HYDRODYNAMIC EFFECTS ON DEVELOPMENT OF MAT-LIKE BIOFILM IN A MICROFLUIDIC ENVIRONMENT

Junghyun Kim[1], Sewoon Han[1], Seok Chung[1*], and Hee-Deung Park[2*]

[1] *School of Mechanical Engineering, Korea University, KOREA,*

[2] *School of Civil, Environmental and Architectural Engineering, Korea University, KOREA*

ABSTRACT

Bacterial cells in hydrodynamic environments tend to form biofilms on surface to protect themselves from harsh external conditions. In microfluidic channel, biofilms grew into multi layered mat like structures. In this work, we revealed the effects of hydrodynamic conditions on development of mat like biofilms. Experimental results showed that shear stress had both positive and negative influences on biofilm developments. This result could be more valuable analysis by dimensional analysis or mathematical approaches.

KEYWORDS

Biofilms, Microfluidics, Shear stress, Mat-like biofilms

INTRODUCTION

In aquatic environment, microorganisms tend to form biofilms on surface to protect themselves from external conditions. The biofilms made by bacterial cells consist of aggregation of cells and extracellular polymeric substances (EPS) that induce improvement of survivability to external hostile environments like high temperature or presence of antibiotic treatments [1]. This characteristic of bacterial biofilms functions as a shield which causes several challenges in clinical applications, such as symptomatic inflammation, antibiotic resistance, recurrence, and the spread of infectious emboli [2-6]. Also, the matrix of biofilms provides more opportunities to cells for proliferation inside the human body [2, 3]. To form these bacterial biofilms, bacterial cells have series of developmental steps [7]. Initial two steps are characterized by the loose adhesion of planktonic cells to a surface and secretion of EPS. Steps three and four accompany the aggregation of bacterial cells and maturation of biofilms. Step five entails diffusion of cells from biofilm matrix and detachment by erosion. At certain condition, biofilm development contain step six, generation of multi-layered mat like biofilms [8, 9]. At all of developmental steps are influenced by physical, chemical, and/or biological environments including shear stress, surface properties, quorum sensing, nutrient concentration, and so on.

Microfluidic approach has been widely used as one of the promising tools for revealing and evaluating these factors above in quantitative way [10-13]. However, compartmentalizing of major determinants (e.g. shear stress) and evaluate their effects still have obscurity. Specially, multi layered mat like biofilms in small scale fluidic environments has not been theoretically accessed because of complex interaction between biofilms and their hydrodynamic conditions. In this work, we revealed the effects of hydrodynamic condition on development of multi layered mat like biofilms through microfluidic approach.

EXPERIMENT

Microfluidic devices were used to observe the interaction between biofilm developments and its hydrodynamic conditions. PDMS and silicon wafer with SU-8 was used to fabricate microfluidic devices that have three different channels. As model bacterium, PA14 sustainably constitutively expressing green fluorescent protein (GFP) was used. As bacterial medium, LB (Luria-Bertani) Broth, Miller (244620, BD, USA) was used. To procure enough and constant cell density, bacterial cells were pre-cultured in shaking incubator at 37℃ and 200 rpm for 24 hours. After that PA14 were suspended in fresh LB medium as OD600=0.1. For inducing bacterial adhesion, the suspension of PA14 were filled into micro-channels and incubated for 2 hours at 37℃. The fresh LB medium was supplied into the microchannel for 48 hours with desired flow rate using a peristaltic pump (74-128-00000, Thermo Fisher Scientific, USA). After bacterial cells formed biofilms in the microchannel, these channels were refreshed by PBS solution to remove suspended cells and unattached biofilms. In these procedures, only difference of hydrodynamic head was used as driving force to minimize shear flow into developed biofilms in the microchannel (**figure 1**). Through a fluorescence microscopy (JuLi, NanoEnTek, Korea), the growth of biofilms was quantified by the change of biofilm coverage in the microfluidic channel.

