# Roles of ionic strength and biofilm roughness on adhesion kinetics of *Escherichia coli* onto groundwater biofilm grown on PVC surfaces

**Supplementary Material**

Submitted to

*Water Research*

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**Bacteria Cell Preparation**

For the adhesion experiment, a single colony was picked from a freshly prepared plate and pre-cultured in Luria-Bertani (LB) broth with 50 µg/L carbenicillin at 37 °C overnight with 200 rpm shaking. This preculture was diluted 100 times in fresh LB media containing carbenicillin and incubated for 8 hours until reaching an optical density of 0.8 at 600 nm ($OD_{600}$). Then, 1 mM IPTG was added to the stock to induce fluorescent expression, and the stock was incubated for 2 more hours to get to 1.2 $OD_{600}$. After 10 hours of incubation, cells were harvested by centrifugation at 17000 × g, cleaned by suspending in 10 mM KCl buffered with 1mM $NaHCO_3$, followed by centrifugation. The cleaning and centrifugation steps were repeated twice. Freshly grown *E. coli* were tested with viability tests using Live/Dead BacLight kit (Invitrogen L7012) in experimental ionic strength (IS) KCl solutions. The stained cells were directly counted under an inverted fluorescent microscope (DM15000 M, Leica, Wetzlar, Germany) with the suitable fluorescent filter set (Chroma Technology Corp.). Phase contrast images of *E. coli* cells were compared with fluorescent images of this same sample using a microscope with an oil objective at 63X magnification to ensure that all cells were emitting fluorescence.

**Control experiments for contact angle measurement**

A control experiment of contact angles was done on 24-week old biofilms at different drying times (from 0 to120 min) (Busscher et al., 1984, van der Mei et al., 1998). As shown in supplementary Figure 1S, contact angle was the largest at 0 min and stabilized after 30 min of measurement. At time 0, we suspected that the contact angle was likely measured on a water layer instead of hydrated biofilm. Because the contact angle was consistent for 30 min up to 120 min of drying time, we used a 30 min drying time for all measurements. This protocol is similar to the one used in Park and Abu-Lail (2011). Contact angle using water was attempted on the biofilm; however, the water drop was quickly absorbed by the biofilm to prevent

consistent measurement. Following the work by Park and Abu-Lail (2011), we obtained Atomic Force Microscopy (AFM) images of the well-dispersed *E. coli* deposited on a glass surface to show that the height of *E. coli* cells was 600 nm (Figure 2S in Supplementary Information). High concentration of *E. coli* cells on the filter for the contact angle measurement caused the cells to aggregate and cover the entire surface of the filter (Figure 3S in Supplementary Information). The roughness of this *E. coli* lawn on the filter cannot be measured with AFM and OCT imaging. However, this roughness should be smaller than the width of *E. coli* cells, i.e., 600nm. Thus, the roughness of the *E. coli* lawn is much smaller than the size of the drop of contact angle probe liquid and should not interfere with contact angle measurements.

**Hamaker constant determination**

Contact angle of diiodomethane on *E. coli* lawn, biofilm and PVC surfaces was first measured and used to calculate the Lifshitz-van der Waals ($\gamma^{LW}$) component of surface energy using equation 1.

Equation 1: $\gamma_s^{LW} = \gamma_L \left( \frac{(1+\cos\theta)^2}{4} \right)$; $\gamma_s^{LW}$ is the surface tension of the surface, $\gamma_L$ is the surface tension of diiodomethane (57 mJ/m$^2$), $\theta$ is the contact angle of diiodomethane on *E. coli* lawn, biofilm, and PVC surfaces.

With the surface tension of each surface we could further calculate Gibb's free energy of *E. coli*-water-biofilm with equation 2.

Equation 2: $\Delta G_{y0}^{LW} = 2\left(\gamma_{water}^{LW} - \gamma_{biofilms}^{LW}\right)\left(\gamma_{bacteria}^{LW} - \gamma_{water}^{LW}\right)$; $\gamma_{water}^{LW}$ is the surface tension of water, $\gamma_{biofilms}^{LW}$ is surface tension of biofilms, $\gamma_{E.coli}^{LW}$ is surface tension of *E. coli*.

