Biomaterials 33 (2012) 5136–5143

Contents lists available at SciVerse ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

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

Xiaolong Luo [a], Hsuan-Chen Wu [a,b], Chen-Yu Tsao [b], Yi Cheng [c], Jordan Betz [b,c], Gregory F. Payne [a,b], Gary W. Rubloff [c,d], William E. Bentley [a,b,*]

[a] Institute for Bioscience and Biotechnology Research (IBBR), University of Maryland, College Park, MD 20742, USA
[b] Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA
[c] Institute for Systems Research (ISR), University of Maryland, College Park, MD 20742, USA
[d] Department of Materials Science and Engineering, University of Maryland, College Park, MD 20742, USA

ARTICLE INFO

Article history:
Received 5 January 2012
Accepted 10 March 2012
Available online 14 April 2012

Keywords:
Biofabrication
Biofilm mimics
Cell signaling
Quorum sensing
Chitosan membrane
Alginate hydrogel

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.

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 cell-communication 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^{2+}). 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

* Corresponding author. Fischell Department of Bioengineering, University of Maryland, College Park, MD 20742, USA. Tel.: +1 301 405 4321; fax: +1 301 405 9953.
E-mail address: bentley@umd.edu (W.E. Bentley).

0142-9612/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biomaterials.2012.03.037