The environment topography alters the way to multicellularity in *Myxococcus xanthus*

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The social soil-dwelling bacterium *Myxococcus xanthus* can form multicellular structures, known as fruiting bodies. Experiments in homogeneous environments have shown that this process is affected by the physicochemical properties of the substrate, but they have largely neglected the role of complex topographies. We experimentally demonstrate that the topography alters single-cell motility and multicellular organization in *M. xanthus*. In topographies realized by randomly placing silica particles over agar plates, we observe that the cells’ interaction with particles drastically modifies the dynamics of cellular aggregation, leading to changes in the number, size, and shape of the fruiting bodies and even to arresting their formation in certain conditions. We further explore this type of cell-particle interaction in a computational model. These results provide fundamental insights into how the environment topography influences the emergence of complex multicellular structures from single cells, which is a fundamental problem of biological, ecological, and medical relevance.

INTRODUCTION

Biological organisms exhibit complex collective behaviors heavily influenced by their intrinsic properties and their interaction with the environment. In the case of microorganisms, experiments have demonstrated that such behaviors are crucially affected by cellular properties such as shape, density, and motion dynamics (1–3). When considering the environment, the substrate’s physical properties have been shown to affect microorganism survival, motility, and collective behavior (4–8). However, those experimental efforts have largely neglected the complexity of the environment where the microorganisms live, favoring experimental conditions that greatly oversimplify ecologically meaningful contexts, partly because of the need to standardize experimental designs (9–12). Recent work in the field of active matter has demonstrated that the topography of the environment can have a major influence on the motion and behavior of nonliving active particles (13–15) and single bacterial cells (8, 16–18), as well as on some collective bacterial phenomena (5, 6, 19–21). Nevertheless, it is still an open question how complex topographies influence microorganism motility and behavior throughout their levels of organization, from single cells to multicellular aggregates and structures.

*Myxococcus xanthus* is a motile rod-shaped soil-dwelling bacterium that glides across surfaces. It is a model system to study the transition to multicellularity, which is one of the most important transitions in the evolutionary history of life (22–24). As part of its life cycle, *M. xanthus* exhibits different types of collective behavior (25–27).

In particular, when nutrients are depleted, *M. xanthus* transits into a developmental stage characterized by cellular aggregation, which culminates in the formation of densely packed multicellular structures called fruiting bodies, containing up to 10⁶ cells, where cell differentiation into spores takes place (22, 27). This developmental process involves different levels of organization, from single cells, to motile cell groups of different sizes, and, lastly, to sedentary aggregates of millions of cells. At the onset of starvation, bacteria come together by colliding and following the slime trails left by other bacteria, thus forming motile aggregates that tend to increase in size and density (22, 25, 26, 28). These aggregates can turn into streams, which sterically confine the cells and eventually permit the formation of three-dimensional stacks and fruiting bodies (Fig. 1) (25, 26, 29).

