Supplementary Materials

Text S1. Calculation of hydrodynamic forces

The corresponding velocity, shear stress, and Reynolds number of each applied flow velocity were calculated by **Equation 1**, **Equation 2**, and **Equation 3**, respectively.

$$V = \frac{Q}{A} \qquad \textbf{Equation 1}$$

The velocity, V (mm/s) of LB medium at each flow velocity was calculated by following the formula above, where Q is the volumetric flow rate (ml/min), and the cross-section area (A) of the flow cell channel (m^2).

$$Re = \frac{\rho V D_h}{\eta} \qquad \textbf{Equation 2}$$

$$D_h = \frac{2wh}{(w+h)} \qquad \textbf{Equation 3}$$

Reynolds number was computed according to **Equation 2**, where ρ is the density of the fluid (kg/m^3), V is liquid velocity obtained from **Equation 1**, and η is the viscosity of the solution. D_h is hydraulic diameter which is calculated based on **Equation 3**, where w is the channel width, and h is the channel height. In this experiment, water at 25 ℃ was assumed as the liquid medium with a dynamic viscosity of 0.0089 dyn-s/cm^2

$$\tau = \frac{6\eta Q}{wh^2} \qquad \textbf{Equation 4}$$

The shear stress τ (dyn/cm^2) was calculated regarding **Equation 4**, where η is the fluid viscosity of the solution, Q is the volumetric flow rate (cm^3/s), w is the channel width, and h is the channel height [65].

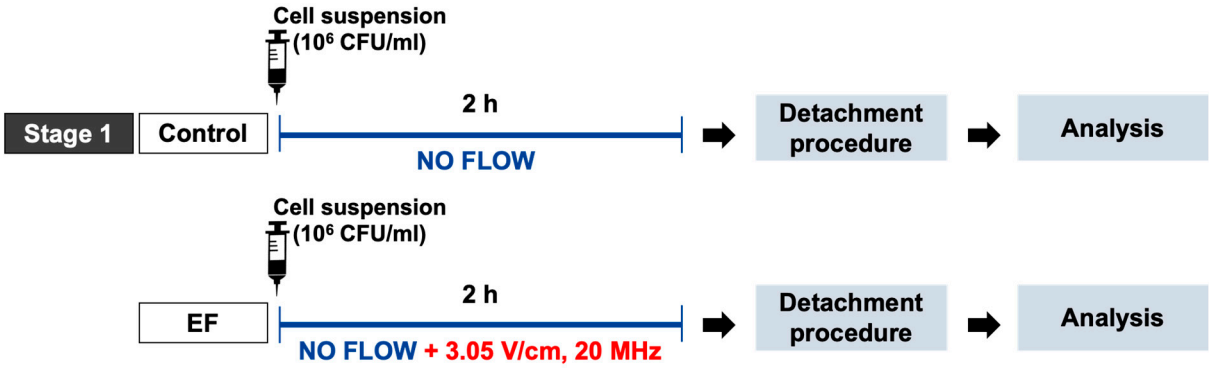
Text S2. Sterilization of the flow cell system

As a measure to prevent unexpected contamination, there is need to sterilize the system after it has been set up and prior to inoculation. This step was carried out by using 0.5 % (v/v) sodium hypochlorite which can decontaminate any microorganisms that is accidentally found within the system. This disinfectant was pumped through the flow system for 2 hours at a flow rate of 3 mL/h/channel. Following that, the system was washed with 0.5 L sterile distilled water at the maximum flow rate to get rid of the sodium hypochlorite and ensuring the bubble trap was completely rendered empty after each wash. The bubble trap can be emptied by pumping in air at high speed until it is free of all the bubbles. Then, the system was filled with sterilized LB medium for at least 30 min to ensure the tubing was saturated with LB medium prior to inoculation.