With Gibb's free energy of the system, Hamaker's constant could be calculated using equation 3.

Equation 3: $A = -12\pi y_0^2 \Delta G_{y0}^{LW}$; $y_0^2$ is the minimum equilibrium distance (van Oss, 1993).

The final result was the Hamaker's constant (A) of the system *E. coli*-water-biofilm.

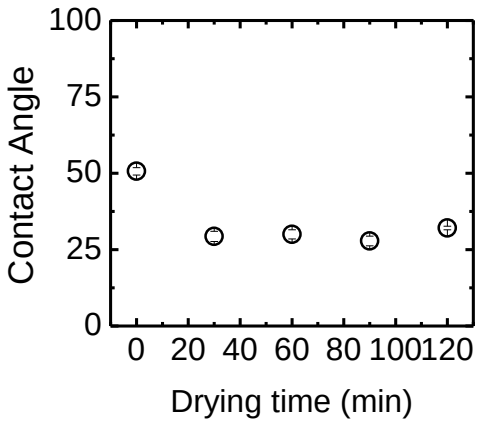


**Figure 1S.** Contact angle of diiodomethane on 24-week old biofilm as a function of drying time.

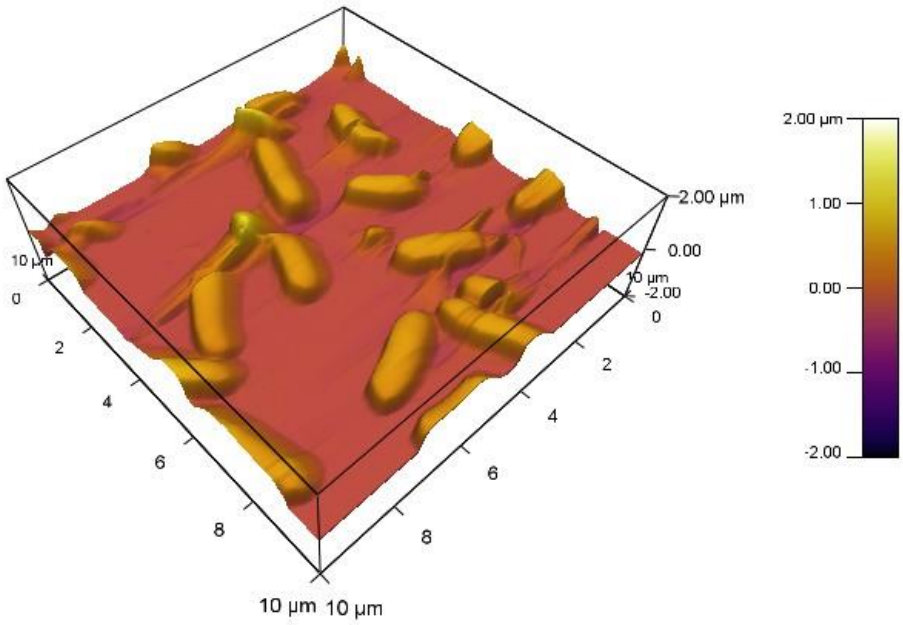


**Figure 2S.** AFM images for *E. coli* cells on a glass slide. All image analysis was done in liquid environment. All measurements were accomplished in a contact mode using a silicon cantilever (Budget Sensors SiNi-30). Images were taken with a small scanning area (2.5×2.5 $\mu m^2$ or 5×5 $\mu m^2$) at a scan rate of 10 Hz and with 256 points per scan line.

