Supporting Information for

Rediscovering Hydrogel-Based Double-Diffusion Systems for Studying Biomineralization

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The supplemental materials contain:

Section S1: A guide for the construction of a Circulating Semi-Infinite Reservoir DDS.

Section S2: The experimental details for Experiments I-III described in the main text.

Section S3: The Matlab code for calculating for the semi-infinite reservoir condition for a Circulating Semi-Infinite Reservoir DDS and additional data for validating the semi-infinite reservoir condition (Experiment IIb).

S1. Construction and Assembly of a Circulating Semi-Infinite Reservoir System

As described in this paper, the Circulating Semi-Infinite Reservoir system can be viewed as multiple Flowing Infinite Reservoir Design Static DDSs stacked in parallel, with longer gels and a circulating, rather than flow-through, reservoir configuration. Solutions in two large vessels, 1 L (custom-modified glass media bottles with hose barbs and stopcocks), are constantly circulated with a peristaltic pump with dual pump heads (Masterflex® L/S® variable speed modular drive by Cole Parmer) creating two turbulent, semi-infinite reservoirs. The system is designed to hold 18 gels for a single set of experiments connected to the reservoirs through a circuit of tubes. The circuit tubing is constructed of tygon (poly-vinyl chloride; PVC) tubing with polypropylene connectors. The various segments of the circuit are connected together through a series of quick disconnects that make assembly, disassembly, troubleshooting, repair and cleaning of the system easy. The connections of the gel tubes to the circuit are made with thin walled vinyl tubing that is pre-stretched to accommodate the gel tubes with a snug connection. The list of materials (all ordered from Fisher Scientific) needed for the construction of one reservoir circuit is (2 reservoir circuits are required for each double-diffusion system):

- **A.** Tygon®/PVC, L/S-15 Masterflex pump tubing, ¼" OD, 16" (Quantity: 1)
- **B.** PVC tubing, 5/8" OD, 3/8" ID
  - **B1.** 9" (Quantity: 1)
  - **B2.** 7" (Quantity: 2)
  - **B3.** 5.5" (Quantity: 1)
  - **B4.** 5" (Quantity: 2)
  - **B5.** 3" (Quantity: 1)
  - **B6.** 2" (Quantity: 11)
- **C.** PVC tubing, ½" OD, 3/8" ID, 20" (Quantity: 1)
- **D.** PVC tubing, 7/16" OD, 5/16" ID, 1¼" (Quantity: 12)
- **E.** Poly-propylene 3/8" tubing tees (Quantity: 13)
- **F.** Quick-disconnects for up to 3/8" ID tubing (Quantity: 5 sets)
- **G.** Quick-disconnects for up to ¼" ID tubing (Quantity: 1 set)
- **H.** Pinch clamp for ½" OD tubing (Quantity: 1)

These parts are assembled into the final reservoir circuit as shown in Figs. S1 - S3. The reservoirs are assembled from commercially available media bottles (1L, Pyrex, Kimax) to which stopcocks with barbs are attached by a glass-blower at a 10° angle to the bottom.

Note about connections: All sizes ID and OD (in inches) are chosen such that connections are snug fits without the need for hose or cinch clamps. To aid in the assembly of the tubing network the ends of the PVC tubing are dipped into acetone to temporarily swell the tubing and then immediately fitted over the polypropylene connectors. The 5/16" ID thin-walled tubing (**D**) used for connecting the hydrogel tubes to the reservoir circuits once cut, are plugged

with #000 rubber stoppers (at either end) and placed in an oven at 70°C for 30-60 min. Every 10 min or so the stoppers in the tubing are pushed deeper into the tubing, stretching the ends of the pre-cut tubes. Once pre-stretched, the tube ends are dipped into acetone to temporarily swell the tubing and then immediately placed on the ports of the reservoir circuit. Only the PVC tubing connections to the quick disconnects are secured with hose clamps and the disconnects are locked together using zip ties, to assure there is no accidental disconnecting of the tubing during handling and operation.

