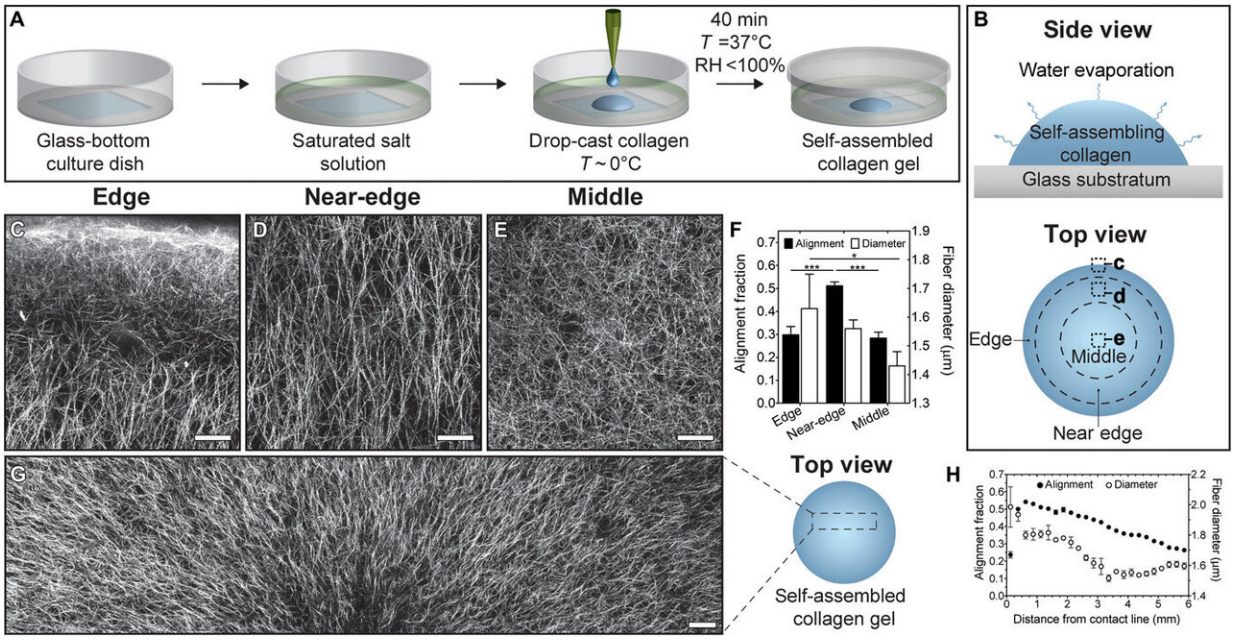
Marangoni flows drive the alignment of fibrillar cell-laden hydrogels

June 25 2020, by Thamarasee Jeewandara



Self-assembly of collagen in evaporating droplets generates aligned networks of collagen fibers. Schematic of (A) drop-casting procedure and (B) top and side views of an evaporating droplet of collagen. CRM images of self-assembled droplet of collagen in the (C) edge, (D) near-edge, and (E) middle regions of interest. Images are oriented such that the top of the image points toward the contact line of the droplet. The location for each image is highlighted in dashed boxes in (B). Scale bars represent 50 μm. (F) Alignment fraction and fiber diameter for drop-cast collagen gels. (G) CRM image of a self-assembled droplet of collagen. Five separate CRM images are stitched together to reveal the radial alignment of collagen fibers. Scale bar, 100 μm. (H) Alignment fraction and fiber diameter as a function of distance from the contact line for drop-cast collagen gels. Collagen solutions (pH 11) were gelled at controlled RH using a

