

## ACCEPTED MANUSCRIPT

**Papillary ductal plugging is a mechanism for early stone retention in brushite stone disease**

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Running head: Early brushite stones form on ductal plugs

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**Abstract** (246 words)

## Purpose:

Mechanisms of early stone retention within the kidney are understudied and poorly understood. To date, attachment via Randall's plaque is the only widely accepted theory in this regard, best described in idiopathic calcium oxalate stone formers. Brushite stone formers are known to have distinct papillary morphology relative to calcium oxalate stone formers. As such, we sought to determine whether stone attachment mechanisms in such patients may be similarly unique.

## Materials and Methods

Patients undergoing percutaneous and or ureteroscopic procedures for stone removal were consented for endoscopic renal papillary examination and individual stone collection. Each removed stone was processed using micro computed tomographic imaging (micro CT) in order to assess three dimensional microstructure and **minerals contained** and to search for common structural features indicative of novel mechanisms of early growth and attachment to renal tissue.

## Results

25 intact brushite stones were removed and analyzed from 8 patients. Video confirmed attachment for 13/25 stones with the remainder believed to have been accidentally dislodged during the procedure. Microscopic examination (light and CT) failed to show evidence of Randall's plaque associated with any stone containing brushite. Conversely, each brushite stone demonstrated microstructural evidence of having grown attached to a ductal plug formed of apatite.

## Conclusions

Three dimensional analysis of small brushite stones suggests overgrowth on ductal apatite plugs as a mechanism of early stone growth and retention. Such findings represent initial supporting evidence for a novel mechanism of stone formation that has previously been hypothesized but never verified.

## Introduction

Small stones that form in the kidney should be passed by a normal urinary system. Indeed, this is the functional principle of shock wave lithotripsy, in which stones in the kidney are broken up into small pieces, which then pass harmlessly from the body.<sup>1</sup> This fact presents a sort of mystery concerning the growth of a kidney stone *de novo*. A stone must begin as a small concrement within the renal urinary space, but in a stone former it must be prevented from being passed before it can grow into a clinically significant stone. Thus, the growth of stones within the kidney must involve some mechanism or mechanisms to retain the early stone while it grows to sufficient size that it cannot be passed.

While it is conceivable that stones could grow within the urinary space so quickly that they would be trapped above the ureteropelvic junction, modeling based on urinary supersaturations does not support the possibility of typical stones growing at such a rapid rate.<sup>2</sup> It is more likely that stones are retained within the kidney by some mechanisms other than size alone during their early growth.

One such mechanism of stone retention in the kidney is that of Randall's interstitial plaque. Some calcium oxalate stone formers have been shown to form their stones solely on Randall's plaques.<sup>3,4</sup> In this kind of stone former, the interstitial plaque becomes exposed to calyceal urine, and a stone is able to grow, quite slowly, as calcium oxalate is deposited onto the plaque, layer by layer.<sup>5</sup> The early stone is retained within the renal calyx by the anchoring of the small stone to the collagenous connective tissue of the renal papilla via the Randall's plaque.<sup>6</sup> Stones growing in this manner can eventually be freed from their papillary anchor<sup>7,8</sup> but they retain within them the mineral signature that shows their early origins on papillary plaque.<sup>9</sup>

But it also seems that some forms of kidney stone can be retained within the calyx by beginning as overgrowths onto ductal plugs.<sup>10,11</sup> In this scenario, mineral is deposited within the renal tubule, becoming lodged within the lumen of the terminal collecting duct. The distal end of such a ductal plug can then be a site for growth of an early stone, as mineral is deposited onto the end of the plug from the calyceal urine. As long as the plug remains lodged in the collecting duct, the nascent stone will be held within the kidney and allowed to grow.

Evidence for this mechanism of early stone retention has been more difficult to collect than it has been for the Randall's plaque mechanism for retaining calcium oxalate stones. In a calcium

oxalate stone, it is relatively easy to see by micro computed tomographic imaging (micro CT) the remnant of Randall's plaque that shows that the stone originated on the papilla [RP paper].<sup>12</sup> In other kinds of stones, the remnant of an apatite plug can be much more difficult to discern.<sup>13</sup>

Thus, although a number of authors have assumed that stones can grow on ductal plugs,<sup>14</sup> and that ductal plugs might be a mechanism for retention within the kidney during early growth,<sup>5</sup> that hypothesis has never been explored in any direct manner. In this paper we studied a series of patients forming brushite stones, and make the case that in this kind of stone former the retention of nascent stones on ductal plugs is most certainly a mechanism of early stone growth.