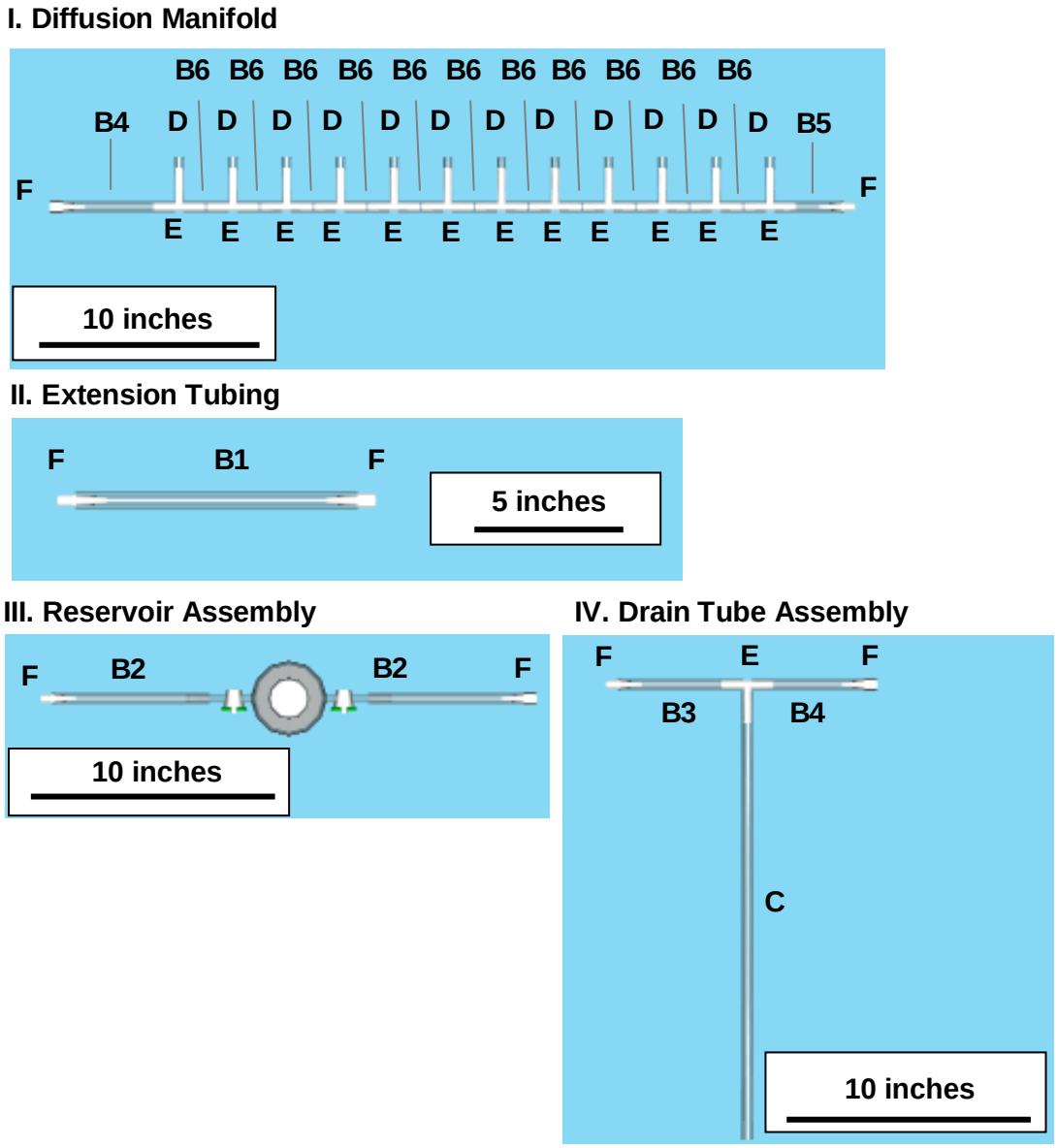

Figure S1. System components (I-IV) assembled using the parts (**A-F**) listed in the text.

