

# 1 Vapor mediation as a tool to control micro-nano scale 2 dendritic crystallization and preferential bacterial 3 distribution in drying respiratory droplets

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## 10 Abstract

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

## 28 1. Introduction

29 Drying patterns formed from the evaporation of droplets of complex biological fluids(such  
30 as tears, synovial fluid or spinal fluid, blood, etc.) are enigmatic and have implications in  
31 biomedical applications[1–3] such as preventing disease transmission[4,5] and  
32 diagnostics[6,7]. Bio-fluid droplets are complex fluids containing several constituents such as  
33 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 crystalization 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  $\mu$ 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  $40 \pm 5\%$ ). This is  
123 referred to as case 1 in this article. Next, a  $2 \mu$ l pendent 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  $0.03 \text{ mm}$  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  $2 \mu$ l pendent ethanol droplet being place 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 pendent 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 pendent ethanol droplet  
132 volume is maintained constant throughout the experiment by maintaining a constant pumping  
133 rate of  $1 \mu\text{l/ minute}$  (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.  $\mu$ -  
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  $\mu$ -PIV  
144 experiments, as shown in Figure 1 (e) (the same was used in our previous work[37]). The  
145 settings for  $\mu$ -PIV experiments are the same as described in our previous work[37].

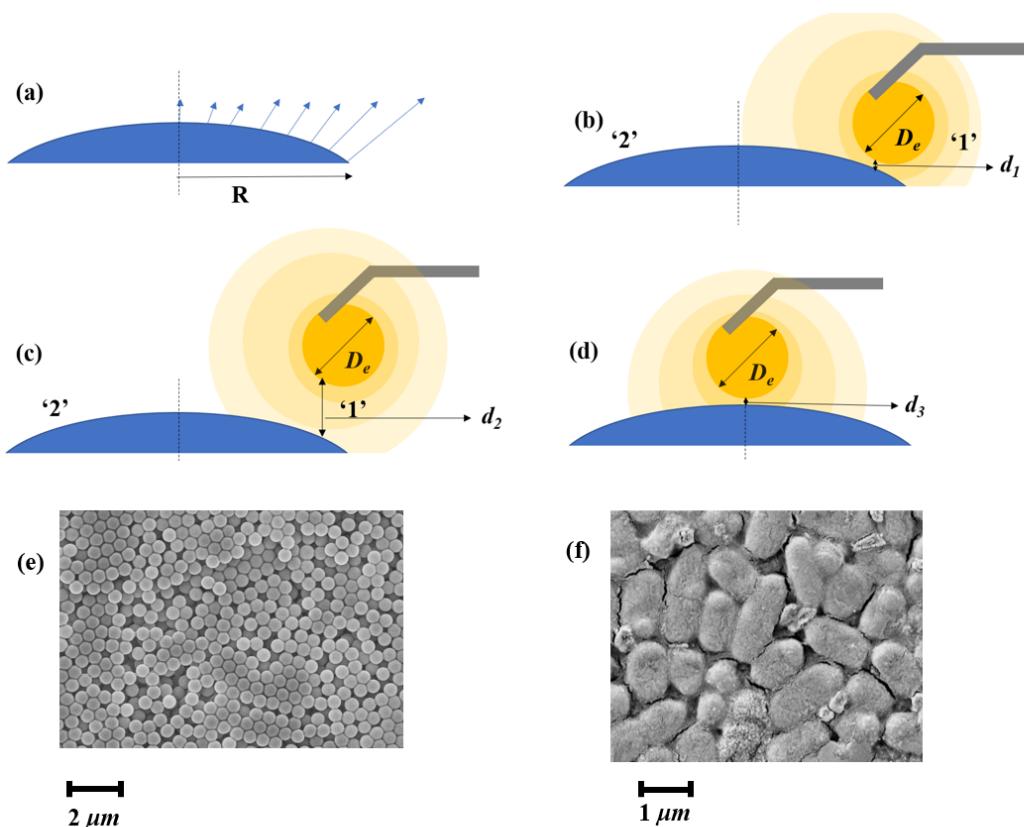
146 **2.4 Viability and Infection Assay**

147 To assess the viability of the bacteria in the dried droplets, the precipitate is resuspended in  
148 40 $\mu$ 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% CO<sub>2</sub>. 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  $\mu$ g/ml for 1 hour to eliminate any  
156 extracellular bacteria. The cells are maintained at 25 $\mu$ 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.

