

Vapor mediation as a tool to control micro-nano scale dendritic crystallization and preferential bacterial distribution in drying respiratory droplets

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Abstract

Deposits of biofluid droplets on surfaces (such as respiratory droplets formed during an expiratory event fallen on surfaces) are composed of the water-based salt-protein solution that may also contain an infection (bacterial/viral). The final patterns of the deposit formed are dictated by the composition of the fluid and flow dynamics within the droplet. This work reports the spatio-temporal, topological regulation of deposits of respiratory fluid droplets and control of motility of bacteria by tweaking flow inside droplets using non-contact vapor-mediated interactions. When evaporated on a glass surface, respiratory droplets form haphazard multiscale dendritic, cruciform-shaped precipitates—using vapor mediation as a tool to control these deposits at the level of nano-micro-millimeter scales. We morphologically control dendrite orientation, size and subsequently suppress cruciform-shaped crystals. The nucleation sites are controlled via preferential transfer of solutes in the droplets; thus, achieving control over crystal occurrence and growth dynamics. The active living matter like bacteria is also preferentially segregated with controlled motility without attenuation of its viability and pathogenesis. For the first time, we have experimentally presented a proof-of-concept to control the motion of live active matter like bacteria in a near non-intrusive manner. The methodology can have ramifications in biomedical applications like disease detection, controlling bacterial motility, and bacterial segregation.

1. Introduction

Drying patterns formed from the evaporation of droplets of complex biological fluids (such as tears, synovial fluid or spinal fluid, blood, etc.) are enigmatic and have implications in biomedical applications [1–3] such as preventing disease transmission [4,5] and diagnostics [6,7]. Bio-fluid droplets are complex fluids containing several constituents such as proteins, surfactants, salts, to mention a few [8]. Due to the many components present in the

34 bio-fluid droplet, the competition between capillary flows driven by continuity and solutal
35 Marangoni flows driven by surface tension gradients (due to differential evaporation of the
36 components) determines the fluid flow inside a droplet[9]. Researchers have observed
37 suppression of the coffee ring effect due to protein adsorption on the surface of the particles
38 in protein droplets containing suspended polystyrene particles[10]. However, the deposit will
39 be at the edge or uniformly distributed over the surface depending on the charge of the
40 protein[11]. The presence of salts in bio-fluids further adds to the complexity leading to the
41 formation of dendritic crystals. The dendritic structure formation is dependent on the salt
42 concentration, drying mode, and particle size and shapes[12,13]. It is clear from several
43 studies[13–16] that the final precipitate formed is dependent on the individual components,
44 the ratio of the components present in the biofluid droplet, the substrate on which the droplet
45 is evaporated, and the environmental conditions. Thus, the final morphology of a deposit
46 depends on the combined effect of several parameters, as discussed, most importantly, mass
47 transport due to fluid flow and the aggregation of colloidal particles within the droplet if
48 parameters such as environmental conditions, substrate, components of the biofluid are
49 maintained constant.

50 The flow inside droplets driven by evaporation is very low ($\sim O(10)\mu\text{m/s}$) and is highly
51 uncontrolled. Since the flow inside the droplet is primarily responsible for the final deposit
52 formed, the final deposit can be controlled by controlling the flow as a corollary. However,
53 most techniques used to control the flow inside droplets, such as acoustic excitation[17],
54 heating[18], magnetic stirring[19], the addition of surfactant[20], are highly intrusive and can
55 lead to denaturing of the biological sample. Hence we propose the non-intrusive vapor
56 mediated interaction[21,22] to control the flow and subsequent patterns formed on drying of
57 the bio-fluid droplet. This is done by placing an ethanol droplet in the vicinity of the bio-fluid
58 droplet (we use a pendent ethanol droplet as shown in Figure 1 (b)), thus creating an
59 asymmetric concentration field of ethanol around the biofluid droplet. The minuscule amount
60 of ethanol vapor in the vicinity of the bio-fluid droplet is adsorbed onto its surface, creating
61 a surface tension gradient across the bio-fluid droplet. This generates vigorous Marangoni
62 flow inside the bio-fluid droplet, whose magnitude and flow direction can be controlled by
63 strategically positioning the ethanol droplet in the vicinity of the bio-fluid droplet (as shown
64 in Figure 1 (b), (c), and (d)).

65 Besides several components present in the bio-fluids, bacteria are also present in the bio-fluid
66 droplets and are generally motile[23], unlike the inert micro/nanosuspension in the