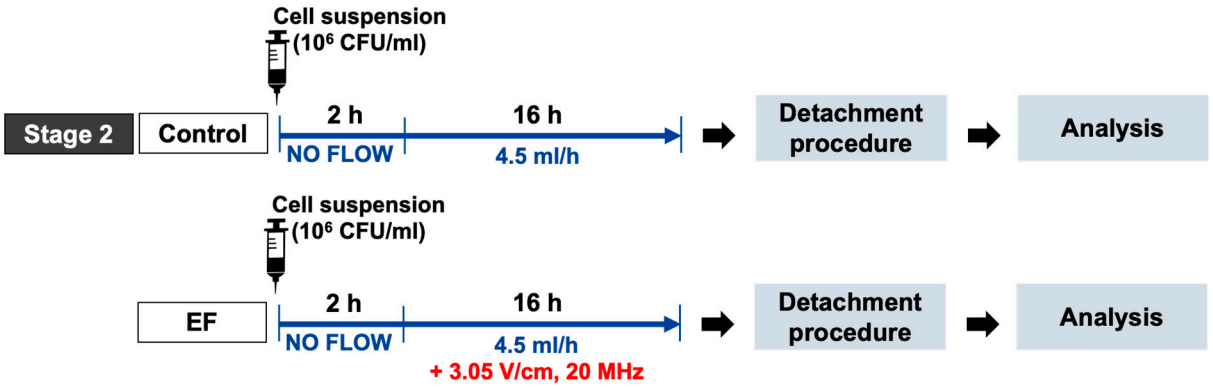
Text S3. Detachment procedure

In each experimental run, the adhered cells were detached from flow cell channel and suspended in PBS solution prior to analysis. Firstly, LB medium in each flow cell channel was drained out slowly to remove non-adherent cells, then replacing with 160 μl of PBS solution. To detach adherent cells, flow cell chamber was shaken by pre-vortex for 5 min, sonicated for 5 min submerging the flow cell in a beaker, and shaken again as post-vortex for 5 min, respectively. Subsequently, the detached cells inside flow cell channel were rinsed out to coning tube with 800 μl of PBS solution.

The suspension of detached cells was used to analyze viable cells measured by standard plate count, protein and polysaccharide content in EPS.

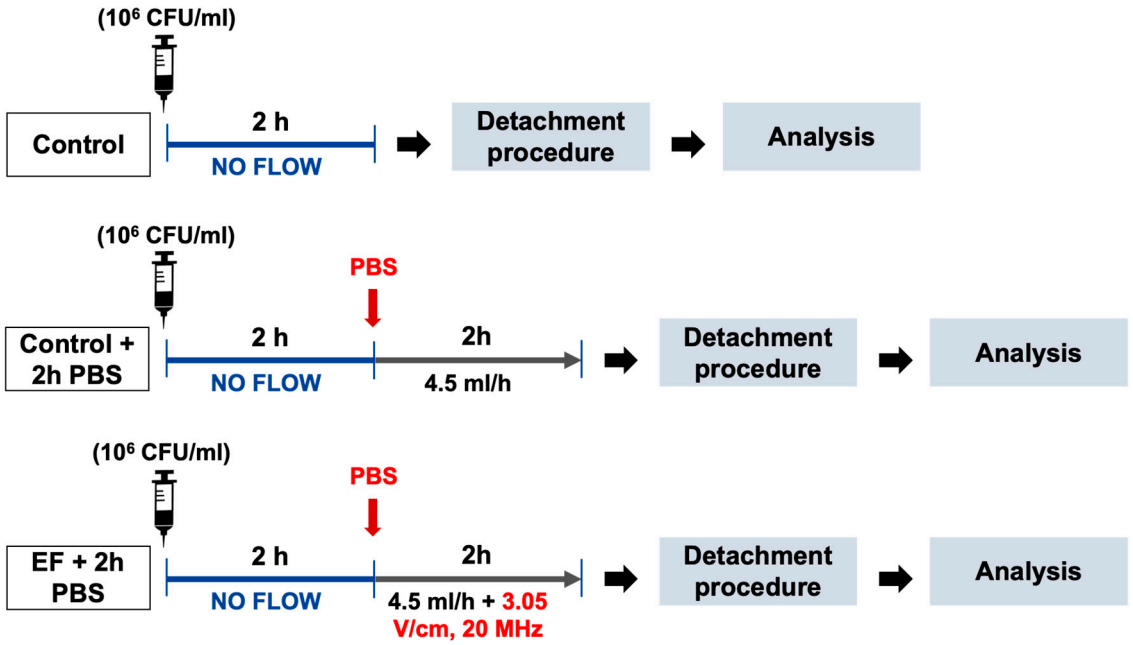


(a)

