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Article

Competing Mechanisms in Bacterial Invasion of Human Colon Mucus Probed with Agent-based Modeling

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ABSTRACT The gastrointestinal tract is inhabited by a vast community of microorganisms, termed the gut microbiota. Large colonies can pose a health threat but the gastrointestinal mucus system protects epithelial cells from microbiota invasion. The human colon features a bilayer of mucus lining. Due to imbalances in intestinal homeostasis, bacteria may successfully penetrate the inner mucus layer which can lead to severe gut diseases. However, it is hard to tease apart the competing mechanisms that lead to this penetration. To probe the conditions that permit bacteria penetration into the inner mucus layer, we develop an agent-based model consisting of bacteria and an inner mucus layer subject to a constant flux of nutrient fields feeding the bacteria. We find that there are three important variables that determine bacterial invasion: the bacterial reproduction rate, the contact energy between bacteria and mucus, and the rate of bacteria degrading the mucus. Under healthy conditions, all bacteria are naturally eliminated by the constant removal of mucus. In diseased states, imbalances between the rates of bacterial degradation and mucus secretion lead to bacterial invasion at certain junctures. We conduct uncertainty quantification and sensitivity analysis to compare the relative impact between these parameters. The contact energy has the strongest influence on bacterial penetration which, in combination with bacterial degradation rate and growth rate, greatly accelerates bacterial invasion of the human gut mucus lining. Our findings will serve as predictive indicators for the etiology of intestinal diseases and highlight important considerations when developing gut therapeutics.

SIGNIFICANCE Our study sheds light on the critical interplay between gut microbiota and the protective mucus barrier in the human colon. By applying agent-based modeling using CompuCell3D, we uncover the pivotal factors driving bacterial invasion into the inner mucus layer, elucidating the mechanisms underlying gut diseases. Our findings not only provide insights into the pathogenesis of intestinal disorders, but also offer predictive ideas for gut therapeutics.

1 INTRODUCTION

The human gut microbiota is composed of trillions of bacteria and other microorganisms that reside in the human digestive tract. The microbiota plays a critical role in promoting homeostatic functions, such as aiding the digestion of complex carbohydrates, stimulating the production of antibodies, and synthesizing various nutrients that the human host requires (1, 2). In return, the host provides sources of energy for microbial growth and habitats to reside in. The colon is one part of the human gut system covered by two layers of mucus that protects epithelial cells from invasion by disease-causing bacteria. The outer layer acts as a residence for bacteria and dietary materials while the inner layer is composed of a denser structure that is mostly free of bacteria (3, 4). The mucus layer also separates the gut contents, including food debris and gut microbiota, from the epithelial cells.

Goblet cells continuously produce and secrete gel-forming mucins to replenish the mucus layer, gradually renewing the mucus system to avoid bacterial aggregation and proliferation (5). Dysfunctions of the mucus layer allow the microbiota to become potentially harmful when they grow in an unregulated manner. Pathogenic biofilms can form on the surface of the intestinal lining and lead to the host's inflammatory response, resulting in severe gastrointestinal diseases, such as inflammatory bowel disease and colon cancer (6). Hence, understanding the mechanisms of bacteria aggregation and penetration in both healthy and diseased guts is very important to attain a clearer view of the causes of these diseases and improve treatment efficacy.

The study of polymicrobial dynamics helped unravel some of the complexity of polymicrobial interactions in the gut. The development of advanced sequencing and imaging techniques

has enabled studies of gut microbial community composition as well as spatial organization. Lin et al. constructed derivatized antibiotic staining probes targeting bacterial surfaces and explored metabolic labeling for imaging gut bacteria on various scales (7). Shi et al. developed high-phylogenetic-resolution microbiome mapping by fluorescence in situ hybridization (HiPR-FISH), a versatile technology that employs binary encoding, spectral imaging and decoding based on machine learning to reveal changes in the intricate spatial structures within the mouse gut microbiome when treated with antibiotics and examined longitudinal stability of spatial architectures in the human oral plaque microbiome (8). These studies leveraged current understanding of microbe-microbe interaction, but they are still limited in exploring how microbe-mucus interactions influence disease.

Although bacteria interact with multiple environmental factors, mucus, a viscous and slimy substance, is a key component that prevents pathogenic biofilm formation and bacterial invasion by creating a physical barrier between the gut and the bacteria that inhabit it (9). Several studies used in-vitro or computational models to examine bacteria-mucus interaction. Kim et al. used a gut-on-a-chip microdevice to simulate the gut environment, including the presence of mucus, epithelial cells, and gut microbiota. They developed a microengineered model of human intestinal inflammation and bacterial overgrowth, and analyzed contributions of the microbiome to intestinal diseases and their mechanisms (10). To model the viscoelastic properties of mucus, Sardelli et al. engineered an in-vitro mucus model (I-Bac3Gel) that was suitable for dynamic bacterial culture (11). Wheeler et al. tested the behavior of bacterium *Pseudomonas aeruginosa* in a 3D laboratory model of mucus. They discovered that glycan molecules can prevent bacteria from connecting with each other and forming harmful biofilms (12). Xu et al. used computational models to simulate bacterial penetration of mucus layer in gastrointestinal. They tested the influence of two common antibodies at mucosal surfaces (secretory IgA and IgG) on the penetrability of mucus layer (13).