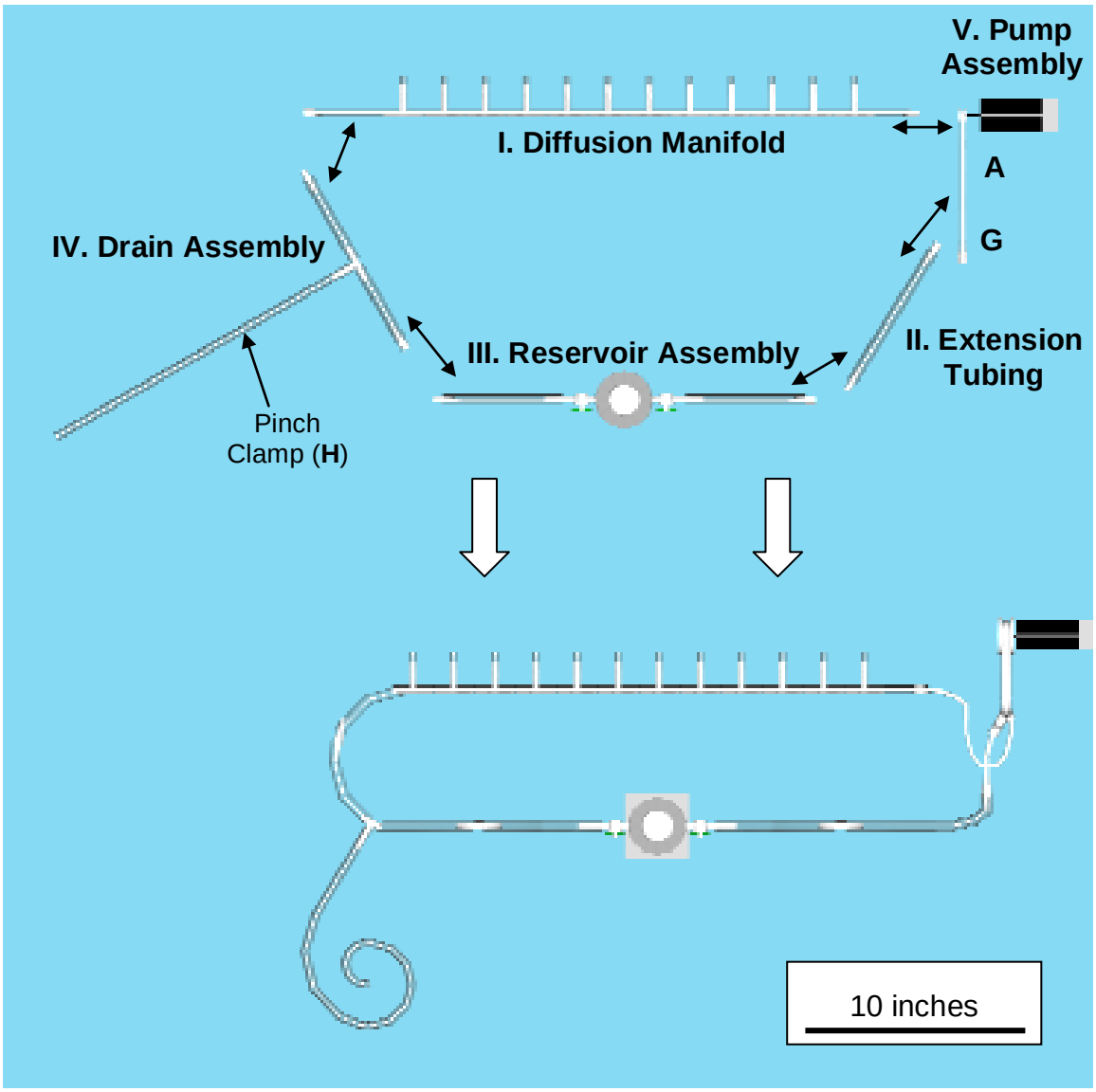

Figure S3. DDS reservoir circuit is assembled from the various system components (**I-IV** in Fig. S1) using quick disconnects (**F** and **G**). Pump assembly not drawn to scale.

