

# **A Three-dimensional Computational Model to Investigate the Influence of Spatial Heterogeneity on the Antibiotic Tolerance of Bacterial Biofilms**

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In this work, we investigate the antibiotic susceptibility of bacterial biofilms with the exclusion of genetic factors using a 3D cellular automata model for biofilm growth. Each cell is treated as an individual entity, and is tracked separately as the simulation marches forward in time. The model incorporates processes of nutrient and antibiotic transport, cell growth, division, death, and detachment. In addition, the dynamics and spatial distributions of slow- and fast-growing cells were also monitored to the level of individual cells, and cell clusters. The model predicted the formation of a mosaic-like architecture comprising of metabolically dormant cellular microniches embedded within faster growing cell clusters and EPS. These inactive cells were less susceptible to killing by antibiotic. We propose that (i) the surrounding high-activity cell clusters act as a reaction-diffusion barrier, thereby restricting antibiotic penetration to the low growth-rate clusters, and (ii) low-activity cells consume antibiotics at a diminished rate, thereby reduced efficacy of treatment. The antibiotic response exhibited three distinct phases. In the first phase that lasted  $\approx 4$ h, the total biomass reduced dramatically ( $\approx 40\%$  reduction). In this phase, the biofilm was dominated by subpopulations of slow-growing cells. There was a strong correlation between dead cells and fast-growing populations. The fraction of inactive cells increased with time, reaching a peak after 4h of treatment. The second phase lasted for  $\approx 8$ h, and was characterized by a decrease in the number of slow-growing cells, owing to increased nutrient availability. Complete eradication of the biofilm was observed in the third phase of treatment. Interestingly, we observed a threshold for the antibiotic concentration below which the treated biofilm exhibited increased lifetimes compared to the untreated one. Quorum-sensing (QS) biofilms were more tolerant, because of the production of EPS. This was further validated by the observation that the average diffusion distances were much higher for the QS-positive biofilm. Taken together, our results indicate that spatial heterogeneity of bacterial cells and EPS contribute to the antibiotic tolerance of biofilms in the absence of genetic triggers. A systematic investigation of the structural properties of these microniches of the biofilm, and their response to treatment may shed light on the biophysical mechanisms of antibiotic resistance of bacterial biofilms.