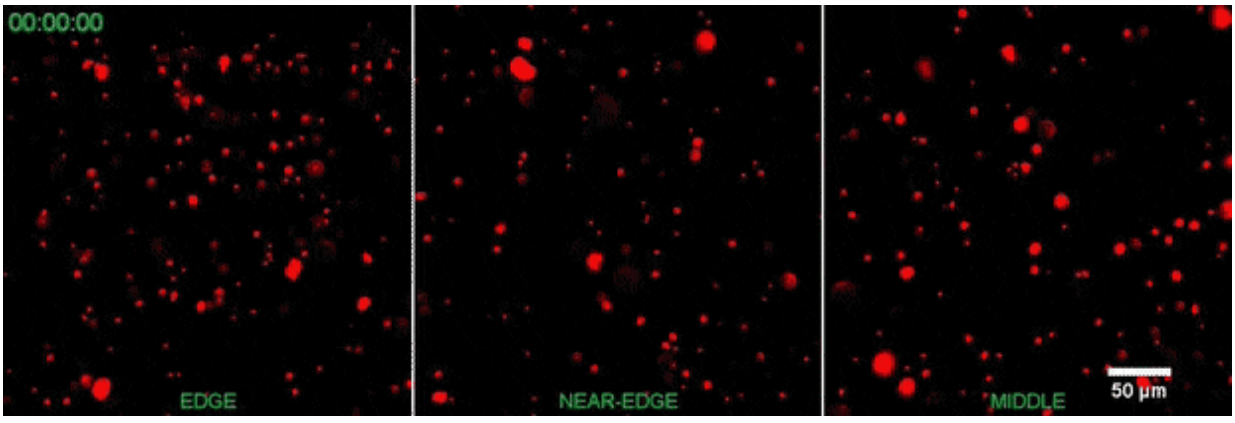
saturated solution of MgCl2 (RH ~ 31%) on UVO-treated glass. *P ≤ 0.05 and ***P ≤ 0.001. Credit: *Science Advances*, doi: 10.1126/sciadv.aaz7748

When a stationary droplet containing a solute in a volatile solvent evaporates, the flow in the droplet can assemble into complex patterns. Researchers have examined such transport in evaporating sessile droplets in solvents. In a new report now published on *Science Advances*, Bryan A. Nerger and a team of scientists in chemical and biological engineering, and molecular biology at the Princeton University, U.S., demonstrated flow in evaporating aqueous sessile droplets containing the self-assembling polymer type I collagen. The material can be used to engineer hydrated networks of aligned collagen fibers. The team noted the Marangoni effect (a term originating from the spread of oil droplets on water) to direct the assembly of collagen fibers across millimeter-scale areas relative to environmental humidity and the geometric shape of the droplet. Nerger et al. incorporated and cultured skeletal muscle cells into the evaporating droplets to observe their collective orientation and subsequent differentiation to myotubes in response to the aligned networks of collagen. The work demonstrates a simple, tunable and high-throughput approach to engineer aligned fibrillar hydrogels to create cell-laden biomimetic materials.

The myriad of solid deposition patterns that arise from evaporation-driven fluid flow was first reported by Robert Brown in 1828 and subsequently investigated for a variety of present-day applications, including microfabrication and ink-jet printing. The coffee ring or outward radial flow can also occur when the solvent is volatile, and the Marangoni flow driven by the latent heat of evaporation is supressed. Marangoni flows arising from thermal or solute-driven gradients in surface tension can also generate recirculating flows. Researchers have described flow in evaporating droplets mainly in the context of particles

suspended in solvents that completely evaporate. In this work, Nerger et al. showed how flow in evaporating droplets could regulate the rate of protein self-assembly and control the alignment of fibrous cell-laden protein networks. The team demonstrated that flow in evaporating aqueous droplets of neutralized type I collagen generated aligned collagen fiber networks.

The thermal and solute-driven Marangoni effects allowed radial flow in the evaporating droplet to orient collagen fibers through self-assembly. The scientists tuned the orientation of fibers by changing the rate of self-assembly, environmental humidity and geometry of the droplet. The skeletal muscle cells incorporated into the evaporating droplets oriented and differentiated into multinucleated myotubes in response to the alignment of collagen fibers and only a fraction of the water evaporated from the droplet, giving rise to a cell-laden hydrogel construct. The resulting hydrogel has broad applications to design biomimetic scaffolds for studies in tissue engineering, developmental biology and self-assembling materials.



