[Original] Matsumoto Shigaku 19 : 10~16, 1993
key words : calcified bodies — histopathology — ultrastructure — electron probe microanalysis — postoperative maxillary cyst

Observations on Calcified Bodies in the Cyst Wall of a Postoperative Maxillary Cyst

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Summary

Light and scanning electron microscopic observations and electron probe microanalysis were carried out on granular calcified bodies that appeared in the wall of a postoperative maxillary cyst in a 59-year-old man. The granular bodies, stained slightly with hematoxylin, were scattered in the cyst wall. They reacted positively to von Kossa's stain indicating calcification. Based on scanning electron microscopic observation, secondary electron images revealed them to be compact bodies, and they appeared as light spots in composition images. Electron probe microanalysis revealed that the bodies were ccomposed mainly of calcium and phosphorus. Some of the large bodies had a membranous calcification core, which was observed especially in von Kossa's stained specimens. Therefore, we believe that these calcification core must be generated cell debris, cell membrane, nuclear membrane or subcellular organelles.

Introduction

Pathological calcium deposition in various tissues mainly occurs in connection with necrotic and/or degenerative changes[1,2]. Therefore, these calcifications are generally characterized as deposits that the needle like or amorphous in shape depending upon the underlying structures. Although granular calcified bodies commonly occur in meningiomas and adenocarcinomas, some papers have also described microcalcifications, especially microcalculi in salivary glands. In oral neoplasms, this type of microcalcification appears mainly in salivary gland tumors[3~7], particularly in pleomorphic adenomas.

During our histopathological survey of surgically removed tissues at the Clinical Division of Matsumoto Dental College Hospital, we discovered a case of postoperative maxillary cyst having granular calcified bodies scattered in the cyst wall. We describe herein the histopathological and ultrastructural features of these bodies.

Presented in part at the 35th meeting of the Matsumoto Dental College Society held on November 7, 1992. (accepted for publication on February 5, 1993)

Materials and Methods

Materials examined in this study were obtained from a postoperative maxillary cyst in a 59-year-old man (MDC 082-91). The specimens were drawn from the patient during surgery, and for histopathological analysis, selected portions were fixed in 10% formalin solution, dehydrated through a graded ethanol series, and then embedded in paraffin. After sectioning, the specimens were stained with hematoxylin-eosin (H-E) and von Kossa's stain and then observed under a light microscope.

For scanning electron microscopy (SEM) and electron probe microanalysis (EPMA), sectioned specimens were mounted on a carbon block and deparaffinized with a xylene solution. They were then processed by critical-point drying, coated with carbon by the cathodic sputtering method, and then examined with a JEOL JCXA 733 super probe.

Results

Histopathological findings:

Light microscopic observation revealed that the cyst wall consisted mainly of collagen bundles having limited lining epithelium (Figs. 1, 2), and in part of the wall signs of reactive bone formation were evident (Fig. 1). The cyst wall connective tissue was partially hyalinized, which showed poor cellular areas, and in these areas some granular and irregular sized bodies stained slightly with hematoxylin were present (Figs. 3, 4). The large masses were globular in shape, and the small ones were sand-like granules. These bodies seemed to have no relationship with the above mentioned reactive bone formation.

These globular masses and sand-like granules reacted strongly with von Kossa's stain, indicating the occurrence of calcification (Figs. 5, 6). Regarding the large globular bodies, their positively stained portion appeared to be laminated, and the area around (Fig. 5 arrows), the periphery (Fig. 6 arrows) and the central area of the bodies were strongly positive. In contrast, the entire small sand-like granule was stained uniformly strong. No positive stainings were found in the surrounding hyalinized tissue.

Scanning electron microscopic findings:

Examination by scanning electron microscopy under low magnification showed several light spots in the composition images which corresponded to the von Kossa's-positive material and their distribution in various parts of the hyalinized collagen bundles (Figs. 7, 8). The secondary electron images revealed that the sectioned surface of the cyst wall was relatively solid, and globular-shaped bodies were scattered in the cyst wall (Figs. 9, 10). These globular structures were approximately 1 to 20 μm in diameter (Figs. 9, 10).

