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Time-Resolved Dynamics of Struvite Crystallization: Insights from the Macroscopic to Molecular Scale

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Abstract: The crystallization of magnesium ammonium phosphate hexahydrate (struvite) often occurs under conditions of fluid flow, yet the dynamics of struvite growth under these relevant environments has not been previously reported. In this study, we use a microfluidic device to evaluate the anisotropic growth of struvite crystals at variable flow rates and solution supersaturation. We show that bulk crystallization under quiescent conditions yields irreproducible data owing to the propensity of struvite to adopt defects in its crystal lattice, as well as fluctuations in pH that markedly impact crystal growth rates. Studies in microfluidic channels allow for time-resolved analysis of seeded growth along all three principle crystallographic directions and under highly controlled environments. Having first identified flow rates that differentiate diffusion and reaction limited growth regimes, we operated solely in the latter regime to extract the kinetic rates of struvite growth along the [100], [010], and [001] directions. In situ atomic force microscopy was used to obtain molecular level details of surface growth mechanisms. Our findings reveal a classical pathway of crystallization by monomer addition with the expected transition from growth by screw dislocations at low supersaturation to that of 2-dimensional layer generation and spreading at high supersaturation. Collectively, these studies present a platform for assessing struvite crystallization under flow conditions and demonstrate how this approach is superior to measurements under quiescent conditions.

Introduction

The crystallization of struvite ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) is relevant for nutrient (nitrogen and phosphorus)^{1, 2} recovery in water purification³⁻⁸ and scale formation in pipelines^{9, 10}, and it is a primary component of so-called *infection stones* arising from urinary tract infections¹¹⁻¹³. The vast majority of studies focused on struvite have overlooked fundamental aspects of crystal nucleation and growth at near molecular level. Knowledge of these processes can aid in the design of conditions that either promote or inhibit struvite formation, depending on the application. The primary motivation for this study is derived from the dearth of information regarding the factors that mediate the formation of struvite infection stones in urolithiasis, which is one of the most difficult and dangerous stone diseases due to the large size and rapid growth of the crystals, and high recurrence rate¹⁴. Infection by urease-positive bacteria, predominantly *Proteus mirabilis*, leads to a cascade of reactions that elevate

urinary pH, as well as ammonium and phosphate ion concentrations, thus resulting in struvite formation^{11, 15, 16}. Struvite crystals, without proper treatment, can develop into large staghorn calculi ($>2,500 \text{ mm}^2$), with the capacity to fill the entire intra-renal collecting system, irritating the bladder and blocking the flow of urine^{17, 18}. Moreover, crystalline biofilms composed of struvite complicate the care of patients undergoing long-term catheterization due to catheter encrustation and blockage, which impacts approximately 50% of catheter users^{13, 19-21}. Due to the emergence of antibacterial resistance and reported recurrence rates of 50%, the standard care involving removal/replacement of catheters and the use of antibiotics is becoming less effective²²⁻²⁵.

Understanding processes of crystallization is crucial for development of improved therapeutics for struvite infection stones. Several factors (e.g. saturation state, pH, and the presence of growth modifiers) can influence struvite formation^{12, 26-31}. Growth modifiers ranging from ions and small molecules to large macromolecules can impact crystal growth by altering supersaturation (i.e. forming modifier-solute complexes), physically blocking solute attachment by binding to crystal surfaces, or disrupting the local environment around crystal-solute interfaces³². Prior studies of modifiers in other systems, such as antimalarials used to avert hematin crystallization³³ and acids used to treat calcium oxalate kidney stones³⁴, have been shown to inhibit crystallization through preferential binding to crystal surfaces. In some cases, modifiers have been shown to possess the ability to dissolve crystals in supersaturated conditions³⁵⁻³⁷. Similarly, studies have identified a select number of modifiers (e.g. citrate²⁷, phosphocitrate²⁸, and pyrophosphate²⁶) that influence struvite crystallization. The majority of studies that test putative modifiers of struvite formation employ bulk crystallization assays under quiescent conditions where solute depletion is tracked by changes in solution properties (conductivity, pH), or the net change in average crystal size and morphology is evaluated *ex situ* by microscopy; however, these techniques are typically time consuming and have limitations that include (but are not limited to) their inability to elucidate mechanisms of growth and modifier action, and to simulate flow conditions encountered in the practical applications described above. The use of flow systems to study mineral formation is purported as a promising approach³⁸⁻⁴¹, especially for infection stone formation, as they mimic the flow conditions where struvite naturally forms (e.g. pipelines, urinary tract systems, etc)^{42, 43}.