## Materials and Methods

Among the same population of patients from which we have studied previous stone conditions,<sup>3, 10, 11</sup> we have had 28 who produced stones that contained at least some brushite, and these patients were studied for evidence of early mechanisms of stone retention via attachment to the renal papilla.

Briefly, patients were recruited who were undergoing ureteroscopy and/or percutaneous nephrolithotomy, and consented for digital endoscopic study of the papillae during surgery, as well as detailed collection of stone specimens. Stone specimens were collected as much as possible one-by-one and on a papilla-by-papilla basis. A key part of the experimental approach in this study was obtaining intact stones, as we reasoned that damage to a stone could easily obscure clues as to stone formation. We used micro CT to confirm the intact nature of the stones, as well as to identify minerals and their relationships within these small stones. We have documented the ability of micro CT to identify minerals in kidney stones.<sup>15-17</sup>

Each stone specimen was scanned using micro CT<sup>17</sup> using voxel sizes of 2-14  $\mu\text{m}$ , depending on the specimen size. Micro CT scans were used to judge mineral types and mineral identifications were confirmed in every patient using infrared spectroscopy.<sup>18</sup> Apparent ductal plugs on small stones were identified by shape and size using micro CT slices and 3D surface renderings.

Statistics. Simple comparisons were done using the Wilcoxon signed-rank test, assuming significance with  $P < 0.05$ .

## Results

In 20/28 patients making stones with brushite, no small brushite stones were collected. Patients who make brushite stones tend to have large stone burdens,<sup>19</sup> and it may be that the mass of stone material removed in these patients was so large that any nascent stones (difficult to collect under any circumstance) were overlooked or simply dislodged with fragments of the larger stones. Indeed, in the 20 patients with no brushite stones that appeared to have been attached to papillae, the total volume of stone removed and scanned by micro CT averaged  $1.7\pm 2.2$  cm<sup>3</sup>. However, in the 8 patients in which at least one stone was collected that had apparently been attached to a papilla, the total volume of stone removed was only  $0.5\pm 0.9$  cm<sup>3</sup>. Thus in these 8 patients the amount of stone material may have been low enough to allow the visualization and collection of nascent stones.

The 8 patients included 4 men and 4 women,  $45\pm 9$  y.o., with only 1 reporting a family history of stones. All 8 patients had undergone bilateral stone removal, and the number of collection units (stone groups, fragment sets, or individual stones) averaged  $10.1\pm 6.9$ , showing the care with which specimens were collected in these patients. Serum and urine analyses of the 8 patients were unremarkable for calcium stone formers, except that the urine was relatively alkaline ( $6.19\pm 0.28$ ) in the range previously reported.<sup>11,19</sup>

Figure 1 shows representative stones from 6 patients in which stones were removed from the tips of renal papillae and collected immediately. These stones thus were documented as being attached to the papilla, and all of them—despite their obvious diversity of structure in many respects—show growth on what appear to be ductal plugs of mineral. On 15 of the 16 stones from these 6 patients, the ductal plugs were composed of apatite. The apatite making up the ductal plugs was sometimes highly layered (as in Figure 1, patient #5) and other times rather solid (as in Figure 1, patient #4), much as has been described for the variation in morphology of kidney stones formed from apatite.<sup>20</sup> One stone (Figure 1, patient #3) contained both apatite and calcium oxalate as part of the apparent ductal plug.

In 2 other patients small stones containing brushite were retrieved that appeared to be intact, and these also showed apparent ductal plugs (Figure 2). Patient #7 yielded 9 such stones, all showing apparent plugs composed of apatite, and patient #8 only 1. From 7 of the 8 patients in Figures 1 and 2 (21 of the 25 stones) calcium oxalate was the mineral in contact with the ductal plug, with brushite crystals extending from the calcium oxalate. Only in patient #5 (Figure 1) were the brushite crystals apparently growing directly onto the apatite plug, and this pattern was seen in all 4 stones from patient #5.

The average width of the plugs in 25 stones from the 8 patients was  $580\pm 254$   $\mu$ m, with the smallest diameter 214  $\mu$ m. These widths are consistent with the plugs having filled the lumens of dilated terminal collecting ducts. Note that the nominal width of a renal papilla is approximately 1 cm, so the size of these plugs is significant. Plug length averaged  $1097\pm 518$   $\mu$ m, and maximum dimension of the stones averaged  $3.3\pm 1.6$  mm.

