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Solution rheological parameters modulate calcium phosphate mineralization in a microfluidic device

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ABSTRACT

Mineralization of calcium phosphate and other materials in vivo and in natural water sources occurs in solutions that are not stagnant, but are flowing. Flow conditions could influence solution mixing and, therefore, mineralization kinetics or mechanism. This work describes the design and characterization of a multi-stream parallel flow microfluidic device that allows for controlled solution mixing and indirect control of laminar flow by altering the microfluidic device width, shape, length, flow rate, and flow velocity. Measurement of solution mixing was accomplished using the protonation of quinine to produce a fluorescent molecule and the rate of calcium phosphate mineralization was monitored by optical microscopy and analysis with Image J software. Experiments were designed to hold the flow rate constant, allowing the solution velocity to vary and to hold the velocity constant, allowing the flow rate to vary. It was found that small changes in laminar flow conditions do not correlate to mineral growth, but solution velocity and flow rate have a substantial effect on calcium phosphate mineralization. AFM and SEM characterization of the mineral produced shows an amorphous material and varying degrees of mineralization possibly due to variation in supersaturation conditions across the solution mixing area. This microfluidic device and analysis procedure allows for improved study of mineralization and the effect of flow conditions relevant to those seen in biological settings.

1. Introduction

Mineralization in biomaterials, ranging from calcium phosphate and calcium carbonates to silica and magnetite, occurs in dynamic, natural fluid environments where mechanical stimuli, interstitial fluid flow, and laminar flow can influence chemical reactions [1–4]. These environments can develop on large scales in natural ocean and fresh waters [5] or on small scales with solution flow in blood vessels, capillaries, and extracellular matrix [6–8]. Recent studies in mineralization have focused on nucleation and growth mechanism [9], template activity [10–12], supersaturation parameters, and confinement [13,14] with great success, but have largely focused on static environments rather than flow conditions. Rheological or flow parameters should be considered as an essential component in approximating natural environment reaction conditions [15]. Furthermore, it is likely that the mechanism of mineralization, the effect of a template molecule, or the effect of confinement could change dramatically from static to laminar to turbulent flow conditions.

Analytical methods in mineralization that allow for dynamic, real-time studies in solution flow have not been widely developed, though would provide an important complement to traditional, static, diffusion-limited, end-point techniques [16–18]. A drawback of existing static techniques is that they could produce non-specific and uncontrolled mineralization due to a lack of control over solution flow and mixing, as previously reported [17]. The solution and mixing parameters required for new analytical methods can be gained through carefully designed and characterized microfluidic devices. Recent advances in the use of microfluidics to control and explore mineralization reactions have been promising. Several researchers have explored nanoreactors and microfluidic droplet engineering to control and parallelize mineralization reactions [14,19,20], while others have focused on protein crystal nucleation and growth using microfluidics [21,22]. The Meldrum group has used microfluidics paired with light polarization microscopy and synchrotron FTIR to study the influence of confinement on calcium carbonate mineralization [13,23], Castro, et al. [24] used single-stream laminar flow in the continuous-flow precipitation of calcium phosphate in a microreactor, and Antebi, et al. [25] used perfusion-flow for biomimetic collagen-hydroxyapatite mineralization. The first multi-stream parallel laminar flow devices were introduced by Kamholz, et al. [26], but have only recently been applied to the field of mineralization with calcium carbonate [27], calcium phosphate [18], and calcium oxalate [28]. Laminar flow in a multi-stream device

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provides a reactive liquid-liquid interface where precursor ions can react and precipitate to form mineral. The increased control over when and where solutions first mix provides a useful advantage in the analysis of the forming mineral. Integration of optical microscopy, light polarization microscopy, infrared and Raman spectromicroscopy, and quartz crystal microbalance technology provides convenient in situ characterization and analysis tools. A remaining question is in the effect that rheological or flow parameters have on precursor mixing and therefore mineralization.

The goal of this work was to determine if changes in microfluidic device dimensions and rheological properties alter calcium phosphate mineralization behavior. Rheological parameters were indirectly controlled by altering height and width of the microfluidic flow cell and by altering flow rate, solution flux, and solution velocity. Linear velocities of approximately 300 to 1000 μm/s, used in this study, are intermediate to velocities observed in biological system which range widely from 100 cm/s to 1 μm/s [29,30]. Extent of mixing between the parallel-flow streams was analyzed with fluorescence where quinine and sulfuric acid solutions react to protonate the quinine, enhancing quinine fluorescence. Mineralization proceeded by reaction of calcium chloride and sodium phosphate (pH 7.4) precursor solutions to precipitate the calcium phosphate materials. This constitutes a simplified model for mineralization in order to explore the effects of rheological parameters in a microfluidic device.

2. Materials and methods

2.1. Chemicals

Calcium chloride dihydrate (≥99.5%) and sodium phosphate dibasic anhydrous (≥99.5%) were purchased from Sigma-Aldrich. Quinine sulfate dihydrate (≥99.0%) was purchased from Fluka. All other chemicals were reagent grade and were used as received.