Various concepts from the field of soft and active matter have already been used to understand the mechanisms underlying *M. xanthus* transition to multicellularity (1, 24, 26, 30–32). For example, the dynamic formation, shrinkage, and growth of cellular aggregates that ultimately produce fruiting bodies has been, to some extent, described as a droplet formation process in thin liquid films (31) and as a phase separation driven by cells that change their motility over time (26, 32). Furthermore, the transition to the various phases that characterize *M. xanthus* development has been proposed to result from the regulation of motility factors, which are usually associated with genetic or strain-specific features of the cells, namely, their speed, reversal frequency, and slime production rate (1, 33, 34). However, myxobacterial aggregation has been recently described in terms of the interplay of self-propulsion and the steric interactions due to their hard-rod shape, showing that steric interactions may be sufficient to induce some types of collective behavior, even in the absence of biochemical attraction among cells (35, 36). In addition, some environmental properties can have a major influence on the collective behavior of *M. xanthus*. For example, the chemical and physical properties of the substrate (e.g., stiffness and tension) substantially affect the behavior of *M. xanthus* and its multicellular development in terms of its fruiting body number, size, shape, and spatial distribution (6, 17, 37–39). Moreover, although the natural populations of *M. xanthus* live in highly heterogeneous soil...
we realized some complex environments by randomly distributing 10-μm-diameter silica particles onto flat agar substrates. Specifically, we assayed the wild-type (WT)-DZF1 *M. xanthus* strain at six cellular densities [measured by their optical density at 550 nm (OD$_{550}$); cellular densities: 0.01, 0.02, 0.06, 0.1, 0.3, 0.7 OD$_{550}$] and seven different topographic conditions (measured by the particle area packing fraction, i.e., the fraction of the substrate area covered by the particles: 0, 0.7, 4.2, 7.5, 24, 36, and 45%; see the “Experimental growth and developmental conditions on heterogeneous substrates” section in Materials and Methods). In each condition, we took micrographs at 0, 24, 72, and 96 hours (see the “Macroscopic experimental measurement and data analysis” section in Materials and Methods). Even a small amount of particles was sufficient to drastically modify *M. xanthus* multicellular development: A packing fraction of just 0.7% was sufficient to hinder the development of the fruiting bodies, as can be seen from their greatly reduced number in Fig. 1B, while a larger packing fraction of 7.5% completely prevented their development, as can be seen from their absence in Fig. 1C.

The development of fruiting bodies as a function of cellular density and particle density is shown in Fig. 2A and movie S1. From this phase diagram, it is clear that the formation of fruiting bodies decreased both by decreasing the cellular density and by increasing the particle packing fraction. Notably, for low cellular densities and high particle packing fractions, a complete arrest of fruiting-body formation occurred (lower left corner of Fig. 2A, encircled by the pink line). If they managed to form, then the number of fruiting bodies increased with the cellular density for each environmental topography (Fig. 2B). The average size of the largest fruiting bodies decreased as the cellular density increased, while they featured a weaker dependency on the packing fraction of particles (Fig. 2C). By contrast, the average size of the smallest fruiting bodies decreased as the particle packing fraction increased, while featuring a weaker increase as the cellular density increased (Fig. 2D). Last, we observed that the shape of the fruiting bodies became more elongated as the particle packing fraction increased (the fruiting-body shape is measured as circularity $c = 4\pi \times$ area/perimeter$^2$, where $c = 0$ indicates an elongated shape and $c = 1$ indicates a perfect circle; Fig. 2E). These results demonstrate that the cellular density and the environment topography jointly affect fruiting-body maturation, number, size, and shape, which are important developmental traits upon which natural selection may act.

**The particles disrupt the early aggregation dynamics by attracting and sequestering cells**

To understand the microscopic mechanisms underlying the changes induced in the multicellular organization of *M. xanthus* by heterogeneous topographies, we studied the local cell-particle interaction during the early stages of aggregation. *M. xanthus* populations at intermediate cellular and particle densities (0.1 OD$_{550}$, 24%; Fig. 3A) were recorded for 2 hours and then tracked to obtain the spatial coordinates and speed of the individual cells (see the sections “Microscopic local observation” and “Tracking analysis of individual cells” in Materials and Methods; Fig. 3, B and C, and movie S2). Over the course of hours, individual and aggregated cells moved toward nearby particles. When reaching the particles, the cells got arranged tangentially to the particles within the aqueous menisci that were formed around the particles (gray rings surrounding particles in Fig. 3A, defined between $r_p$ and $r_s$ in Fig. 3B). These menisci are formed by capillary action at liquid film interfaces (Fig. 3B). These menisci form also around cells (Fig. 3C), although, in this case, they

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**Fig. 1. *M. xanthus* fruiting-body development in homogeneous and heterogeneous topographies.** *M. xanthus* cells come together to develop multicellular fruiting bodies, which can be identified as dark spots on agar plates. (A) On a homogeneous substrate and at a cellular density of 0.01 OD, fruiting bodies start forming at 72 hours and are completely mature by 96 hours (some mature fruiting bodies are indicated by the arrows). (B and C) Even a relatively small amount of silica particles randomly distributed over the agar surface can (B) hinder (0.7% particle packing fraction) or (C) completely prevent (7.5% particle packing fraction) the fruiting-body formation. Scale bar, 1 mm.

