Bio-inspired flow-driven chitosan chemical gardens
Electronic Supplementary Material

Pawan Kumar, Dezső Horváth, and Ágota Tóth*

*Department of Physical Chemistry and Materials Science,
University of Szeged, Rerrich Béla tér 1., Szeged, H-6720, Hungary. and
Department of Applied and Environmental Chemistry,
University of Szeged, Rerrich Béla tér 1., Szeged, H-6720, Hungary.*

Chemicals

All solutions were prepared with deionized water (Purite RO100 instrument). The chemical reagents chitosan (low molecular weight, Sigma-Aldrich), acetic acid (VWR, 99-100 %), and sodium hydroxide (Honeywell Fluka, pellets) were used for all the experiments.

Experimental methods

Two different chemical compositions were used: solution L (lower chitosan concentration) and solution H (higher chitosan concentration). Into 50 mL of CH_3COOH (0.2 M) solution, 0.250 g or 0.375 g of chitosan was added and the mixture was stirred for 5 hours at 23 ± 2 °C, producing solution L and H, respectively.

For the study of tubular or budding growth, and complex shapes, we used solution L and H where the inner volume of a Plexiglas cuvette of 1 × 1 × 10 cm^3 (having the inlet hole in the center) was filled with 4 mL of (1.0 M, 3.0 M, and 5.0 M) NaOH solution. The chitosan solution was injected with a peristaltic pump (Ismatec Reglo) either through a Tygon tube (i.d.: 0.76 mm) and needle (i.d.: 0.38 mm) or for solution H, Tygon tube (i.d.: 1.42 mm) and needle (i.d.: 0.6 mm) (see Figure S1). For the periodic patterns, a different cuvette was considered with an inner volume of 1 × 1.5 × 20 cm^3 (having an inlet hole near the y-z plane) and filled with 15 ml of 1.0 M NaOH solution. Chitosan solution H was then injected into the hydroxide solution through the Tygon tube (i.d.: 1.42 mm) and needle (i.d.: 0.6 mm) (see Figure S1). Moreover, we also created periodic patterns on the surface of the gel using a syringe pump (KD Scientific) in order to validate that the formation of patterns is invariant to the type of injection applied (see Figure S7).

The gelation experiments were performed by injecting the chitosan solution continuously into the cuvette. Images of the pattern formation were recorded using a Unibrain fire-i 630c camera in color mode with Vivitar extension tubes. Image data were analyzed by ImageJ software. The flow rate was determined by measuring the mass of the deionized water as a function of time at different rpm values of the peristaltic pump.

The viscosity of 500 mL of solution L and H was measured with a rotational viscometer (Anton Paar ViscoQC-300) and the density of the reactants with a density meter (Anton Paar DMA-500). The viscosity of solution H lowered after 20 days to 33.00 mPa s.

Name	Composition	ρ (g/cm^3)	η (mPa s)
Solution L	0.50 w/V%	1.0005	19.80
Solution H	0.75 w/V%	1.0014	43.49
NaOH	1.0 M	1.0390	1.13 [1]
NaOH	3.0 M	1.1135	1.73 [1]
NaOH	5.0 M	1.1795	2.84 [1]

Table S1: The chemical composition of chitosan in 0.2 M CH_3COOH. Densities and viscosities of the reactants. The viscosities of the NaOH solution are from reference [1].

Tomography reconstruction

For the three-dimensional view of periodic patterns, we used a test tube (o.d.: 1.63 cm and length 9.45 cm) and a thin transparent polyvinyl plate (0.2 mm thickness) placed vertically in the center of the tube. The needle was adjusted alongside the sheet, such that the periodic patterns grew in the center of the sheet. After the structure formation, the tube was filled with NaOH solution to avoid space in the tube and covered from the upper and lower side with parafilm. We then placed the tube on a flat surface in front of a black background and images were recorded

* atoth@chem.u-szeged.hu

with 15 images/s rate by slowly rolling the tube without slipping. A total of 546 images were captured for a full rotation of the tube. The rotating angle was calculated from the rolling distance and the tube circumference. For tomography reconstruction, we used 99 images, which had an approximately equal angle following the work of Knoll et al.[2] A free download MATLAB script [3] was used for the tomography reconstruction of folding and symmetric (see Video S4) patterns. The details of the method are reported in studies by Winfree, Müller and Steinbock.[4–7]

Particle image velocimetry (PIV)

Fluid flow motion inside and outside of the tube was analyzed using particle image velocimetry (PIV).[8] For the inside region, we added latex beads (SD6A Sigma, in aqueous suspension with 6.4 μm mean particle size) and dispersed 50 μL of latex beads solution in 1 mL of chitosan solution. A green laser beam (Roithner LaserTechnik with 532 nm wavelength and 100 mW electric power) was oriented vertically with the help of thin convex lens (Techspec), such that it fell on the side view of the hydrogel tube and made a right angle with the camera. The time delay for the image recording was set to 66 ms. For the outer region (near the head and cusps of the tube), we dispersed 1.2 mg of fine grind black pepper in 1 mL of NaOH (1.0 M) solution. The green laser was positioned vertically in the center of the front view of the pattern. Images were recorded with a time delay of 66 ms. The velocity vector was analyzed using the FFT deformation technique of PIVlab open-source software [9, 10] in MATLAB, wherein interrogation area 128 px^2 and step size of 64 px was set (Figure S5).