67 droplets[9,10]. Bacterial motility in these droplets is key to its navigation[24], biofilm
68 formation[25], and self-assembly[26] and is crucial for its survival in the dried
69 precipitates[27]. Bacteria also respond to the change in the environment[28] and the surfaces
70 in their vicinity[29]. Experimental observations in the literature indicate that the bacteria
71 exhibit swimming motion in droplets that can even propel the droplets[30]. Several methods
72 have been devised to engineer the bacterial motion[31], which can potentially be used to
73 develop biosensors[32] and other microfluidic devices[33]. However, microbial motility with
74 respect to its physical environment, most notably to fluid flow in its surroundings, is often
75 neglected in the literature. In order to see the effect of flow on the deposition and aggregation
76 of live bacteria in the bio-fluid droplets, we seed rod-shaped *Salmonella enterica* serovar
77 Typhimurium as a model system in the bio-fluid. Although *Salmonella* Typhimurium (STM),
78 a gut pathogen, is transmitted by the orofecal transmission route via contaminated food and
79 water, many studies have demonstrated the aerosol transmission of specific serovars
80 of *Salmonella enterica*, such as *Salmonella* Typhimurium, *Salmonella* Agona, etc.[34,35].
81 With this as a physiological significance, STM is used in the experiments as a model system.
82 In the present work, surrogate respiratory fluid (SRF) (for components, see section 2.1)
83 droplets containing bacteria (*Salmonella enterica* serovar Typhimurium) are considered a
84 model bio-fluid system to demonstrate the control of crystallization and bacterial deposition
85 through non-intrusive vapor mediation. In a practical scenario, respiratory droplets generated
86 during an expiratory event from an infected host may fall on the ground, can form fomites,
87 and have a potential for secondary disease transmission[5]. In this article, vapor mediation is
88 used as a tool for preferential bacterial deposition on the evaporated deposit without
89 diminishing its viability and simultaneously retaining its pathogenesis. In addition, we report
90 the transformation of spatio-temporal and topological regulation of crystals formed in bio-
91 fluid droplets via controlled Marangoni convection. Multiscale dendritic cruciform-shaped
92 precipitates are formed on the drying of a single surrogate respiratory droplet (without the
93 presence of vapor). We have demonstrated that the dendrite orientation and size can be
94 morphologically controlled, and subsequently, we can suppress cruciform-shaped crystals
95 using vapor mediation. The nucleation sites are also controlled via preferential transfer of
96 solutes in the droplets; thus, achieving control over occurrence and crystal growth
97 dynamically.

98 2. Experimental Section

99 2.1 Preparation of Surrogate Respiratory fluid (SRF)

100 The surrogate model of respiratory fluid used in experiments consists of dissolved salts and
101 alveolar surfactants emulating reality[7]. The respiratory fluid composition used in this article
102 is the same as Vejerano et al. reported[36]. The process of preparation of the surrogate fluid
103 is detailed as follows: 0.9 % by wt. of NaCl, 0.3% by wt. of gastric mucin (Type III, Sigma
104 Aldrich), and 0.05 % wt. of di-palmitoyl phosphatidyl-choline (DPPC (Avanti Polar Lipids))
105 is added in deionized water. The final composition is sonicated for 15 minutes to create a
106 homogeneous solution. Next, the homogenized solution is centrifuged at 5000 RPM for 15
107 minutes to pellet the impurities present in the liquid. The pH value of the prepared solution
108 is 6 or greater.

109 **2.2 Preparation of bacterial culture**

110 Fluorescently labeled mCherry wild type (WT) *Salmonella enterica* serovar Typhimurium
111 strain 14028 (S. Typhimurium) is grown in Luria broth with appropriate antibiotic
112 concentrations under shaking conditions at 37°C. Overnight cultures prepared from a single
113 colony from a freshly streaked plate are used for the experiments. 1.5ml of overnight culture
114 was centrifuged at 6,000 RPM to pellet the bacterial cells and washed once with autoclaved
115 MilliQ water. The resulting pellet is then resuspended into a freshly prepared surrogate
116 respiratory solution (SRF) and serially diluted such that each SRF droplet of 0.5
117 μ l approximately contains $\sim 10^3$ bacteria. The approximate size and shape of the bacteria used
118 are shown in Figure 1(f).

119 **2.3 Experimental set-up**

120 $0.5 \pm 0.1 \mu$ l droplet of surrogate respiratory fluid (SRF) (with bacteria) is gently placed on the
121 clean glass substrate as shown in Figure 1 (a) and allowed to evaporate in controlled
122 laboratory conditions (temperature $27 \pm 3^\circ\text{C}$, and relative humidity at 40 ± 5 %). This is
123 referred to as case 1 in this article. Next, a 2μ l pendant ethanol droplet is brought near side
124 '1' (see Figure 1(b) and (c)) of SRF droplet at a distance of $d_1 = 0.085 \pm 0.01 \text{ mm}$ and $d_2 = 0.21 \pm$
125 0.03 mm referred to as case 2 and case 3, respectively (refer to Figure 1 (b) and (c), Side '1'
126 is referred to the side where the ethanol is placed and side '2' is the side opposite to the
127 ethanol). Case 4 consists of the 2μ l pendant ethanol droplet being placed close to the SRF
128 droplet near the center of the SRF and the distance between them being $d_3 = 0.085 \pm 0.025 \text{ mm}$
129 (refer to Fig. 1 (d)). The distances mentioned above (d_1 , d_2 , d_3) are the distance between the
130 pendant ethanol droplet's surface and the surface of the SRF droplet, which is maintained
131 constant until the SRF droplet evaporates (as shown in Figure 1). The pendant ethanol droplet
132 volume is maintained constant throughout the experiment by maintaining a constant pumping
133 rate of $1 \mu\text{l}/\text{minute}$ (for the given laboratory conditions) equivalent to ethanol evaporation.

134 All experiments are conducted at least four times to maintain repeatability and
135 reproducibility.

136 The contact line dynamics and the droplet's height are obtained from shadowgraphy images
137 using a NIKON D7200 camera attached to a Navitar zoom lens. The volume of the droplet is
138 estimated, assuming the droplet to be of a spherical cap. The crystallization dynamics are
139 captured at 2.19 *fps* from the top-view, imaged using a high-resolution CCD camera
140 (PCO2000) mounted on a BX51 Olympus frame (See Video 7.1, Video 7.2, Video 7.3, Video
141 7.4). A halogen-based light source (TH4 200, Olympus) is used for top-view illumination. μ -
142 PIV experiments are done to study the flow field within the droplet qualitatively and
143 qualitatively. Neutrally buoyant monodisperse polystyrene particles are used for μ -PIV
144 experiments, as shown in Figure 1 (e) (the same was used in our previous work[37]). The
145 settings for μ -PIV experiments are the same as described in our previous work[37].