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environments, the role of the substrate properties in *M. xanthus* motility and development has been studied only on smooth agar substrates. Thus, the question of how the environmental topography comes into play at different scales during unicellular-to-multicellular transitions, specifically in *M. xanthus*, remains to be explored.

Here, we experimentally demonstrate that the environment topography alters both single-cell motility and multicellular organization in *M. xanthus* colonies. Specifically, we demonstrate the effect of heterogeneous topographies, created by randomly placing silica particles over agar plates, on cellular motility, as well as on the number, size, and shape of multicellular fruiting bodies. We find that the particles attract some individual cells, whose trails are then followed by other cells. This results in the sequestration of a sufficiently large number of cells to alter the formation of aggregates, effectively hindering the formation of multicellular fruiting bodies, especially at low cellular densities. We support our results with numerical simulations of early aggregation and discuss them in terms of the interplay between the physical and the biological processes involved in the development of multicellular fruiting bodies under ecologically meaningful conditions.

**RESULTS**

**The substrate topography alters *M. xanthus* development and fruiting-body formation**

In the homogenous environment provided by a smooth agar plate, the *M. xanthus* colonies reach their complete development, fully maturing their fruiting bodies, in 96 hours, as shown in Fig. 1A. The fruiting bodies can be recognized by their shape and size and by their dark color conferred by the presence of differentiated spores (see, e.g., arrows in Fig. 1A). To determine the contribution of the environment topography to the collective dynamics of *M. xanthus*, we realized some complex environments by randomly distributing...
are much smaller in comparison to those formed around particles (cf. Fig. 3B). Individual cells also migrated and collided into growing aggregates with a nematic arrangement, which, in turn, fused with other nearby aggregates or moved toward particles (Fig. 3C and movie S2).

Cell migration could target either nearby particles or cellular aggregates (Fig. 3, B and C, respectively). Initially, cells featured an exploratory behavior characterized by short displacements in seemingly random directions, but as they came closer to a target, they started moving straight toward it (movie S2). As quantified in Fig. 3 (D and E), individual cells increased their speed, exhibiting a drastic acceleration when they reached the triple-phase interface line of the particle’s or the cellular aggregates’ aqueous meniscus \( r_p \) and \( r_b \) in Fig. 3 (D and E)]. This strongly suggests that bacteria are pulled into the meniscus because of capillary attractive forces (40–42), which have been proposed to be a dominant factor of colloid deposition at interfaces and are one of the drivers of \( M. xanthus \) motility (41, 43, 44). The speed of cells moving toward particles (Fig. 3D) was significantly larger than that of cells moving toward other cells (Fig. 3E). Moreover, since the particle meniscus is larger than the cell meniscus, particles exerted a greater attraction over a larger area. We also observed that, once cells entered a particle’s meniscus, they remained there for the rest of the time (Fig. 3, B and C, and movie S2). Last, some cells left a visible slime trail of their trajectory, which was later followed by other cells (Fig. 3, B and C, and movie S2). The existence of this trail-marking and trail-following mechanism in \( M. xanthus \) motility is well known (27, 28, 45). Over time, the number of trails approaching a particle increased, which, in turn, further increased the effective strength and domain of the attraction exerted by the particle. Consequently, the space between particles was gradually depleted of cells, the majority of which accumulated around the particles (Fig. 3B and movie S2). This pronounced trapping effect does not occur with bacteria-bacteria interactions since individual and aggregated bacteria are always moving and reorganizing during this early stage, inhibiting the formation of persistent trails.

We did not find evidence that the cells surrounding the particles, although somehow aggregated, could give rise to fruiting bodies. These observations show that the heterogeneous substrates that we designed disturb the aggregation and developmental dynamics of \( M. xanthus \) because the cells are attracted by particles with more...
strength than by other cells. Furthermore, as cells continuously modify the substrate by leaving trails that other cells can follow, these cellular trails reinforce the attraction of individual cells and groups of cells toward the particles, effectively enabling longer-distance attraction. Therefore, the combined action of attraction and subsequent trail following renders large areas around the particles depleted of cells. As a consequence, the particles successfully compete with the cells, managing to attract a great number of cells and preventing the sequestered cells from forming fruiting bodies.