Supporting Videos

VideoS1: Thin tubular structural formation of the chitosan hydrogel, shown at 12x real time. The experimental conditions are Q: 0.306 mLmin^{-1}, [NaOH]: 3.0 M and solution L.

VideoS2: Growth of the budding structure, shown at 32x real time. The experimental conditions are Q: 0.147 mLmin^{-1}, [NaOH]: 1.0 M and solution L.

VideoS3: Growth of the wrinkling instability on the surface of the tube, shown at 4x real time. The experimental conditions are Q: 0.858 mLmin^{-1}, [NaOH]: 1.0 M and solution H.

VideoS4: Tomography reconstruction of the upper portion of the tube (see Figure 5(b-c)). The experimental conditions are Q: 0.858 mL min^{-1}, [NaOH]: 1.0 M and solution H.

VideoS5: Fluid motion near the head and the periodic patterns of the tube determined by PIV analysis shown at 0.27x real time. Further details are in the particle image velocimetry (PIV) section.

---

[1] P. M. Sipos, G. Hefter, and P. M. May, J. Chem. Eng. Data **45**, 613 (2000).
[2] P. Knoll, A. V. Gonzalez, Z. C. McQueen, O. Steinbock, Chem. Eur. J. **25**, 13885 (2019).
[3] https://www.chem.fsu.edu/steinbock/software.html.
[4] A. T. Winfree, S. Caudle, G. Chen, P. McGuire, Z. Szilagyi, Chaos **6**, 617 (1996).
[5] U. Storb, C. R. Neto, M. Bär, S. C. Müller, Phys. Chem. Chem. Phys. **5**, 2344 (2003).
[6] T. Bánsági Jr., O. Steinbock, Phys. Rev. Lett. **97**, 198301 (2006).
[7] T. Bánsági Jr, O. Steinbock, Chaos **18**, 026102 (2008).
[8] M. Raffel, C. E. Willert, F. Scarano, C. J. Kähler, S. T. Wereley, J. Kompenhans, Particle image velocimetry: a practical guide (Springer, 2018).
[9] W. Thielicke, E. J. Stamhuis, Journal of Open Research Software, **2**, 30 (2014).
[10] W. Thielicke, PIVlab - particle image velocimetry (PIV) tool 2020, (https://www.mathworks.com/matlabcentral/fileexchange/27659-pivlab-particle-image-velocimetry-piv-tool).

Additional table and figures

[NaOH] (M)	Q (mL/min)	H_v at 360 s (cm)	d (cm)
1.0	0.051	1.91 ± 0.23	
1.0	0.147		
1.0	0.244		0.30 ± 0.03(*)
1.0	0.306		0.31 ± 0.01
1.0	0.368		0.31 ± 0.02
1.0	0.462		0.31 ± 0.01
1.0	0.560		0.31 ± 0.01
3.0	0.051	1.48 ± 0.20	
3.0	0.147	2.72 ± 0.28	
3.0	0.244		0.16 ± 0.01
3.0	0.306		0.16 ± 0.01
3.0	0.368		0.16 ± 0.01
5.0	0.051	1.18 ± 0.11	
5.0	0.147	1.30 ± 0.26	

Table S2: The vertical height (H_v) and the outer diameter (d) of the chitosan hydrogel at different NaOH concentrations and flow rates for gels prepared with solution L.