While there is significant progress in developing human gut models, due to the complexity and dynamic evolution of mucus and microbiota, there is still a lack of robust biological models for testing the conditions that prompt bacteria to invade the inner mucus layer. To probe these conditions, we develop an agent-based computational model with the aid of the CompuCell3D software to simulate the behavior of bacteria in the mucosal environment. Previous research used Compucell3D to simulate the growth patterns of biofilm, demonstrating the utility of computational models in simulating the dynamics of gut microbiota (14). In our model, we simulate the growth of both bacteria and mucus, and by modifying different input parameters, we mimic the different gut environments and unveil the tipping point between healthy and pathogenic bacteria.

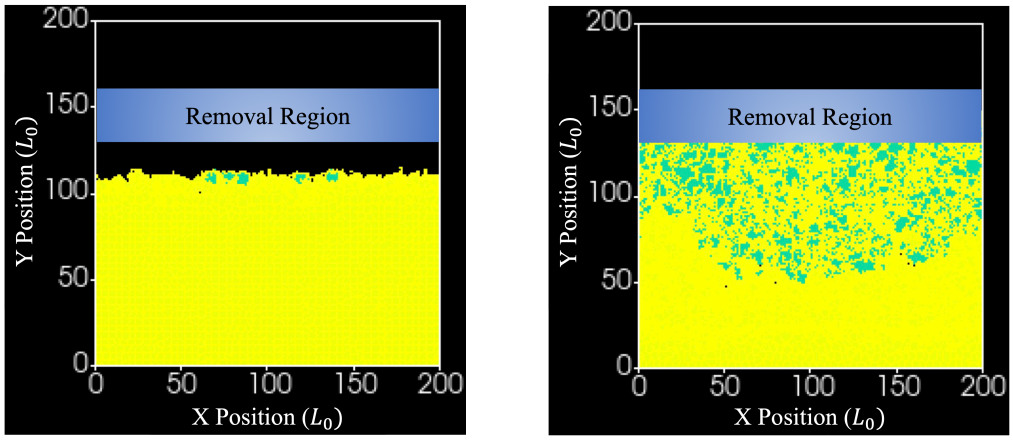
2 METHODS

2.1 Agent-based modeling

Systems biology involve the use of mathematical modeling and computational simulations to predict the behavior of biological systems and potentially use these behaviors for purposes of biological design (15). There several major agent-based frameworks for modeling and simulating cell behavior and dynamics. NetLogo is user friendly, but less suitable for complex biological simulations due to existing constraints in model customizations. NetLogo lacks features for bacterial modeling, such as specific cell-cell interactions (16, 17). Another platform, CellDesigner, is primarily focused on graphical modeling of gene-regulatory and biochemical networks, and it does not include a simulation engine itself or database integration module, which is not well-suited for simulating bacterial dynamics (18). Similar to CellDesigner, BioNetGen is a powerful tool in complex biochemical networks such as enzymatic activities and drug deliveries, but it is not suitable for population-level dynamics (19). RePast offers advanced agent-based modeling capabilities, but the coding process is much more complicated as it has fewer limitations. It works primarily for social and economic systems, so it does not include modules specifically for bacterial modeling such as cell growth, metabolism, or interactions (20). In contrast to these platforms, CompuCell3D has the right set of modeling tools for investigating bacterial dynamics.

CompuCell3D (CC3D) provides a platform for bacterial simulations using the Glazier-Graner-Hogeweg (GGH) model, a cell-oriented framework designed to simulate growth and pattern formation due to biological cells' behaviors (14). CC3D can be used simulate and predict gut bacteria behavior, thus addressing experimental difficulties when dealing with actual bacteria and mucus in the human gut. In our models, both bacteria and inner layer mucus are represented as individual agents. Since the mucus is usually cross-linked, the average length between nodes is modelled as the cell size and the voids between cells represent the pores in inner layer mucus. The outer layer is simply modeled as the media surrounding the bacteria and the inner layer mucus.

To minimize the computational resource needed to simulate the large-scale dynamic behavior of cellular systems, 3D models in CompuCell3D are usually reduced to their 2D analogs (21). Figure 1(a) depicts the construction of the model in CC3D in length unit L_0, which equals to 0.125 μm for each L_0. The unit settings will be discussed in detail in next section. To initiate the simulations, a few bacterial cells are added randomly on the interface between the mucus inner layer and outer layer. The bacteria then grow and reproduce together with mucus. Following the mucus renewal process, which is important for its protective function, the outer layer of mucus with microbiota is easily removed (i.e., sloughed off) due to the movement of stools or flushed away by liquid in the gut (22). To model this dynamic removal of mucus in the human gut, cells are removed in a pre-specified region



(a) (b)