In this study, we employ a microfluidics platform for the rapid screening of struvite growth under flow at varying conditions. Our findings demonstrate the advantages of this platform over more traditional bulk crystallization assays at quiescent conditions, owing in part to the ability to capture time-resolved dynamics of crystal growth. In the microfluidics device, crystal seeds are grown under the continuous supply of solutions maintained at a fixed composition. Time-resolved studies of struvite growth are performed to quantify the kinetics of crystallization and assess changes in morphology as a function of supersaturation. In parallel, we conducted *in situ* scanning probe microscopy measurements to elucidate the molecular mechanisms of struvite growth. Collectively, our findings reveal that struvite growth occurs *via* a classical mechanism that is governed by ion/molecule addition.

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Figure 1. (a) The effect of relative supersaturation, σ , on the bulk crystallization of struvite. Two representative (Rep1 and Rep2) optical micrographs of struvite crystals obtained from multiple batches are shown for each value of σ (1.1 – 4.0). All scale bars are equal to 100 μm . (b) Schematic representation of the coffin-like habit of struvite with labelled crystallographic faces⁴⁴. Adapted with permission from Prywer and Torzewska⁴⁴. (c) Scanning electron micrograph (SEM) of an x-shaped struvite crystal synthesized at $\sigma = 3.3$. (d–f) Time-elased optical micrographs of struvite growth capturing the overgrowth of individually nucleated crystals. (g) SEM of spicule-like struvite crystal synthesized at $\sigma = 3.3$. (h–j) Time-elased optical micrographs capturing the outward growth of crystals into the hierarchical structure presented in (g).

Results and Discussion

Bulk Crystallization Assays. We first tested the effect of supersaturation on struvite crystallization using *ex situ* bulk crystallization, which is a conventional means of evaluating the independent or combined effects of different parameters by tracking the changes in overall size, morphology, and number density of crystal populations. The relative supersaturation, σ , of growth solutions was adjusted by altering the pH from 8.0 to 9.3 (Figure S1), which yields values of $1.4 < \sigma < 4.0$, respectively (where $\sigma = (\text{IAP}/K_{\text{sp}})^{1/3} - 1$ and $\text{p}K_{\text{sp}} = 13.26$ at 25°C)⁴⁵. In assays with $\sigma \leq 1.0$, there were no crystals detected within the typical timeframe of measurements (ca. 36 h). At low supersaturation ($\sigma = 1.0 - 1.5$), a small population of crystals with a coffin-like morphology, analogous of struvite crystals found in living organisms, was observed^{46, 47}. Scanning electron micrographs (Figure S3) revealed that the coffin-shaped crystals exhibit multiple facets with the following surface terminations (Figure 1b): (001), (00 $\bar{1}$), (012), (0 $\bar{1}$ 2), (101), and ($\bar{1}$ 01) faces⁴⁴. At moderate supersaturation ($\sigma = 1.5 - 3.0$), the population of crystals was predominantly twinned and/or polycrystalline, producing x-like and spicule-like crystals, respectively. The latter are formed by

two general processes. The first involves either the agglomeration or overgrowth of individually nucleated crystals (Figure 1c). Time-resolved optical micrographs from the microfluidics analysis, which will be described in more detail later, have captured the overgrowth of adjacent crystals (Figure 1d – f). The second process involves the growth of spicule-like particles comprised of multiple crystals growing in random directions from an apparent common core (Figure 1g). Time-resolved images (Figure 1h – j) have been able to capture the outward growth of crystals in these hierarchical structures. Collectively, these complex features render the analysis of anisotropic crystal growth more challenging.

For all bulk crystallization assays in this study, comparisons among different batches revealed wide variations in crystal size, shape, and number density. Examples of two representative (Rep) batches are shown in Figure 1a for each supersaturation tested. In some instances (e.g. $\sigma = 2.1$), there were substantial differences between batches, whereas others were more consistent (e.g. $\sigma = 4.0$); however, at high supersaturation ($\sigma > 3.0$) the frequency of hierarchical structures was increased (Figure S2). Moreover, the crystals can develop irregular morphologies, as shown in Figure 1a (Rep2, $\sigma = 3.3$), that

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resemble either rough growth or defective crystals (e.g. intergrowths). There are several factors that potentially contribute to the heterogeneity of struvite crystals grown under quiescent conditions. The first is a correlated reduction in pH (by 1 – 2 pH units) with struvite formation, which has a concomitant effect on supersaturation (Figure S1). In addition, the fact that struvite is composed of three ionic constituents⁴⁷ suggests that diffusion limitations in quiescent assays could lead to local variations in supersaturation. Irrespective of the causes, the resultant heterogeneity makes it difficult to quantify systematic trends in