Video collected during endoscopy showed visual evidence of ductal plugging with mineral in all 8 patients. Images of papillae from patient #6 are shown in Figure 3 as typical. In the uppermost left panel, the small stone shown in Figure 1 was found attached, and this nascent stone is shown in more detail in Figure 4. Micro CT study of this stone revealed a cylindrical piece of apatite that appears to have been broken, and which was located on the tissue side of the stone as it was attached. The diameter of this apatite cylinder measured 214  $\mu\text{m}$ , a size consistent with the cylinder having been part of a ductal plug, as terminal collecting ducts have a nominal diameter of  $\sim 150 \mu\text{m}$ . The apparently upper end of the apatite plug (away from the break) was imbedded in calcium oxalate (CaOx), along with some more apatite. Brushite crystals were growing at the surface of the CaOx/apatite region.

Figure 5 shows the stone that was obstructing the upper ureter in patient #6. This large stone (maximum dimension 9.1 mm) was composed primarily of brushite, but had an extension on one side that looked roughly cylindrical, and which contained apatite. High-resolution examination of this region revealed an elongated rod of apatite with width varying from 210-300  $\mu\text{m}$  and an overall length of  $\sim 1400 \mu\text{m}$ . The distal end of this presumed apatite plug was covered with brushite, but the end near the body of the stone was associated with the only region of CaOx in the stone, which in turn was apparently the site of growth of the brushite that made up the bulk of the stone. Thus this stone shows the same pattern as most of the stones in Figure 1 and 2: Initiation of calyceal mineral deposition onto the presumed ductal plug was CaOx, with brushite growth following.

## Discussion

Brushite stone formers tend to have large stone burdens and recurrent stone events.<sup>19</sup> Additionally, papillae in brushite stone formers exhibit dramatic damage and extensive ductal plugging,<sup>21</sup> but the link between this papillary pathology and the clinical events has been only conjectural.

Herein we propose a likely explanation: Ductal plugs formed of apatite (which manifest as both gross yellow deposits<sup>22</sup> and likely papillary damage via crystal nephropathy<sup>21</sup>) come into contact with the urinary space and in turn serve as nidi for small stones that are then retained within the kidney and allowed to grow to clinical size. Among 28 patients with brushite stones we were able to identify 8 with a total of 25 apparently nascent stones. All of these stones showed micro CT evidence of apatite extensions that likely had been ductal plugs, some demonstrably so, with the ductal plug visible as the stone was removed from the papilla. Most of the stones (21 of 25) showed deposition of CaOx onto the apatite, followed by brushite. These nascent stones averaged 3.3 mm in maximum dimension, approaching the size expected to be clinically relevant, and an additional stone with internal architecture showing a recently covered ductal plug (Figure 5) was the symptomatic stone leading to the patient's procedure.

Ductal plugs of mineral have been known to exist for some time,<sup>23</sup> but the relevance to clinical stone formation has been difficult to establish. It seems likely that the formation of a mineral plug in a papillary collecting duct would require an initial adherence of mineral to the epithelium lining the duct,<sup>24</sup> but there are conditions in which agglomerated crystals might form rapidly enough to become lodged within the terminal collecting duct.<sup>25</sup> Ductal plugs are known to exist in a number of different kinds of stone formers,<sup>26</sup> but only limited evidence of their possible role in early stone growth has been presented.<sup>11</sup>

Demonstrating a connection between ductal plugs and early stone growth has been made difficult because of their size and composition. Ductal plugs are almost always less than 1 mm in diameter, and they are usually composed of apatite, which is a common mineral in the stones of almost all of the kinds of stone formers in which ductal plugs have been identified.<sup>26, 27</sup> Thus, if a stone forms on a ductal plug, and then is released from the papilla and receives mineral overgrowth to hide the plug, identifying the plug within the stone can be quite challenging, especially if stone overgrowth mineral includes additional apatite.<sup>13</sup>

A strength of the present study is the use of micro CT, which allows the exploration of stone structure at high resolution. Moreover, the appearance of apatite by micro CT is unique relative to CaOx or brushite, making the identity of minerals in the present study easy to distinguish. However, the spatial resolution of micro CT is inversely proportional to the size of the specimen, so that identifying apatite plugs within larger specimens is more difficult. Additionally, larger stones must be broken up to be removed, making it more unlikely that the stone nidus will be preserved. Thus the surgical technique in the present study was also essential: Harvesting tiny stones is technically challenging, and visualizing such stones in place and then retrieving them intact is even more difficult.