161

162



163

164 **Figure 1 Schematic representation of the experimental cases. The Surrogate Fluid**  
165 **Droplet (SRF) is allowed to evaporate on the glass surface in the following**  
166 **configurations (a) case 1: A single SRF droplet is placed, (b) case 2: a pendent ethanol**  
167 **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**  
168 **'1', (c) case 3: a pendent ethanol droplet is placed at a farther distance ( $d_2 \sim 0.21 \pm 0.03$**   
169 **mm) to the SRF droplet on side '1', (d) case 4: a pendent ethanol droplet is placed very**  
170 **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**  
171 **image of (e) 860 nm inert polystyrene microspheres, (f) rod-shaped STM bacteria.**

172

### 3. Results and Discussions

173

#### 3.1 Spatio-temporal control of crystallization

174

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

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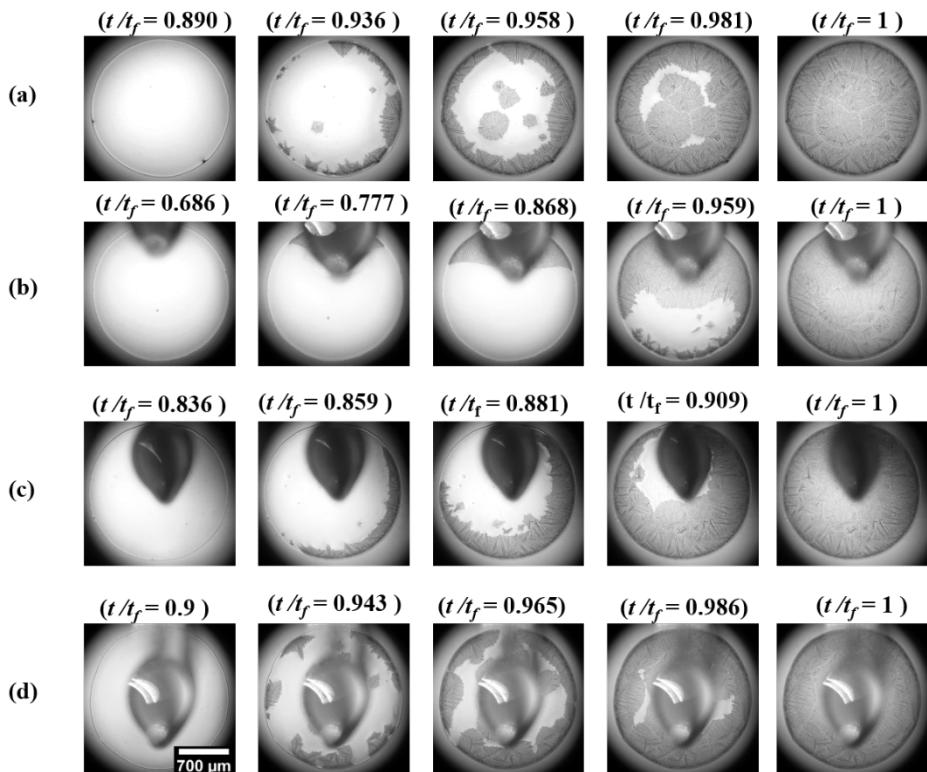
181

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  $d_2$  ( $d_2 > d_1$ , 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).