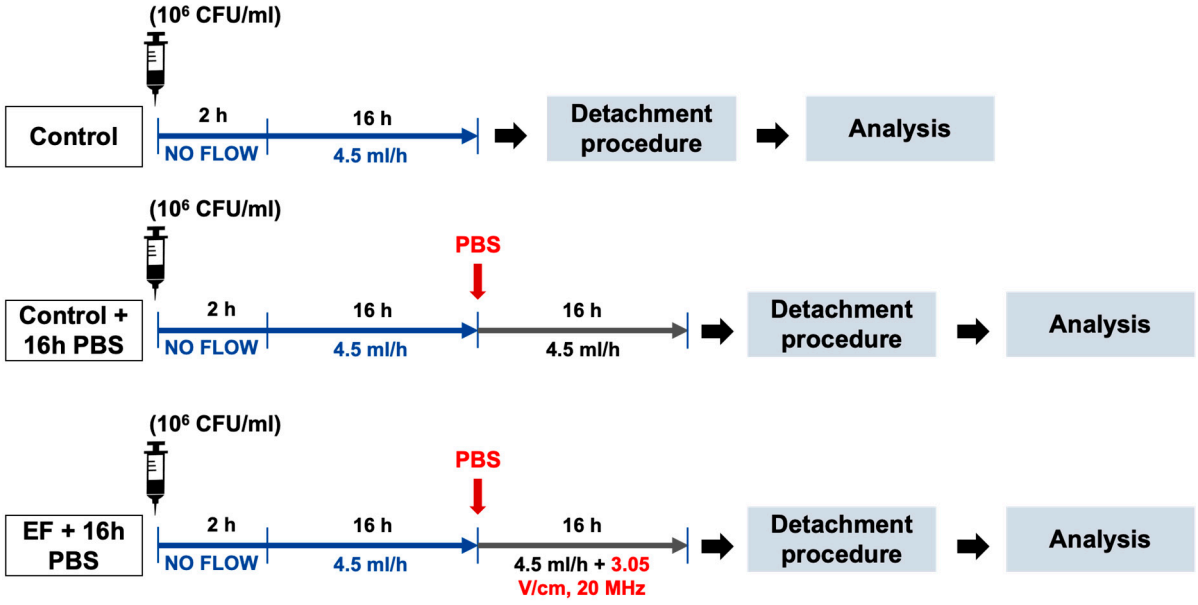


(b)

Figure S1. Experimental conditions of alternating electric field exposure during biofilm development processes: (a) stage 1 and (b) stage 2



(a)



(b)

Figure S2. Operating conditions of alternating electric field exposure during cell detachment (a) after stage 1 and (b) after stage 2

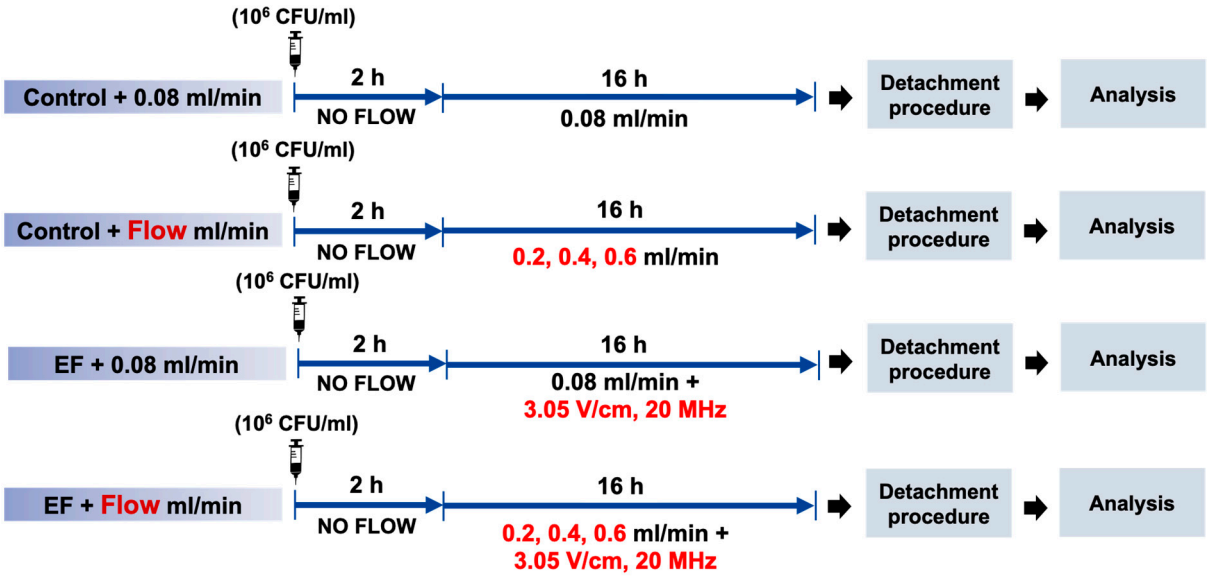


Figure S3. Experimental conditions for investigating the combined effect between alternating electric fields and hydrodynamic shear.