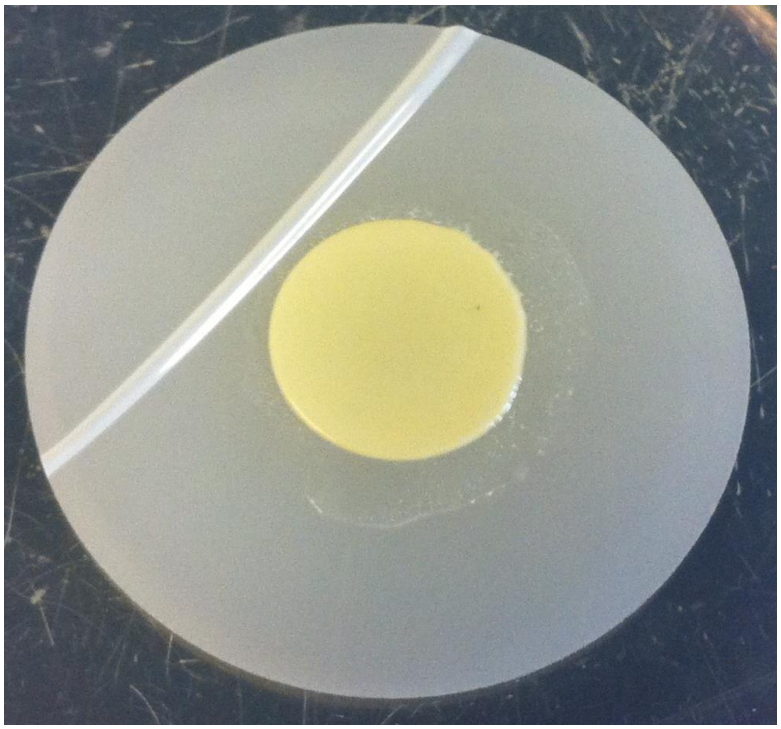

**Figure 3S.** Image of *E. coli* lawn on a 0.45 μm membrane filter for contact angle measurement. This filter was kept on top of a 10% agar plate, containing 20% glycerol, to keep the cell lawn hydrated.