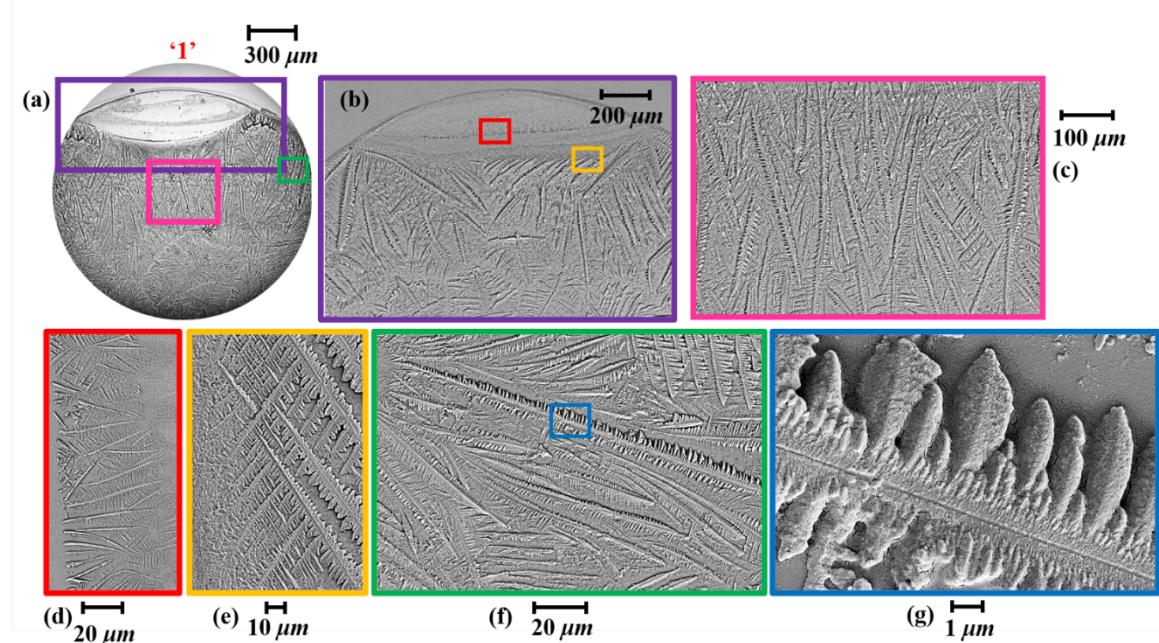
230  
231 **Figure 2 Dynamics of crystallization.** Snapshots with non-dimensional time  $t/t_f$  (given at  
232 top of every image) for (a) case 1, (b) case 2, (c) case 3, (d) case 4. The scale bar given  
233 in the bottom left image in (d) is valid for all images in the Figure.  $t$  is the instantaneous  
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  $\sim O(1) \mu\text{m}$  to  $\sim O(10^3) \mu\text{m}$ . 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  $\sim O(1) \mu\text{m}$  to  $\sim O(10) \mu\text{m}$  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  $\sim O(10^2) \mu\text{m}$  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).  
265

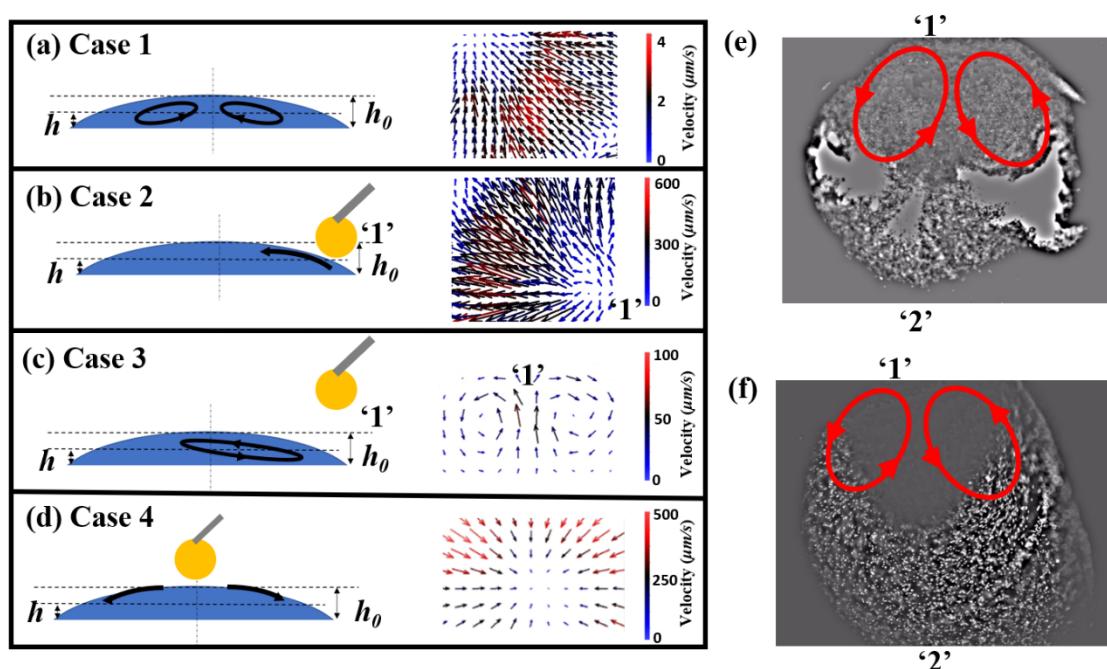
266 We observe that the cruciform-shaped crystals are suppressed near side '1' even for case 3.  
267 The directional flow from side '1' to '2' in the droplet creates elongated parallel dendrites  
268 with directional orientation from side '1' to side '2' (refer to Figure S4 in the supplementary  
269 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 (~2-5  $\mu$ m) and bigger dendrites (~100-300  $\mu$ 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  $\mu$ -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**