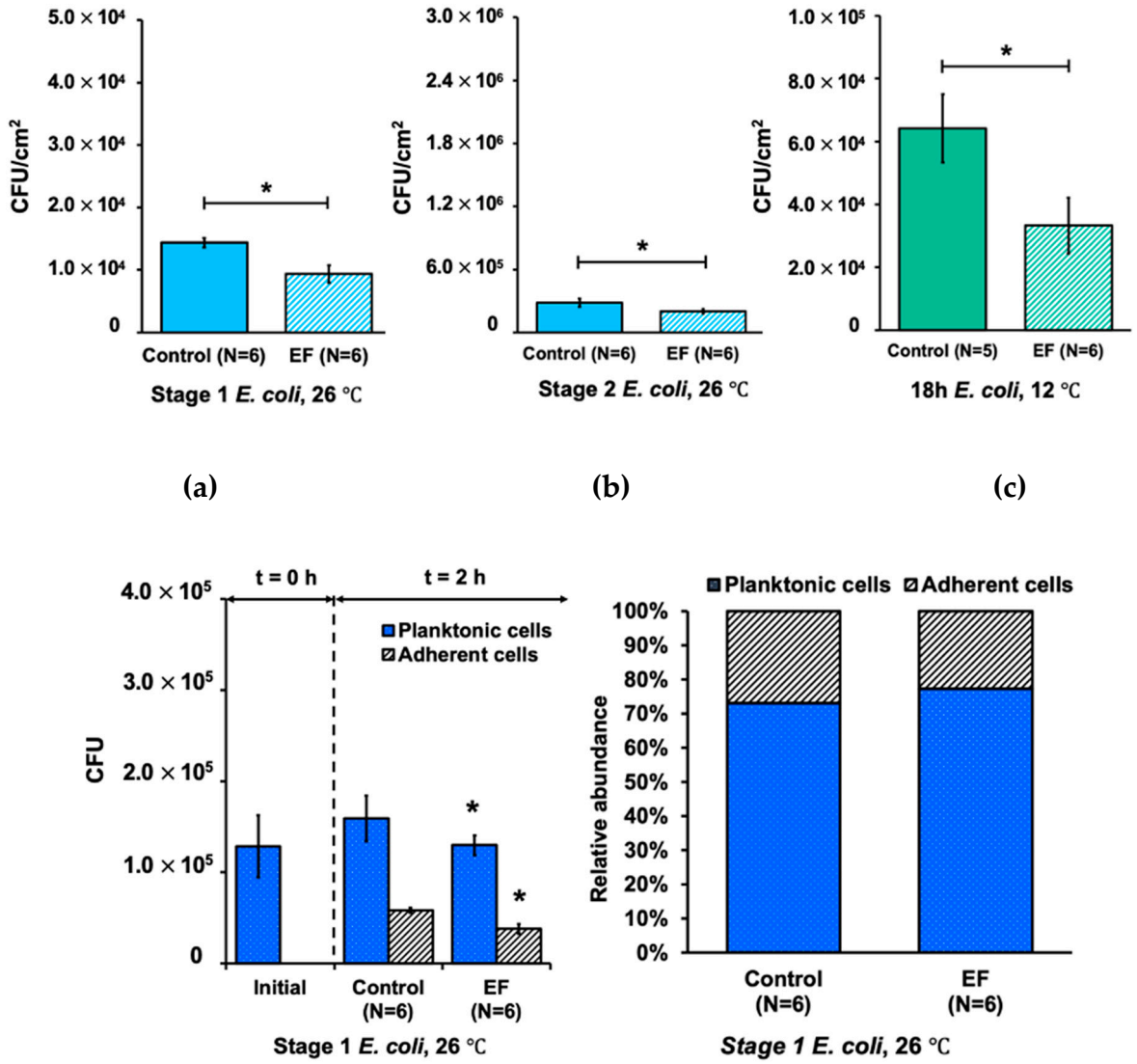


Figure S4. Effects of alternating electric fields on E. coli biofilm. Number of adherent cells after (a) stage 1, (b) stage 2, and (c) 18-h continuous exposure (stage 1 + stage 2). (d) Total number of planktonic and adherent cells in CFU after stage 1. (e) Relative abundance of planktonic and adherent cells. EF represents biofilm group under alternating electric field exposure. * denotes statistically significant difference with $p < 0.05$.