**Particle-cell attraction is crucial to alter cell aggregation**

The experimental results presented in the previous sections show that *M. xanthus* cells are attracted to particles, mainly via capillary attraction forces and reinforcing trail following, even more than they are attracted to other cells. To further test the overall effect of this cell-particle attraction on cell aggregation, we used a qualitative Glazier-Graner-Hogeweg computational model (46), which is commonly used to model cellular and developmental dynamics and has successfully reproduced different aspects of *M. xanthus* collective behavior (46–48). This model considers elongated and semiflexible simulated cells capable of secreting trail-forming slime and of adhering to other cells forming stable aggregates (see the “Computational model” section in Materials and Methods; Fig. 4A). The heterogeneous substrate was simulated, adding particles at various densities [0, 1, 5, 15, and 25% packing fraction; Fig. 4, B, C, and D, respectively]. The attractive effect of the aqueous meniscus observed in the experiment was modeled through a particle-cell attraction gradient given by a decreasing logarithmic function. The model parameters were selected on the basis of previous reports and our own experimental observations (Table 1).

We simulated three types of substrate: (i) a smooth substrate with no particles (Fig. 4A), (ii) a substrate with randomly distributed 10-µm particles acting as steric obstacles (without attractive force) (Fig. 4B), and (iii) a substrate with randomly distributed particles attracting individual or aggregated cells (Fig. 4C). In each one of these substrates, we introduced a population of simulated cells and allowed them to organize until steady aggregates were formed (around 5000 Monte Carlo steps). We performed 10 replicates for each condition and counted the number of aggregated cells that were not trapped by the particles (Fig. 4D). The attractive effect of the aqueous meniscus observed in the experiment was modeled through a particle-cell attraction gradient given by a decreasing logarithmic function. The model parameters were selected on the basis of previous reports and our own experimental observations (Table 1).
almost the same as in the reference case, with a slight decrease when the particle density increased to 25% of the area. In contrast, when attractive particles were added (Fig. 4, C and D, and movie S3), the average number of aggregated cells that were not trapped by a particle sharply decreased with particle density. This sequestration effect was still observed in additional simulations in which the virtual particles had different sizes (5 and 15 μm) and arrangements ranging from ordered to random (fig. S1). It was also observed in simulated mutants with reduced reversals and slime trail following (fig. S2). However, aggregates were still formed as slime trail following was decreased [in agreement with (36)], but the sequestration effect was attenuated with reduced slime trail following and it appeared to be lost in its absence, further highlighting the key reinforcing role of this type of cellular attraction. While the features of heterogeneous media are often considered solely as obstacles for living active particles [e.g., (16, 18, 49)], our simulation results support that attractive particles may sequester nearby cells, diminishing the local cellular density in the remaining area and hindering or completely preventing their fusion into bigger aggregates that could eventually give rise to fruiting bodies. More generally, attractive particles such as those constituting the topography of our experimental setup establish a qualitatively different interaction with cells compared to steric obstacles, resulting in completely different dynamics and behaviors.

