PHOSPHATE TYPE KIDNEY STONE (BRUSHITE) FORMATION IN GEL: A MORPHOLOGICAL STUDY ON GROWTH PATTERNS

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INTRODUCTION

Gallstones, gout and urinary stone diseases are associated with the presence of crystals contribute to tissue damage followed by successive aggregation, crystal growth, blockage of ducts and pain. Urolithiasis is a common clinical problem with high recurrence. Urinary stones are composed of insoluble crystalline compounds which include inorganic phases such as oxalate, phosphate and urate salts while organic matrix include proteins, lipids, polysaccharides and cellular components such as cystine, xanthine, calcium carbonate or hippuric acid. The damaged glycosaminoglycan layer favors the attachment of calcium phosphates, glycoprotein aggregates and cellular debris to the urothelial surface. Successive adhesion and aggregation results comparatively larger particle to form urinary stones. Calcium phosphate urinary stones which are about 15% of urinary stones are found in the form of brushite, dahlite, hydroxyapatite, whitlockite and octacalcium phosphate. Brushite crystals form urinary stone, promote whewellite (calcium oxalate) stone formation and act as a precursor to form apatite and octacalcium phosphate type urinary crystals. Among all urinary phosphate crystals, brushite has the greater hardness and difficult to remove surgically by the shock wave and ultrasonic lithotripsy. Brushite crystals grow rapidly with high recurrence rate and cause a significant degree of renal tissue injury as compare to other forms of stone. Brushite crystals induce apoptosis of renal proximal tubular epithelial cells and collecting ducts which create sites for crystal attachment followed by growth and maturing into brushite and whewellite stones.

Crystal growth in gel is a simple and inexpensive in vitro technique which provides direct access to observe the crystallization process resembled to human physiological conditions. Gel technique has been successfully applied to grow pathologic crystals. Although, brushite crystals has already been grown in gel. Severe renal tissue injury, high recurrence rate and difficulty in surgical removal of brushite stone potentiate us to study their different morphological features as an applied approach to evaluate risk factors and natural prophylactic management in future.

MATERIALS AND METHODS

Chemicals

Calcium chloride dihydrate, orthophosphoric acid and sodium silicate solution (Merck, Germany).

Instruments

Glass test tubes of 25mm diameter and 150mm length; IR Prestige-21 FTI-R Spectrophotometer Shimadzu; JSM-6380A Scanning Electron Microscope and EDS EX-54175 JMU, JEOL Japan; Nikon Eclipse E 400 binocular microscope, Japan.

Crystal growth and characterization

The single diffusion gel method was used with some modifications. Sodium meta silicate solution of 1.06 specific gravity and 1M orthophosphoric acid solution were mixed to prepare gel medium. After gel formation, 20ml of 1M calcium chloride solution was gently poured along with the wall of test tube. The test tube was capped and observed at 7th and 14th day. The carefully recovered crystals were washed, dried and characterized by FT-IR, SEM and EDS.

Statistical analysis

Number, size and weights of crystals are expressed as mean ± standard error of mean and were analyzed by unpaired student t-test.
Table 1: Growth patterns of brushite crystals

<table>
<thead>
<tr>
<th>Growth periods (days)</th>
<th>Number</th>
<th>Weight (mg)</th>
<th>Size (mm)</th>
<th>On the basis of number</th>
<th>On the basis of length</th>
</tr>
</thead>
<tbody>
<tr>
<td>07</td>
<td>7.40±0.50*</td>
<td>31.76±0.46*</td>
<td>2.91±0.27*</td>
<td>N2c and R2a &gt; N1a and R1a &gt; C1c &gt; N2a and R1c &gt; P7a &gt; C1d.</td>
<td>N2c &gt; R1c &gt; R2a &gt; C1c &gt; R1a &gt; N2a &gt; P7a &gt; N1a &gt; C1d.</td>
</tr>
<tr>
<td>14</td>
<td>8.80±0.96*</td>
<td>86.93±0.33*</td>
<td>5.87±0.88*</td>
<td>N1e &gt; C1g &gt; N1c &gt; N2c &gt; P1b &gt; C1h and P7a &gt; N1a.</td>
<td>N1a &gt; C1k &gt; N2c &gt; P1b &gt; C1g &gt; N1e &gt; P7a &gt; N1c.</td>
</tr>
</tbody>
</table>

C1c: solid bullet, C1d: hollow bullet, C1g: solid thick plate, C1h: thick plate of skeletal form, N1a: elementary needle, N1c: elementary sheath, N1e: long solid column, N2a: combination of needles, N2c: combination of long solid column, P1b: sector plate, P7a: radiating assemblage of plates, R1a: rimed needle, R1c: rimed plate or sector, R2a: densely rimed plate or sector. All values represent mean ± SEM of n=5; *P<0.01 showing significant values using unpaired student’s t-test.