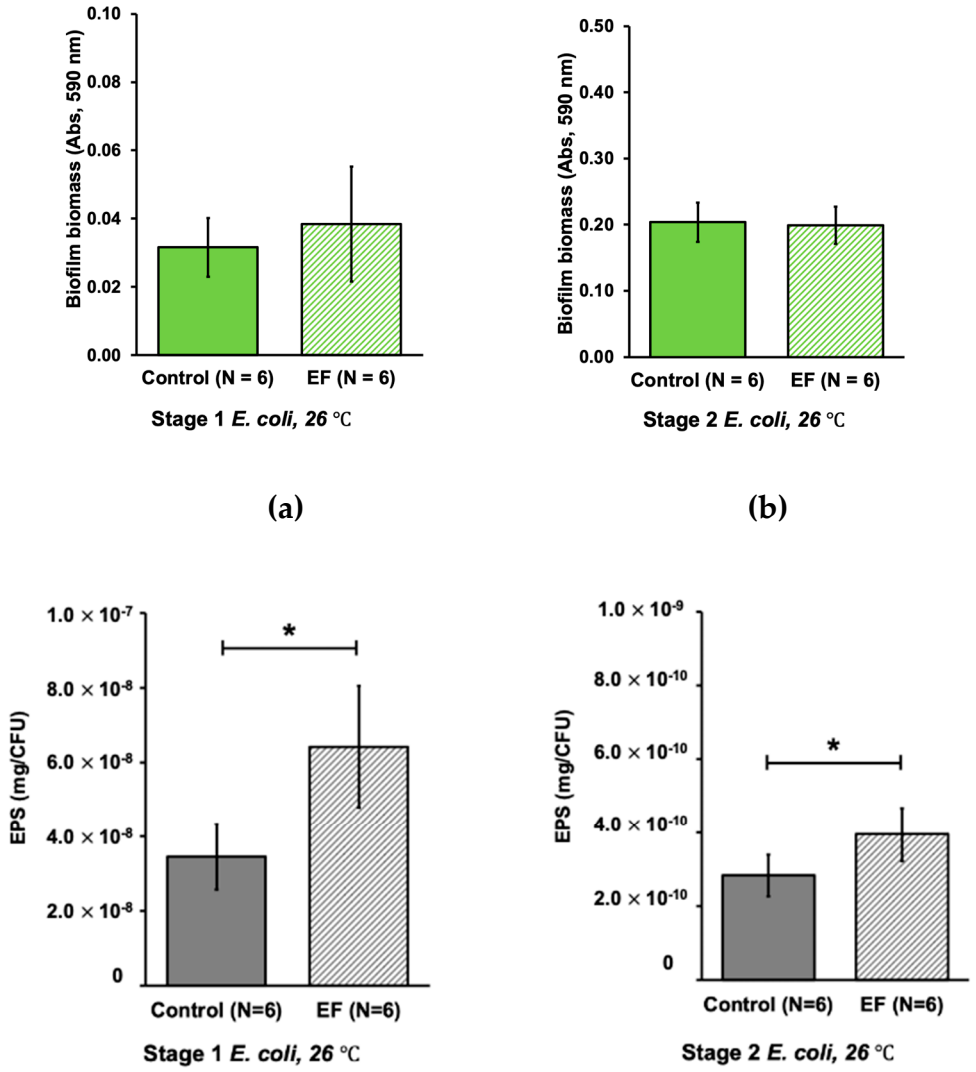
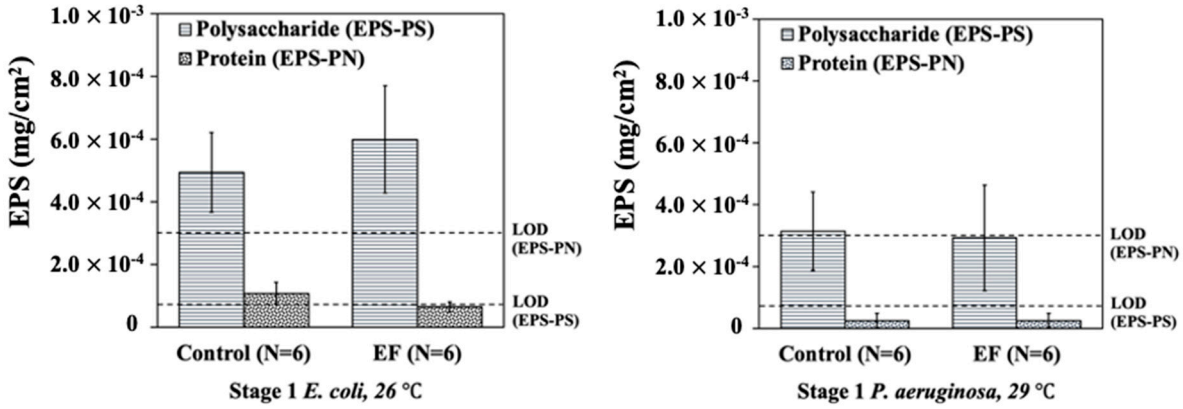
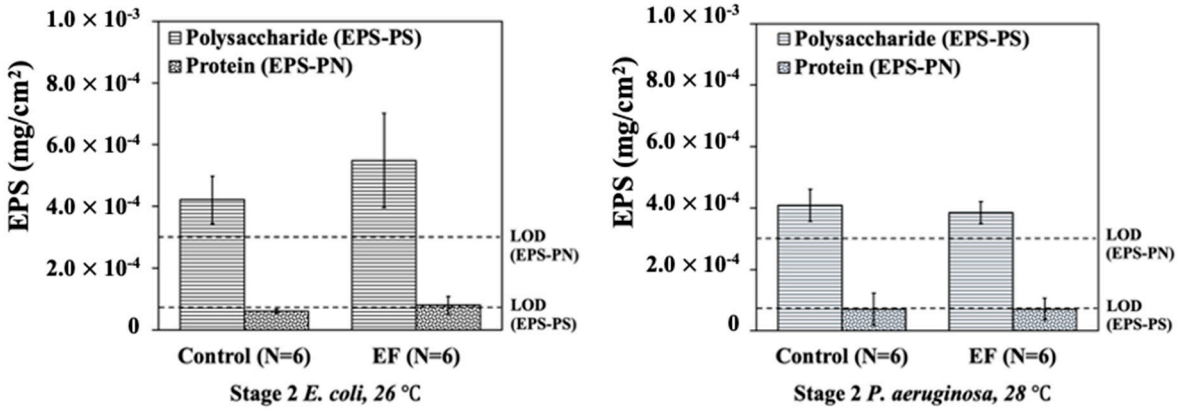