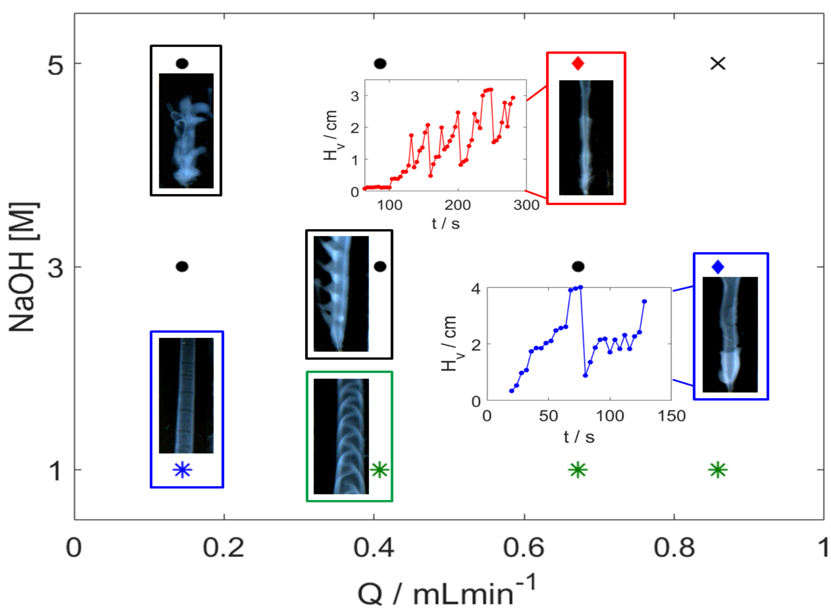

Figure S1: Phase diagram of the organic gardens. Solution H is injected with needle (0.60 mm) and tube (i.d.: 1.42 mm). The field view of thin and thick tubes is 0.93×2.24 cm^2. The inset figure of the red and blue curve represents the growth profile (H_v) of the thinner (0.672 mL min^{-1}) and thicker (0.858 mL min^{-1}) tubes, respectively.

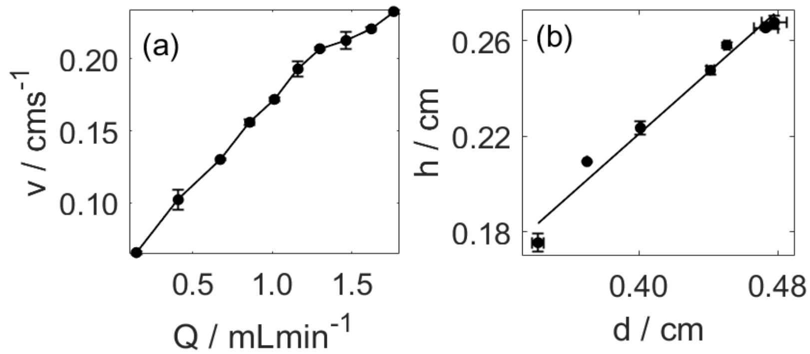

Figure S2: (a) Flow velocity (v) of the tubular hydrogel as a function of flow rate (Q). (b) The distance from the wall (h) as a function of the tube diameter (d). Solid line is the fitted straight line with slope = 0.643 ± 0.005 and intercept = -0.035 ± 0.002.

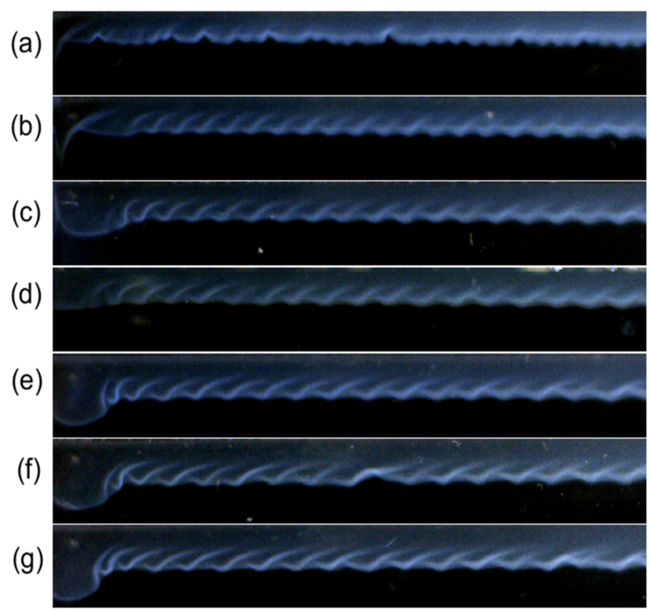

Figure S3: (a-g) Images of the side view (z-x plane) of the tube with flow rates in mL min^{-1} of a) 0.408, (b) 0.672, (c) 0.858, (d) 1.013, (e) 1.162, (f) 1.299, and (g) 1.464. The field view of all the frames is 3.95 × 0.47 cm^2.