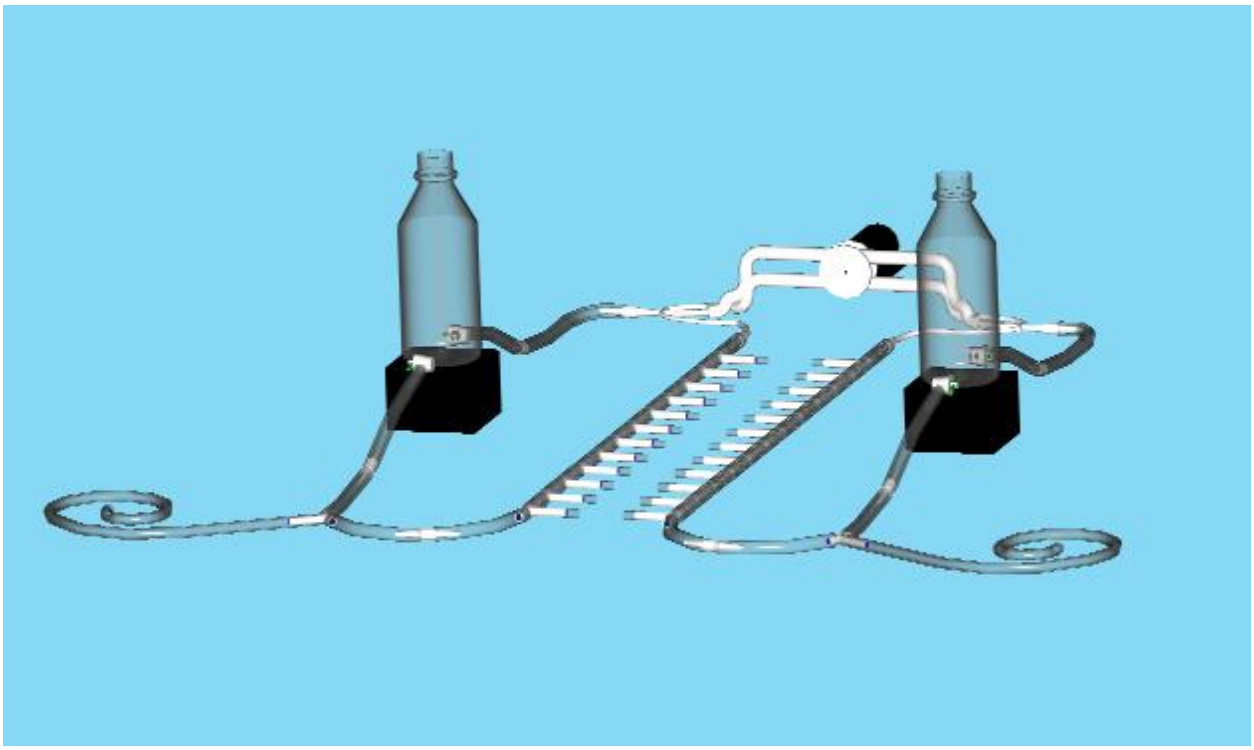

Figure S3. Once assembled with a stir plate under each reservoir, two reservoir circuits (Fig. S2) make a complete DDS. Gel tubes are then placed into the system as needed for a given experimental set-up.

S2. Experimental Procedures for Experiments I-III.

S2.1 General Methods

S2.1.1 Solutions:

A pH 7.4 150 mM Tris Buffer stock solution is prepared with 0.5% sodium azide (for 1 L: 19.83 g Tris HCl [Sigma], 2.91 g Tris Base [Sigma], 0.4 g sodium azide [Fisher]). This stock solution is used to prepare gelatin solutions and as a hydrating solution for diffusion gels. 100 mM Calcium Chloride Dihydrate +99% (Sigma-Aldrich) in 150 mM Tris Buffer (pH 7.4) with 0.5% sodium azide is used as the calcium source, the phosphate source is 115 mM Ammonium Phosphate Monobasic (Sigma-Aldrich) in 150 mM Tris Buffer (pH 7.4) with 0.5% sodium azide.

S2.1.2 Gelatin Gels:

Gelatin 10 w/v% in Tris Buffer pH 7.4, is prepared with gelatin powder, 225 Bloom Type-A (MP-Biomedicals), in pre-prepared Tris buffer with 0.5% sodium azide. The solution is heated to 55°C in a hot water bath while stirring. Once the solution has been made it can be immediately used or stored in the refrigerator (up to 3 weeks). Pre-prepared gels can reheated to 40ºC and used at this temperature. At temperatures above 50ºC the gel solution becomes frothy with bubbles and can lead to defects in the gel.

S2.1.3 Tube Preparation and Filling:

Tubes are prepared from disposable polystyrene serological pipettes (10 mL pipettes, 3/8" OD, 5/16" ID, Corning Costar) cut to length with a hotwire cutter, washed, rinsed with DI H_2O, and dried in the oven at 70°C. Tubes are capped with polypropylene caps punched from the pre-cleaned lids of food containers using a 9-10 mm cork borer, and sealed with Parafilm.

Tubes are capped and filled with warm (40-50°C) gelatin solution in such a way that the highest number on the pipette will be closet to the calcium source or furthest from the phosphate source. (This convention makes it easy to identify which ends of the tube have been in contact with which ion solution).

Once the tubes are filled, they are overfilled so that the meniscus of the gelatin provides a mushroom-cap-like appearance as the gel protrudes out of the tube. The tubes can either be set on the counter or placed in the refrigerator to cure the gel. Once cured, the caps on the tubes are either removed (for double diffusion tubes) or left on (for single diffusion tubes). Further treatment of the tubes is described for each experiment.