Figure S5. Effect of alternating electric fields in term of total biofilm biomass of E. coli biofilm after (a) stage 1 and (b) stage 2. Content of EPS after (c) stage 1 and (d) stage 2. EF indicates biofilm group under alternating electric field exposure. * denotes statistically significant difference with $p < 0.05$.

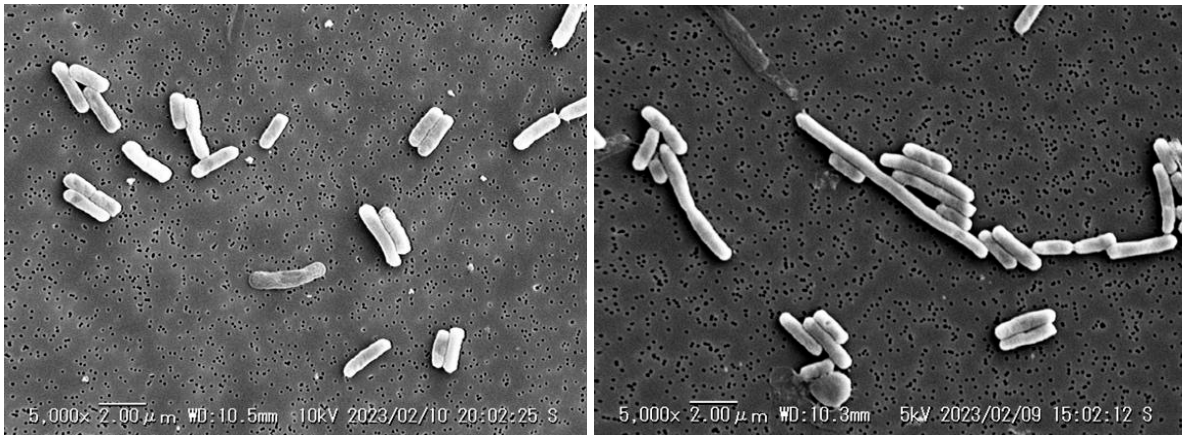


(a) Stage 1

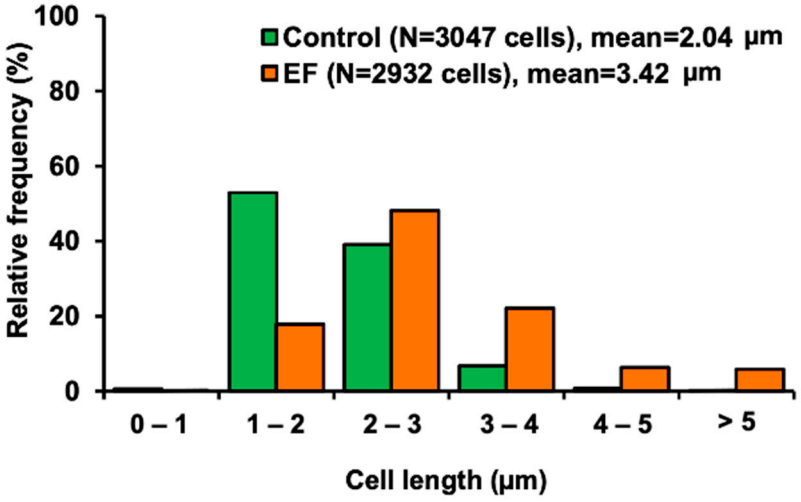


(b) Stage 2

Figure S6. Total EPS amount (mg EPS per cm2) after (a) initial surface attachment stage (stage 1) and (b) early developmental phase (stage 2) of E. coli and P. aeruginosa under exposure to alternating electric field. EF