Figure 2. Schematic of the microfluidic device (not drawn to scale). (a) *In situ* seeding method. A solution containing Mg^{2+} , NH_4^+ , PO_4^{3-} (solution 1) is mixed in-line through a Y-connector with a solution of NaOH and NaCl (solution 2). The combined solution flows through two mixing chambers for complete mixing. The mixing chambers consist of microchannels of cross-sectional area $400 \times 200 \mu\text{m}^2$ and length 11 cm, whereas the growth chamber consists of microchannels of cross-sectional area $400 \times 400 \mu\text{m}^2$ and length 5 cm. (b) Struvite seed crystals are generated in a 20-ml synthesis vial and directly delivered to the growth chamber. (c) Representative optical micrograph of a channel containing struvite seeds of $40 \mu\text{m}$ average length in the (101) direction.

the growth and/or physical properties of struvite crystals as a function of synthesis conditions. To this end, we designed and implemented a microfluidics platform to address these limitations.

Microfluidic Platform for Struvite Crystallization.

Microfluidics is most commonly used as a microbatch system in the form of droplet microfluidics to monitor crystal nucleation and growth in nanoliter volumes^{48–52}. To our knowledge, few crystalline materials have been studied in microfluidic devices under continuous flow of a growth medium^{38, 39, 53, 54}. In this study, we used a modified design of an established microfluidics platform⁵³ for measurements of struvite crystallization. The configuration used in the microfluidic system has several advantages: it delivers a growth medium at fixed pH and supersaturation to crystal surfaces, allows for the analysis of anisotropic growth in multiple crystallographic directions, and is an efficient method for gathering statistically-significant data over a range of synthesis conditions. This platform characterizes struvite crystallization in flow conditions that mimic the urinary tract system, which is relevant for biomedical applications, and provides a well-defined laminar flow profile for assessing the effects of mass transfer. Moreover, seeded growth in microfluidics platform under continuous flow isolates the effects of growth from that of nucleation at fixed solute supersaturation.

The procedure for introducing crystal seeds into the microfluidics device required optimization. Our first attempt involved *in situ* seeding whereby a highly supersaturated solution was mixed in stream and flowed through a growth chamber to nucleate and retain crystals within microchannels (Figure 2a). In this configuration, a mixture of magnesium ammonium phosphate

(solution 1) was mixed in-line with an aqueous solution of NaOH and NaCl (solution 2) in ratios that generated a combined growth solution of predetermined pH and supersaturation. An undesirable outcome of this set up was the generation of high solute concentrations at the boundaries of the two inlet solutions (prior to the generation of a homogeneous solution within the mixing chamber). This consequently led to rapid struvite

Figure 3. *In situ* secondary growth of a seed crystal that was generated within the microfluidics device using the configuration in Figure 2a. (a) A seed crystal grown under continuous flow (24 ml min^{-1}) of supersaturated ($\sigma = 0.74$) growth solution. (b – d) Images of the same seed crystal at later imaging times as the concentration of solute was incrementally adjusted to higher supersaturation: (b) $\sigma = 1.10$, (c) $\sigma = 1.51$, and (d) $\sigma = 1.97$.

crystallization that clogged the inlet tubing and the micrometer-sized channels of the mixing chamber. The pre-emptive formation of seed crystals led to their random and scattered deposition on glass interfaces throughout the microfluidics device. An interesting observation was also made when attempting to conduct secondary growth of seeds. Notably, a significant fraction of seed crystals would not grow, irrespective of supersaturation, which we attribute to potential defects leading to strain. For instance, it is reported that crystal defects cause strain on the lattice, increase the free energy of the crystal and reducing supersaturation, thereby facilitating growth cessation⁵⁵. We posit that the unusually high local concentrations at the point of in-line mixing increases the probability of generating highly defective seed crystals. This effect was not observed in prior studies of crystals grown under flow, such as our previous work on barite (BaSO_4)⁵³ that led to the initial design of the microfluidic device.

Among the fraction of struvite seed crystals that were observed to grow within the microchannels when placed into contact with a secondary growth solution (Figure 3a), higher supersaturation led to abnormal phenomena. This included asymmetrical growth along particular crystallographic directions (Figure 3b), and the formation of crystals that resemble those in Figure 1a ($\sigma = 3.3$) when the growth solution introduced into the microfluidics device reached $\sigma \geq 1.5$ (Figure 3, c and d). The purportedly defective seed crystals retained within the growth chamber tended to be one of two general types: those that lacked visible signs of defects (similar to Figure 3a) and those that had notable irregularities (similar to Figure 3c). Our studies of varying

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supersaturation were intended to assess our hypothesis that local increases in solute concentration increased the probability of generating irregular (or defective) seed crystals. Indeed, our findings indicate that higher supersaturation does result in these apparent features; therefore, based on these observations, all *in situ* growth measurements reported herein were performed at lower supersaturations ($\sigma < 1.1$).