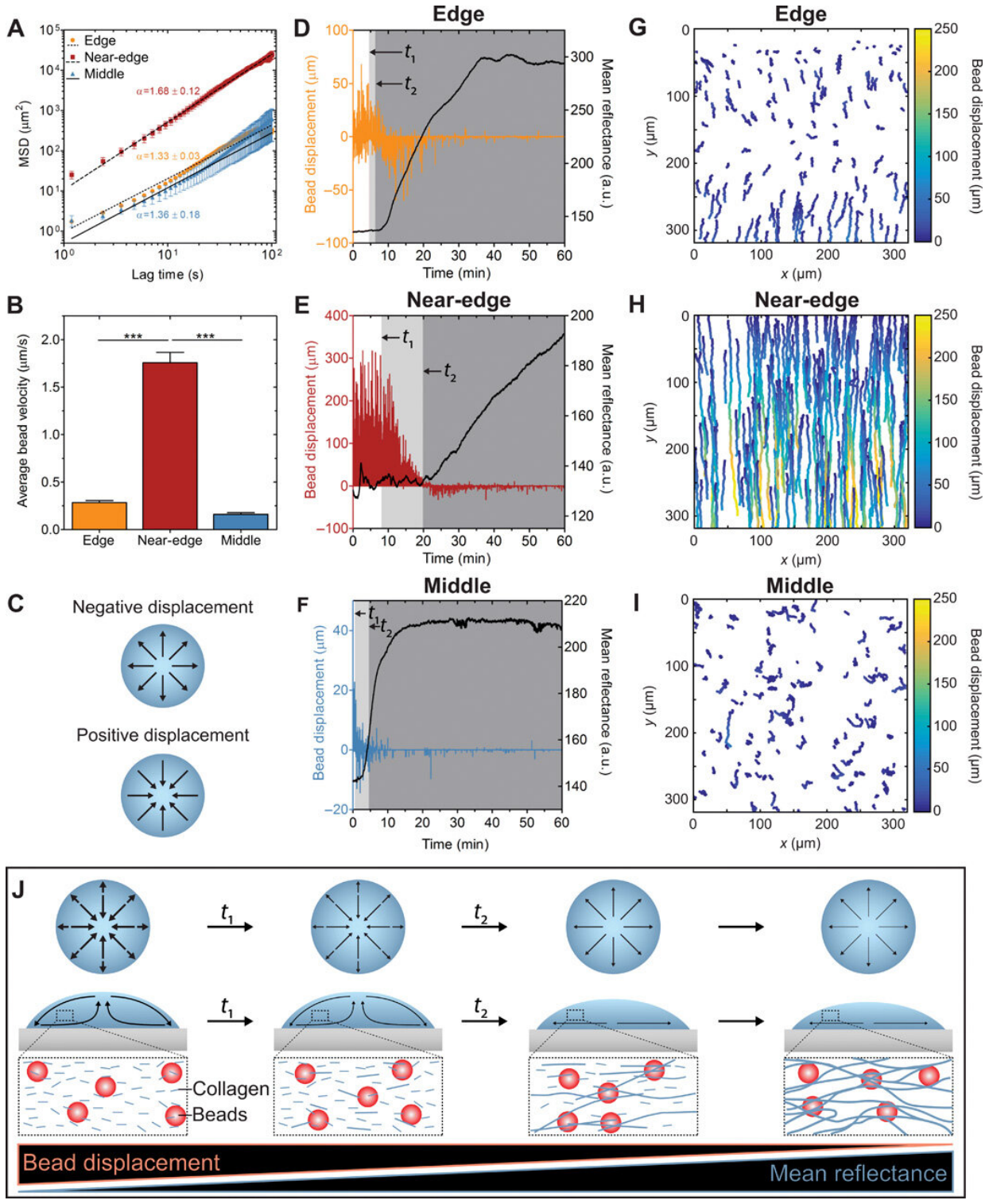
Representative time-lapse CRM videos of bead movements in the edge, nearedge, and middle regions of drop-cast collagen. RH was controlled using a saturated solution of MgCl2 (RH~31%) and collagen solutions were gelled on

UVO-treated glass. Credit: *Science Advances*, doi: 10.1126/sciadv.aaz7748

Self-assembly of collagen fibers in evaporating droplets of collagen

The team drop-casted neutralized solutions of type I collagen onto ultraviolet (UV)/ozone treated glass-bottom culture dishes by controlling the relative humidity (RH) of the culture dishes before depositing collagen inside the dish. They then placed the culture dish in a larger sealed petri dish to initiate collagen self-assembly. The collagen constructs self-assembled while water evaporated from the droplet, and the team visualized the orientation of collagen fibers in three distinct regions of the droplet including the edge, near edge, and the middle. The team observed orientations of the collagen fibers within the droplet and showed their variation within the evaporating droplets.