**DISCUSSION**

We studied the development of the multicellular bacterium _M. xanthus_ as a function of different cellular densities on substrates with varying environmental topographies, artificially built with randomly deposited silica particles. We observed that the environment topography had a strong impact on the development of its multicellular fruiting bodies. We found that fewer fruiting bodies were formed as the particle packing fraction increased. This trend was particularly pronounced at low bacterial densities, for which the formation of fruiting bodies could even be completely inhibited, as shown in Fig. 2 (A and B).
Moreover, the presence of particles was associated with the formation of smaller fruiting bodies than in homogeneous substrates (Fig. 2, C and D) and with the change in fruiting-body shape from circular to elongated (Fig. 2E). Our results are in line with previous studies reporting that a critical cell packing fraction is required for the emergence of multicellular structures. Considering that studies had not addressed the impact of the substrate topography on the emergence of smaller fruiting bodies than in homogeneous substrates (35) and that the substrate physical properties are able to modify the cellular spatial arrangement (36), our results are in line with previous works reporting that a critical cell packing fraction is required for the emergence of multicellular structures, even arresting it in certain conditions. Further experimental efforts could help understanding the type of media, developmental stage, and strain conditions and range and strength of capillary action could be certainly altered, although these forces may have contrasting effects depending on the type of media, developmental stage, and strain conditions and may interact in complex ways with other physical and biological processes (41). For example, in submerged or desiccating media, the slime trail following on bacterial motility and aggregation, especially in thin liquid films formed on solid substrates in contact with air, such as those thought to prevail in their natural environments (57). However, these forces may have contrasting effects depending on the type of media, developmental stage, and strain conditions and may interact in complex ways with other physical and biological processes (41). For example, in submerged or desiccating media, the range and strength of capillary action could be certainly altered, although slime-media or other liquid-liquid interfaces may still play a role in the aggregation dynamics. Therefore, one limitation of our study is that, although we explored different topographies and virtual mutants in our simulations (figs. S1 and S2), experiments were performed only for one type of strain, topography, and environmental condition. Further experimental efforts could help understanding

Table 1. Parameters used to simulate M. xanthus early aggregation. The referenced parameters are based on previously reported M. xanthus models or based on our experimental data.

| Parameter | Remark | Value | References |
|-----------|--------|-------|------------|
| $T_m$     | Amplitude of cell-membrane fluctuation | 10   | (46)       |
| $\lambda_L$ | Cell elongation constriction value | 30   | This work  |
| $L_n$     | Cell target length | 6 μm; 12 pixels | (28)       |
| $C$       | Connectivity penalty value | $10^{11}$ | This work  |
| $\lambda_{CV}$ | Cell volume constriction value | 5    | (46)       |
| $V_{ct}$  | Cell target volume | 3 μm$^3$; 12 pixels | (28)       |
| $\lambda_B$ | Curvature constriction value | $10^6$ | (61)       |
| $\lambda_S$ | Slime following value | 10   | (46)       |
| $k_S$     | Slime decay rate | 0.006 | (63)       |
| $F_S$     | Uniform slime secretion | 0.9  | (63)       |
| $\lambda_P$ | Cell-particle attraction value | 200  | This work  |
| $k_P$     | Particle attractive gradient, particle domain of attraction | 0.003 | This work  |
| $F_P$     | Uniform renewal of particle attractiveness | 0.9  | This work  |
| $J_{cc}$  | Cell-cell adhesion | 10   |           |
| $J_{cp}$  | Cell-particle adhesion | 7; 50 (attractive; non-attractive particles) | (46) and this work |
| $J_{pp}$  | Particle-particle adhesion | 50   |           |
| $J_{pm}$  | Particle-medium adhesion | 10   |           |
| $J_{mm}$  | Substrate-medium adhesion | 0    |           |
| $J_{mc}$  | Substrate-cell adhesion | 20   |           |

models and observations pointing to surface tension as a force driving M. xanthus motility (43, 44, 54). These observations are in line with previous findings showing that M. xanthus gliding motility is associated with surface tension, which facilitates cellular adhesion to the substrate and generation of pushing forces (43, 44, 55, 56). The magnitude and range of the attraction exerted by the particles was larger than the attraction exerted by other cells (Fig. 3 and movie S2), inhibiting the cells from forming large cell-only aggregates, which are a precursor of the fruiting bodies.
the contribution of capillary action and slime trail following in different physical contexts, for example, using different substrates, humidity conditions, and particle sizes or materials.