Electron probe microanalytic findings:

Analytically, these materials were calcified (Figs. 11, 12) as judged from their composition determined from radiographic (Ca-Kα and P) images (Figs. 13, 14). EPMA demonstrated that the calcified masses consisted mainly of calcium and phosphorus (Fig. 15), indicating calcium phosphate; in contrast, the surrounding tissues showed no trace of these elements (Fig. 16).

Discussion

According to the literature, pathological calcification, which is composed mainly of hydroxyapatite crystals, is often associated with membranous cellular debris, and these crystals are

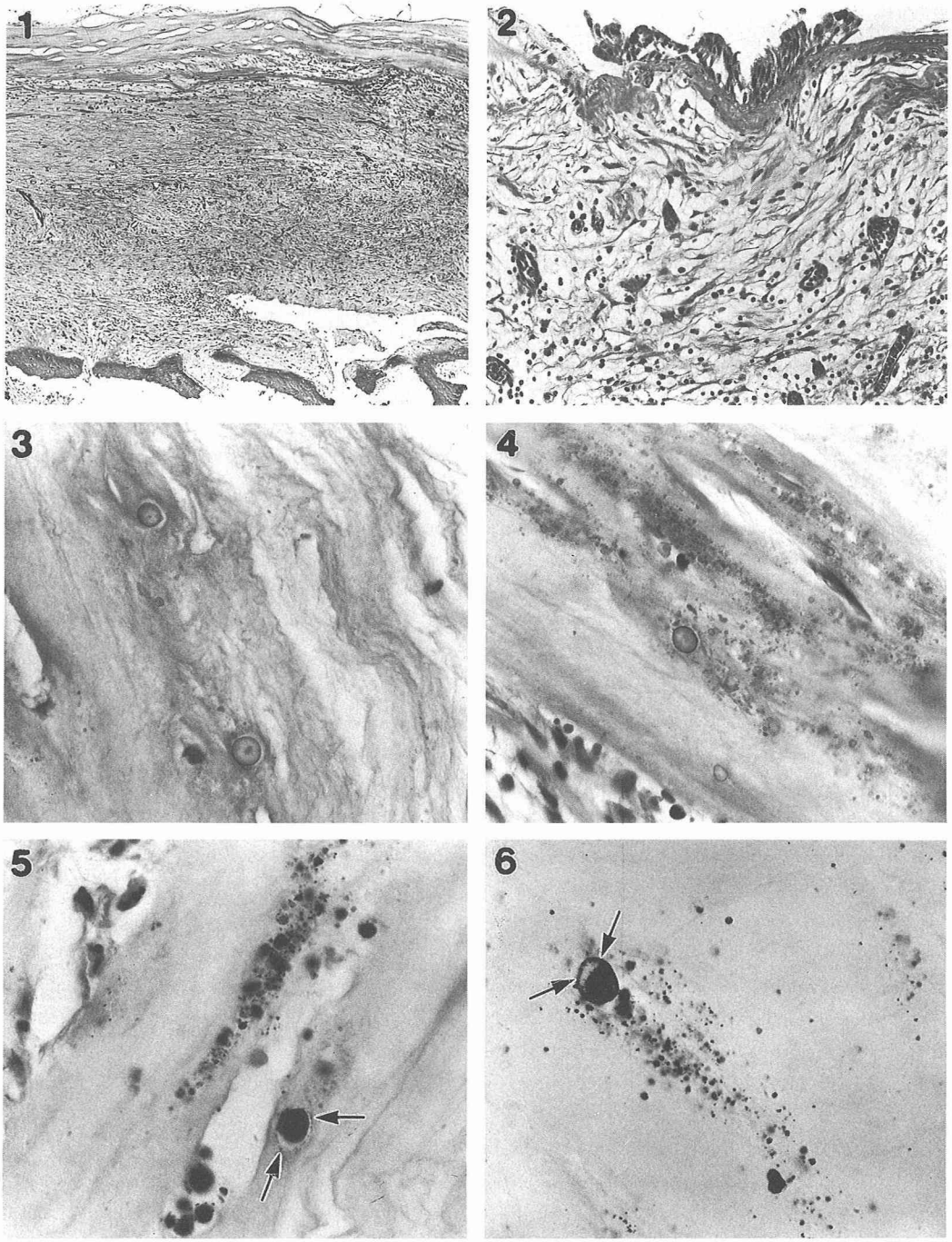

Fig. 1 Histopathological view of the cyst wall (H-E, ×40).
Fig. 2. Lining epithelium of the cyst wall (H-E, ×150).
Fig. 3. Collagenous tissue of the cyst wall (H-E, ×480).
Fig. 4. Globular bodies and sand-like materials in the tissue (H-E, ×480).
Fig. 5. Arrows showing the von Kossa's-positive stainings around the large body (×600).
Fig. 6. Positive stainings existing in the periphery (arrows) and the center of the large bodies (von Kossa's, ×600).