The main limitation of the present study is that we cannot specify how generalizable these data are. That is, although all of the brushite stones we examined in this study showed evidence of their growth beginning on ductal plugs, we cannot extend such a statement to all stones growing in this kind of patient. Specifically, although we never saw brushite on a stone growing on Randall's plaque (and at least one of the patients did have a CaOx stone growing on Randall's plaque but with no brushite), we cannot rule out such a possibility. This study also does not provide resolution to important questions of why, for example, apatite forms in certain situations rather than another calcium salt (such as brushite, or even CaOx). The occurrence of apatite as the mineral in ductal plugs is very common, even when the bulk urine is too acidic to allow apatite precipitation.<sup>26</sup> The same kind of spatial separation was seen in the present study—between the mineral forming in ductal plugs (apatite) and mineral forming from the urine (brushite)—but we have no explanation of this phenomenon.

## Conclusions

Ductal plugs composed of apatite are common in patients forming brushite stones, and such plugs can form the nidus for early stone growth. If the end of the ductal plug extends into the calyceal urine, deposition of brushite can occur, and such stones can grow to clinically significant size. In 84% of the stones with ductal plugs studied, overgrowth from calyceal urine was initially CaOx, then followed by brushite, but the significance of this mineral pattern is unclear. We conclude that ductal plugs formed of apatite are the likely mechanism by which brushite stones are retained in the kidney during early stone growth.

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## Legends

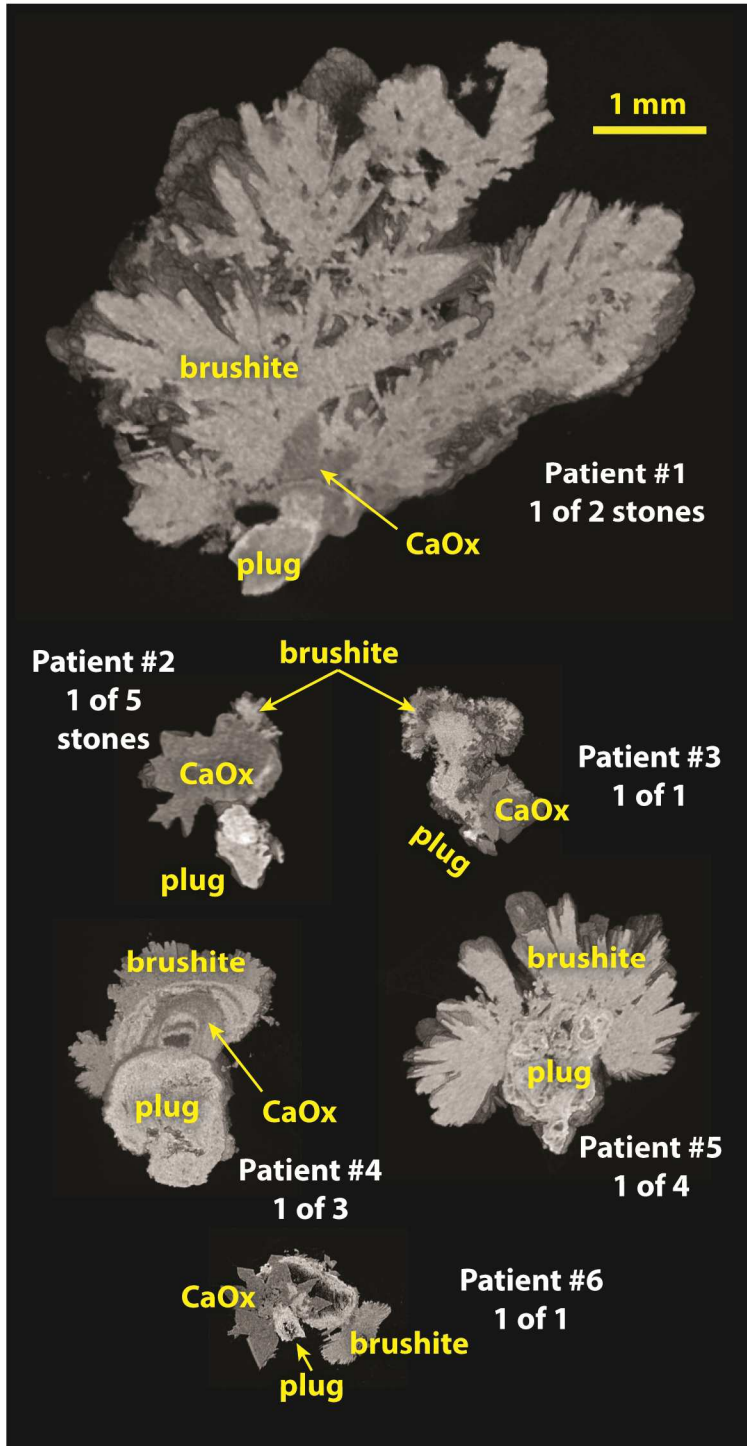
Figure 1. Representative nascent stones from brushite stone formers, each of which was visually seen to have been adherent to the tip of a renal papilla. Each image shows a 3D surface rendering with micro CT image stack cut away to show presumed ductal plug. Numbers—such as ‘1 of 5’ at top center—indicate the number of small stones in that patient for which the one shown is representative. CaOx: calcium oxalate.