S2.1.4 Setting up the Diffusion System:

If the diffusion system is dry, the sample tubes can be place on the system before the fluid is added. Fill the reservoirs with their respective solutions. Open the reservoir valves, (if these valves are not opened when the pump is turned on, pressure will build in the system and a rupture will occur) and make sure that the drain valve/pinch-clamp is closed, turn on the peristaltic-pump, turn up the setting to level number 3 (42 mL/min) for Masterflex® L/S® 15 tubing. This setting was chosen based upon a predetermined path-length for what we considered the "edge" of the gel. Then using Eq. 1 from the text,

$\tau >> L^2/D$, was used to determine the maximum amount of time allotted before new solution should make contact with the gel, to maintain steady state at this edge. The specific time will vary depending on the gel type, and path-length of this gel edge as agreed upon by the user. The flow rate also coincided with the maximum allowable speed that did not lead to dislodging the gel. As the pump moves fluid through the tubes any bubbles will be displaced by the fluid. Removing the air bubbles can be assisted by raising and lowering the tubing assembly to help guide the air pockets out through the reservoir. Once the air has been displaced from one half of the tubing (for each side, calcium/phosphate), turn the pump to '0', switch the direction on the pump, turn the pump back up to '3' and remove the air from the second half of the tubing. Once the air has been removed from all the junctions between the tubes the manifold, and the fluid in the tubes is in full contact with the gels then the system is considered "started".

To stop an experiment, the pumps are stopped and the valves on the reservoirs are closed. Then fluid is drained from the system. Once fluid is no longer in contact with the gel tubes the system is considered "stopped".

S2.1.5 Handling of the gels for analysis

Once gel-filled tubes have been removed from the DDS they are placed in a refrigerator (30-45 min) depending on the initial temperature of the gels, to firm up the gels for handling. To remove the gels from the plastic tubes, the polystyrene tube can be cracked open using a pair of pliers. Once made bare, the gels are cut into even slices using a custom-designed tool to cut every slice to the same length (0.667 cm length; 0.333 mL volume) all at one time.

S2.1.6 Inductively Couple Plasma Atomic Emission Spectroscopy (ICP-AES)

The gel slices are hydrolyzed as described in each experiment. The hydrolyzed samples are then run through an axially viewed ICP trace analyzer emission spectrometer (model ICAP 61E trace analyzer, Thermo Electron, Waltham Ma). The transfer optics had been replaced with a short depth of field transfer optics to reduce matrix effects. The resulting concentration from each solution was reported in ppm for calcium and phosphorous and subsequently converted to mM.

S2.2 Experiments

S2.2.1 Experiment I: Effect of the boundary conditions, $\Delta c_0=0$, $\Delta x_0=0$, and $\Delta t_0=0$, on the kinetics of mineral precipitation.

Dynamic DDSs were run with varying boundary conditions, all 12 tubes in the system are DD (double diffusion) tubes. The experimental conditions used were: a non-circulating system with non-hydrated gels (Condition A), a non-circulating system with hydrated gel (Condition B), a circulating system with non-hydrated gels (Condition C) and a circulating system with hydrated gels (Condition D). The time of the initial precipitation event was recorded in all of the experiments for all tubes.

Condition A was made by filling twelve, 6 cm diffusion tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. The gels were left to set at 20 °C for 30-45 minutes. Once the gel was firm, the plastic caps used when filling the tubes were removed and the gel was cut flush to the diffusion tube using a razor blade. Tubes were

then placed on the DDS, and once the bubbles in the reservoir circuits were removed using the peristaltic pump, the system was disconnected from the pump, the stirplates were turned off, and the system was run for 5 days.

Condition B was made by filling twelve, 6 cm diffusion tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. The gels were left to set for 30-45 minutes. Once the gel was firm the plastic caps used when filling the tubes were removed and the tubes were placed in 15 mL centrifuge tubes and pre-filled (to avoid the trapping of air bubbles) with ~6 mL of Tris buffer pH 7.4 (so the entire tube is submersed). Tubes were then placed in a water bath at 25°C for 2 days. After pre-hydration the tubes were removed and the gels cut flush to the diffusion tube using a razor blade, then placed on the DDS, and once the bubbles in the reservoir circuits were removed using the peristaltic pump, the system was disconnected from the pump, the stirplates were turned off, and the system was run for 5 days.

Condition C was made by filling twelve, 6 cm diffusion tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. The gels were left to set for 30-45 minutes. Once the gel was firm the plastic caps used when filling the tubes were removed and the gel was cut flush to the diffusion tube using a razor blade. Tubes were then placed on the DDS with a circulating peristaltic pump and stirplates, and run for 5 days.

Condition D was made by filling twelve, 6 cm diffusion tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. The gels were then pre-hydrated as described for "Condition B." After pre-hydration, the gels were cut flush to the diffusion tube using a razor blade, then placed on the DDS with a circulating peristaltic pump and stirplates, and run for 5 days.