It has been shown that aggregation can result from pure physical interaction among active particles even without considering slime trail following (36). However, our results also reveal a synergy between capillary attraction forces and the slime trail following, typical of *M. xanthus* motility (27, 45). Because cells leave behind slime trails as they are attracted toward a particle, an increasing number of trails pointing toward the particle meniscus build up over time. This self-reinforcing mechanism gradually increases both the net attraction strength and the attraction domain of particles over cells. In nature, this mechanism could underlie different types of attractive interactions among living and nonliving elements of the environment. From an ecological perspective, our observations highlight the role of living active matter in continuously shaping its environment. The resulting bidirectional interaction involves several physical and biological interplaying aspects.

We have now shown that fruiting-body formation can be arrested because the cells are sequestered by the particles. It is known from the literature that, as part of multicellular development, cells form stacks that eventually lead to the three-dimensional organization of fruiting bodies (26, 29). Furthermore, models for cellular differentiation within fruiting bodies suggest that spores are formed after the accumulation of certain molecules at the center of these cellular aggregates (48, 58). Taking this into account, we speculate that the cellular sequestration by particles prevents both cellular differentiation and formation of three-dimensional structures. This is also in agreement with the lack of evidence for fruiting-body formation around particles in our experimental setup despite the local accumulation of cells.

The mechanism of cell sequestration that we postulate requires attraction exerted by the particles on the cells, in contrast with mechanisms involving particles that act just as steric barriers or obstacles (16, 49). We have investigated this aspect by developing a computational model to compare the effect of particles with and without an attractive field on the motility and aggregation of simulated cells (Fig. 4A and movie S3). This model gives rise to qualitatively different patterns of aggregation for attractive and non-attractive particles, whose trends coincide with those observed in our experiments: While non-attractive particles act only as obstacles that increase the local cellular density (Fig. 4B), attractive particles sequester cells and prevent the formation of aggregates that could eventually come together to form fruiting bodies (Fig. 4C). These simulations highlight the important role of cell-particle attractive interactions in determining the trajectories and spatial arrangement of groups of cells, biofilms, and other active materials.

In conclusion, we have shown that a heterogeneous topography greatly affects the formation of multicellular *M. xanthus* fruiting bodies and have provided empirical and numerical evidence for an underlying mechanism based on the sequestration of cells. Specifically, we postulate a combination of physical and biological processes that lead to cell sequestration by particles and the concomitant alteration of multicellular development into fruiting bodies, especially at low cellular densities. This contributes to our understanding of how *M. xanthus* cells can respond to the physical attributes of their environment and how the intricate organism-environment interactions are shaped. More generally, this work advances our understanding of the role of the environment topography not only on the general organization principles of active particles but also on the ecological challenges involved in the origin and development of multicellular aggregates in complex ecological contexts.

**MATERIALS AND METHODS**

**Experimental growth and developmental conditions on heterogeneous substrates**

Following the protocol described by Yang and Higgs (59), DZF1 strain was taken from a frozen stock by spotting 50 μl onto a casitone yeast extract (CYE) agar plate (1% Bacto Casitone, 10 mM tris-HCl (pH 7.6), 0.5% yeast extract, 10 mM Mops (pH 7.6), and 4 mM MgSO₄) and incubated at 32°C for 2 days. Cells from the resulting colony were transferred to 25 ml of CYE liquid medium and incubated at 32°C, shaking at 250 rpm overnight. The culture dilution was grown from 0.1 OD₅₅₀ until it reached 0.7 OD₅₅₀ (nutrient-rich liquid culture), taking a sample each 0.1 OD₅₅₀. Dilutions from the 0.1 OD₅₅₀ sample were made to obtain 0.01, 0.02, and 0.06 OD₅₅₀ samples. Before the bacteria development assays, cells were harvested by spinning them at 8000 rpm for 5 min. The resulting pellet was washed with a tris phosphate magnesium (TPM) medium solution [10 mM tris-HCl (pH 7.6), 1 mM K₂HPO₄, and 8 mM MgSO₄] and resuspended in 1/10 of the original volume. Fifteen microliters was spotted onto the heterogeneous substrate. After the spots dried, the plates were incubated at 32°C for 96 hours. The 0.01 OD₅₅₀ and 0.02 OD₅₅₀ samples were conducted five times, while 0.06 OD₅₅₀ to 0.7 OD₅₅₀ samples were conducted in triplicate for statistical support.

