LETTER TO THE EDITOR

Ultrafine structure of calcium oxalate monohydrate renal calculi

Estructura ultrafina de cálculos renales de oxalato cálcico monohidratado

Dear Editor,

Calcium oxalate monohydrate (COM) calculi may be divided into two types: papillary calculi with an attachment area to the papilla, and calculi developed in a renal cavity with no detectable sites of attachment. Papillary calculi have a core located near the attachment site and compact radially striated peripheral layers formed by columnar crystals. Calculi developed in cavities have a central core serving as nidus for development of columnar crystals emerging from the core.1 Compact layers of columnar crystals are concentrically laminated, i.e. individual layers of columnar crystals are separated by a thin concentric layer of different color and appearance. Thin concentric layers are apparently composed of tiny crystals,2 although their structure and composition have not yet been scrutinized since it was not possible with the available instrumentation. We aimed at clarification of the structure and composition of these thin concentric layers using atomic force microscopy (AFM).

Two COM calculi from our collection, one papillary and the other developed in a cavity, were selected. Both had smooth surface, dark gray color, rounded and elongated shape and had about 4mm in diameter. The calculi were cut into half with a surgical knife. One piece was placed in a vacuum chamber of a scanning electron microscope (SEM, Hitachi S3400N) and the cross-section morphology was observed. The second half was cast in epoxy resin, cured for several hours at 40°C, and the resulting blocks were cut at −70°C in nitrogen atmosphere using a cryoultramicrotome (MicroStar Technologies, USA) equipped with a diamond knife. The cutting plane was inclined approximately 45° from the perpendicular to the surface of the cross-section due to calculus hardness. Several smooth slices, few micrometers thick, were prepared and observed by AFM (Dimension Icon, Bruker, USA) in a peak Force QNM imagining mode which provided topographic and adhesion maps of the surfaces.

Based on images obtained from SEM and AFM, it was impossible to differentiate between concentric thin layers occurring in COM papillary calculi and those formed in a cavity, as their structural and compositional features were nearly identical.

SEM image showed that layers of columnar crystals composed of blocks of juxtaposed sheet-like crystals were interrupted by thin, approximately 2μm thick, concentric layers with different structure, but identical to the two types of calculi (Fig. 1A and B). Arrangement of crystalline matter in thin layers was clearly different to that of layers of columnar crystals. Details of structure on a nanometre scale were provided by AFM. Topographic 3-D map (Fig. 1C) shows a 25 μm² area of the thin concentric layer separating two adjacent layers of columnar crystals, beginning on the left and right side corners of the image.

The depicted part of the thin concentric layer is compact (non-porous) with a slightly undulated surface. This layer is composed of juxtaposed thin plate-like particles of various thicknesses with different spatial orientations from those of the columnar crystals forming the adjacent layers. A 3-D adhesion map (Fig. 1D) of the same area in fig. 1C demonstrates that the surface of the particles is covered by a soft material (yellow color), presumably of organic origin. The interiors of particles are composed of stiff material of inorganic origin, as demonstrated by dark brown discoloration of the steps formed when particles were vertically broken. The fact that these structural features are similar in both types of COM calculi, papillary and formed in cavities, is because urinary composition in which both types of calculi develop is analogous, and the concentric thin layers would correspond to periods of interruption of growth of the columnar crystals of the striated layers.


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Figure 1  General image of thin concentric layer dividing blocks of columnar crystals (A) in a calcium oxalate monohydrate papillary renal calculus and (B) in a calcium oxalate monohydrate renal calculus formed in a renal cavity. (C) 3-D topographic map of the concentric layer. Scan 5 μm × 5 μm. (D) 3-D adhesion map combined of the same area as depicted in C. Scan 5 μm × 5 μm.

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References


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