Nerger et al. incorporated fluorescent beads into the droplets to understand if the orientation of collagen fibers correlated with the internal patterns of flow during evaporation. They then observed the motion of the beads and the self-assembly of collagen to suggest that Marangoni flow drove recirculation within the evaporating droplets. The bead movements were consistent with the patterns of collagen fiber alignment throughout the droplet. The scientists quantified the flow by calculating time- and ensemble-averaged parameters, including mean-squared displacement (MSD), total displacement, and velocity of bead trajectories. The measurements showed increased mobility for beads in the near-edge region of the droplet, while the average velocity of beads were five- to 10-fold higher in the edge or middle regions.



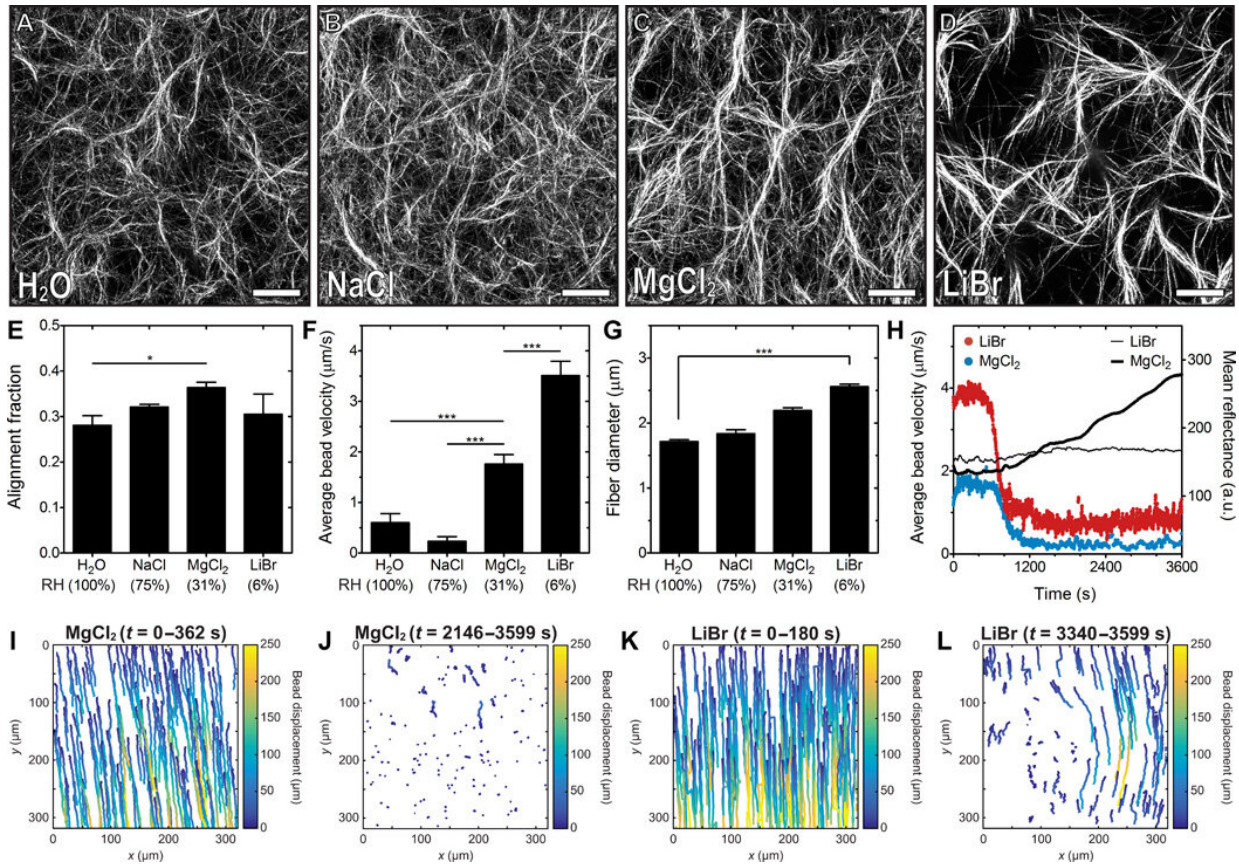
Evaporation drives distinct regional patterns of flow, which are attenuated by the self-assembly of collagen. (A) Time- and ensemble-averaged MSD for bead trajectories. Trajectories in the middle region exceeding 300 frames in length