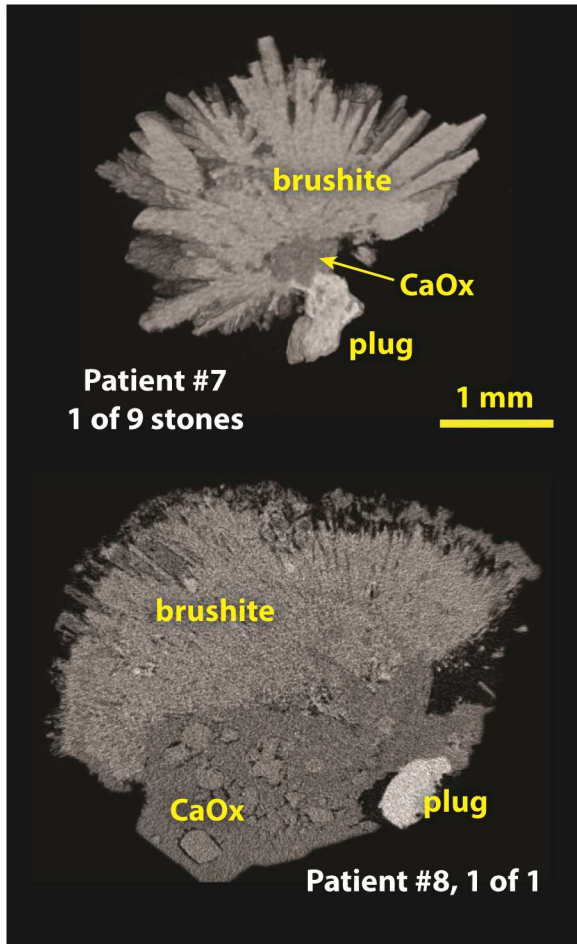
Figure 2. Nascent stones from brushite stone formers that were found loose in the urine during a procedure. These stones presumably had been anchored to a renal papilla, but were dislodged during the process of removing larger stones in the kidney. Images and numbers as in Figure 1.

Figure 3. Images of the 8 papillae mapped in kidney of brushite stone former (patient #6). A great deal of mineral is apparent, as well as deformations of papillary tips. Some apparent ductal plugs are marked with arrowheads. Circle in image of upper pole 1 indicates small stone that is the subject of Figure 4.