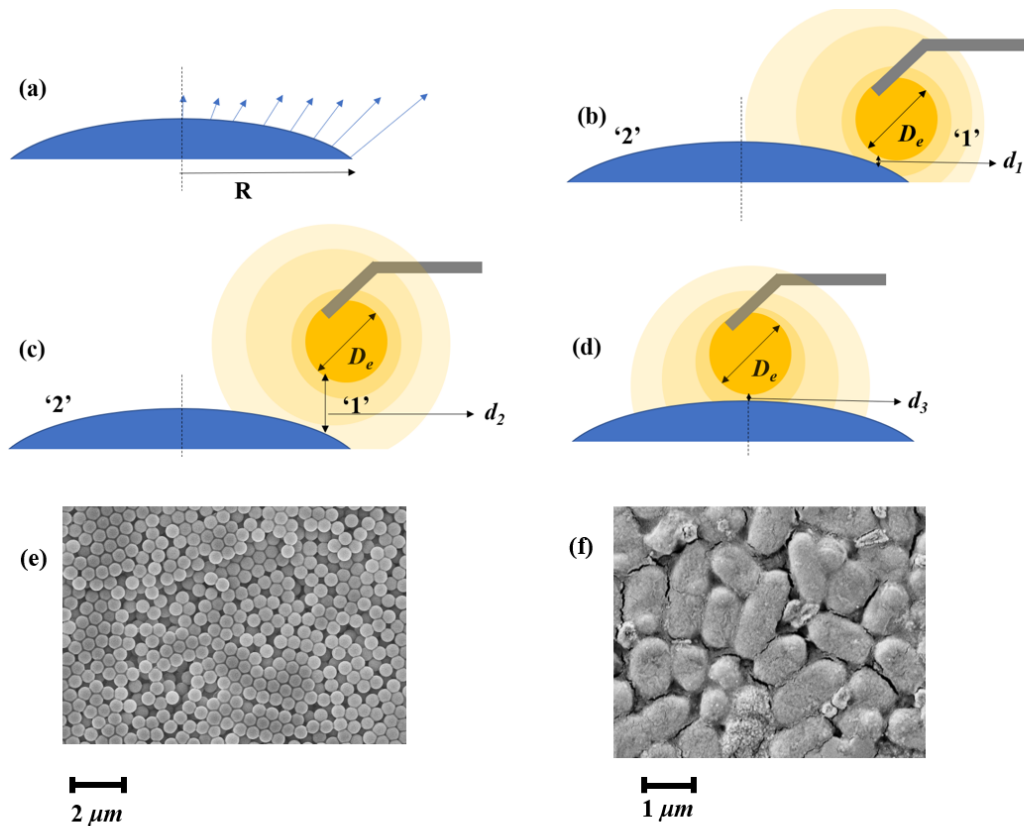
146 **2.4 Viability and Infection Assay**

147 To assess the viability of the bacteria in the dried droplets, the precipitate is resuspended in
148 40 μ l of PBS and plated onto *Salmonella-Shigella* agar (SS agar) at appropriate dilution. The
149 viability of bacteria is calculated by multiplying with dilution factor in terms of CFU/ml.
150 Further, to measure the pathogenicity of these bacteria in dried droplets, the resuspended
151 droplets are subjected to murine macrophages RAW 264.7. Further, 24 well plates are
152 centrifuged at 500-700 RPM to enhance bacterial attachment to host cells and incubated for
153 25 minutes at 37°C and 5% CO₂. The media containing bacteria is discarded and washed thrice
154 with 1X phosphate buffer saline (PBS). The cells are further subjected to Gentamicin
155 treatment (dissolved in DMEM) at a concentration of 100 μ g/ml for 1 hour to eliminate any
156 extracellular bacteria. The cells are maintained at 25 μ g/ml gentamicin containing DMEM for
157 the entire experiment. Finally, infected cells are lysed using 0.1% Triton-X 100 at 2 hours and
158 16 hours post-infection, and appropriate dilutions spread on SS agar plates.

159 The fold proliferation is calculated as follows: CFU at 16 hours is divided by CFU at 2 hours
160 to obtain fold replication of intracellular bacteria.

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Figure 1 Schematic representation of the experimental cases. The Surrogate Fluid Droplet (SRF) is allowed to evaporate on the glass surface in the following configurations (a) case 1: A single SRF droplet is placed, (b) case 2: a pendent ethanol droplet is brought very close to the SRF at a distance of $d_1 \sim 0.085 \pm 0.01 \text{ mm}$ on the side '1', (c) case 3: a pendent ethanol droplet is placed at a farther distance ($d_2 \sim 0.21 \pm 0.03 \text{ mm}$) to the SRF droplet on side '1', (d) case 4: a pendent ethanol droplet is placed very close to the SRF droplet at a distance $d_3 \sim 0.085 \pm 0.025 \text{ mm}$ at the center of the drop. SEM image of (e) 860 nm inert polystyrene microspheres, (f) rod-shaped STM bacteria.