Figure 1: The schematic of CC3D model and simulation setup. (a) Initial state of the model: the inner mucus layer has a thickness of 100 L_0, with a few bacteria put randomly on the top and a removal region in the range of 130 - 160 L_0. (b) After 100 T_0, bacteria grow, divide, and finally penetrate the mucus layer, and cells within the removal region are sloughed off.

at the outer-inner mucus interface, in the range of 130 - 160 L_0. Since this layer is constantly removed from the inner mucus layer under normal biological conditions, if bacteria can penetrate or proliferate in this portion of the mucus layer, they may then go on to penetrate the whole inner mucus layer or at least pose a threat to the epithelial cells underneath. There are two types of nutrient sources for bacteria in the human gut: the mucus and the food debris. Some bacteria can degrade the mucus structure by secreting mucolytic enzymes, which ease bacterial invasion of the mucus (23). Hence we model the bacteria as absorbing nutrients by digesting mucus and food debris to grow and divide together with mucus.

Disruptions in mucosal homeostasis increase the number of mucus-consuming bacteria and allow them to penetrate the mucus layer more easily as mucus becomes less sticky (24). Correspondingly, we would like to explore the effects of three variables in the simulation: bacterial growth rate, contact energy between bacteria and mucus, and the rate of mucus decomposition by bacteria. We explore the circumstances where bacteria can successfully penetrate the mucus layer. If the bacteria grow and divide more rapidly compared with mucus, they may finally fully penetrate the mucus layer, as seen in Figure 1(b).

2.2 Bacteria and mucus cells construction

Each individual bacterium is modeled as a single cell in CC3D. Mucus is a slippery aqueous secretion produced by and covering mucous membranes (25). It is gel-like, mainly consisting of MUC2 mucin (highly organized glycoprotein network) produced by goblet cells (26). As shown in Figure 2, mucin forms a polymeric net-like mucus layer that is attached to the epithelial cells. Mucus is continuously secreted from underneath to refresh the mucus layer (3). The average mucus cell size can be set as the average length between nodes of the crosslinked mucins, with the voids between cells viewed as pores.

In CC3D, we set the unit length L_0 to be 0.125 μm and a unit time step t_0 is 1 sec. According to references (9, 27, 28), all the values of parameters are then converted into the reference units in CC3D:

$$\text{Bacteria Size} \sim 1\mu m = 8L_0$$

$$\text{Mucus Size} \sim 0.5\mu\text{m} = 4L_0$$

$$\text{Mucus Thickness} = 100L_0 = 12.5\mu\text{m}$$

$$\sim \frac{1}{8}\text{real thickness of mucus inner layer}$$

Due to the size limitations of our model to balance the computational intensity, we model just the top 1/8th surface of the actual thickness of the mucus inner layer (29). Even so, we anticipate that if bacteria can penetrate the top region of the inner layer, bacteria are very likely to propagate through the whole layer eventually or at least survive in the inner mucus layer, thus causing diseases. From our simulations, we find that there is no condition allowing for the reversal of bacterial propagation after penetration under the same initial condition, even after very long simulation times.

2.3 Contact Energy

The intercellular contact energy is the adhesion energy between neighboring cells to determine how strongly cells stick to each other. The energy is based on a matrix specifying all contact interactions between cell types:

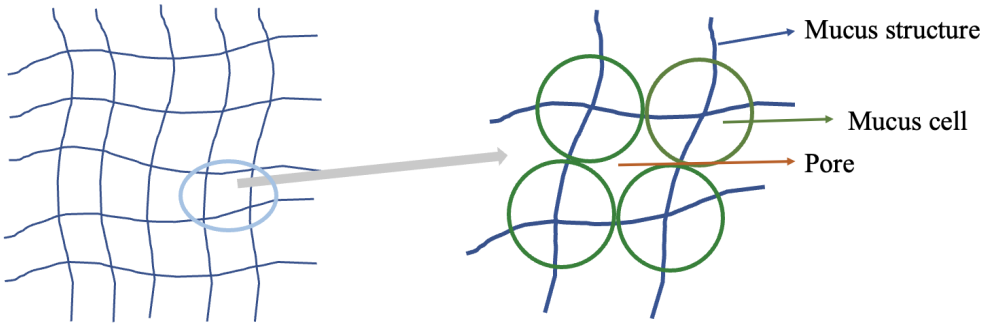


Figure 2: Microstructure of mucus layer composing of grid-like MUC2. The circles at nodes can be viewed as mucus cells, and the vacant space among them are pores.

$$\boldsymbol{J} = \begin{bmatrix} J_{A,A} & J_{A,B} & J_{A,C} \\ & J_{B,B} & J_{B,C} \\ & & J_{C,C} \end{bmatrix}$$

A,B and C are the cell types. When the surface area of a cell changes, the contact energy of the cell is recalculated based on the difference in surface areas compared to other cell types (30):

$$E_{\text{Contact}} = \sum_{i}^{\text{all pixels}} \sum_{j}^{i} J\left[\tau\left(\sigma_i\right), \tau\left(\sigma_j\right)\right]$$

Subscripts i and j denote two neighboring lattice sites, σ denotes cell IDs, and τ denotes cell types. The first summation in the equation is for all pixels that make up a particular cell, and the second summation is over the pixels around the pixel i. Large contact energies between cells make it harder for cells to stick to each other, while low energies promote contact. With lower contact energies between bacteria and mucus, the mucus becomes less repulsive to bacteria, resulting in easier bacterial invasion.

