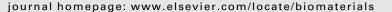
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# Biofabrication of stratified biofilm mimics for observation and control of bacterial signaling

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#### ABSTRACT

Signaling between cells guides biological phenotype. Communications between individual cells, clusters of cells and populations exist in complex networks that, in sum, guide behavior. There are few experimental approaches that enable high content interrogation of individual and multicellular behaviors at length and time scales commensurate with the signal molecules and cells themselves. Here we present "biofabrication" in microfluidics as one approach that enables *in-situ* organization of living cells in microenvironments with spatiotemporal control and programmability. We construct bacterial biofilm mimics that offer detailed understanding and subsequent *control* of population-based quorum sensing (QS) behaviors in a manner decoupled from cell number. Our approach reveals signaling patterns among bacterial cells within a single biofilm as well as behaviors that are coordinated between two communicating biofilms. We envision versatile use of this biofabrication strategy for cell–cell interaction studies and small molecule drug discovery.

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### 1. Introduction

Within bacterial communities, signaling molecules interspersed among individual cells cue population-scale behavior [1–5], including pathogenesis [6–8]. While denoted quorum sensing (QS) because the multicellular phenotype is often mapped onto and coincides with high cell number [9], the molecular basis for multicellularity remains somewhat obscure [10,11]. In particular, biofilms are the aggregate of microorganisms embedded within a self-produced matrix of extracellular polymeric substances (EPS) that account for over 90% of the dry mass [12], with the precise and molecular interactions of the EPS components undefined and poorly understood [13]. Efforts to study biofilm behaviors have been limited in part because the spatial features and cellcommunication phenomena are difficult to track *in situ* and in real time. Alternatively, biofilms are virtually impossible to otherwise assemble in a pre-determined manner that would enable interrogation at cellular length scales.

Here, biofabrication [14-17] uses programmed pH and chemical gradients locally generated within microfluidic networks for the assembly of cell-polysaccharide composites in spatially localized and physically separated hydrogel layers. Depicted in Fig. 1, our approach takes advantage of two of nature's stimuli-responsive polysaccharides (Fig. 1a), chitosan (its film forming properties are pH-dependent) and alginate (its eggbox structure is divalent cation-dependent, e.g., Ca<sup>2+</sup>). While these polysaccharides have been electrodeposited onto microfabricated devices by programmed electrical signals [18–23], we now demonstrate programmability via pH gradients generated in situ using fluidics [24]. In Fig. 1b, a freestanding chitosan membrane is synthesized by generating a stable pH gradient at the interface of two converging and concurrent flows – the first being an acidic chitosan solution, the second a basic buffer solution [24]. The resultant membrane extends from the point of convergence (left) to the point of divergence (right) and it adheres to the channel endpoints. 3D vertically aligned alginate hydrogel films, with or without cells, are then assembled onto one side of the chitosan membrane by allowing calcium ions to diffuse through the chitosan membrane from the





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