3. Results and Discussions

3.1 Spatio-temporal control of crystallization

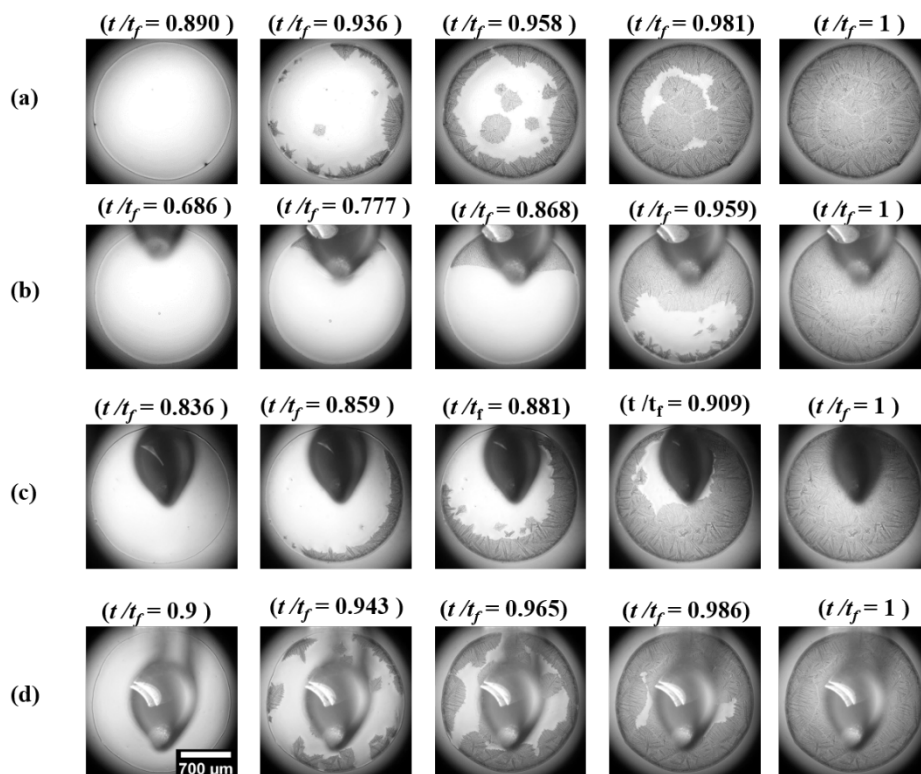
In this section, we describe the global dynamics of crystallization as obtained from the experimental observations. In the SRF, 70% of the solute mass is NaCl. On drying of NaCl solution, cuboidal crystals are expected to form[37]. However, due to the presence of other colloids (mucin, surfactant), a gelatinous mixture is formed at the later stages of evaporation due to solvent desiccation. The salt solution is non-homogeneously dispersed and is embedded in the gelatinous matrix. The formation of dendrites is due to the non-homogenous distribution of salt in the droplet. The role of mucin in the present study is analogous to agar forming dendrites, as observed by Goto et al.[38]. The inception of crystallization occurs

182 when the solution attains supersaturated conditions due to the drying of the droplet. For Case
183 1, the solute is accumulated more near the edge of the droplet; thus, the rim of the droplet
184 gets saturated faster. The optical profilometry data of the dried droplet shows a ring deposit
185 with a ring thickness of $\sim 3\text{-}4\ \mu\text{m}$ (refer to Figure S6 in supplementary information). As a result
186 of supersaturation near the rim of the droplet, the onset of crystallization always occurs from
187 the droplet's rim, which acts as a nucleus, and thereby, there is sustained growth of crystals
188 connected to the saturated solution that propagates to the center [9] (Refer to Video 1 in the
189 supplementary information, Figure 2 (a)). Crystallization can start anywhere from the rim (as
190 it is an instability), and there is no control over it with the natural evaporation of the droplets.
191 The instantaneous length of the dendrite (l) is considered from the point of nucleation until
192 the tip of the growing front.

193 We now show that by changing the flow inside the droplet through vapor mediation, we can
194 preferentially segregate the solute within the droplet, thus control the inception of
195 crystallization. A pendent ethanol droplet brought close to the SRF droplet at a distance d_1
196 (case 2, refer to Figure 1 (b)). Due to the proximity of the ethanol droplet, there is vigorous
197 Marangoni flow induced in the sessile droplet [22], which leads to a contact line slip from side
198 '1' (see Video 2, Figure 2 (b)) (flow dynamics inside the droplet are further described in detail
199 in section 2.3, and we will currently focus on the global contact line and crystallization
200 dynamics in this section). The contact line slip was observed in salt solution droplets in our
201 previous work [37]. However, in the present work, although the slip occurs from the side '1',
202 it leaves behind a gelatinous substance at the side '1' near the initial contact line due to
203 colloids present in the SRF droplet. As the fluid entirely moves towards side '2', leaving
204 behind trace solute near side '1', the region near side '1' attains saturation. Thus the inception
205 of crystallization for case 2 always happens from side '1' (Video 7.2, Figure 2 (b)). However,
206 since the contact line motion is due to liquid flow towards side '2' and not as a consequence
207 of evaporation, there is a considerable amount of liquid present in the side '2'. Hence,
208 although the inception of crystallization occurs very fast, the growth of crystallization is slow
209 compared to other cases (refer to Figure S1 in the supplementary information). Due to the
210 contact line slip, an uneven ring deposit with a lesser thickness $\sim 1.7\ \mu\text{m}$ at side '1' and more
211 thickness $\sim 5\ \mu\text{m}$ at side '2' is formed in case 2 (refer to Figure S7 the supplementary
212 information).