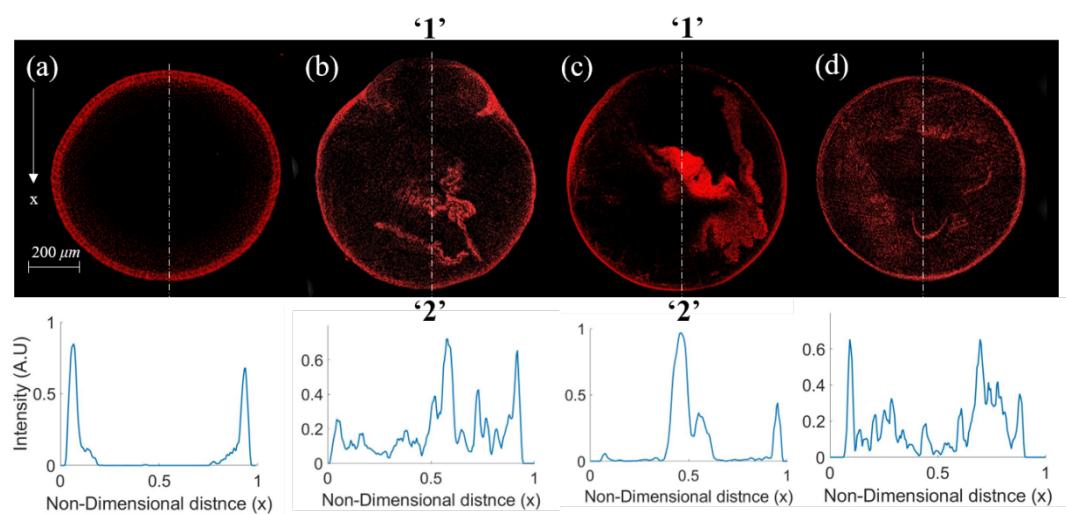
303 **nm inert nanoparticles (refer to video 6) where the particles do not aggregate (are**  
304 **discrete). The red arrows in (e) and (f) represent the flow direction.**

305

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  $\mu$ -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.

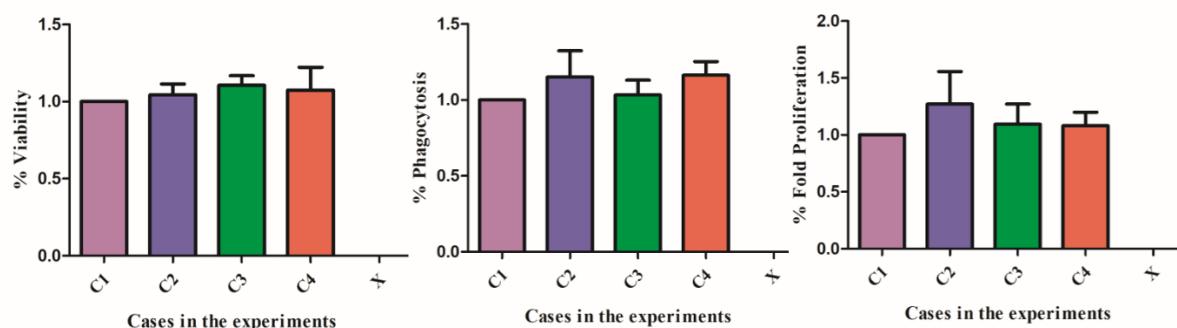
340 **3.5 Bacterial distribution and viability of the dried precipitate**

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  $\mu$ 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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