2.4 Cell Growth

We use reproduction rate to represent bacterial growth, which is the doubling time of bacterial population. Initially, some bacteria cells are randomly placed at the surface of the inner mucus layer, and they grow and divide together following the reproduction rate (31, 32):

$$\text{Bacteria Reproduction Rate} \sim 20min$$

$$\text{Mucus Growth Rate} \sim 240\frac{\mu m}{h} = \frac{1}{15}\frac{\mu m}{s} = \frac{8}{15}L_0/t_0$$

Nutrient absorption is key to cell growth. Under healthy physiological states, mucus grows at a constant rate. Bacteria mostly consume the food debris for nutrient, and only a small amount of them can degrade the mucus structure. However, physiological changes, such as those arising from dietary compositions lacking in fiber, can affect bacterial penetrability and the growth rate of the inner layer of mucus. Approximately, the value of normalized penetrability is doubled and mucus growth rate decreases to one-fifth compared with mucus production in healthy states (33). Moreover, there is an increase in the number of bacteria from species that thrive on mucus consumption. Due to these myriad reasons, bacteria can penetrate the inner mucus layer more easily, potentially leading to diseases of the gut.

2.5 Uncertainty quantification and sensitivity analysis

Uncertainty quantification (UQ) is a technique used to characterize and estimate the uncertainty in model predictions. UQ helps to identify the sources of uncertainty, which can arise from various factors such as measurement errors, model approximations, or variability in input parameters. While errors are unavoidable and inevitable, they can always be reduced through identifying the sources and quantifying the nature of these errors (34). Sensitivity analysis is a technique of UQ that focus on how uncertainties in the output of a mathematical model or system can be divided and allocated to different sources of uncertainty in its inputs (35). In combination with a statistical tool known as the Monte Carlo method, which is often used to evaluate the uncertainty of measurements, sensitivity analysis can identify which input parameters in the given range have the most significant impact on the output following the formula below (36):

$$\text{Sensitivity} = \frac{\text{Percentage change in output}}{\text{Percentage change of input}}$$

Through sensitivity analysis, we can understand the relations between the input parameters and the output values. Since the absolute values of the variables cannot be easily determined, we select a reasonable range for each parameter and vary the values within this range after each test following a uniform dis-

tribution. We then apply UQ and sensitivity analysis to test the correlation between the variables and bacterial penetration.

2.6 CC3D System setup

There are several assumptions in our agent-based models. We assume that bacteria cells have uniform sizes of 1 μm and that there are two nutrient sources: food debris and mucus. Mucus is modeled as cells with dimensions of 0.5 μm. We model just the top 1/8th surface of the mucus inner layer as we find that this is more than sufficient to identify tipping points that lead to bacterial penetration during our simulations. The removal region above the mucus inner layer models the mucus outer layer that is being sloughed off repeatedly under normal biological conditions. With this model, we then proceed to explore the conditions needed for bacterial invasion into the inner mucus layer.

To minimize computational resources and simulation time, 3D cubic boxes in CC3D are usually simplified to 2D slabs. This model reduction significantly reduces the model's complexity (21). Our simulation box is set to be 200×200, which is periodic in the x-direction and has a fixed boundary in the y-direction. We enlarge the neighbor order to be four to reduce lattice anisotropy (37). We select rectangular slabs as the initial cell layout to create layer-like cell clusters. We create two types of cells: bacteria and mucus. Two chemical fields (mucus and food) are also added to represent the two nutrient sources available to the bacteria. Lastly, to capture the cell properties and behaviors, we model the contact energy between cells, and allow both bacteria and mucus cells to grow and divide at specified rates. In addition, mucus cells can secrete nutrients for bacteria to absorb, hence modeling the degradation of the mucus by bacterial mucolytic enzymes.

In the initial state, we create several bacteria in random positions on the mucus layer. We set the initial cell size to be 1 μm and allow the cells to grow and divide at our specified reproduction rate. Mucus cells grow with a constant growth rate and since the bacteria uptake nutrients for food, the growth of bacteria is directly influenced by both the food and mucus chemical fields. Bacteria will degrade mucus cells upon contact following our specified degradation rate. Bacteria and mucus within the removal area are constantly removed to model the mucus clearance mechanism in human gut. Data containing bacterial positions and field values are stored for further analyses.