213 The strength of the Marangoni flow is reduced when the ethanol droplet is placed at a distance
214 d_2 (as $d_2 > d_1$, see section 2.3), which is insufficient to cause contact line slip. However, more
215 solute is deposited on the side '2' on every flow circulation due to Marangoni flow. This is a

216 similar type of deposition observed in our previous work[21]. As a result, the inception of
217 crystallization is reversed (starts from side '2') due to supersaturation at side '2' of the droplet
218 (refer to Figure 2 (c) and Video 3). Thus, although there is no contact line slip, there is a
219 preferential transfer of solute to side '2'. This is reflected in the optical profilometry data with
220 a thicker deposit at side '2' (refer to Figure S8 in the supplementary information).
221 When the ethanol droplet is brought near the center of the sessile droplet (case 4, Figure 1
222 (d)), the crystallization occurs from the center as well as the rim of the droplet, merging to
223 join each other (refer to Figure 2 (d) and Video 3). As an experimental constraint, it is not
224 possible to observe crystallization from the center of the droplet due to the hindering pendent
225 ethanol droplet (refer to Figure 2 (d)). Hence the time for the inception of crystallization is
226 taken as the time when we first see the crystals from the droplet's rim (Figure S1(b) in the
227 supplementary information). As the fluid and solute flow outwards from the center, the
228 central region of the dried precipitate has the most negligible thickness, and the thickness of
229 the deposit increases radially (refer to Figure S9 in the supplementary information).



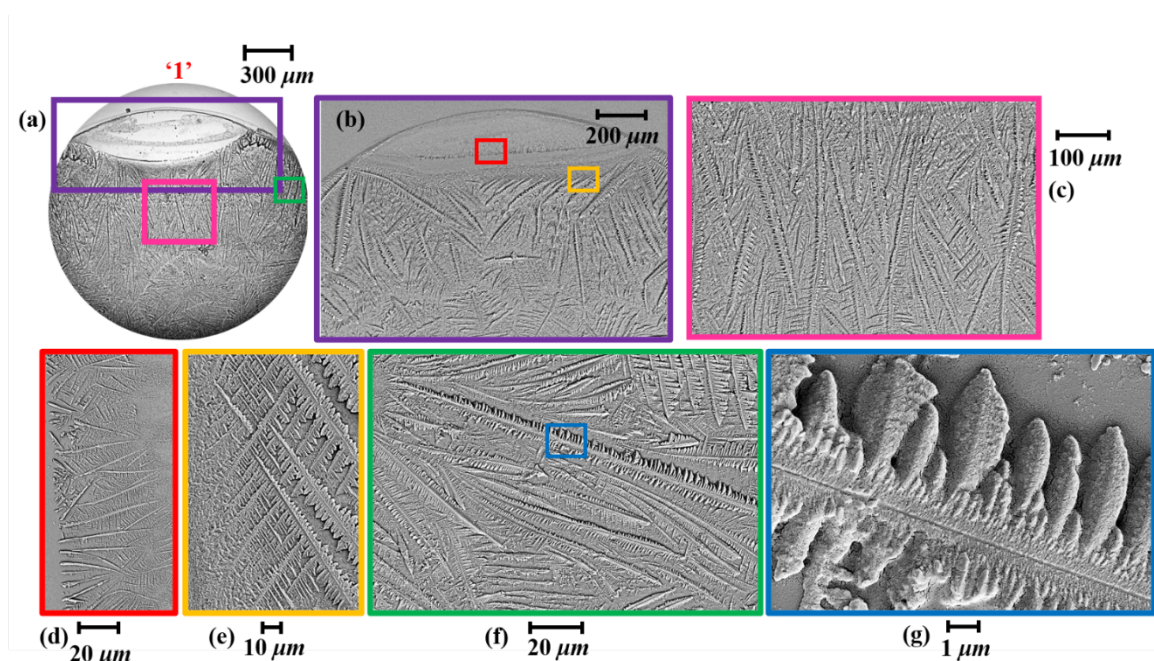
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231 **Figure 2 Dynamics of crystallization.** Snapshots with non-dimensional time t/t_f (given at
232 top of every image) for (a) case 1, (b) case 2, (c) case 3, (d) case 4. The scale bar given
233 in the bottom left image in (d) is valid for all images in the Figure. t is the instantaneous
234 time, and t_f is the total time of evaporation.

235 3.2 Multiscale dendritic patterns

236 SEM Micrographs reveal that the dendritic crystals on the dried SRF droplet are of size
237 ranging from $\sim O(1) \mu m$ to $\sim O(10^3) \mu m$. The crystal pattern form in a haphazard way from
238 randomly formed nucleation sites for case 1. Crystals grow from the inner nucleation site in
239 cruciform dendritic shape with branches radiating outwards(refer to Figure S2 in the
240 supplementary information).

241 The formation and growth of dendritic crystals depend on competition between solvent loss
242 (which increases the concentration of salt) and diffusion of salt ions towards the leading edge
243 of the tip (which reduces the concentration of salt in solution)[9]. With the natural evaporation
244 of the droplet, there is no control over the length, shape, or dynamics of crystallization. As a
245 result, crystals of different shapes are formed everywhere on the deposit.

246 It has been observed from the experiments that a graded distribution of crystal sizes and
247 controlled directional orientation can be obtained by controlling the flow in droplets using
248 vapor mediation. When the ethanol droplet is placed very close to the SRF droplet (case 1),
249 fine tiny crystals of $\sim O(1) \mu m$ to $\sim O(10) \mu m$ are observed in the slip region at side '1' (refer
250 to Figure 3 (d)). Suppression of cruciform-shaped crystals is observed near side '1'. With
251 the crystallization starting from side '1', elongated dendrites greater than $\sim O(10^2) \mu m$ with
252 their orientation away from the side '1' are formed (refer to Figure 3 (c)). Certain crystals
253 form at an inclined orientation from side '1' (refer to Figure 3 (b), (e), and (f), which could
254 be due to a non-controlled vapor source in the very vicinity, leading to vigorous Marangoni
255 in different directions. A controlled vapor field, as done by Volpe et al.[39] could reduce
256 such aberrations, which is out of the scope of the present work as we are dealing with only
257 the proof-of-the-concept. Since the region near side '2' is less affected by the vapor field,
258 we see the dendritic crystals are similar to case 1, such as the haphazard formation of
259 cruciform crystals (refer to Figure S3 in supplementary information).