Figure 4. Structure of struvite along the [010] zone axis, according to data reported by Prywer *et al.*⁵⁶, where the dashed line indicates the mirror plane. The view on the left is an ordered arrangement where the callout (right) illustrates the degrees of freedom of ammonium groups that represents the disordered structure.

We performed structural analysis to look for possible signatures that may make struvite prone to defects. Several crystal structure determinations of struvite have been published, based on X-ray and neutron diffraction data^{56–60}. While clues of subtle disorder are present in each of the published results, in every case except one it was not reported. Whitaker *et al.* proposed that the ammonium ion was partially disordered, but they lacked the technology in 1970 to conclusively prove it⁵⁷. In order to verify inherent disorder in the structure, we collected our own high resolution X-ray data⁶¹. Our data are consistent with the previous studies and confirm that the ammonium group truly is disordered across a mirror plane (Figure 4). There is also strong evidence that several of the water molecules are disordered as well. The various ionic species comprising solid struvite do not fit together in an orderly fashion. Such structural characteristics can predispose the crystals to growth defects, especially under a highly supersaturated solution. Since all room temperature and very low temperature studies to date show the same evidence of internal disorder, the method by which the crystals are grown would not seem to be a factor in removing this disorder within individual single crystals; however, changing the growth conditions could conceivably alter the thermodynamics of crystallization and significantly reduce the tendency of twinning and/or fracturing.

Using the microfluidic configuration in Figure 2a, we tested a range of supersaturations ($1.5 < \sigma < 4.0$) for seeding by two different routes: altering pH (pH 7–9) and directly adjusting solute concentration (2.1 – 7 mM). In all cases, we observed heterogeneity among seed crystals with respect to their size and apparent defects, which manifested in a broad range of growth rates. This confirmed the necessity of utilizing an alternative seeding method. Previously, Jensen and coworkers⁵⁴ reported a process whereby they seeded glycine crystals in a microfluidics device by first synthesizing seed crystals in a continuously stirred system and then delivering them into the device using pressure-driven flow. Herein we adopted a similar approach (Figure 2b) using seed crystals prepared by stirring a supersaturated solution ($\sigma = 1.5$) with a stir bar at 600 rpm for 15 sec. This relatively short timeframe limits the nucleation density and results in mostly single crystals of approximately homogeneous size. This solution was then immediately transferred to a syringe and delivered directly to

the growth channels of the microfluidic device. With this method, we were able to introduce ca. 20 to 30 well-faceted crystals (35 – 45 μm in length) along the channel (Figure 2b, callout). This approach also circumvented the problem of uncontrolled precipitation in Figure 2a that led to clogged microchannels and crystal heterogeneity. Another advantage of the direct injection of seeds is an observed uniformity of growth rates among populations of crystals (as described in the following sections).

Figure 5. Time-elapsing optical micrographs of struvite growth under flow (24 ml/h) of supersaturated solution ($\sigma = 0.95$) at room temperature. Macroscopic growth rates of struvite crystals were imaged normal to the (a) $\vec{a}$ direction and (b) $\vec{c}$ direction. Images are shown at times 0 min (left) and 35 min (right).

Analysis of Struvite Growth under Flow. Seed crystals orient along the microchannels in one of two configurations with their faces normal to either the $\vec{a}$ direction (Figure 5a) or $\vec{c}$ direction (Figure 5b). An approximate equal percentage of both configurations enables the tracking of struvite growth along all three principal crystallographic directions. Prior experimental and computational fluid dynamics analyses⁵³ of the microfluidic device have shown that the mixing chamber results in complete mixing of solutes without detectable gradients along the width of the microchannels in the growth chamber. For all measurements reported herein, the supersaturated solutions used for growth studies were set below the metastability limit ($\sigma < 1.1$) to eliminate the possibility of nucleation within the solution.