represents biofilm group which exposed to alternating electric field. LODEPS-PS and LODEPS-PN indicate the detection limit of polysaccharide and protein



(a) **(b)**



(c)

Figure S7. Representative SEM images (5000× magnifications) of adherent E. coli cells after alternating electric field exposure in (b) stage 2 and (a) control condition (porous structure in the background is the surface of track-etched polycarbonate membrane). (c) Cell length distribution of adherent with and without exposure to alternating electric field.

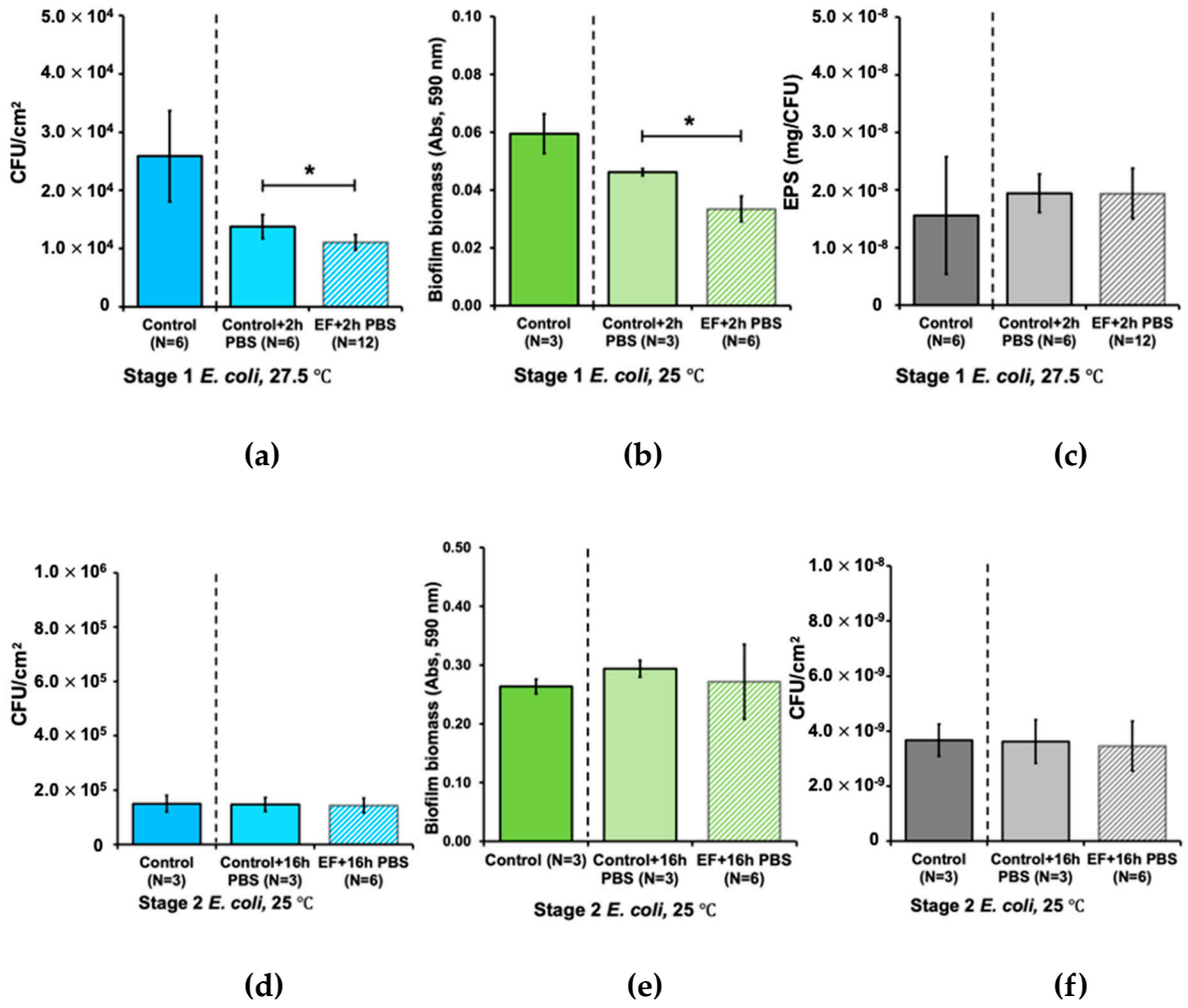
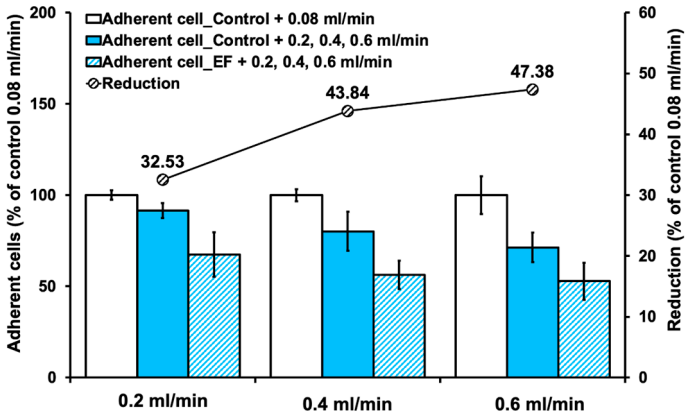
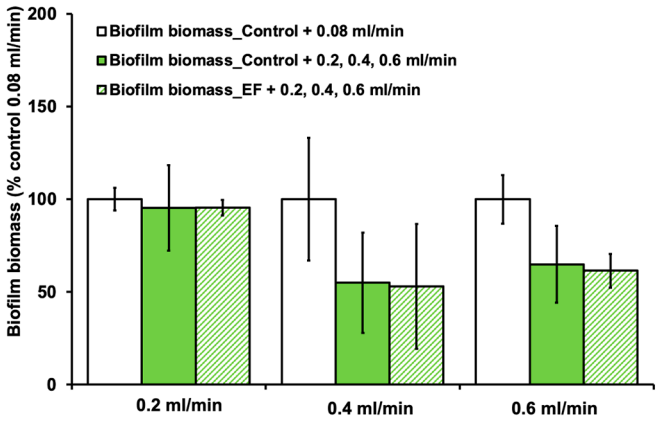