3 RESULTS AND DISCUSSION

3.1 Bacterial Elimination Under Healthy Mucus Layer

Under healthy states, the inner mucus layer has a good barrier function and is usually free of bacteria. The gel-like mucus has high viscosity, making it hard for bacteria to go through. The gut microbiota with the mucus system correspondingly has sufficient turnover to avoid bacterial penetration and pathogen growth. The growth rate of mucus is approximately 240 $\mu m/h$, and the reproduction rate of bacteria is around 20 min. Bacteria mostly consume fiber from food debris as their primary energy source and they rarely degrade mucus under normal healthy circumstances. To model this behavior, we vary the three parameters of bacterial reproduction rate, contact energy between bacteria and mucus, and degradation rate of bacteria to mucus, within reasonable physiological ranges. Intestinal bacteria have two types of nutrient sources, which means the bacterial growth is influenced by the availability of food debris and mucus.

We find that under healthy conditions, all bacteria will eventually be eliminated from the inner mucus layer within two minutes. To determine how these parameters affect bacterial elimination time, we repeat the simulations five times for each case and average the elimination time to quantify the standard deviation shown in Figure 3. The default values of the parameters are:

$$\left.\begin{array}{l} GF_{\text{bac-food}} = 0.0008 \\ GF_{\text{bac-muc}} = 0.0002 \end{array}\right\} \text{Reproduction Rate } \sim 20 \text{ min}$$

$$\text{Contact Energy }_{\text{bac-muc}} = 6J/pix$$

$$\text{Decomposing Rate } = 4\%$$

GF is the growth factor defined in CC3D. As bacterial growth rate increases, the time taken to fully shed bacteria fluctuates. This fluctuation indicates that there is no obvious influence of bacterial reproduction rate to the elimination time. In healthy physiological states, we expect that the inner mucus layer grows rapidly with sufficient time to refresh and replenish itself. Changes in bacterial reproduction rate, relative to the mucus growth rate, is not sufficient for overwhelming the shedding of the inner mucus layer. However, for the other two parameters of degradation rate and contact energy, there are obvious changes in the elimination time. From Figure 3, increasing bacterial degradation rates will result in longer persistence time of the bacteria, leading to linearly increasing elimination times. In comparison, increased contact energy leads to a logarithmic decrease in the elimination times. Overall, in these two cases, although all bacteria are removed eventually, they have a stronger tendency to degrade mucus and penetrate into the mucus layer, which allows them to survive longer in mucus before elimination.

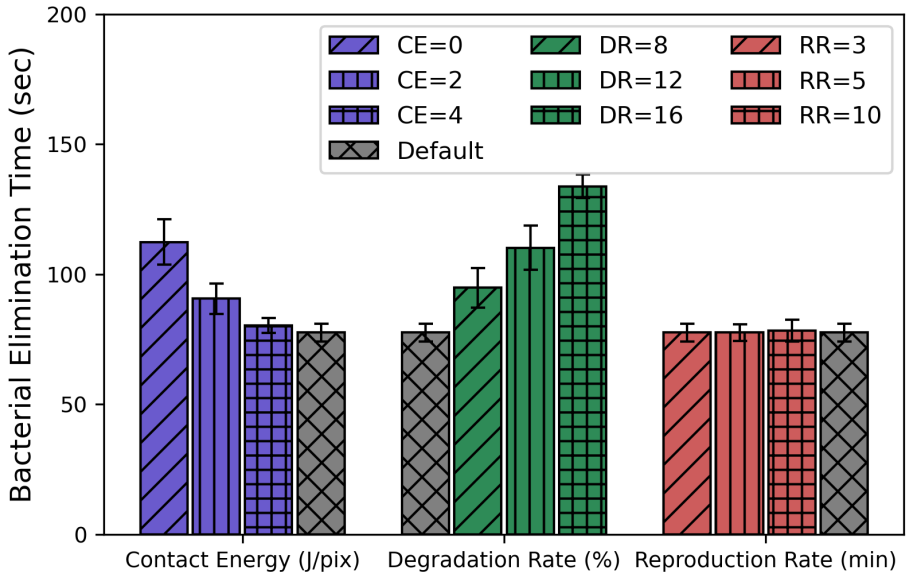


Figure 3: Bacterial elimination time when bacteria are on colon inner mucus layer of normal functionalities with changing parameters of contact energy between bacteria and mucus, bacterial degradation rate and reproduction rate. The default values are $CE = 6, DR = 4, RR = 20$.

3.2 Bacterial Invasion of Dysfunctional Mucus Layer

Under diseased states, we expect that the mucus layer has poorer barrier functions and the mucus turnover is disrupted, thus bacteria may be able to penetrate more easily. Bacteria can then accumulate and aggregate together to form biofilm, which is adverse to the gut system. From previous literature, the mucus growth rate can decrease to about one fifth of the original rate and mucus viscosity will also be greatly reduced (33). There will be more mucus-degrading bacteria and fewer bacteria that degrade dietary fibers, and bacterial reproduction rates are also altered to a large degree (24, 38). In this case, bacteria may fully penetrate the inner mucus layer and be in contact with the epithelium, leading to inflammation and disease development. If all bacteria in contact with the inner mucus layer will degrade mucus, there are three variables that we consider in our simulations: the bacterial reproduction rate, the contact energy between bacteria and mucus, and the rate of mucus degradation by bacteria. Each variable is changed one at a time to discover the boundary conditions needed for bacterial penetration. We average the data from five independent simulations of each case to quantify the standard deviations. Changes in the penetration depth of bacteria with these variables are shown in Figure 4. The default values of the parameters are:

$$GF_{\text{bac-muc}} = 0.001(\text{Reproduction Rate} \sim 20min)$$
$$\text{Contact Energy}_{\text{bac-muc}} = 6J/pix$$
$$\text{Decomposing Rate} = 4\%$$