260

261 **Figure 3 SEM images of dried precipitate for case 2 near side ‘1’ (colored). (a) Image of**
262 **the deposit near side ‘1’. Zoomed-in image of the region within the box, (b) with purple**
263 **border in (a), (c) with pink border in (a), (d) with a red border in (b), (e) with a yellow**
264 **border in (b), (f) with green border in (a), (g) with a blue border in (f).**

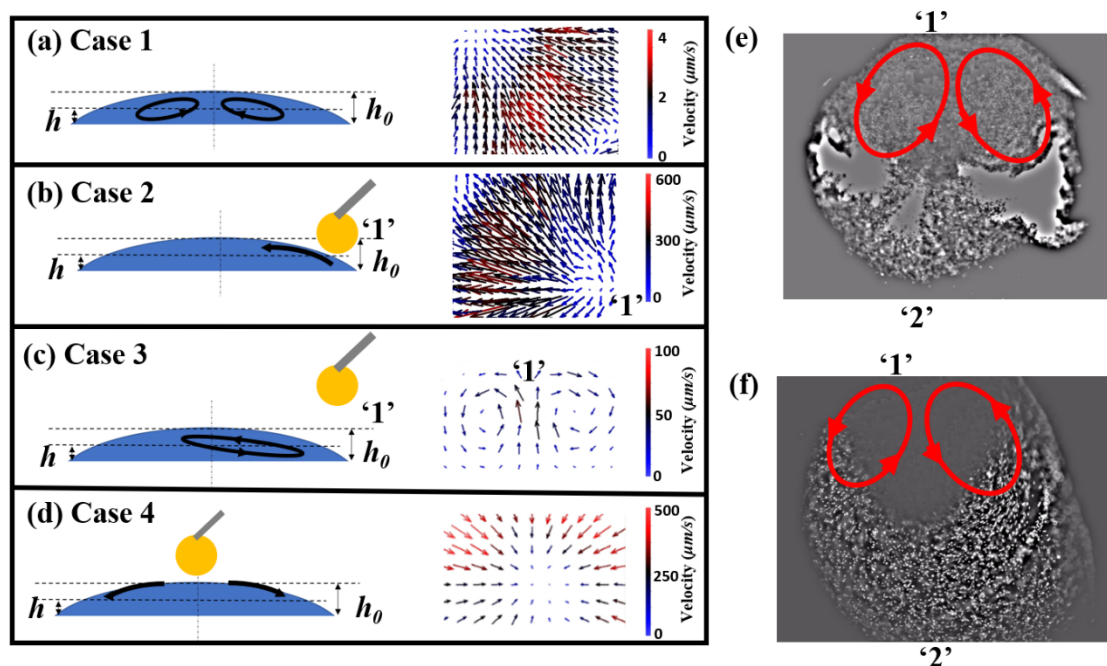
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266 We observe that the cruciform-shaped crystals are suppressed near side ‘1’ even for case 3.
267 The directional flow from side ‘1’ to ‘2’ in the droplet creates elongated parallel dendrites
268 with directional orientation from side ‘1’ to side ‘2’ (refer to Figure S4 in the supplementary
269 information). Similar to case 2, cruciform-shaped crystals are found near side ‘2’ for case
270 3. In case 4, a central hole is formed at the droplet in the end stages of evaporation, similar
271 to a hole formed in thin films due to vapor-mediated Marangoni convection[40]. We again
272 report a graded size distribution of the dendrites for case 4, with the smallest being near the
273 center ($\sim 2\text{-}5\ \mu\text{m}$) and bigger dendrites ($\sim 100\text{-}300\ \mu\text{m}$) as we move radially outwards. The
274 dendrites are oriented towards the radial direction (to Figure S5 in the supplementary
275 information).

276 **3.3 Flow dynamics inside droplet under the influence of vapor mediation**

277 The flow inside the droplet is measured using μ -PIV. The flow inside an SRF droplet (Case
278 1 in this chapter) was analyzed by Abdur et al.[9]. Flow is visualized in the SRF droplet by
279 seeding fluorescent particles of 860 nm procured from Thermo Fischer (refer to Video 6).
280 All measurements are taken below the midplane of the droplet ($h/h_0 < 0.5$). Double toroidal
281 Marangoni flow is observed initially in the SRF droplet, which later transforms into the

282 capillary flow near the evaporation. The magnitude of flow is $\sim O(10) \mu\text{m/s}$ (refer to Figure 4
 283 (a)). The flow in case 2 is initially circulatory and becomes unidirectionally away from side
 284 '1' later (refer to Figure 4 (b)). At later times the surface tension gradient is very high, and
 285 due to the unidirectional flow, the contact line slips. The explanation for this was given in
 286 our previous studies [37].
 287 The magnitude of flow velocity in case 2 is found to be $\sim O(10^3) \mu\text{m/s}$. In case 3, the
 288 Marangoni convection is insufficient to cause a slip, but circulatory flow with a lower
 289 magnitude of flow $\sim O(10^3) \mu\text{m/s}$ occurs. There is unidirectional flow at the end of
 290 evaporation in case 3, similar to case 2; however, the flow is insufficient to cause a slip. The
 291 flow inside SRF droplet in case 2 and case 3 is qualitatively similar; only the magnitude of
 292 velocity differs by one order of magnitude. In case 4, there is a circulatory flow to maintain
 293 the continuity; however, as the droplet thickness becomes significantly less in the end,
 294 strong radially outward flow from the center creates a dent at the center. The mechanism of
 295 formation of holes was previously explained by Kim et al. [40] is similar to case 4.