Evaluation: To monitor the timing for precipitation events in each system, a DDS with each condition was run once and the precipitation in the gels was checked every 6-12 hours until precipitation was first seen in any tube. Once a rough time frame for the onset of precipitation was determined, a second DDS with each condition was run and monitored (starting 12 hours before the rough time frame for each condition was established) every 15 minutes until every diffusion tube had precipitation. The time when precipitation occurred in each tube was recorded for each tube.

S2.2.2 Experiment II: Evaluating the semi-infinite condition for the sample length and reservoirs.

For this experiment, we used the "best practices" boundary conditions (Condition D) from Experiment I. Both 6 cm single diffusion (SD) tubes and 8 cm SD tubes were tested for 5 days. In evaluating the semi-infinite condition for the reservoirs as ion sources, each reservoir was sampled every-other day of the experiment (total time of 5 days).

S2.2.2.1 Evaluating the boundary condition of "semi-infinite sample length"

A DDS was run with six, 6 cm SD tubes and six, 8 cm SD tubes for each reservoir circuit, for a total of 12 tubes per circuit and 24 tubes for the whole DDS. The system was setup and run as described for "Condition D" in Section 2.2.1.

After 5 days the DDS was stopped, the exact time run was recorded, the tubes removed from the system, and prepared for analysis as described in Section 2.1.5. From each tube, the gel slices located at x = 1.995 cm, 2.665 cm, and 3.335 cm from the

diffusing ion source, were placed in 10 mL of 0.8 M nitric acid, for a total dilution of 31:1. The end slices from each tube were placed in 5 mL of 0.8 M nitric acid, for a total dilution of 15.5:1. The slices in nitric acid were sealed in 15 mL centrifuge tubes and placed in the oven at 70°C for two days to hydrolyze the gels. Once hydrolyzed the solutions were then analyzed by ICP-AES. The average concentration value for a location x was determined across each set of 6 tubes for each tube length and diffusing ion.

Diffusivities of ions through each tube were determined using the estimation $x = 1.6\sqrt{Dt}$ of the diffusion penetration distance from a point source where the concentration has fallen to 25% of the concentration at x = 0. Each concentration value for the three sections at x = 1.995 cm, 2.665 cm, and 3.335 cm, from the diffusing ion source were plotted against the distance of the middle of each slice from the source. An exponential fit line was applied to the points and x was solved for $c = c_0/4$. Once x was found for $c = c_0/4$ that value was plugged into $x = 1.6\sqrt{Dt}$ at a constant time t (recorded at the end of the experiment).

These diffusivities were used to subsequently calculate the values of ion concentrations (using Eq. S1) at the end of their respective tubes and the center of the 6.67 mm wide end-slice. These calculated values were then compared to measured values of ions within that end slice.

$$c(x,t) = c_0 erfc\ \frac{x}{2\sqrt{Dt}} \qquad \text{Eq. S1}$$

S2.2.2.2 Evaluating the boundary condition of "semi-infinite reservoir"

To probe the semi-infinite volume condition (c > 99.9% of c_0) for the 1 liter reservoirs, sample concentrations of the reservoirs were taken at zero, three and five day time points, from the calcium and phosphate reservoirs in a DDS running only SD tubes (12 tubes per reservoir). Each reservoir sample (0.1 mL) was diluted to 10 mL in 0.8 M nitric acid. The solutions were then analyzed by ICP-AES.

S2.2.3 Experiment III: Empirically measuring the effect of changing x_0, t_0, c_0, on the measured ion product.

S2.2.3.1 Baseline Experiment

Dynamic DDSs were run with varying boundary conditions with either all DD tubes or a combination of DD and SD tubes. The two extremes from Experiment I, conditions A and D, were used. An all DD system was run under Condition D to establish the supersaturation threshold window for this gel (10 w/v % 225 Bloom Type-A gelatin) in this system.

Three sets (0 hour, +1 hour, and +2 hour time points) of three, 6 cm diffusion tubes were prepared as described for Condition D, Section 2.2.1. Each set of three tubes was placed on the DDS one hour apart, with a circulating peristaltic pump and run until precipitation was seen in the first set of tubes. Based on the data from Experiment I the system was monitored every 15 minutes starting 6 hours before precipitation was expected to occur. Once precipitation occurred in the first set of tubes (started at 0 hour), the DDS was stopped and the tubes removed. Once removed only the first set of tubes (71.43 hours) showed signs of precipitation, the other two remaining sets of tubes were

clear. After removal from the system the tubes were placed in the refrigerator for 45 minutes to firm-up before handling and cutting. After 45 minute the two remaining sets of tubes began to show signs of precipitation but fainter than the first set of tubes. The gels were then scanned on a flatbed scanner (Canon 4440F). After imaging, the gels were prepared for analysis as previously described. The middle section (center of the section at x = 3.0 cm, which contained the precipitate) of each gel was placed in 10 mL of 0.8 M nitric acid, for a total dilution of 31:1.