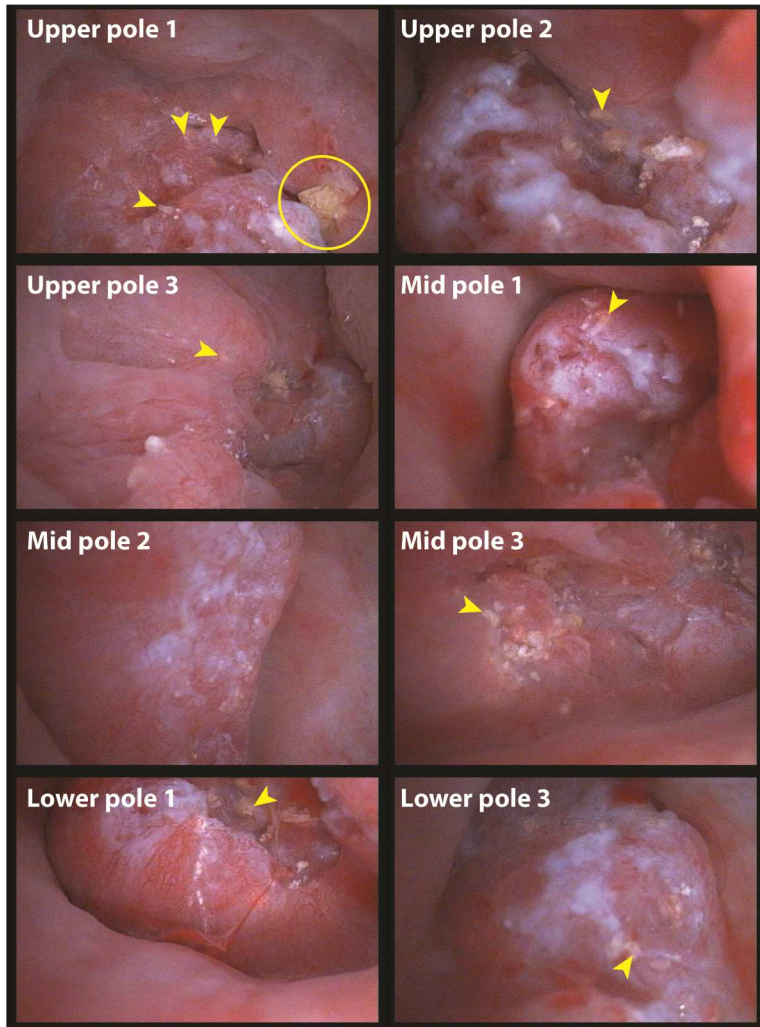
Figure 4. Small, attached stone removed from upper pole 1 (Figure 3). A: Photo of stone on mm graph paper. B: Three-dimensional (3D) reconstruction of micro CT scan of stone, cut away to reveal what appears to be a stub of an apatite plug. When surface rendering was rotated in 3D, the apatite region clearly showed an irregularly flat end with the appearance of a broken rod. C: Micro CT slice of stone in a plane similar to that of cut-away in B. The apparent ductal plug is indicated, with apatite identified by its characteristic brightness surrounding dark voids. At the distal (upper) end of the apatite plug grew calcium oxalate dihydrate crystals (COD), which in places show conversion to monohydrate (COM). Upper right is a small mass of apatite, apparently grown from the urine, which shows characteristic brightness with a rather large x-ray lucent interior. Finally, brushite is seen ‘blooming’ on the surface; brushite is identified by its brighter-than-COD appearance and its thin, radially oriented crystals.

Figure 5. Symptomatic stone from same patient as Figures 3 and 4. A: Stone on mm graph paper. B: Surface rendering of stone from micro CT scan. C: Maximum intensity projection (MIP) of micro CT image stack, showing the brightest voxels through the stone. The bulk of the stone is pure brushite. Dashed oval at lower right indicates region of what appears to be a rod of apatite that originated as a ductal plug. D: High-resolution micro CT slice through plug region (dissected off of stone for this scan). Apatite rod, which may have formed as a ductal plug, is oriented horizontally across lower part of the image. Mineral near the distal (urine) end of the presumed plug is dominated by calcium oxalate. Brushite appears on the surface of the COD crystals, and also on the apatite of the presumed ductal plug. Dashed line indicates plane of section for panel E. E: Slice across the apparent ductal plug. Cross-section of apatite rod shows brushite crystals radiating out from its surface. Gray region close to apatite rod suggests initial growth of CaOx before brushite, as described in Figures 1 and 2.