Struvite crystals were grown under both quiescent and flow conditions. Struvite grows fastest in the $\vec{b}$ direction, thus leading to its characteristic coffin-like shape with a truncated rectangular pyramid morphology (see Figure 1b). Under quiescent conditions, struvite crystals increase only slightly in size before reaching a plateau (Figure 6, purple triangles) due to the rapid depletion of solution, thus reducing the driving force for struvite growth. Furthermore, struvite precipitation causes a reduction of pH which accelerates the drop in supersaturation. These effects restrict bulk crystallization analyses to measurements of final crystal size and morphology. Conversely, a continuous flow of supersaturated solution maintains a constant driving force (supersaturation) that allows for kinetic studies of struvite growth in all crystallographic directions. As shown in Figure 6 (blue circles), measurements of crystal length in the $\vec{b}$ direction under flow result in a linear advancement with time. Using this data, we can then extract the growth rate, r , from the slope of the line.

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The microfluidics platform allows for facile analysis of parameters such as supersaturation, solution pH, and flow rate with statistical certainty. It is well established that crystal growth can be either transport or kinetically limited⁶². The former is dominated by the mass transport of solute from the solution to the crystal solid-liquid interface, whereas the latter is limited by the energetics of solute incorporation into the crystal lattice. The relative importance of each rate limiting step can be tuned by

Figure 6. Continuous seeded crystallization in the microfluidics device. Temporal change in struvite crystal length, $\bar{b}$, in the microchannels at quiescent and flow conditions (flow rate = 24 ml/h). Dashed lines are guides to the eye and error bars equal two standard deviations for measurements of 30 or more crystals over multiple batches.

varying the flow rate. An increasing flow rate results in a decreasing boundary layer thickness surrounding the crystal interface, thereby reducing the time for solute to diffuse through the solution to the crystal surface. In a purely kinetic regime, the crystal growth rate reflects the rate of solute (ion or molecule) adsorption/desorption at the crystal surface, which is tuned by the supersaturation. Here, we investigated the relative importance of transport versus surface kinetics by varying the flow rate in the microfluidic device. For these measurements we focused exclusively on the fastest growing direction ($\bar{b}$ axis). As shown in Figure 7, the rate of crystal growth increases linearly with flow rate below 24 ml h⁻¹, which corresponds to the region of transport limitation. At flow rates higher than 24 ml h⁻¹, the rate of struvite growth is approximately constant (i.e. plateaus at 0.9 μm h⁻¹), thus signifying the onset of the kinetic regime. For measurements reported herein, we operate at flow rates within the kinetic regime to minimize the effects of mass transport limitations.

Growth kinetics were examined over a range of supersaturations. A unique aspect of the microfluidic platform is the ability to track macroscopic growth along all principal dimensions of a crystal, which is not possible with techniques such as atomic force microscopy (AFM) or ellipsometry, which are limited to specific interfaces (i.e., often faces with the largest surface area). Struvite growth rates along the $\bar{a}$, $\bar{b}$, and $\bar{c}$ directions showed a superlinear dependence over the range of supersaturations investigated (Figure 8). This dependence is indicative of surface growth by layer generation and spreading where models predict a power law behavior. It has been established that the growth of faceted crystal surfaces generally proceeds layer by layer through spiral growth triggered by screw dislocations, and/or nucleation and growth of 2D islands⁶³. The normal growth rate (r) of a face resulting from a screw dislocation

is related to step velocity v and mean step density, h/l , where l is the characteristic spacing between steps and h is the step height:

$$r = (h/l)v \quad (1)$$

When the separation between the turns of spirals is sufficient (i.e. when the diffusion fields of the turns do not overlap), the relationship between normal growth rate and supersaturation is $r \sim \sigma^2$. For overlapping or dense hillocks,^{64, 65} this dependence is reduced to $r \sim \sigma$. The growth rate of faces with 2D nucleation is

Figure 7. Effect of flow rate on the seeded growth of struvite. The growth rate was measured by linear regression of changes in crystal length, $\bar{b}$, with time from continuous imaging in the microchannels (cross-sectional area = 400 × 400 μm²). The growth solution consisted of 2.3 mM MgCl₂ · H₂O and 2.3 mM NH₄H₂PO₄ at pH 8.62 with a relative supersaturation of $\sigma = 0.89$. Dashed lines are guides to the eye. Error bars equal two standard deviations obtained from measurements of 30 or more crystals in a single batch.

