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Micro-3D printing of microscopic bacterial communities

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Bacteria within the human body commonly thrive within structured three-dimensional (3D) communities composed of multiple bacterial species. Organization of individuals and populations within bacterial aggregates is believed to play key roles in mediating community attributes, affecting, for example, the virulence of infections within the cystic fibrosis lung, chronic wounds, and oral cavity. To gain insights into how geometry may influence pathogenicity, we have developed a strategy for 3D printing bacterial communities in which physically distinct but chemically interactive populations of defined size, shape, and density can be organized into essentially any arrangement. In this multiphoton lithographic technique, microscopic containers are formed around selected bacteria suspended in gelatin via focal cross-linking of polypeptide molecules. After excess reagent is removed, trapped bacteria are localized within sealed cavities formed by cross-linked gelatin, a highly porous material that supports rapid growth of fully enclosed cellular populations and readily transmits numerous biologically active species, including polypeptides, antibiotics, and quorum-sensing signals. Using this approach, we show that resistance of one pathogenic species to an antibiotic can enhance the resistance of a second species by virtue of their 3D relationship.