2.2. Fabrication of microfluidic flow cells

Flow cells were designed and fabricated as previously described [18], but with constant length and variable width, height, and shape (Fig. 1, Supporting Information, S1). The adhesive double-sided tape provided a convenient and low-cost material that was easily cut to the correct channel dimensions using a commercially-available craft cutter [31]. Two heights of 0.12 mm and 0.36 mm were achieved due to the dimensions of the commercially-available tape. For the straight-channel device, widths of 5, 6, and 7 mm were used. The changes in width and height systematically altered the channel perimeter and cross-sectional area. The curved-channel device, termed the "fish", had a smallest width of 5 mm and a largest width of 7 mm. The fish device shape was studied to determine the effect of a continuously changing perimeter or cross section. The gold-coated glass substrate provided a hydrophobic surface with an ability to adsorb forming calcium phosphate materials, as previously demonstrated [18]. At calcium chloride (15.0 mM), sodium phosphate (8.0 mM), and pH (7.4 pH) concentrations used in these experiments, it is expected that homogenous mineralization will dominate and mineral particles will precipitate onto the hydrophobic gold surface. This is in contrast to templated heterogeneous nucleation that occurs at the solid-liquid interface [17,18]. A glass slide was drilled to add inlet and outlet holes and the device was aligned and assembled by hand. Solution flow was systematically varied in the design of two specific experiments. In constant velocity (CV) experiments, solution flow rate was adjusted for each device to maintain a constant velocity of ions moving through the microfluidic channel. The average flow rates of 25–106 μL/min were used to achieve a constant average velocity of 700 μm/s (Fig. 2). The average velocity (v) was calculated as:

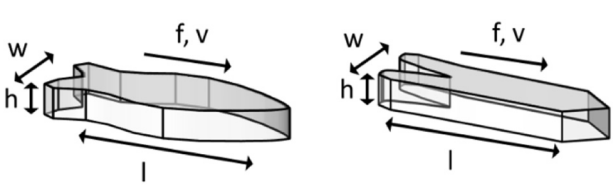

Fig. 1. Flow cell design shows the dimensions and operation parameters for the fish and straight-channel device (not to scale). The channel depicted here is cut out of adhesive double-sided tape and sandwiched between a gold-coated substrate and a glass slide. **w**) channel width, 5–7 mm; **h**) channel height, 0.12 or 0.36 mm; **l**) channel length, 15 mm; **f**) flow rate, 25–106 μL/min; **v**) average velocity, 317–1333 μm/s. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

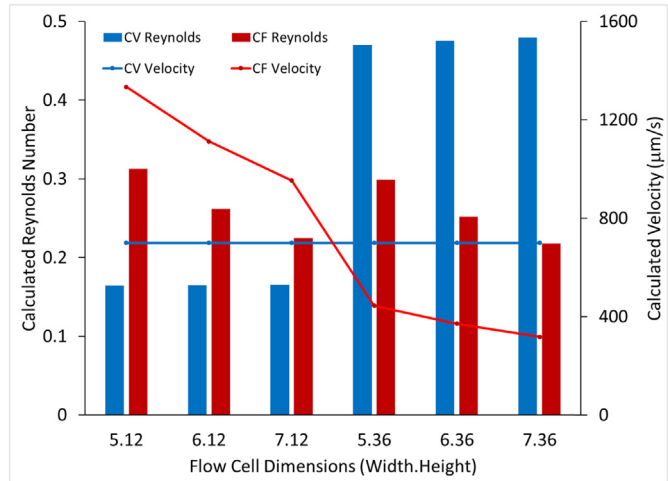

Fig. 2. Calculated Reynolds numbers (bars) and velocity (line) for each device dependent on reaction conditions of constant velocity (CV) or constant flow (CF) experimental design. Re was calculated according to Eq. (2) and varies with device cross-sectional area and flow velocity.

$$v = \frac{Q}{A} \tag{1}$$

where, Q is the average flow rate in $\mu m^3/s$ and A is the cross-sectional area in μm^2. CV experiments maintained the residence time in the device to 21 s, but resulted in significantly different Reynold's numbers (Re) between flow cells of 0.12 and 0.36 mm height (Fig. 2). Re [26,32–34] was calculated as:

$$Re = \frac{4Q}{\nu P} \tag{2}$$

where, the fluid density (ρ) and absolute viscosity (μ) are substituted for the kinematic viscosity of water (ν), which is $1.004 \times 10^3\ \mu m^2/s$ at 20 °C, and P is the perimeter (μm). In all experiments Re was less than one, so are all considered to constitute bulk solution laminar flow, though likely to different degrees. In constant flow (CF) experiments, the average flow rate was held constant at 48 μL/min which resulted in various average velocities based on the changing cross-sectional areas. Velocities ranged from 317 to 1333 μm/s, so residence times for an ion in the device ranged from 11 to 47 s. In CF experiments there is a constant decrease in Re with increasing width of the device (5 mm to 7 mm width), but not a substantial difference between different height devices (0.12 and 0.36 mm height). Both CV and CF experiments can be considered constant composition experiments [35] because the continuous flow constantly replenishes the solution at the mineralization site, keeping the solution supersaturation ratio constant throughout the experiment. The variety of these device designs and experimental parameters constitutes eighteen unique rheological conditions explored in both mixing and mineralization experiments below. This provides a comprehensive and useful, though not exhaustive, overview of the rheological effects.