Figure 8. Growth rate, r , in the $\bar{a}$ (orange), $\bar{b}$ (blue), and $\bar{c}$ (grey) directions measured in microchannels at a fixed flow rate (24 ml/h) as a function of relative supersaturation, σ . Dashed lines are guides to the eye. Error bars equal two standard deviations obtained from measurements of 30 or more crystals in a single batch. Inset: plot of the same data in log-scale. Solid lines are linear regression fits to the regime at lower supersaturation.

determined by the size of the nuclei and their rates of formation. According to classical nucleation theory (CNT), the rate of 2D nucleation of new layers (J_{2D}) is determined by the free-energy barrier for formation of the 2D critical nucleus, ΔG^*_{2D} :

$$J_{2D} = \exp(-\Delta G^*_{2D}/k_B T) \quad (2)$$

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Estimates of the barrier for nucleation, $\Delta G_{2D}^* = \pi R_c h \gamma$, are dependent upon the critical radius of the nucleus, $R_c(\sigma)$, which is governed by the Gibbs-Thompson relation, $R_c = \Omega \gamma / k_B T \sigma$ (where h is the layer thickness; γ is the surface free energy of the layer edge; and Ω is the volume of a formula unit). Based on the decrease in R_c with increasing σ , the resulting growth rate for the 2D nucleation regime is expected to follow an exponential dependence upon σ .

Replotting the growth rate data as $\ln r$ versus $\ln \sigma$ (Figure 8, inset) reveals a linear region corresponding to the quasiparabolic rate law of screw dislocations with the expected power law dependency in the range $1 < n < 2$ (i.e. $n_{\bar{a}} = 1.1$, $n_{\bar{b}} = 1.6$, $n_{\bar{c}} = 1.5$). At higher supersaturation, the reaction order increases ($n > 2$), indicating an exponential dependence that is commensurate with the transition to 2D nucleation (the corresponding linearized plot is provided in Figure S4 with $n_{\bar{a}} = 5.6$, $n_{\bar{b}} = 8.2$, and $n_{\bar{c}} = 6.3$). In order to validate the transition between these two modes of growth, we monitored struvite crystallization at a microscopic length scale using *in situ* AFM to capture layer-by-layer growth in real time, as described in the following section.

Analysis of Struvite Surface Growth. *In situ* AFM has proven to be a valuable technique for elucidating structural and dynamic characteristics of crystal growth mechanisms^{66–68}. Previously, it has been observed that struvite nucleation and growth on a brucite, $\text{Mg}(\text{OH})_2$, surface occurs *via* a particle-mediated process suggesting a deviation from classical crystallization⁶⁹; however, the physicochemical properties of these particles remain elusive, as well as the exact mechanism of struvite crystallization, which could potentially involve multiple pathways, including classical ion/molecule addition. In order to gain a fundamental understanding of various modes of growth, we used AFM to track the dynamics of growing struvite surfaces. The liquid cell used for *in situ* AFM measurements is equipped with inlet/outlet ports to allow growth solution to be delivered at a constant flow rate, analogous to the design of the microfluidics platform.

Struvite crystals with their (101) surfaces oriented normal to the plane of imaging were placed in the AFM liquid cell in contact with growth solutions prepared with low supersaturation ($\sigma = 0.43$). From the time-resolved images extracted from Movie S1 we observed a classical growth mechanism wherein spirals of trapezoidal morphology bound by (012), (0 $\bar{1}$ 2), (001), and (00 $\bar{1}$) steps initiate from dislocation sources (Figure 9, a and b). The average height of steps forming hillocks (Figure 9c, $h = 0.59 \pm 0.02$ nm) is approximately equal to the unit cell dimension (0.58 nm, Figure 9d). Identical measurements performed with growth solutions at higher supersaturation ($\sigma = 1.38$) showed the transition to 2D layer nucleation and spreading (Figure 9e). Snapshots taken from Movie S2 show the nucleation of new layers (arrows in Figure 9f) occurring at random locations on the substrate surface. The advancement of layers on the (101) interface is associated with growth along the $\bar{a}$ direction. Based on the macroscopic growth rates obtained from the microfluidics device (Figure 8), the transition to 2D layer generation and spreading occurs around $\sigma = 0.86$, which is consistent with AFM measurements conducted at supersaturations below and above this threshold.

The classical modes of layer growth in Figure 9 do not show evidence of the particle-mediated process that was postulated by Hövelmann and Putnis⁶⁹; however, it is important to note that the system being analyzed in their study is markedly different from the one presented here. For instance, the source of Mg^{2+} in the study by Putnis is derived from the dissolution of a brucite ($\text{Mg}(\text{OH})_2$) surface in contact with a $\text{NH}_4\text{H}_2\text{PO}_4$ solution. The exact supersaturation that mediates heterogeneous nucleation and growth of struvite on the brucite surface is unknown, while the 100 mM $(\text{NH}_4)_2\text{HPO}_4$ solution (pH 8.5) employed in their crystallization assays is significantly more concentrated than those used in our

study (< 10 mM). Our attempts to perform microfluidic and AFM studies at higher supersaturation were unsuccessful owing to the rapid rate of struvite nucleation and growth, which led to the plugging of inlet/outlet ports as well as the deposition of crystallites (from solution) to the surfaces of growing crystal substrates. However, it is evident from our studies at more moderate conditions that struvite growth can occur *via* a classical mechanism.