The biofilms in microfluidic environments with shear flow had a unique characteristic, accumulation into mat-like structures growing toward the center of channel from the corners. These thick biofilms filled from the bottom of channel to the top of that, and remained after PBS washing procedures. In hydrostatic condition (e.g. no flow condition), biofilms in the channel easily washed out by the soft refreshment due to lack of adhesive force. Experimental result showed that development of the mat like biofilms was determined by three major factors, shear stress, nutrient concentration, and aspect ratio of the channel ($r=w/h$) (**figure 2**). Development of biofilms had parabolic relationship with amount of shear stress due to shear stress had either positively and

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negatively effects. Shear stress helped to supply nutrient and diffuse cells, and at the same time induced erosion detachment of biofilm matrix. This result could be more valuable analysis through dimensional analysis or theorizing a mathematical model.

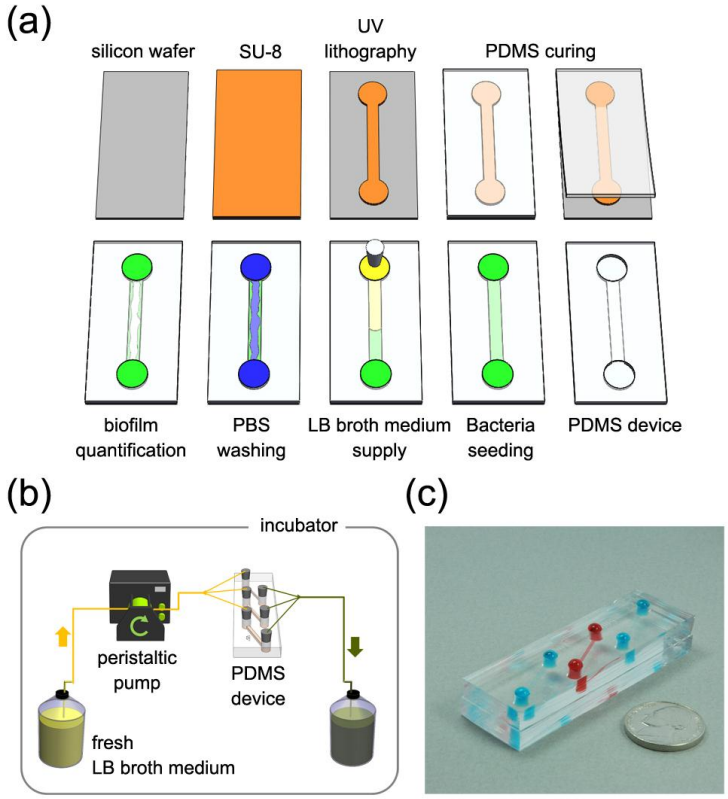

Figure 1. Experimental procedures. (a) Fabrication of microfluidic devices and experimental steps. (b) A schematic diagram of experimental equipment. (c) PDMS microfluidic device.

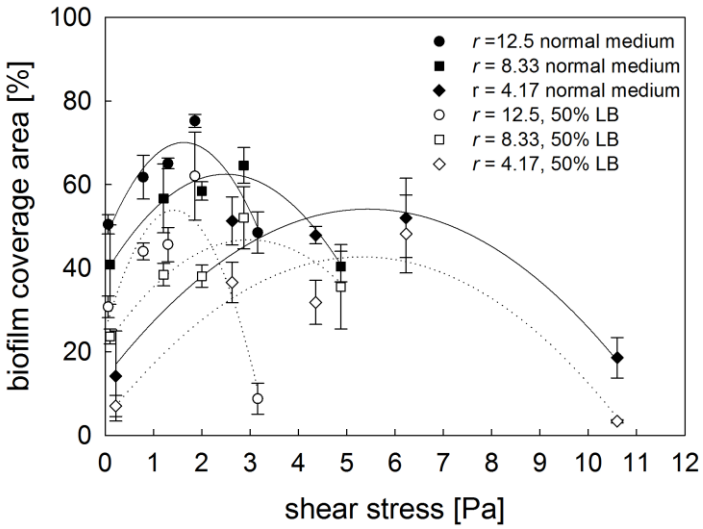

Figure 2. Effects of shear stress on bacterial biofilm development.

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CONTACT
Junghyun Kim +82-2-3290-3761 or coolkj@gmail.com