Heterogeneous substrates were fabricated, randomly distributing 10-μm-diameter silica particles onto the flat agar TPM substrate. To this end, TPM agar plates were first prepared by filling each with 30 ml of TPM/agar media (1.5% agar concentration) and storing them overnight at 32°C before use. Then, ~7.9 mg of dry silica particles was resuspended in 1 ml of deionized water. Dilutions of 2:3, 1:3, 1:10, 1:20, and 1:100 were obtained from this concentrated particle stock, and 40 μl of each was spotted onto the TPM agar plates.

**Macroscopic experimental measurement and data analysis**

To study the aggregation at the population level, micrographs of the resulting fruiting bodies were taken at 370.8 pixels/mm using a Leica M50 stereomicroscope with an Achro 0.63 objective lens and a Canon EOS Rebel T3i camera. To avoid border effects, the fruiting bodies developed at the edge of the population were not considered. For image processing, micrographs were binarized into black/white images and phenotypic traits were measured using FIJI (ImageJ) software version 2.0.0 (60). The changes in development due to the substrate heterogeneity and the cellular density were quantified and plotted using heatmaps for each trait (Fig. 2, B to E).

**Microscopic local observation**

To examine the cell-cell and the cell-particle local interactions, a sample at 0.1 OD₅₅₀ cellular density and 24% particle density was observed using an optic microscope with an Olympus UPlanSApO 20× lens and recorded with a Basler acA 1600-20uc camera for 16 hours at 23°C. To prepare the sample, 40 μl of TPM/agar media (1.5% agar concentration) were deposited over a coverslip and, 10 min later, 3 μl of the cellular culture was spotted onto the TPM substrate. Ten minutes later, the sample was sealed using a coverslip and vacuum grease, and it was then observed for 2 hours at 23°C at a sampling rate of 0.5 frames s⁻¹.
Tracking analysis of individual cells

For the tracking analysis shown in Fig. 3, a semi-automatic tracking was performed following morphological properties of the cells with a homemade routine in MATLAB. For each individual trajectory, the distance, speed, and spatial coordinates were obtained.

Computational model

To explore *M. xanthus* early aggregation in different topographies, we built a Glazier-Graner-Hogeweg model (also known as the Potts model) using the CompuCell3D 3.7.8 platform (46). Our model is based on *M. xanthus* experimental data and previously published models for this organism (28, 31, 47, 61–63). The cellular properties and their interactions were implemented by a Hamiltonian equation whose minimization, through the Monte Carlo algorithm, drives the dynamics of the system (Table 1) (46)

\[ H_{GGH} = E_{\text{stretch}} + E_{\text{bending}} + E_{\text{slime}} + E_{\text{particle}} + E_{\text{adhesion}} \] (1)

The *M. xanthus* rod shape was represented by 12 continuous segments (47) that hold their shape through the stretch energy

\[ E_{\text{stretch}} = \left( \lambda_l \sum_{i} (L_{i} - L_{0})^2 \right) + \left( \sum_{i} \lambda_{cV} (V_{cV} - V_{c_{\text{ref}}})^2 \right) \] (2)

where the first term refers to the elongation *L* and the second term refers to the volume *V* (area in our model). \( L_{0} \) is the current length of a cell \( \sigma \), \( L_{0} \) is the reference length, and \( \lambda_{l} \) is the elongation constriction value. The same notation applies to the second term of the equation. Cell breakdown was avoided by introducing a connectivity penalty value \( C \) (64). The simulated cells were randomly added on the substrate.