Figure 9. *In situ* AFM imaging of layered growth on (101) surfaces. (a and b) Deflection mode images taken at (a) 26 sec and after (b) 52 sec of continuous imaging in a supersaturated growth solution ($\sigma = 0.39$). Time-resolved images from Movie S1 capture the advancement of layers emanating from a screw dislocation. (c) Height profile measured along the growth hillock in panel a. (d) One unit cell of struvite showing an idealized rendering of a step on the (101) surface with a height equivalent to those measured in panel c. Color coding of atoms: O (red); P (orange); Mg (green); H (grey); and N (light blue). (e and f) Deflection mode images taken at (e) 105 sec and after (f) 131 sec of continuous imaging in a growth solution at higher supersaturation ($\sigma = 1.38$). Time-resolved images from Movie S2 capture the generation (yellow arrows) and growth of 2D islands.

Conclusions

In summary, we evaluated the growth of struvite under flow using a microfluidics device. Flow conditions are often encountered in clinical and industrial settings, such as urinary catheters and waste water treatment. The platform used to assess simple growth media in this study can serve as a foundation for future analyses of increasingly more complex systems (i.e., artificial

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urine solutions, *in vitro* bladder model). In the absence of flow, our findings reveal that bulk crystallization leads to a heterogeneous distribution of crystal size and shape with populations of crystals that often differed from batch to batch. X-ray diffraction of struvite crystals reveals an inherent disorder attributed to the ammonium ions, which is qualitatively consistent with the visible appearance of crystal defects (particularly at high supersaturation).

A shift from quiescent to flow conditions allowed for the analysis of struvite growth along all major crystallographic planes. The resulting kinetics of crystal growth are in agreement with the bulk habit in that the growth along the *b*-direction is much faster. Characterization of macroscopic growth rates in the microfluidic channels as a function of solute supersaturation resulted in a typical profile corresponding to distinct modes of growth. This was confirmed by time-resolved *in situ* AFM measurements that reveal a transition from microscopic growth by spiral dislocations at low supersaturation to 2D generation and spreading of layers at high supersaturation. Based on our observations, we posit that struvite growth (under the conditions tested in this study) predominantly occurs by a classical pathway involving monomer addition.

The microfluidics device used for these analyses is a versatile platform for evaluating struvite crystallization under conditions of flow, which is most relevant for environments where struvite mineralization occurs naturally. The microfluidics platform also offers the unprecedented ability to rapidly screen and visualize crystal growth at macroscopic length scales while maintaining a constant supersaturation (i.e. driving force for crystal growth), which is difficult to achieve by conventional methods. Ongoing work in our group is focused on expanding this platform to include studies of struvite growth in the presence of additives that function as modifiers to inhibit the rate of crystallization. Collectively, these studies aim to elucidate new routes to control, and ultimately suppress, struvite formation in diverse growth media.

Experimental Section

Materials. The following reagents were purchased from Sigma Aldrich (St. Louis, MO, USA): magnesium chloride hexahydrate (BioXtra, $\geq 99.0\%$), ammonium dihydrogen phosphate (99.999% trace metals basis), and sodium hydroxide ($\geq 98.0\%$). Sodium chloride (ACS reagent, $\geq 99.9\%$) was purchased from Alfa Aesar (Heysham, UK). Millex 0.22 μm membrane filters were purchased from Millipore Ltd (Cork, Ireland). Deionized water used in all experiments was purified with an Aqua Solutions RODI water purification system (18.2 M Ω). All reagents were used as received without further purification.

Bulk Crystallization. Batch crystallization was performed in 20-ml glass vials by dissolving NaCl in deionized water, then adding 1.4 ml filtered equimolar stock solutions of each solute: 50 mM $\text{NH}_4\text{H}_2\text{PO}_4$ and 50 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$. A clean glass slide was placed at the bottom of each vial to collect crystals. The pH of the growth solution was adjusted to values ranging from pH 7.5 to 9.3 by the addition of appropriate volumes of 1 M NaOH solution. The vials were vortexed for 5 sec followed by a subsequent pH measurement. The final composition of the growth solution was 7 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$:7 mM $\text{NH}_4\text{H}_2\text{PO}_4$:150 mM NaCl. Crystallization was performed at room temperature (20°C) for 36 h at static conditions (without stirring or agitation). Crystals grown on glass slides were analyzed with a Siemens D5000 X-ray powder diffractometer using a $\text{CuK}\alpha$ source (40 kV, 30 mA). Struvite formation was confirmed using X-ray diffraction (XRD) reference

patterns provided by the database of the RRUFF™ project with ID: R050540.1. In addition, the final crystals were observed in the solution by optical microscopy using a Leica DMI8 instrument and scanning electron microscopy (SEM) using a FEI 235 dual-beam focused ion beam instrument. SEM samples were prepared by attaching glass slides to carbon tape and coated with 15–20 nm gold to reduce electron beam charging.