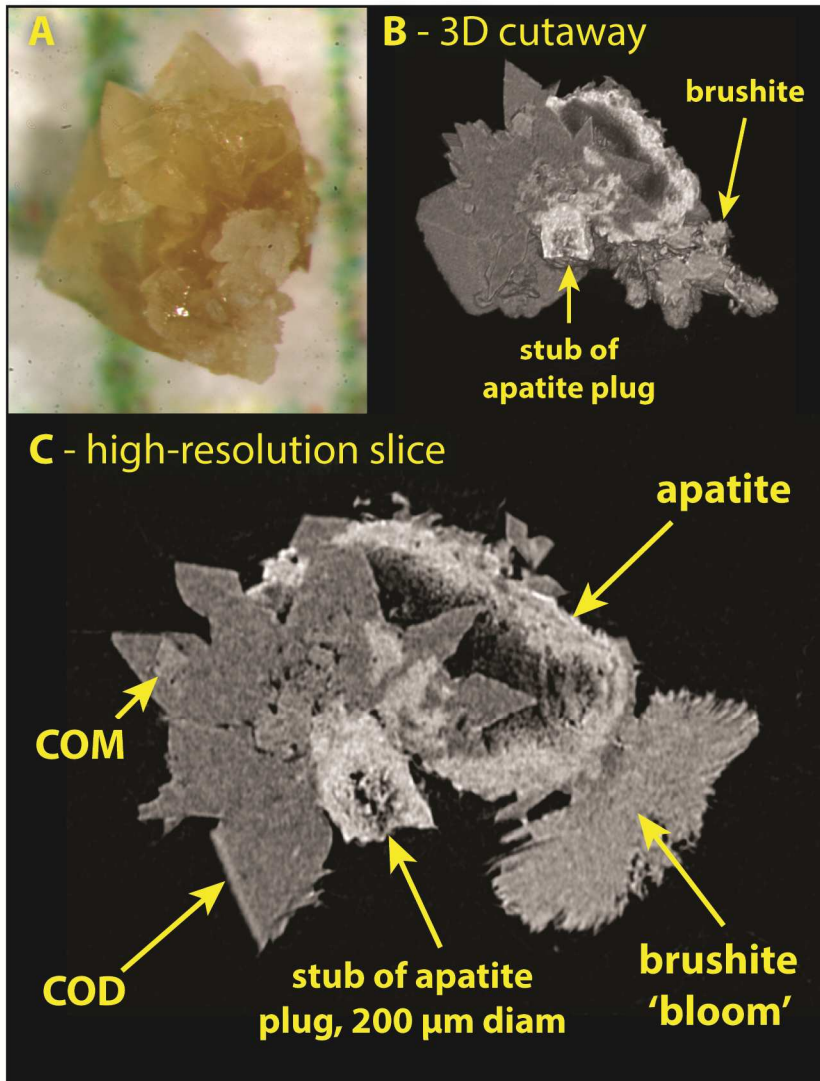


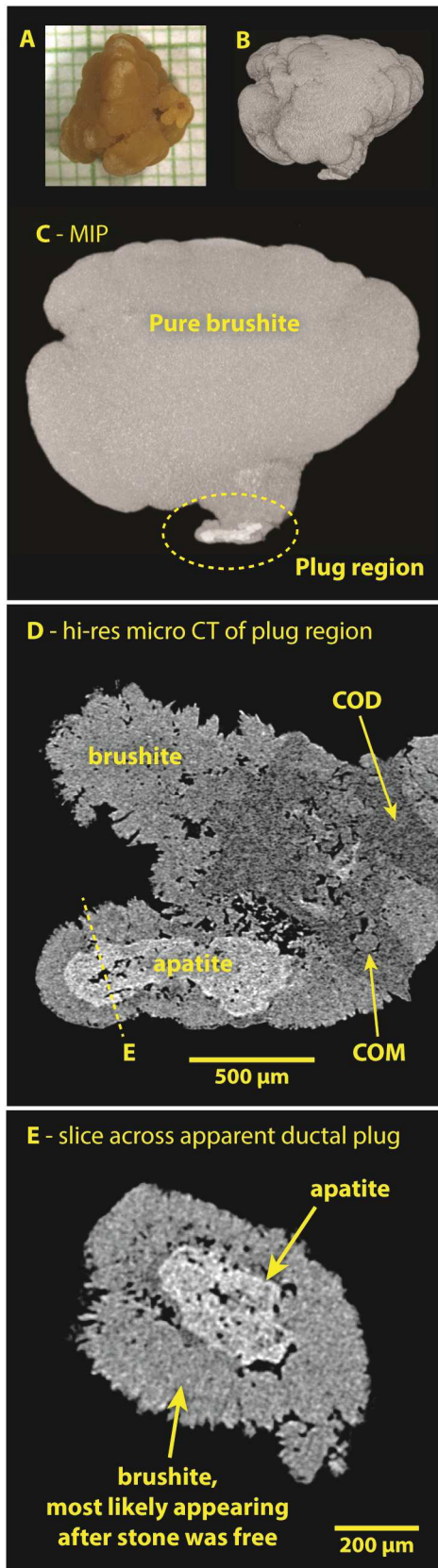


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Abbreviations

Micro CT, micro computed tomographic imaging

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