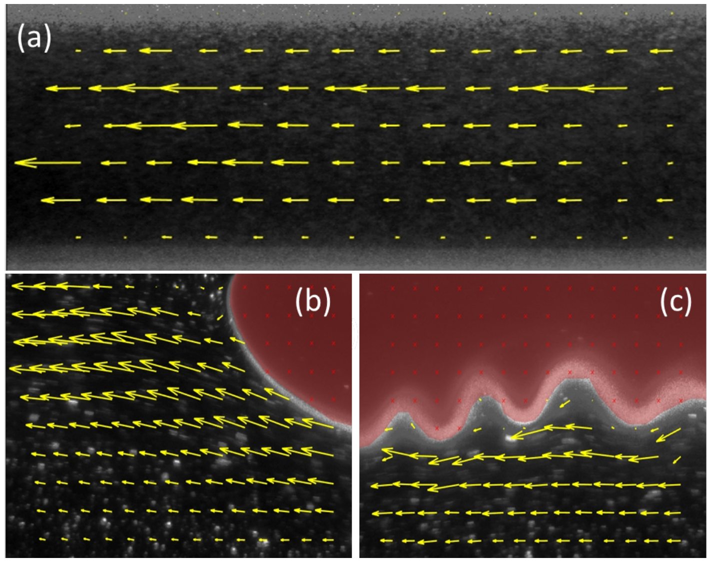

Figure S4: Particle image velocimetry (a) inside the tube, (b) near and front of the head and (c) near the wavy region outside the tube. The linear flow velocity of the particles is in the same direction as the tube growth. Flow rate is 0.858 mL min^{-1}.

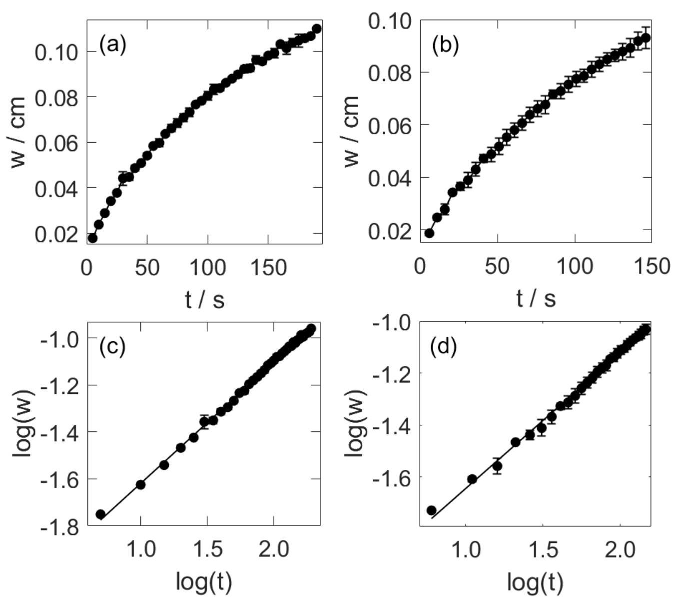

Figure S5: (a-d) The temporal evolution of tube thickness (w). Flow rate (a, c) 1.013 mL min^{-1}, and (b, d) 1.299 mL min^{-1}. The straight line fitting to the log-log plots (c-d) gives the slope of (c) 0.518 $\pm$ 0.002 and (d) 0.514 $\pm$ 0.012. The diffusion coefficients obtained: (c) (5.43 $\pm$ 0.18) $\times$ 10^{-5} cm^2s^{-1} and (d) (5.70 $\pm$ 0.11) $\times$ 10^{-5} cm^2s^{-1}.

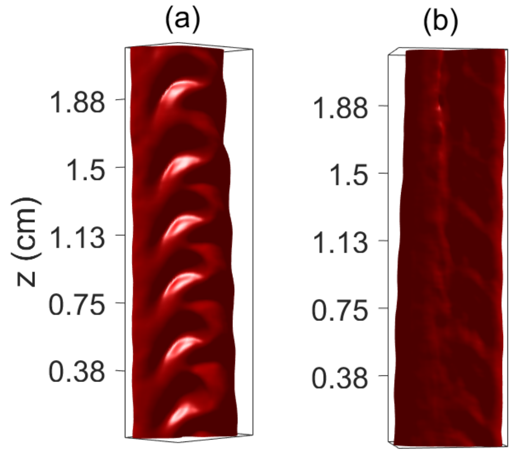

Figure S6: Tomography reconstruction of the lower portion of the tube at two different angles (a) 45° and (b) -100°. Flow rate: 0.858 mL min^{-1}.

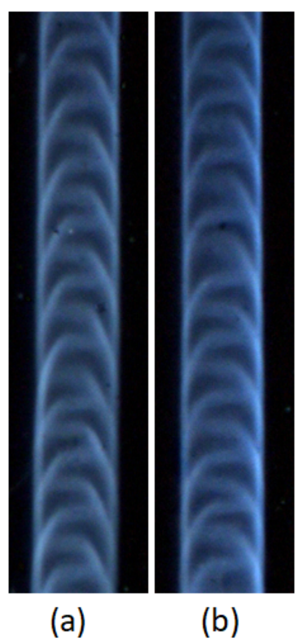

Figure S7: Chitosan tubes formed with injection by a peristaltic pump (a) and a syringe pump (b).
Flow rate: 0.858 mL min^{-1}.