The solutions were then analyzed by ICP-AES. The average concentration value for each ion was determined across each set of 3 tubes for each middle section. The average concentration values of calcium and phosphate for each middle section for each set of tubes were then multiplied together to give a supersaturation value for each time point. The minimum possible supersaturation value was found by taking the lowest value, by standard deviation, for calcium and phosphate concentrations in the earliest time point (69.40 hrs.) and multiplying them together. The maximum possible supersaturation value was found by taking the highest value, by standard deviation, for calcium and phosphate concentrations in the latest time point (71.43 hrs.) and multiplying them together.

S2.2.3.2 Ion product for Condition A and Condition D

Once the point in time at which precipitation occurred was established (71.43 hrs.), this time point was used as a marker for the next experiment. Two systems were run looking at each condition (Conditions A and D from Experiment I), six DD tubes were run, three for Condition A, three for Condition D, and twelve SD tubes were also run, six for calcium (Conditions A and D), six for phosphate (Conditions A and D). The two systems were stopped 2 hrs. ± 0.1 hrs. before precipitation was expected to occur for Condition D.

Condition A tubes were made as described in Section 2.2.1 by filling three, 6 cm DD tubes and six, 8 cm SD tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. Tubes were then placed on the DDS, and once the bubbles in the reservoir circuits were removed using the peristaltic pump, the system was disconnected from the pump and run until 69.36 hrs.

Condition D tubes were prepared as described in Section 2.2.1 by filling three, 6 cm DD tubes and six, 8 cm SD tubes with 10 w/v % 225 Bloom Type-A gelatin in Tris buffer pH 7.4. After pre-hydration the tubes were removed and the gels cut flush to the diffusion tube using a razor blade, then placed on the DDS with a circulating peristaltic pump and run until 69.36 hrs.

Once the experiments were complete, the tubes were removed from the system and prepared for analysis as previously described. The three sections of each gel located at x = 1.995 cm, 2.665 cm, and 3.335 cm, from the diffusing ion source were selected and placed in 10 mL of 0.8 M nitric acid, for a total dilution of 31:1. The slices in nitric acid were sealed in 15 mL centrifuge tubes and place in the oven at 70°C for two days to hydrolyze the gels. Once hydrolyzed the solutions were analyzed by ICP-AES. The average concentration value for a location x was determined across each set of 9 tubes for each tube length and diffusing ion. The ion product for each set of conditions A and D was found by multiplying together the average concentration of calcium and phosphate from the middle sections of each set of DD tubes. The maximum and minimum possible ion product values were found by taking the highest and lowest values, by standard

deviation, for calcium and phosphate concentrations in the middle sections and multiplying them together. Calculated ion products were found by using the ion concentrations found at x = 3.0 cm for the SD tubes for each condition. The error values were found by taking the highest and lowest values of the standard deviation, for calcium and phosphate concentrations in just the middle sections and multiplying them together.

S3. Calculating and Verifying the Semi-Infinite Condition for Reservoirs.

A key boundary condition for a Circulating Semi-Infinite Reservoir Dynamic DDS is the semi-infinite reservoir condition. Using the reported literature values for the fastest moving ion (D_{Ca} = 6.0 x 10^{-6} cm^2/s),[1] the timing (5 days) and the length of the diffusion tube (6 cm), we determined the semi-infinite sink and source condition for a reservoir. To calculate the total amount of material leaving a source reservoir and entering a sink reservoir, the total flux of material at each point (in and out of the diffusion tube) must be determined. Such a calculation is based on the second derivative of Fick's first law (or the first derivative of Fick's second law). Rather than find the integral of an *erfc* function at two points along a tube for 432,000 seconds, we chose instead to write a Matlab® script to perform the necessary calculations in a loop.

For the semi-infinite sink condition, the total flux of material V out of the diffusion tube is calculated from the first point y, 6.0 cm (end of the tube) to the second point z, 6.1 cm (1 mm into the sink reservoir). The total time of the system t is 432,000 seconds (5 days), and the concentration f of an ion at the first point y and the concentration s at the second point z are calculated using the solution to the diffusion equation for each second, and the diffusivity D found in literature for calcium in a 10 w/v % 275 Bloom Type-A gelatin. The instantaneous flux for each time point j is calculated by Fick's 2nd law (see equation 3 in main text). This calculation is made by finding the difference in concentration between the two points (y and z) and dividing by the distance between the two points, converting the volume in liters to cm^3 and multiplying this value by the diffusivity D of the system. This calculation yields a solution for j in $mmol*cm^{-2}*s^{-1}$. Each value found for j at each time point is then successively added to each other for all time t, producing at total flux of material V per unit area (cm^2).

