affect the IUDs in situ as well as the nature of the crust that is formed on their surfaces. Light microscopic examination of the used IUDs suggested that a prolonged exposure to the endometrial environment resulted in gross disintegration of the IUD surface. While the surface of an unused IUD is smooth and glistening, an IUD that has been in utero for 36 months or longer appears pitted with flaking material on the surface [3, 4]. The true nature of the flakes and pits is clarified by SEM analysis of the used IUDs [11, 12]. The present and earlier SEM studies [2, 9 13, 14] clearly demonstrate that the flakes are

actually dried crust coming off the IUDs, it is the result of encrustation of the IUD surface with biological and calcific materials.

Earlier microscopic and crystallographic studies [2, 6, 9, 10, 15, 17] of the surface crust of used IUDs have shown that the crust contains calcium carbonate, various types of rod shaped and filamentous bacteria and a number of cells, most of which are macrophages. This study is in general agreement with these earlier findings. Microanalysis of the crust showed the presence of calcified material which was identified as calcium carbonate by x-ray diffraction [8]. A hitherto unreported crystalline phase of calcium phosphate was also identified. Calcium phosphate was detected by energy dispersive x-ray microanalysis, where it is possible to analyze individual crystals. These crystals were identified as apatite based on their characteristic morphology [16]. Calcium phosphate may have escaped detection in earlier studies because it appears to be present in only a small quantity which may not be enough for detection by commonly used techniques. X-ray diffraction failed to detect calcium phosphate in the IUD crust, even in this study. The probable reason why calcium phosphate escaped detection is because it is present adherent to the IUD surface, under layers of cellular and acellular material, comprising the bulk of the crust. Thus, it may not have been removed with the crust when the latter was scraped for crystallographic analysis. In this study residual calcium phosphate crystals were found attached to the IUD surface after the crust was removed.

The association between IUDs and pelvic inflammation of bacterial origin has been recognized, even though the presence of bacteria in the IUD crust has rarely been reported. In a light and transmission electron microscopic study Potts and Pearson [10] found bacteria in only 2 of 55 IUDs studied. The bacteria were present within 0.1 micron of the surface. The bacteria on IUD surfaces may be a contaminant from the endogenous genital flora, since most devices are removed through the cervix and vagina. Schmidt et al. [13, 14] were first to report the establishment of microbial growth on the IUDs and suggested that it was one of the foremost changes in IUDs in utero. In a scanning and transmission electron microscopic study, Marrie and Costerton [9] illustrated bacteria of various morphologic types, ranging from rod shaped to filamentous, inhabiting the IUD crust. The bacteria were present in densely packed and highly organized microcolonies. Results of the studies described here fully agree with the findings of Schmidt et al. and Marrie and Costerton. In the present study, although some bacteria were found on the surface, most of the IUD associated bacteria were present deeply embedded in the crust. The crust had to be crushed and teased apart to see these bacteria. Some of the surface bacteria and other biological material may have been contaminants, adhered to the IUDs during extraction from the uterus. Most bacteria and other cellular and fibrillar material however, appeared to be an integral part of the crust.

Two basic types of IUDs are currently in use [3]. Inert IUDs are made of plastic alone, while medicated ones have plastic bodies which have a copper wire wound around them or are hollow tubes containing a slurry of progesterone which is released by membrane controlled diffusion. Medicated devices may have enhanced contraceptive capability over inert IUDs. Their greater efficacy depends on the release of copper or progesterone. Once the IUD's surface becomes coated, as this study and previous studies have shown, the rate of release of these substances may decrease. This may explain, in part, the reported progressive decrease in the release rate of copper from the copper devices over time [2]. The prolonged use of a medicated IUD results in a loss of its efficiency, not only due to the slow loss of medication, but also probably to the encrustation.

The information about IUD calcification reported and discussed here was obtained by using a standard scanning electron microscope with an energy dispersive x-ray microanalysis attachment. No complicated preparation techniques were involved. No sectioning was required, that would be difficult because of the hardness of the IUD and the associated crust. The use of related techniques of backscattered imaging and SEM histochemistry will give more information about IUD calcification and these are planned. This study further demonstrates the effectiveness of SEM analytical techniques in the area of biomedical research.

Acknowledgments

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Discussion with Reviewers

P.K. Gupta: Does the nature of surface and crust change with time of IUD usage? Do the changes stabilize after a period of time?
Authors: It would appear so. We found calcium phosphate crystals near the IUD surface while the top layers were generally calcium carbonate.

P.K. Gupta: Are there any quantitative changes in copper peak with usage? Could these be defined and used as indications for IUD usefulness?
Authors: If you are asking whether there is any depreciation in the amount of copper present in the IUD through usage we are unable to answer the question because we have not done any quantitative microanalysis of the IUD. However we would like to point out that microanalysis of encrusted copper IUDs did not show any copper peaks even though the copper wire was still present beneath the crust. Gosden et al. (Text Ref. #2) made similar observations. The encrustation may interfere with the availability of copper and limit its release from the IUD thus making the IUD less efficient. Thus, the quantitative changes in the amount of copper in the IUD may not be as critical for its contraceptive effectiveness, as the availability of copper.

T.J. Marrie: Did the thickness of the crust covering the IUD correlate with the duration that the device was in utero? What was the average thickness of the crust? Did the thickness of the crust vary according to the type of IUD?
Authors: We did not measure the thickness of the crust. It did appear that the IUDs that had a longer stay in utero had more crust material than those that were in utero for a shorter period of time.

T.J. Marrie: Did the amount of calcium and phosphorus present within the crust correlate with the duration that the IUD was in place?
Authors: We are unable to answer this question because we did not do any quantitative microanalysis, and we are not sure whether we can answer this question by the techniques we are using.

P. Frasca: Do you have any clues for mechanisms leading to the reported calcification?
Authors: The calcification phenomenon appears similar to plaque formation in teeth and encrustation of catheters. First an amorphous coat is laid down on the IUD surface. This is followed by deposition of fibrillar material, to which various types of cells and cellular debris adhere. The latter are then calcified using calcium, bicarbonate and phosphate present in the intrauterine fluid.

P. Frasca: Is it possible that the crystals shown in Fig. 6 are artifacts due to formalin fixation? Freeze drying would avoid possible artifacts, due to chemicals and water. Have you considered using freeze drying instead of your reported sample preparation method?
Authors: The crystals shown in Fig. 6 are so well formed and so characteristic of basic calcium phosphate (apatite) that their chances of being artifacts are very remote. Freeze drying is a good idea for sample preparation and we would use it in future studies.

G.M. Roomans: Why have the authors limited themselves to the study of fixed IUDs, from which soluble material has been lost? A better chemical characterization of the material investigated could have been obtained if the authors had analyzed unfixed freeze-dried or even air-dried material. Better counting statistics would have made it possible to detect minor components of the IUD crust.
Authors: We are interested in calcification of IUDs and we think that the biological material adherent to the IUD surfaces plays an important role in the process. To identify the IUD-adherent material we wanted to properly fix the specimen. The procedure utilized in the text was the best we could do. Moreover, we are also studying the crust by transmission electron microscopy, the same crust we examined by SEM. We agree that freeze-drying of the specimen and better counting statistics could have made it possible to detect soluble as well as minor components, if present.