The cell semiflexible movement was described by the bending energy avoiding abnormal contractions by

\[ E_{\text{bending}} = \kappa_B \sum_{x_i} (1/R_{\text{curve}}(S_{\sigma(x_i)}, S_{\sigma(x_{i+1})}, S_{\sigma(x_{i+2})})^2 \] (3)

\[ R_{\text{curve}}(S_{\sigma(x_i)}, S_{\sigma(x_{i+1})}, S_{\sigma(x_{i+2})}) = \frac{|S_{\sigma(x_{i+2})} - S_{\sigma(x_{i+1})}|}{2 \sin \theta(S_{\sigma(x_{i+1})} - S_{\sigma(x_{i+2})}, S_{\sigma(x_{i+2})})} \] (4)

where \( \kappa_B \) is the curvature constriction value and \( R_{\text{curve}} \) is the curvature radius of three consecutive cell segments \( x_{i+1}, x_{i+2} \) of a cell \( \sigma \) (62).

The secretion of slime trails and their tracking by other cells was implemented using

\[ E_{\text{slime}} = -\lambda_S (c_j - c_i) \] (5)

\[ \frac{\partial c}{\partial t} = -\kappa_S c + F_S \] (6)

where \( c_i \) and \( c_j \) are the slime concentration at sites \( i \) and \( j \), \( \lambda_S \) is the slime secretion rate, \( F_S \) is the uniform slime secretion, and \( \kappa_S \) is the logarithmic decay rate. Thus, the cells tended to move from lower to higher slime concentration sites.

To simulate the heterogeneous media, attractive particles were modeled through

\[ E_{\text{particle}} = -\lambda_P (c_j - c_i) \] (7)

\[ \frac{\partial c}{\partial t} = \nabla^2 c + (-k_P c) + F_P \] (8)

where \( c_i \) and \( c_j \) are the attraction values at sites \( i \) and \( j \), \( \lambda_P \) is the particle attraction coefficient, \( F_P \) is the uniform renewal of the particle attractiveness, and \( k_P \) is the attractive gradient of particles. Ten-micrometer-diameter particles were randomly added; the volume and position of the particles were static throughout the simulation (“freeze” function in CompuCell3D).

The adhesion of the model elements (cell, particle, and substrate) was defined by

\[ E_{\text{adhesion}} = \sum_{i,j,\text{neighbors}} f(t_{\text{pair}}(i,j)) \left( 1 - \delta_{\sigma(i)}(\sigma(j)) \right) \] (9)

\( f \) represents the adhesion value between two contiguous pixels \( i \), \( j \) that belong to a particular item \( \sigma \), \( \sigma' \), and an element \( \tau \). The second term prevents considering pixels that are not at the boundary of each item with a Dirac function \( \delta \) (46).

Simulation execution and analysis

The model was executed for a cellular density of 0.03 cells/\( \mu \text{m}^2 \) and five particle densities (0, 1, 5, 15, and 25% packing fraction) simulating two scenarios, with and without cell-particle attraction. In each case, the total number of aggregated cells within and outside the particle attraction domain was obtained for all conditions. This was done for 10 replicates at 5000 Monte Carlo steps (Fig. 4). We considered as biologically relevant aggregates those groups of cells formed by at least three cells. This parameter was chosen examining the frequency of the number of adhered cells at the beginning and at the end of the simulation without particles. Aggregates of three cells were infrequent at the beginning of the simulation and increased their frequency at the end, suggesting that their presence is not due to chance but to the aggregation process. Because we hypothesized that experimental aggregates within the particle menisci did not contribute to eventual fruiting bodies, we excluded those simulated aggregates. The number of aggregated cells was normalized with respect to the number of aggregated cells in homogeneous condition (Fig. 4A).

Analyses were conducted in R (version 4.0.2) using RStudio (65, 66). The ggplot2 package version 3.0.0 was used for visualization (67).

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/35/eabh2278/DC1

View/request a protocol for this paper from Bio-protocol.

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**Data and materials availability:** All data needed to evaluate the conclusions in the paper and the code generated for computational simulations during the current study are available in the GitHub repository [https://github.com/lapeco1a/Myx_heterogeneous_data_exp.git](https://github.com/lapeco1a/Myx_heterogeneous_data_exp.git) and [https://github.com/lapeco1a/Myx_heterogeneous_media_model/tree/master/Myxoliquid](https://github.com/lapeco1a/Myx_heterogeneous_media_model/tree/master/Myxoliquid), respectively.

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