were eliminated to improve computational efficiency. The slope, α, represents the power-law exponent that was fitted to the data. (B) Average radial bead velocity for 500 bead trajectories identified in each of three replicates. (C) Direction of radial flow corresponding to positive or negative displacement. Radial bead displacement in the (D) edge, (E) near-edge, and (F) middle regions of an evaporating droplet of collagen. Black lines represent mean reflectance at 488 nm. Characteristic times associated with the formation of free-flowing collagen fibers, t1, and the formation of a stable network of collagen fibers, t2, are annotated on plots (D to F). a.u., arbitrary units. Single-bead trajectories color-coded based on bead displacement for the (G) edge, (H) near-edge, and (I) middle regions of an evaporating droplet of collagen. The first 500 trajectories that exceeded 20 frames in length in each region of interest are plotted. (J) Flow fields observed in an evaporating droplet of collagen containing fluorescent beads. Collagen solutions were gelled at controlled RH using a saturated solution of MgCl2 (RH ~ 31%) on UVO-treated glass. ***P ≤ 0.001. Credit: *Science Advances*, doi: 10.1126/sciadv.aaz7748

Tuning collagen fiber alignment and diameter

Nerger et al. next explored the collagen fiber alignment in the droplets where the velocity of Marangoni flow was proportionate to the rate of evaporation. The alignment of collagen fibers depended on the flow-induced shear rate. Therefore, the team hypothesized that they could tune collagen alignment by adjusting the RH (relative humidity) variation. The process allowed them to also control the rate of droplet evaporation. They tested this using pure water and saturated salt solutions in the culture dish and used confocal reflection microscopy (CRM) to show that collagen fiber alignment decreased under the high RH conditions provided by water or sodium chloride (NaCl). When they decreased the RH using lithium bromide (LiBr), the alignment fraction decreased, while increasing the collagen diameter due to reduced kinetics of collagen self-assembly. The RH regulated the alignment of

collagen fibers by regulating the flow rates. Sufficiently large flow rates could therefore disrupt the formation of a stable collagen network. The team also varied the pH of the solution and deduced collagen fiber alignment to be a function of the kinetics of self-assembly in an evaporating droplet. The scientists could control the pattern of collagen alignment by controlling the geometry of the droplet.