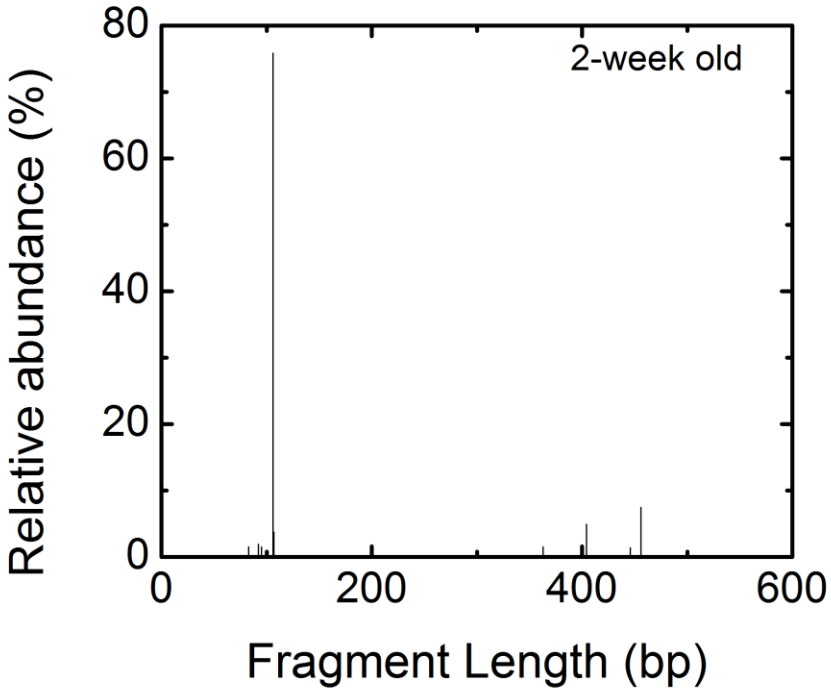


**Figure 4S-1.** T-RFLP profiles analyzed by Genemapper V 4.0. for 2-week old biofilm.

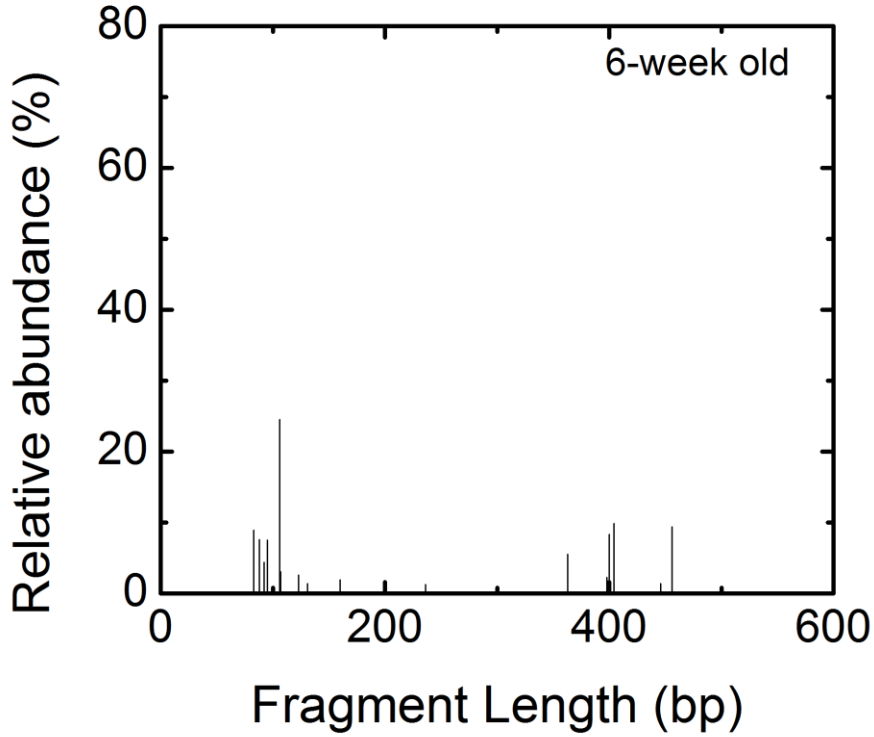


**Figure 4S-2.** T-RFLP profiles analyzed by Genemapper V 4.0. for 6-week old biofilm.

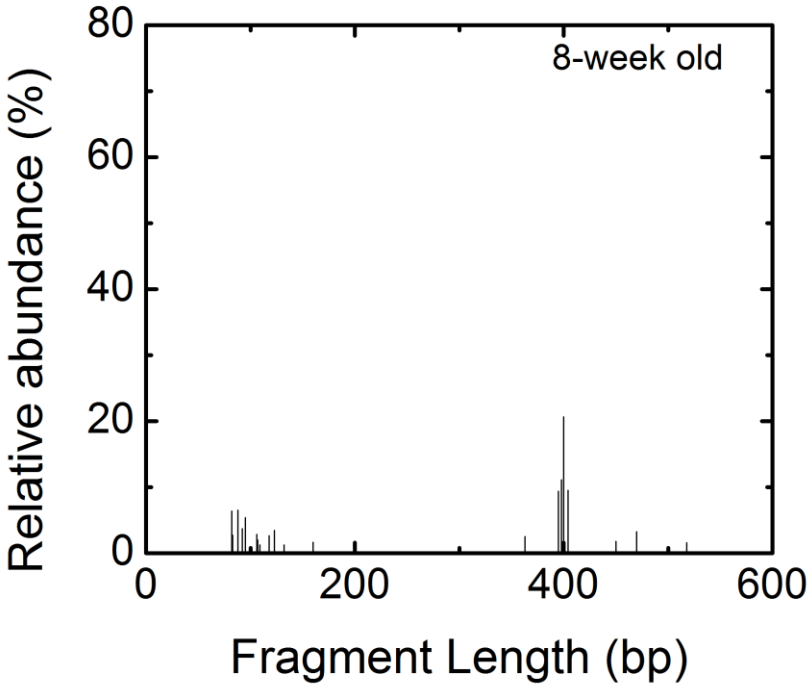


**Figure 4S-3.** T-RFLP profiles analyzed by Genemapper V 4.0. for 8-week old biofilm.