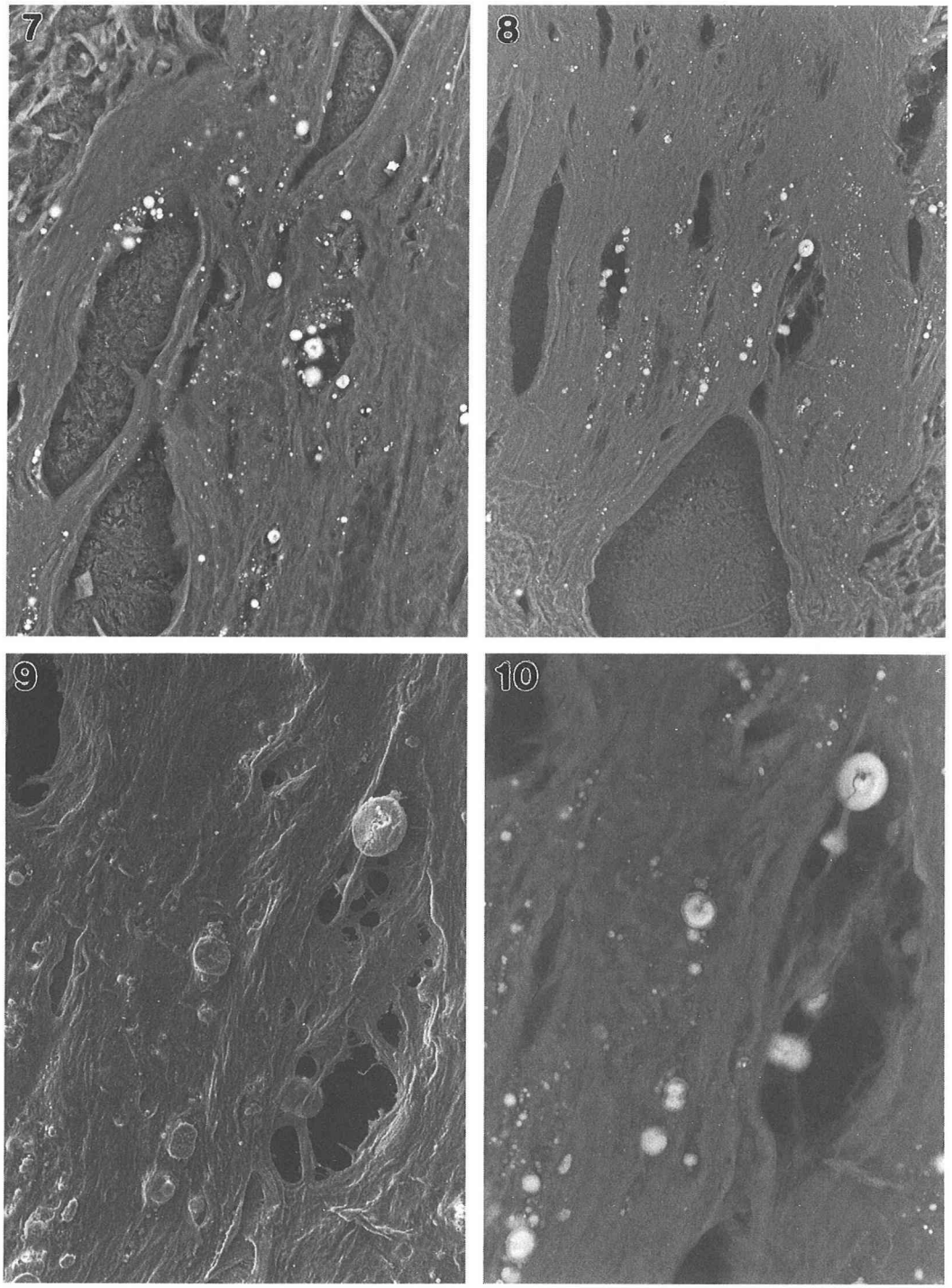

Fig. 7. Composition image showing numerous light spots in the tissue (×600).
Fig. 8. Composition image showing light spots in the tissue (×360).
Fig. 9. Enlarged photograph of the area shown in Fig. 8 (Secondary image, ×1,200).
Fig. 10. Composition image of the same area of Fig. 9.