***In situ* Characterization of Growth.** A microfluidics device was used to examine struvite growth at macroscopic length scales. The device (poly-di-methyl-siloxane (PDMS) on glass) was fabricated in house using a reported protocol⁵³. The device contains microchannels with a $400 \times 200 \mu\text{m}^2$ cross-sectional area and a length of 4.4 cm. The device used for these experiments was designed with one inlet and one outlet on each end of the channel. This system was monitored under continuous supply of a growth solution using a semi-automatic inverted light microscope (Leica DMI8 equipped with PL Fluotar 5x, 10x, 20x, and N Plan L 50x objectives).

Struvite seed crystals were prepared in a 20 ml vial containing a solution of composition 4 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$:4 mM $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 8.5). The order of reagent addition was identical to the procedure described above, using continuous stirring (600 rpm). The solution was mixed with a stir bar for a short time (ca. 30 sec) to minimize nucleation, followed by its immediate transfer (by syringe) to the growth chamber of the microfluidics device.

Growth solutions for microfluidics studies were prepared with molar concentrations of 2.3 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$:2.3 mM $\text{NH}_4\text{H}_2\text{PO}_4$ and a chosen range of pH 8.0 – 9.0 to selectively control the supersaturation. The growth solution was delivered to the microchannel at flow rates of 0 – 60 ml/hr using a dual syringe pump (Chemxy, Fusion 200) and two syringes (plastic BD syringe, 30 ml) with an in-line mixing configuration. Solution 1 contained magnesium chloride mixed with ammonium dihydrogen phosphate, and Solution 2 contained sodium hydroxide. Combinations of the two solutions resulted in a final concentration of 2.3 mM of each component and the desired pH after exiting a mixing chamber placed prior to the entrance of the growth chamber (refer to Figure 2). For *in situ* time-resolved studies, images were acquired every 5 min at multiple positions along the microfluidics channel. Optical micrographs of crystals were analyzed using ImageJ (NIH) for the average length along the $\vec{a}$, $\vec{b}$, and $\vec{c}$ directions. The growth rate was measured by linear regression of crystal length versus time data.

In situ atomic force microscopy (AFM) was performed to examine the temporal changes in topographical features on struvite crystal surfaces. Struvite crystals prepared by the bulk crystallization method described above were mounted on an AFM specimen disk (Ted Pella) covered with a thin layer of thermally curable epoxy (Loctite, China). The epoxy was first partially cured in an oven for about 6 min at 60°C prior to gently pressing the glass slide collected from bulk crystallization to the specimen disk to immobilize struvite crystals on the partially-cured epoxy. The sample was then dried in air overnight to completely cure the epoxy. All AFM measurements were performed in a Cypher ES instrument (Asylum Research, Santa Barbara, CA) using silicon nitride probes with gold reflex coating and a spring constant of 0.15 N/m (Olympus, TR800PSA). The liquid cell (ES-CELL-GAS) contained two ports for inlet and outlet flow to maintain constant supersaturation during AFM measurements. Several concentrations of magnesium chloride and dihydrogen ammonium phosphate ranging from 1.9 to 2.3 mM, as well as pH values spanning pH 8 – 10, were used to test a range of supersaturations. The growth solution was delivered to the liquid cell using an in-line mixing configuration where the two solute solutions were combined immediately before being introduced into the cell (similar to the microfluidics configuration). Freshly prepared growth solutions were used for each experiment (within an hour of their preparation). Continuous imaging was performed

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at ambient temperature in contact mode with a scan rate of 10 – 78 Hz at 256 lines per scan.

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Conflict of Interest

The authors declare no conflict of interest.

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Entry for the Table of Contents

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Going with the flow: A microfluidics platform for assessing crystal growth under flow conditions is used to characterize struvite crystallization over a range of synthesis conditions. A combination of *in situ* microfluidic assays and atomic force microscopy measurements reveal that struvite predominantly grows by a classical pathway involving layered surface growth *via* the addition of solute ions/molecules.

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Time-Resolved Dynamics of Struvite Crystallization: Insights from the Macroscopic to Molecular Scale