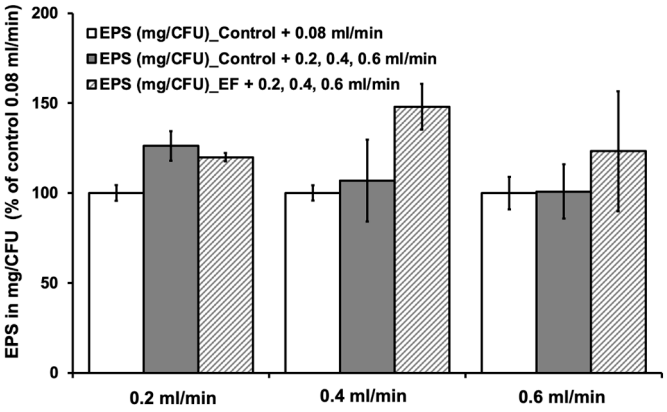
Figure 8. Effects on cell detachment for *E. coli* after stage 1 and stage 2 under different conditions and applied parameters. The conditions for testing the effect on cell detachment after stage 1 consisted of 2 h of LB medium (control) and 2 h of LB medium followed by 2 h of PBS in the absence of an electric field (control + 2 h PBS) and 2 h of LB medium followed by 2 h of PBS with an alternating electric field (EF + 2 h PBS). The conditions for testing after stage 2 were as follows: 16 h application of LB medium (control), 16 h application of LB medium followed by 16 h of PBS in the absence of an electric field (control + 16 h PBS), and 16 h application of LB medium followed by 16 h of PBS with an alternating electric field (EF + 16 h PBS). In each condition, the effects after stages 1 and 2 were investigated in terms of the number of adherent cells (a,d), total biofilm biomass (b,e), and number of EPSs in mg per CFU (c,f), respectively. * represents a significant difference according to our statistical test between the control with PBS and EF with PBS ($p < 0.05$).



(a)



(b)



(c)

Figure S9. Change in (a) adherent cells and percent reduction, (b) biofilm biomass, and (c) EPS content in mg per CFU of E. coli, after exposing to alternating electric field assisted by different flow velocities (0.2, 0.4 and 0.6 ml/min). The data show in percentage percentage normalized by the results of control 0.08 ml/min in absence of alternating electric field. * represents statistical difference compared to non-exposed adherent cells in each flow rate ($p < 0.05$).