Table 2: FT-IR wave numbers and vibrations assignment of brushite crystals

<table>
<thead>
<tr>
<th>Wave numbers (cm⁻¹)</th>
<th>Standard</th>
<th>Grown</th>
<th>Reported values*</th>
<th>Bonds / vibrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>3541.31</td>
<td>3541.31</td>
<td>3539.80,3543.16,3544</td>
<td>O-H stretching (weakly H bonded OH vibrations) of water</td>
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<tr>
<td>3485.37</td>
<td>3487.30</td>
<td>3487.10,3487.57,3489</td>
<td></td>
<td></td>
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<tr>
<td>3290.56</td>
<td>3282.84</td>
<td>3282.79,3284</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2370.51</td>
<td>2372.44</td>
<td>3161.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2370.51</td>
<td>2372.44</td>
<td>3166.70,3167</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1653.00</td>
<td>1649.14</td>
<td>1648.30,1650.29,1652</td>
<td>H-O-H Symmetric bending vibrations</td>
<td></td>
</tr>
<tr>
<td>1552.70</td>
<td>1558.48</td>
<td>1214.20,1211.02,1217</td>
<td>PO4=P=O associated stretching vibrations</td>
<td></td>
</tr>
<tr>
<td>1136.07</td>
<td>1136.07</td>
<td>1136.70,1129.96,1134</td>
<td>PO4 bond, P=O stretching vibrations</td>
<td></td>
</tr>
<tr>
<td>1064.71</td>
<td>1062.78</td>
<td>1062.30,1064.54,1065</td>
<td></td>
<td></td>
</tr>
<tr>
<td>987.55</td>
<td>987.55</td>
<td>987.20,989</td>
<td></td>
<td></td>
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<tr>
<td>873.75</td>
<td>873.75</td>
<td>874.1,873.10,872</td>
<td>P-O-P asymmetric stretching bond</td>
<td></td>
</tr>
<tr>
<td>790.81</td>
<td>792.74</td>
<td>792.0,705.00,795</td>
<td>(H-O-) P=O bond (strong absorption) acid phosphates</td>
<td></td>
</tr>
<tr>
<td>665.44</td>
<td>661.58</td>
<td>664.00,666</td>
<td></td>
<td></td>
</tr>
<tr>
<td>528.50</td>
<td>528.50</td>
<td>577.1,576.30,577</td>
<td></td>
<td></td>
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</tbody>
</table>

Figure 1: Liesegang rings formation during brushite crystals growth
(A) No ring observed on 1st day, (B) 10 rings on 2nd day and (C) 12 rings on 3rd day.

Figure 2: Brushite crystals grown in gel medium (A) upper portion, (B and C) middle portion and (D) lower portion of test tube.
Figure 3: Measured brushite crystals of different morphologies. Microphotographs with 10x magnification (a-q) and photographs without microscope (r-t).

Figure 4: Different types of brushite crystals with their average number observed at 7th and 14th day

Figure 5: Different types of brushite crystals with their average length (mm) observed at 7th and 14th day
RESULTS AND DISCUSSION

Brushite crystals were observed as bullet, columnar, needle and platy crystals and their aggregates (Figure 1-3). Weight of harvested crystals, their morphology and density (crystal crowd) are shown in Table-1 and Figure 4-5. The SEM images are shown in Figure-6. EDS spectrum of testing and standard samples shows the matched mass percentage of detecting elements. The average mass % of the elements is calcium 20.23, phosphorus 20.50 and oxygen 59.27. The values of standard compounds are calcium 25.97, phosphorus 15.98 and oxygen 58.05% (Figure 7). The FT-IR spectra compared with the spectra of commercially available compound and previously reported values (Table 2; Figure 8).

Morphological features of harvested crystals are described according to Magono and Lee meteorological classification. Brushite crystals were observed in different shapes and sizes. Column shape crystals were dominant in number and needle shaped in length. The diffusion of ions through the gel medium results insoluble precipitates in the form of Liesegang ring. Each ring contains numerous nuclei participative in crystal formation. The number of rings increased with time and total twelve rings were observed. The SEM photographs of brushite shows smooth surface with complete sheet like morphology. The EDS measurements were made at different points on the crystal surface. The results indicate that the crystals are composed of primarily calcium, phosphate and oxygen. The less difference of mass percentages between grown crystals and standard compound verify the grown crystals as calcium hydrogen.
phosphate dihydrate (brushite). The FT-IR resembles with that of commercially available compound and reported values (Figure 8).

The world population of about 12% suffers with urolithiasis. Everywhere throughout the world in various nations and societies, individuals utilize plants, for the aversion and cure of kidney stone as per their ethno pharmacological data. Interest in herbal drugs is growing due to their efficiency and less side effects. However, the understandings of the urolithiasis pathophysiology and pharmacology of natural medicines are of great importance for the development of safe and effective antiurolithiatic medicines. The studies of crystal growth morphology are very important to understand the physico-chemical properties of crystals participating in cholelithiasis, gout and urolithiasis. In case of promotory, modulatory and inhibitory effects of additives, these studies provide an applied approach for the management and cure of these diseases. This study shared detailed morphology, average size, number and weight of brushite crystals. Any type of change in these parameters will provide the sense of phosphate type kidney stone promotion, modulation and inhibition.

CONCLUSION

Brushites were observed with fourteen unique morphologies explained on the basis of Magono and Lee meteorological classification. The acquired information regarding shapes, size and average weight of crystals may be applied by incorporating extracts of natural products during crystal growth. The observed changes as compared to the control experiments will be helpful to evaluate risk factors and also devising the means for the prophylactic management of phosphate type urinary stones. The present study only shares the brushite crystal growth patterns. In future, atomic force microscopy and scanning confocal interference microscopic techniques are recommended to measure growth rates of crystals along different axes to understand the growth kinetics.

REFERENCES


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