Overall, the penetration depth grows with decreasing contact energy and bacterial reproduction rate, or when degradation rate between bacteria and mucus increases. However, when we dig into the influence of the three parameters, we notice that the parameters imbued differing characteristics. For the contact energy, there seems to be a large jump in penetration depth as its value reduces from four to two. When the contact energy is larger than 4 J/pix, bacteria show little tendency to penetrate, and will be eliminated eventually. However, the penetration depth increases dramatically from almost 0 μm to 40 μm when contact energy goes beyond the tipping point. The morphology of the bacterial cells may explain this phenomenon. When the contact energy is 4 J/pix, bacterial cells retain approximately oval shapes. However, when the contact energy is reduced to 2 J/pix, the bacterial cells become irregularly shaped, as shown in Figure 5(a). The irregular shapes of the bacterial cells ease the penetration into the mucus, resulting in much larger penetration depths. Also, in this case, bacteria cells have larger surface area, which means they come into contact with more neighboring mucus cells, thus increasing the degradation of the mucus cells.

As the bacterial degradation rate increases, the penetration depth rises linearly at first, and then remains stable subsequently. When the degradation rate increases to a certain value, around 14% in this case, the bacteria already degrades the neighboring mucus completely, as shown in Figure 5(b). Hence, increasing the bacterial degradation rates does not significantly impact bacterial penetration. The degradation rate is a competing factor with the mucus growth rate, helping the bacteria maintain stasis in the mucus layer.

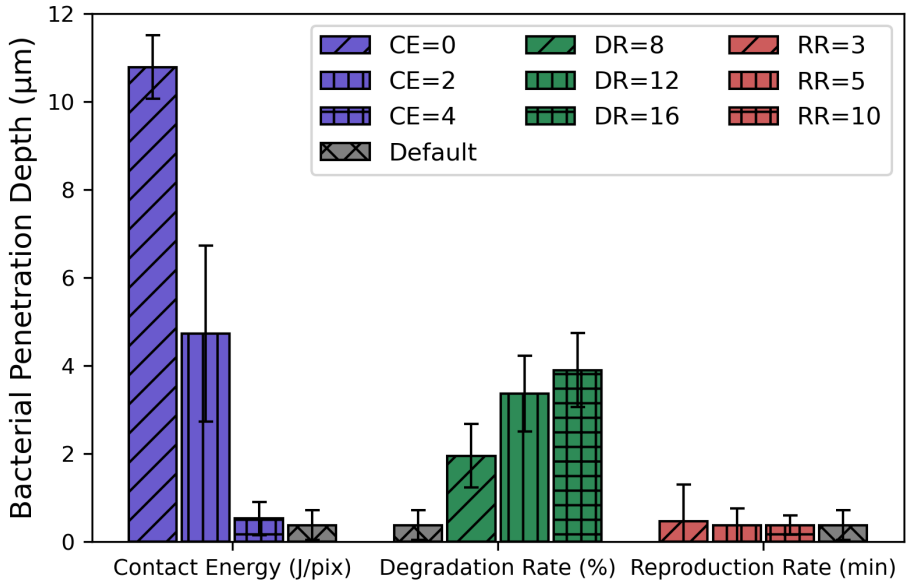
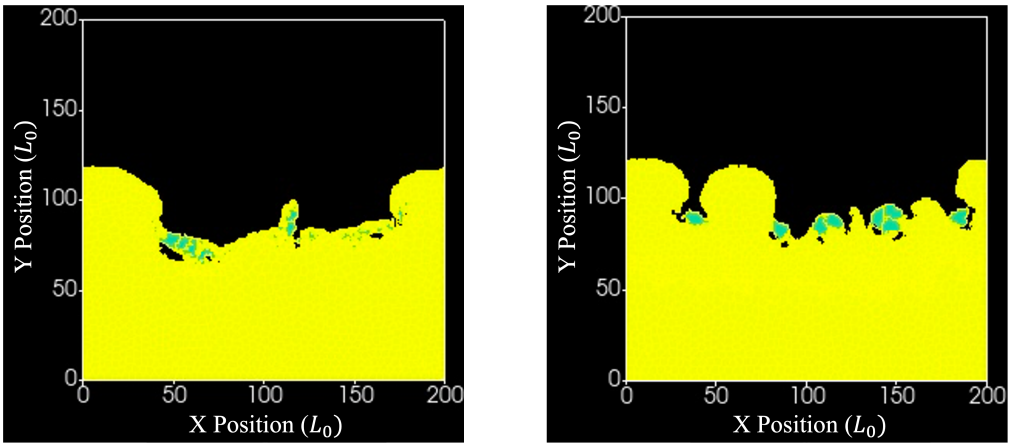


Figure 4: Bacterial penetration depth when bacteria are on dysfunctional colon inner mucus layer with changing parameters of contact energy between bacteria and mucus, bacterial degradation rate and reproduction rate. The default values are $CE = 6, DR = 4, RR = 20$.