296
 297 **Figure 4** Instantaneous vectors of velocity for (measurement $h/h_0 < 0.5$ taken below the
 298 mid-plane of the droplet (a) case 1 (at initial times $\sim t/t_f \sim 0.1$), (b) case 2 ('1' indicates
 299 the side at which the ethanol is placed, measurement at $\sim t/t_f > 0.7$), (c) case 3 ('1'
 300 indicates the side at which the ethanol is placed, measurement at $\sim t/t_f > 0.7$), (d) case 4
 301 (at initial times $\sim t/t_f \sim 0.1$). (e) Flow visualization for case 2 with bacteria (refer to video
 302 5). Bacteria tend to aggregate near side '2'. (f) Flow visualization for case 2 with 860

303 ***nm* inert nanoparticles (refer to video 6) where the particles do not aggregate (are**
304 **discrete). The red arrows in (e) and (f) represent the flow direction.**

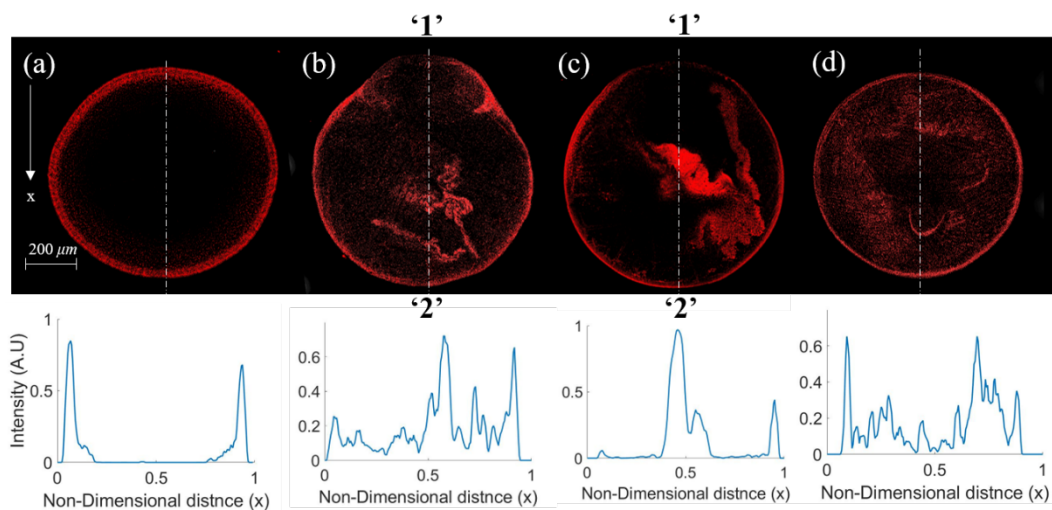
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306 **3.4 Bacterial distribution on the dried deposit**

307 The inertial forces generated by the fluid flow in the droplet ($\sim O(10^3)$ $\mu\text{m/s}$) are very high
308 compared to the bacterial motility. Therefore, it is expected that the bacteria move along with
309 the flow as it would be difficult for them to resist inertia due to the Marangoni flow. At the
310 end stage of evaporation, a gelatinous matrix of colloids formed is highly viscous, and the
311 bacteria are trapped in it. Therefore, the motility of the bacteria will largely be restricted once
312 the droplet dries. With the proposition that the bacteria faithfully follows the flow inside the
313 droplets, we expect that most of the bacteria must lie in the regions of maximum solute
314 deposit (information of the solute deposit in terms of thickness of the deposit is obtained from
315 optical profilometry data). The SEM images do not reveal the final bacterial positions as the
316 bacteria might have been embedded in the crystal matrix. However, since the bacteria used
317 are tagged with a red fluorescent protein, confocal microscopy reveals the final positions of
318 the bacteria in the deposit. Confocal microscopy data shows that most bacteria are found at
319 the midplane of the crystal deposit.

320 Figure 5 (a) shows that the intensity is maximum near the ring because of the high bacterial
321 density in the region. Since most of the solute deposit in case 1 is at the ring, bacteria are also
322 found near the ring. The ring deposit is a consequence of the fluid flow, as discussed in section
323 2.3. Ring deposit due to solutal Marangoni flow was also explained by Marin et al.[41].

324 The bacterial deposit for cases 2 and 3 is less toward side '1' compared to side '2' (Figure 5
325 (b) and (c)). The bacterial deposit is less near the center for case 4 (Figure 5 (d)).



326

327 **Figure 5 Confocal microscopy of the deposit with the fluorescence emission from the**

328 **bacteria, (a) case 1, (b) case 2, (c) case 3, (d) case 4. Plots in the bottom row correspond**
329 **to the intensity variation along the dashed line in the top row.**

330

331 The fluorescent polystyrene particles used in μ -PIV experiments (which are inert and
332 faithfully follow the flow as they are neutrally buoyant and have low stokes number of the
333 particles) show similar deposits as bacteria (refer to Figure S10 in the supplementary
334 information). However, clustered agglomeration is seen in the case of bacteria. Such
335 agglomeration of bacteria could be due to the bacterial response to high shear flow[42]. The
336 clustering of bacteria is seen by fluorescence visualization of the bacteria in the Marangoni
337 flow (see Figure 4 (f) and refer to video 6). Thus the bacteria do not just move along with the
338 flow. However, the confocal images in Figure 5 show that bacterial distribution can be
339 controlled on a global scale using vapor mediation.