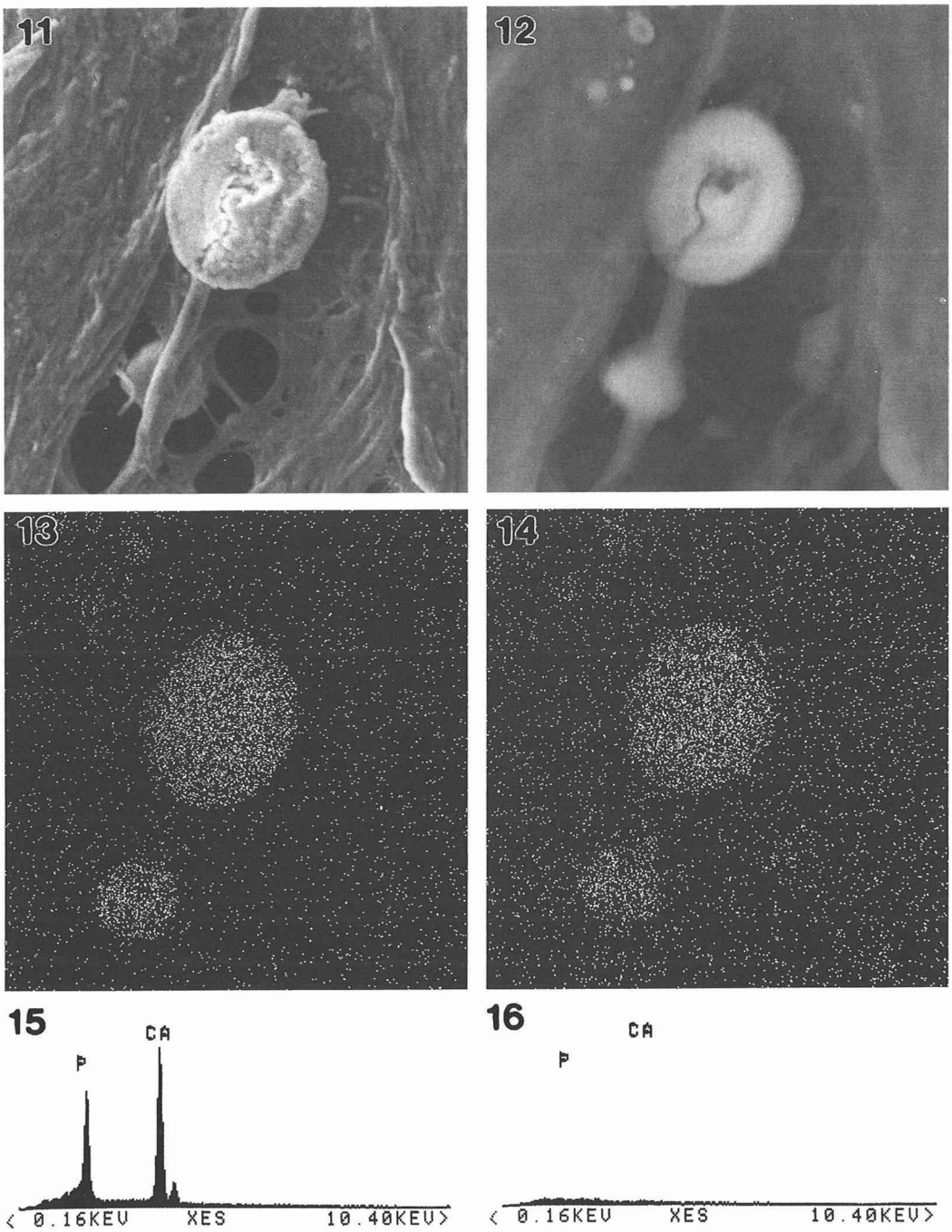

Fig. 11. Enlarged graph of Fig. 9 demonstrating large globular body (Secondary image, ×3,800).
Fig. 12. Composition image of same calcified body seen in Fig. 11.
Fig. 13. Ca-Kα image of the same area shown in Fig. 11.
Fig. 14. Phosphorus image of the same area shown in Fig. 11.
Fig. 15. Result of EPMA of calcified bodies.
Fig. 16. Result of EPMA of surrounding tissues.

thought to have started to form on these membranous structures[1]. Regarding a case of calcinosis universalis, Kawakami et al. (1986)[2] reported that the calcification site was closely related to foci of fibrinoid degeneration ; they proposed that the globular and/or membranous structures seen were derived from degenerating cells. They suggested, therefore, that these globular and/or membranous structures might be involved in the initial calcification in this case. Furthermore, Kawakami et al.[8,9] described membranous structures in the pathologic calcification sites elicited by calcium hydroxide -containing dental material, and discussed the relationship between the membranous structure and initial calcification. We believe that some of these structures have a matrix vesicle-like function, although we think that the calcified bodies are caused mainly by dystrophic changes because of their appearance[10]. Our previous papers described the ultrastructure of these calcifications in salivary gland tumor cases[3~7], and we suggested that the calcium binding capacity of these materials may be closely related to these microcalcifications. Furthermore, in a case of central neurinoma these microcalcifications were also observed[11].

In our present case, the relationship between the calcium depositions and the vesicular membranes was not evident, and we were unable to find obvious membranous structures in the present case despite careful observation. However, we believe that the microcalcifications are closely related to cellular degeneration. In general, the cyst wall is invaded by inflammatory cells