(a) (b)

Figure 5: Morphology of bacterial penetration into the inner mucus layer under (a) low contact energy of 2 J/pix (b) high degradation rate of 14 %, with all the other parameters in default values in each case.

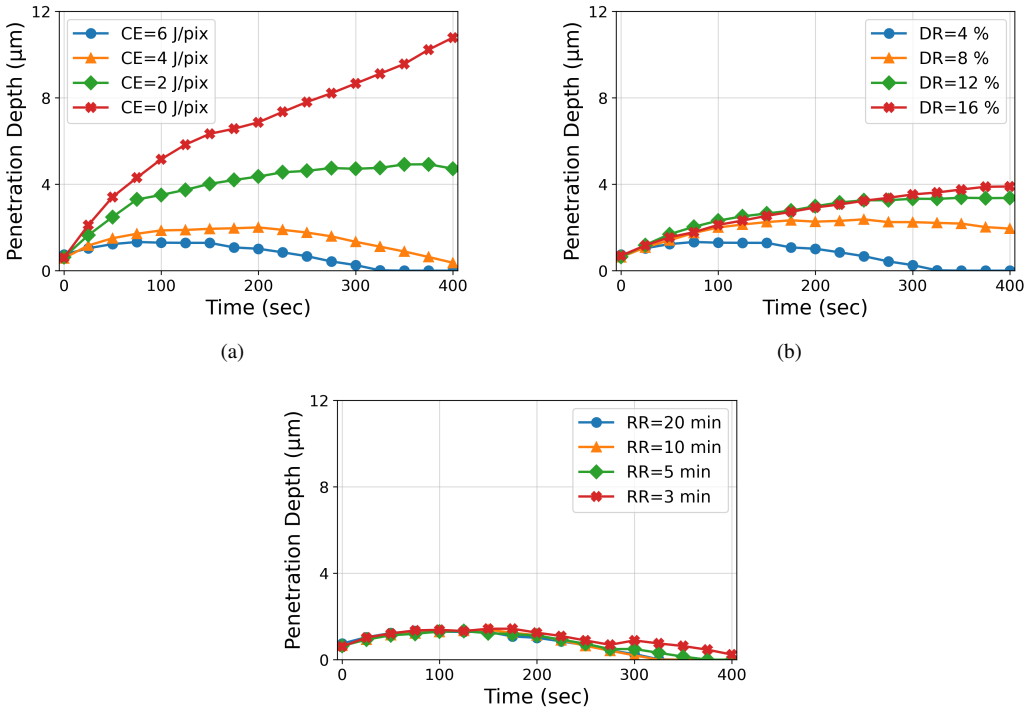


Figure 6: Penetration depth of bacteria on dysfunctional colon inner mucus layer changing with time of different variables (a) contact energy (b) degradation rate (c) bacterial reproduction rate.

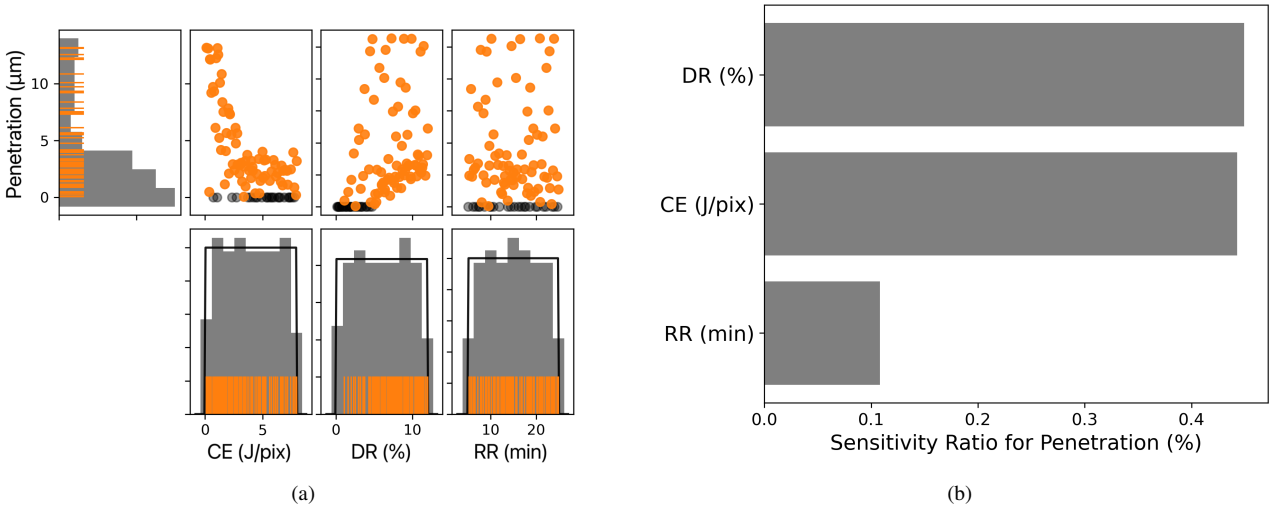


Figure 7: Uncertainty quantification and sensitivity analysis (a) bacterial penetration depth for the changing of parameters: contact energy between bacteria and mucus (CE), degradation rate of bacteria to mucus (DR) and bacterial reproduction rate (RR) (b) sensitivity ratio of the three parameters for penetration.