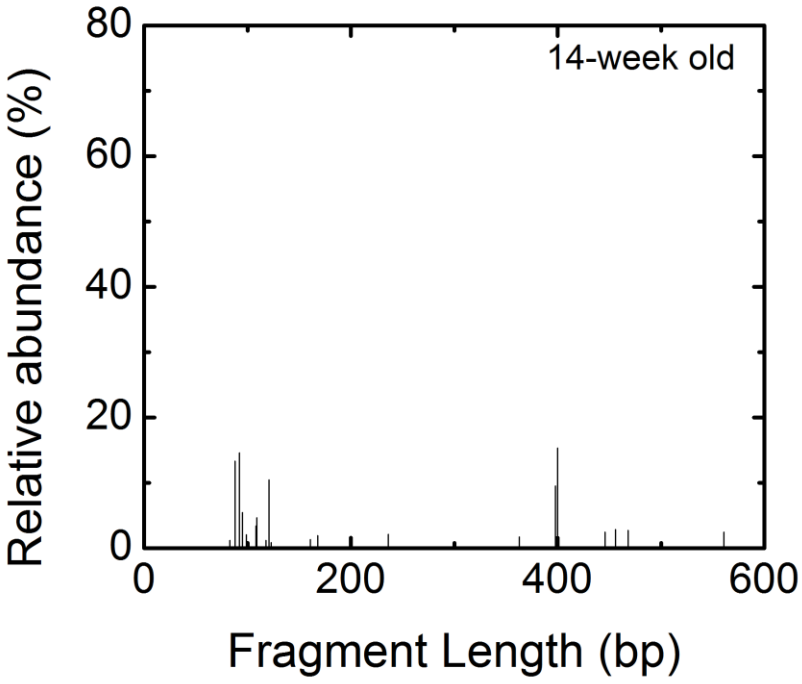


**Figure 4S-4.** T-RFLP profiles analyzed by Genemapper V 4.0. for 14-week old biofilm.

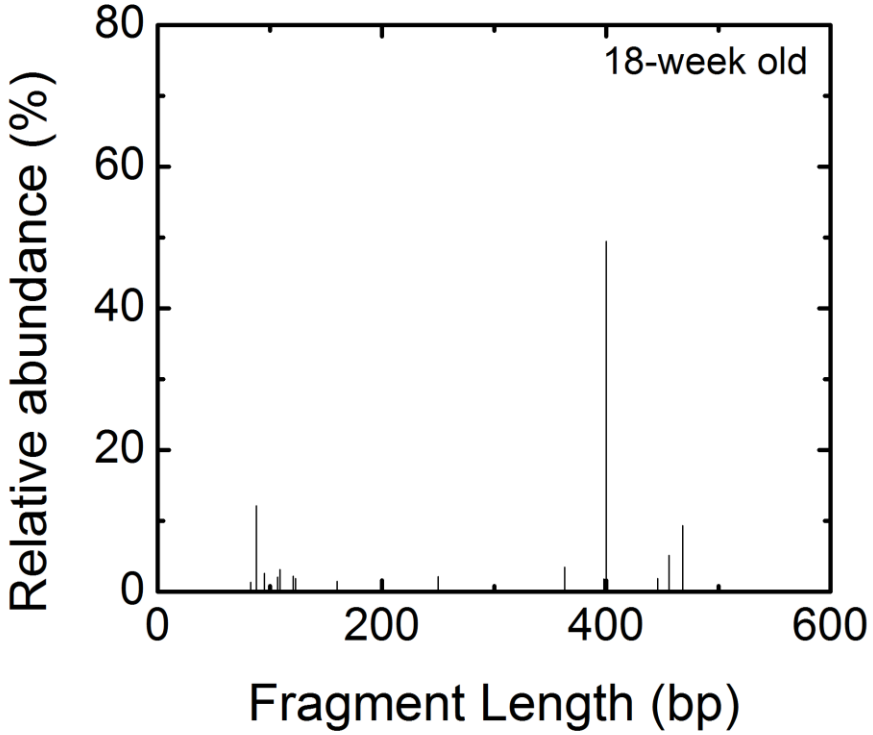


**Figure 4S-5.** T-RFLP profiles analyzed by Genemapper V 4.0. for 18-week old biofilm.

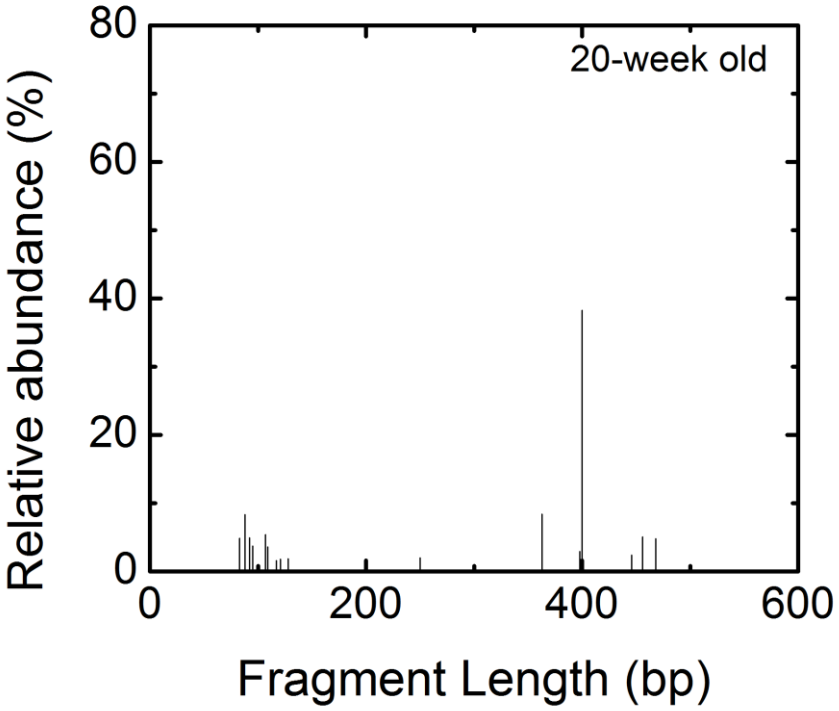