at the early stage of its development but these cells disappear with the passage of time. At the time of inflammatory cell disappearance, some of these cells may degenerate. Thus, we speculate that the initial calcification will occur around these degenerating structures. In fact, we found core structures on the periphery of large calcified bodies by light microscopic observation of von Kossa's-stained specimens and by scanning electron microscopic examination. We believe these membranous calcification cores to be that of generated cell debris, cell membrane or nuclear membrane, as judged from their diameter. However, there were no core structures in the center of the small bodies, suggesting that the microcalcification in them occurred in close relation with the degeneration of subcellular organelles, i. e. mitochondria, rough and smoothed surfaced-endoplasmic reticulum, Golgi apparatus, and so on. According to the results of electron probe microanalysis, the elemental composition of the calcified bodies was similar to that in other cases already described[11,12].

Acknowledgement

The authors wish to express their thanks to Professor M. Yamaoka, Department of Oral and Maxillofacial Surgery II, Matsumoto Dental College, for supplying the surgical material from the patient. Chief Technologist S. Akahane, Laboratory of Electron Microscope, Matsumoto Dental College, is acknowledged for his technical assistance in electron microscopy.

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抄録：術後性上顎嚢胞の嚢壁にみられた石灰化物の観察

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59歳男性に発現した術後性上顎嚢胞で，その嚢壁に石灰化物の存在する1症例を経験したので，光顕的ならびに電子顕微鏡的に追究した．光顕的に嚢胞の裏装上皮は，高度の炎症により破壊されたためほとんど認められず，嚢壁の大部分は硝子化した線維性組織により構成されていた．同部にはヘマトキシリンに淡染した構造物が介在しており，これらの構造物は von Kossa 染色に強く陽性反応を示し，石灰化物であることが確認された．さらに大きな球状石灰化物の一部では，その周囲に膜様に石灰化した構造があった．なお，これらは，SEM の二次電子像では比較的平滑に，組成像では明るく，また EDS では Ca と P が主たる成分であることを示した．したがって，今回の症例の検索では，その形成基盤となった嚢壁は裏装上皮がなくなるほど高度な炎症を起こしていたことなどから，大きな球状石灰化物の基盤となったものは変性・壊死した炎症性細胞などの核膜あるいは細胞膜であり，砂状の石灰化物では細胞内小器官であるものと推察された．