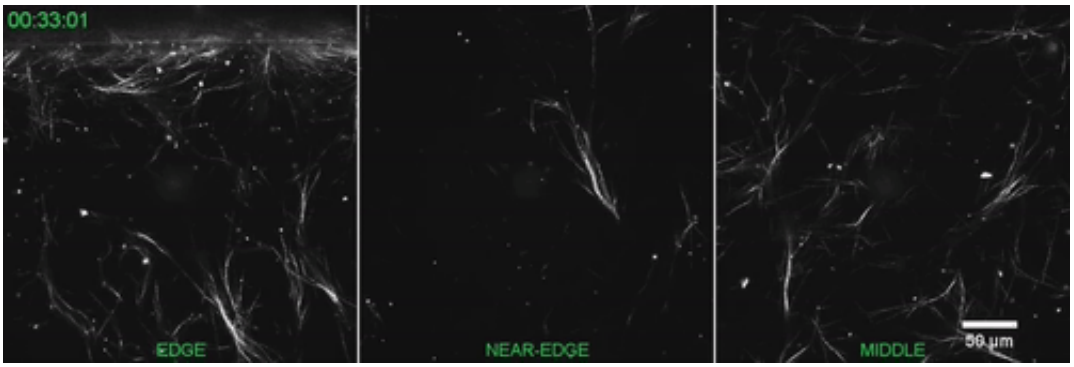


The RH (relative humidity) affects the alignment fraction and geometry of collagen fibers. Representative CRM images in the near-edge region of droplets of collagen self-assembled in the presence of (A) water (RH ~ 100%) and saturated solutions of (B) NaCl (RH ~ 75%), (C) MgCl2 (RH ~ 31%), or (D) LiBr (RH ~ 6%). Scale bars, 50 μm. (E) Alignment fraction of collagen fibers in the near-edge region of droplets of collagen. (F) Average radial bead velocity in the near-edge region of droplets of collagen. Velocity was determined from the

average of 500 bead trajectories. (G) Average collagen fiber diameter in the near-edge region of droplets of collagen. (H) Average radial bead velocity as a function of time in the near-edge region of droplets of collagen. Bead velocity data were smoothed using a moving average of 10. The black lines represent mean reflectance at 488 nm. Bead displacement in the near-edge region of droplets incubated with saturated solutions of (I and J) MgCl2 or (K and L) LiBr. (I) and (K) represent bead trajectories at the beginning of an experiment and (J) and (L) represent trajectories after the characteristic time t2. The total time during which trajectories are plotted is noted above each plot. Collagen solutions were gelled on UVO-treated glass. *P ≤ 0.05 and ***P ≤ 0.001. Credit: *Science Advances*, doi: 10.1126/sciadv.aaz7748

Patterning cell alignment for differentiation

Aligned networks of collagen fibers can typically influence physiological cell and tissue behavior as well as biological processes as a promising avenue in tissue engineering. To determine if cells remained viable on collagen after droplet evaporation, Nerger et al. included human breast cancer or skeletal muscle cells into the solution of collagen before drop casting and evaporation. The breast cancer cells oriented radially along collagen fibers in the droplet, and the skeletal muscle cells oriented in the direction of collagen fiber alignment. After four days in cell culture, the skeletal muscle cells differentiated to form multinucleated myotubes aligned to the direction of collagen fibers throughout the droplet. To confirm the influence of collagen on differentiation, the scientists cultured cells on a glass substrate under the same conditions and noted the sarcomeric structures to be contrastingly smaller and randomly oriented. The data demonstrated how evaporating droplets of collagen patterned cell alignment and differentiation across millimeter length scales.



Representative time-lapse CRM videos of collagen fiber self-assembly in the edge, near-edge, and middle regions of drop-cast collagen. RH was controlled using a saturated solution of MgCl2 (RH~31%) and collagen solutions were gelled on UVO-treated glass. Credit: *Science Advances*, doi: 10.1126/sciadv.aaz7748

In this way, Bryan A. Nerger and colleagues used Marangoni flow generated in evaporating droplets of type I collagen to regulate the self-assembly of collagen and produce three-dimensional (3-D) networks with tunable fiber alignment, diameter and porosity. They prevented complete evaporation of the droplets to form 3-D hydrated collagen fiber networks to support mammalian cell growth and differentiation. The system has potential to generate a simple, high-throughput approach to incorporate tissue explants or organoids in aligned networks of collagen. The approach will allow the production of physiologically relevant aligned tissue constructs for broad applications in life sciences and medicine.

More information: Bryan A. Nerger et al. Marangoni flows drive the alignment of fibrillar cell-laden hydrogels, *Science Advances* (2020). DOI: 10.1126/sciadv.aaz7748

Robert Brown. XXVII. A brief account of microscopical observations

made in the months of June, July and August 1827, on the particles contained in the pollen of plants; and on the general existence of active molecules in organic and inorganic bodies, *The Philosophical Magazine* (2011). DOI: 10.1080/14786442808674769

Robert D. Deegan et al. Capillary flow as the cause of ring stains from dried liquid drops, *Nature* (2002). DOI: 10.1038/39827