The total amount of material E (in mmol) per tube, is calculated by multiplying the total amount of total flux of material V per unit area by the cross-section of the diffusion tube. The total amount of material entering the sink reservoir T is found by multiplying the value for total amount of material E leaving each tube by the total number of tubes. The value of T is then multiplied by 10 to find to target volume N (in liters) for a reservoir that will satisfy the infinite condition of c = 0.1% of c_0.

[1] A. L. Boskey, *J. Phys. Chem.* **1989**, *93*, 1628-1633.

S3.1 Matlab script for calculating the semi-infinite condition for a sink reservoir:

```
V = 0;                                  % Total flux of material per unit area
D = 6.0*10^-6;                          % Diffusion constant
y  = 6.0;                               % Start point in cm
z = 6.1;                                % Final point in cm
for t = 0:1:432000;                     % Time in seconds
f = 100*erfc((y/2*sqrt(D*t)));          % Concentration at the first point y in mM
s = 100*erfc((z/2*sqrt(D*t)));          % Concentration at the second point z in mM
k = (f-s)/0.1;
j = (D*k)/1000;                         % The instantaneous flux
V = V+j;                                % Total flux of material per unit area
end
E = V*((0.397^2))*pi                    % Result: the total amount of material per tube in mmol
T = E*12                                % Result: the total amount of material entering the sink reservoir
n = E*10                                % Result: target volume per tube (in liters)
N = T*10                                % Result: target volume (in liters)
```

In order to calculate the semi-infinite condition for the source reservoir, the same technique is applied as finding the condition for a sink reservoir except the total flux of material is calculated from the first point y = 0.01 cm to the second point z = 6.0 cm (the entire length of the gel diffusion tube).

S3.2 Matlab script for calculating the semi-infinite condition for a source reservoir:

```
V = 0;                                  % Total flux of material per unit area
D = 6.0*10^-6;                          % Diffusion constant
y  = 0.001;                             % Start point in cm
z = 6;                                  % Final point in cm
for t = 0:1:432000;                     % Time in seconds
f = 100*erfc((y/2*sqrt(D*t)));          % Concentration at the first point y in mM
s = 100*erfc((z/2*sqrt(D*t)));          % Concentration at the second point z in mM
k = (f-s)/0.1;
j = (D*k)/1000;                         % The instantaneous flux
V = V+j;                                % Total flux of material per unit area
end
E = V*((0.397^2))*pi                    % Result: the total amount of material per tube in mmol
T = E*12                                % Result: the total amount of material leaving the source reservoir
n = E*10                                % Result: target volume per tube (in liters)
N = T*10                                % Result: target volume (in liters)
```

S3.3 Data from Semi-Infinite Source Experiment (Experiment IIb)

Table S1: Concentration data of uncycled reservoirs from a Single Diffusion system taken at 0, 3 and 5 days.

	Absolute Concentration		**% Relative Change from Day 0**	
Reservoir: Day	**Average (mM)**	**Stdev**	**Average (mM)**	**Stdev**
Calcium: 0	101.34	± 0.38	0	±0.38 %
Calcium: 3	100.14	± 0.91	-1.17 %	± 0.90 %
Calcium: 5	99.6	± 0.56	-1.71 %	± 0.55 %
Phosphate: 0	117.46	± 0.80	0	± 0.67 %
Phosphate: 3	117.82	± 0.14	+0.31 %	± 0.12 %
Phosphate: 5	115.54	± 0.19	-1.63 %	± 0.16 %

S3.4 Changing and Re-filling the Reservoirs During an Experiment

If it has been determined that in order to maintain the semi-infinite condition for an experiment that the reservoirs need to be changed, then the following describes how to change the reservoirs without disturbing the experiment. For all experiments without exclusively double diffusion tubes and no precipitation occurring in less than 3.2 days, we implemented this procedure.

- A few hours before the infinite condition is violated, open the valve of the drain tube while the pump is running and the stir plates are stirring.
- Let the level of the reservoir drop to just above the drain of the reservoir so no air bubbles get into the system.
- Refill the reservoir to 100-150 mL above the normal operating fill line of the reservoir and let the system run/circulate for at least 15-30 min.
- After the system has circulated for that period of time, drain the reservoir to the normal operating fill line and leave running until the next time the reservoir has to be changed.