**Figure 4S-6.** T-RFLP profiles analyzed by Genemapper V 4.0. for 20-week old biofilm.

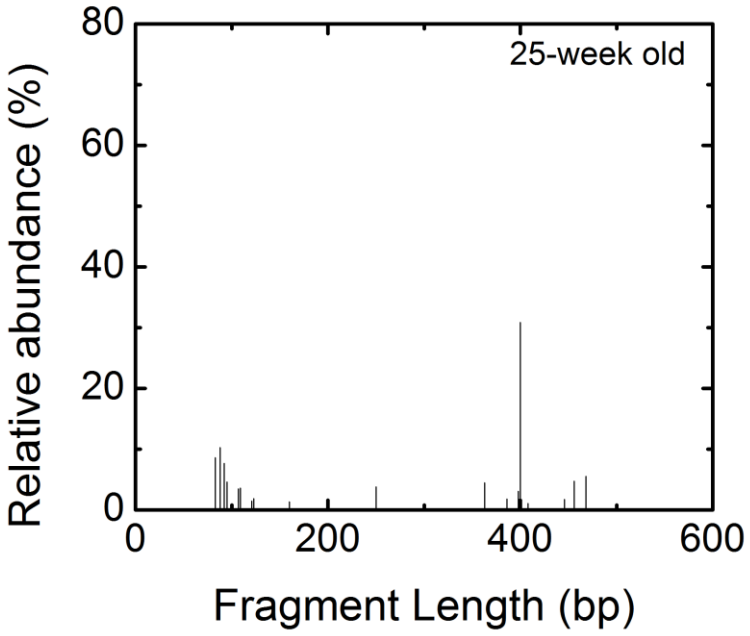


**Figure 4S-7.** T-RFLP profiles analyzed by Genemapper V 4.0. for 24-week old biofilm.

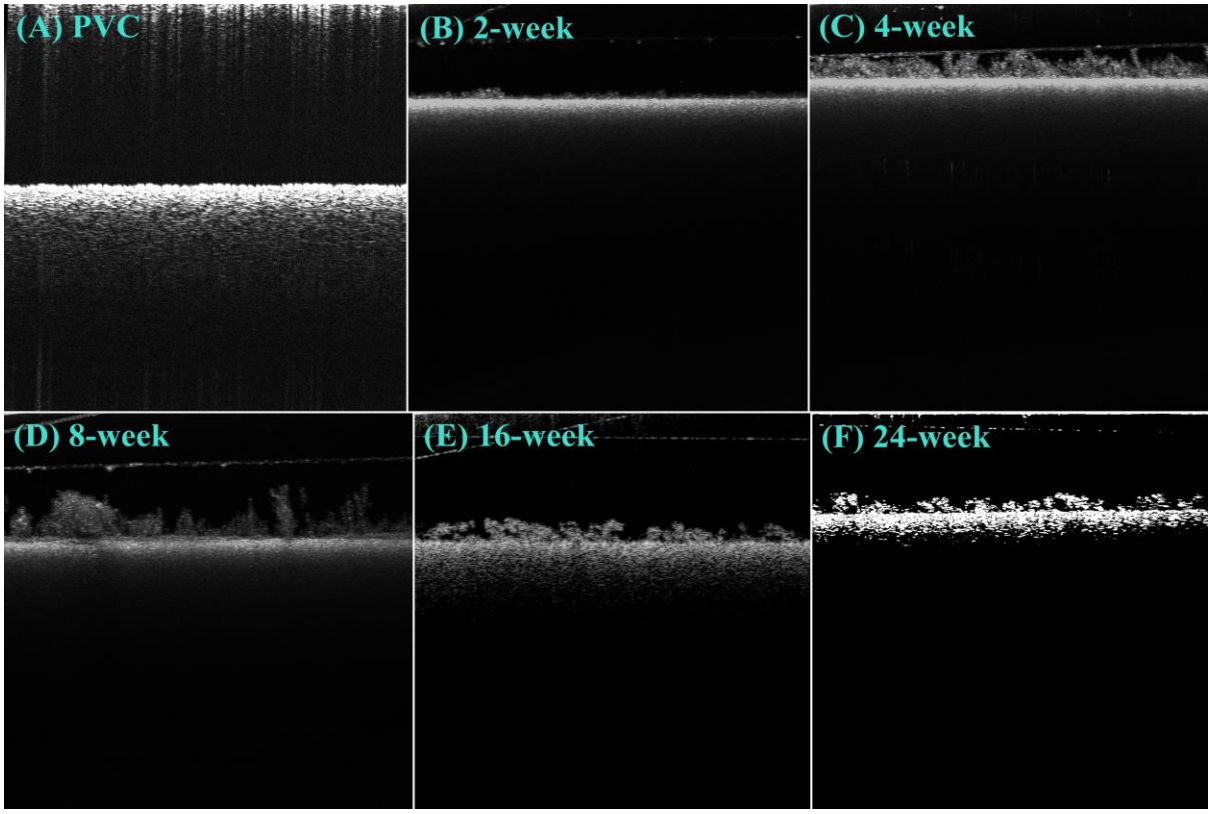


**Figure 5S.** OCT images and biofilm structures of A) PVC, B) 2-week old, C), 4-week old, D) 8-week old, E) 16-week old, and F) 24-week old biofilms.

**References**

Busscher, H.J., Weerkamp, A.H., Vandermei, H.C., Vanpelt, A.W.J., Dejong, H.P. and Arends, J. (1984) Measurement of the surface free energy of bacteria cell surfaces and its relevance for adhesion. Applied and Environmental Microbiology 48(5), 980-983.

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