For bacterial growth rate, we cannot see any obvious relationships from Figure 4. For example, when the bacterial reproduction rate decreases from 20 min to 5 min (i.e., the bacteria is doubling faster), the bacterial population does not have a significant rise before eventual elimination, thereby keeping the penetration depth constant. But if the bacterial reproduction rate keeps decreasing beyond this to an unrealistically fast rate, bacteria will proliferate and grow inside the mucus layer as mucus is the bacteria's only source of nutrients. This will greatly increase their chances of survival. To conclude, bacterial growth rate and degradation rate do not have a strong effect on mucus penetration but can help bacteria survive in the inner mucus layer, while increases in contact energy helps them better penetrate into the barrier.

Figure 6 shows the penetration profile of bacteria for the three parameters. As expected, increasing contact energy and reproduction rate results in the highest rate of bacterial penetration. Bacteria also penetrate the mucus layer at slower speed with increasing degradation rate. Consistent with the results from Figure 4, these two parameters are major factors in bacterial proliferation in the inner mucus layer, while the contact energy stimulates mucosal penetration. To verify the importance of the three parameters to the bacterial penetration depth, we apply uncertainty quantification and sensitivity analysis in our computational model. We conduct 100 tests, each test is run for 400 time steps, with randomized bacterial positions initially. The inputs are the three parameters mentioned above and the output is the depth of bacterial penetration. The three variables are set in reasonable ranges, and change after each single test following the uniform distribution. Figure 7(a) illustrates the distribution of the one hundred samples with the changing of the three variables. The grey dots represent simulations that do not result in bacterial muco-penetration, while the orange points indicate the simulations where bacterial muco-penetration occurred. The contact energy is sampled within the range of 0-8 J/pix, the degradation rate is 0-12% and the bacterial reproduction rate is 5-25 min. Figure 7(b) shows the results of the sensitivity analysis. The contact energy and degradation rate are the dominant factors, which imply that bacteria have a larger probability of muco-penetration if these two values are sufficiently high. In contrast, changes in bacterial growth rate only affects the penetration to a small extent.

4 CONCLUSION

We developed a bacteria-mucus model using the software, CompuCell3D, to simulate the biological process of bacterial penetration into the inner mucus layer in the human gut. With this model, we simulated the bacteria-mucus dynamics under physiologically healthy states to evaluate the impact of three parameters on the bacterial elimination time: bacterial reproduction rate, contact energy between bacteria and mucus, and the rate of bacterial degradation of mucus. Decreasing contact energy or increasing degradation rate extended elimination time, while bacterial reproduction rate had negligible effect. In addition, we conducted simulations to test bacterial penetration depth under physiologically diseased states, which was modeled as a dramatic drop in the mucus growth rate. The results showed that bacterial reproduction rate and degradation rate helped the bacteria survive in the mucus layer, while the contact energy could strongly stimulate bacterial penetration. Lastly, we applied uncertainty quantification and sensitivity analysis, which revealed contact energy and degradation rate as key factors affecting bacterial penetration.

There are two primary limitations in our model. Firstly, CC3D is a cell-based modeling software, so we constructed liquid-like mucus using individual cells, potentially neglecting its inherent fluidic properties. Also, due to constraints of computational resources and simulation time, our model do not consider bacteria variety and nutrition sources, which may oversimplify the microbial diversity and complexity, and neglect some important microbial interactions. To improve these limitations, future research can integrate fluid dynamics models within CC3D to capture the liquid-like properties of mucus. Perhaps with additional computational resources or more efficient algorithms, multiple bacterial types and diverse nutrition sources can be modeled.

For future validations of the relations between the parameters and bacterial penetration, experimental groups could use gut-on-a-chip models to replicate the structure of human gut. Mucus-like hydrogels can help with such validations. Bacteria can then be cultured and transferred into the microchannels of the chip, while introducing the necessary nutrients (39). With the help of such in-vitro models, we can better relate the simulation results with it and eventually verify our hypotheses. Regardless, our findings can still serve as predictive indicators for the etiology of intestinal diseases and our work highlighted important considerations when developing gut therapeutics.

5 AUTHOR CONTRIBUTIONS

Z.Y. and J.Y. conceived the research. Z.Y. designed and conducted the experiment(s). Z.Y. and J.Y. analyzed the results. J.Y. acquired the funding and resources. All authors reviewed the manuscript.

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7 DECLARATION OF INTERESTS

The authors declare no competing interests.

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