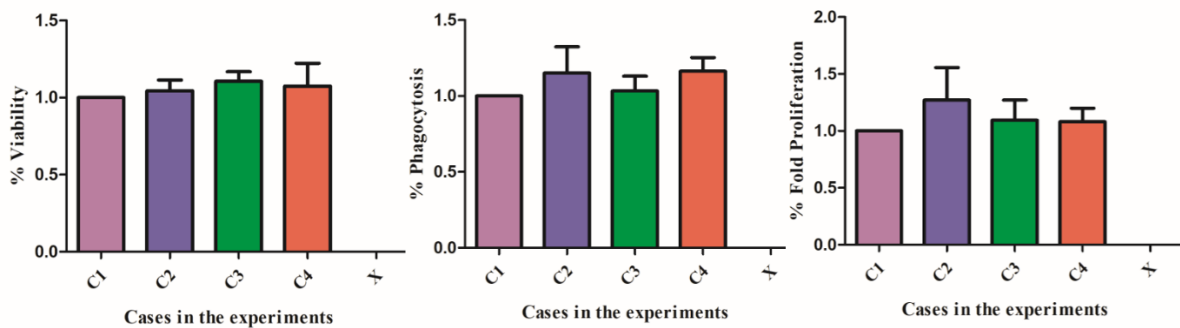
340 **3.5 Bacterial distribution and viability of the dried precipitate**

341 We have described in our previous works that the process of vapor mediation is non-intrusive
342 as a minimal amount of ethanol is adsorbed on the surface of the droplet[21,43]. This can be
343 seen from the volume regression curves of the SRF droplet (refer to Figure S11 in the
344 supplementary information). The evaporation characteristics of the SRF droplet remain
345 unchanged irrespective of the presence of ethanol droplet in its vicinity for all the four cases
346 described (refer to Figure S11 in the supplementary information). This section investigates if
347 there is any effect of vapor mediation on bacterial viability and its pathogenicity.

348 Bacterial reminiscent in the deposit survives over hours and days[44]. To see the effect of
349 vapor mediation on the deposit, we conduct viability tests as described in section 2.3. The
350 deposit after two hours is plated, and viable bacteria count is noted. Case 1 is considered as
351 a control. The viability count in case 1 is found to be $\sim 5 \times 10^4$ CFU/ml. Percent viability is
352 calculated by non-dimensionalizing the viability count of a particular case to that of Case 1.
353 Viability for case 2, case3, and case 4 remain the same as case 1, as shown in Figure 6 (a).
354 Thus, the bacterial viability is invariant of ethanol vapor present in the vicinity of the SRF
355 droplet. However, when an ethanol droplet of $0.5 \mu\text{l}$ is directly cast onto the dried deposit of
356 case 1, bacteria loses its viability. This case where ethanol comes in direct contact is labeled
357 as case x. Thus it is clear that vapor mediation is non-intrusive to the viability of the bacteria.
358 The uptake of bacteria by murine macrophages RAW264.7 (phagocytic cell) is observed, and
359 the percentage of phagocytosis is calculated. The percentage of phagocytosis is the same for
360 all cases (refer to Figure6 (b), case 1 is taken as a control, and values of other cases are non-
361 dimensionalized by case 1). The viable bacteria have the potential to infect the host through

362 the oro-fecal route. The bacteria, which has now been internalized, will be able to replicate
363 inside the host cell. The fold proliferation data reveals the survival and virulence of the
364 bacteria after entering the host cells. Fold proliferation of all four cases is retained at the same
365 level (refer to Figure 6 (c)).

366 A new culture is prepared (4 times each), and the bacteria resuspended in the SRF is drop-
367 casted onto the surface, and drying experiments are conducted for all four configurations of
368 Case 1,2,3 and 4 with four trials each. Thus, considering multiple freshly prepared cultures
369 and multiple droplets cast onto the surface of each freshly prepared culture for all
370 configurations give us the same results. Therefore, the error bar plotted in Figure 6 is
371 calculated, taking into account the trials mentioned above.



372
373 **Figure 6 Plots of (a) percentage viability, (b) percentage phagocytosis, (c) percentage**
374 **fold proliferation of bacteria for different cases. Case x represents when 0.5 μ l of**
375 **(volume of ethanol droplet is equivalent SRF droplet deposited) of 99% ethanol droplet**
376 **is poured onto the deposit and allowed to dry.**

377

378 4.Conclusions

379 Manipulation or segregation of particles in drying droplets[45], controlling motion of droplets
380 using vapor mediated interaction is shown by several studies[21,39,46–48]; however,
381 controlling an active living matter such as bacteria using a non-intrusive vapor mediation
382 technique has not been studied. We have experimentally investigated the control of the
383 distribution of bacteria within the deposit using vapor mediation without affecting bacterial
384 viability and pathogenesis. The flow inside the SRF droplet is altered by strategic positioning
385 of ethanol droplet leading to spatio-topological control of self-assembly, organization of
386 biomolecules, and crystallization is demonstrated using vapor mediated interaction of
387 droplets. Multiscale dendritic patterns can be formed and dynamically controlled using this
388 technique. This study provides valuable insights into the vapor-mediated non-contact

389 mechanism to control the pattern formation in complex solutions like biofluids. These
390 findings provide a preliminary understanding of the bacterial interaction with the fluid flow
391 inside the droplet. Proof of concept presented in this work can be used as a tool to control
392 bacterial motility, its segregation, and patterns of bio-fluid deposits which may have wide-
393 ranging implications in clinical infection scenario and biomedical engineering.

394

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