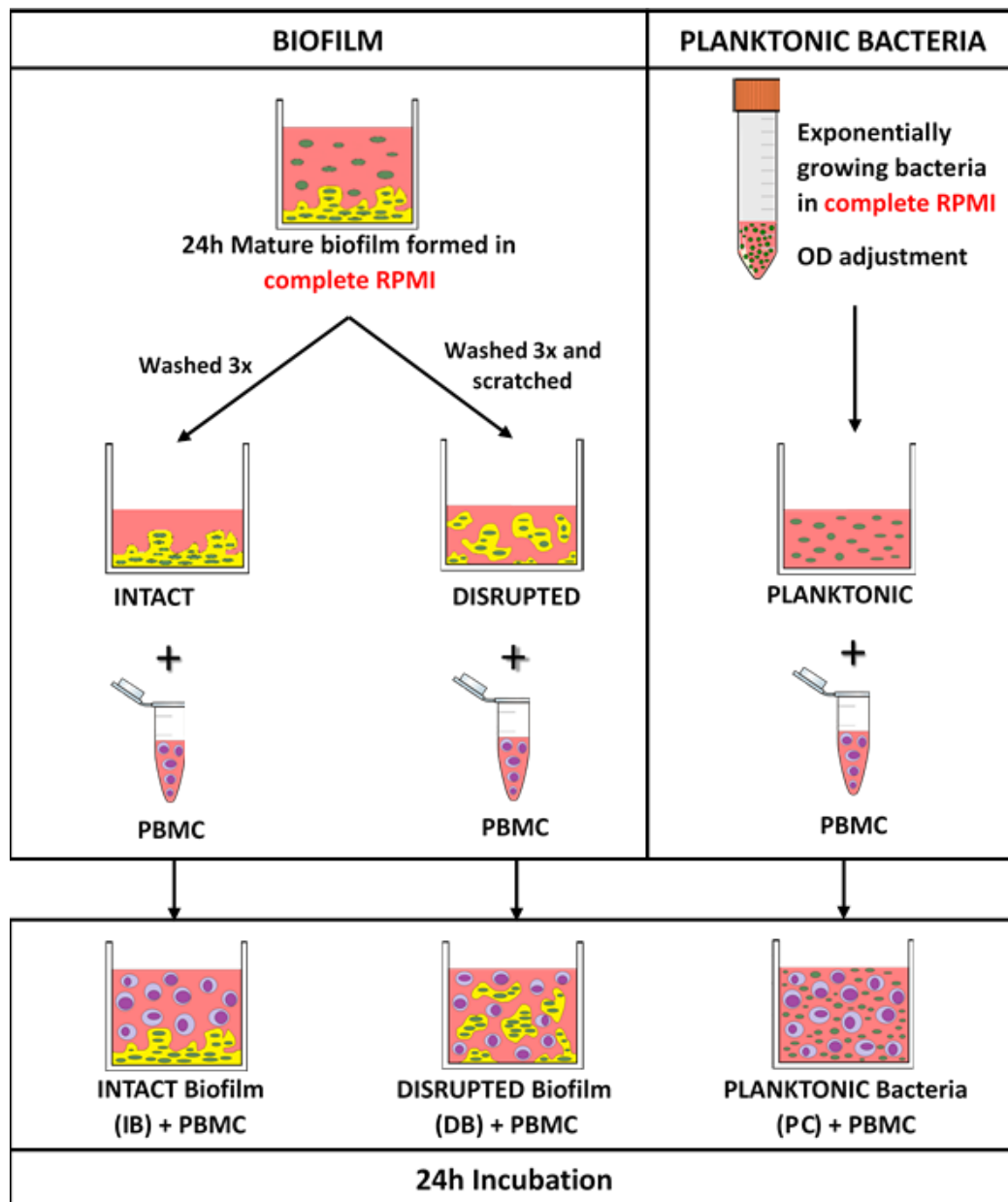


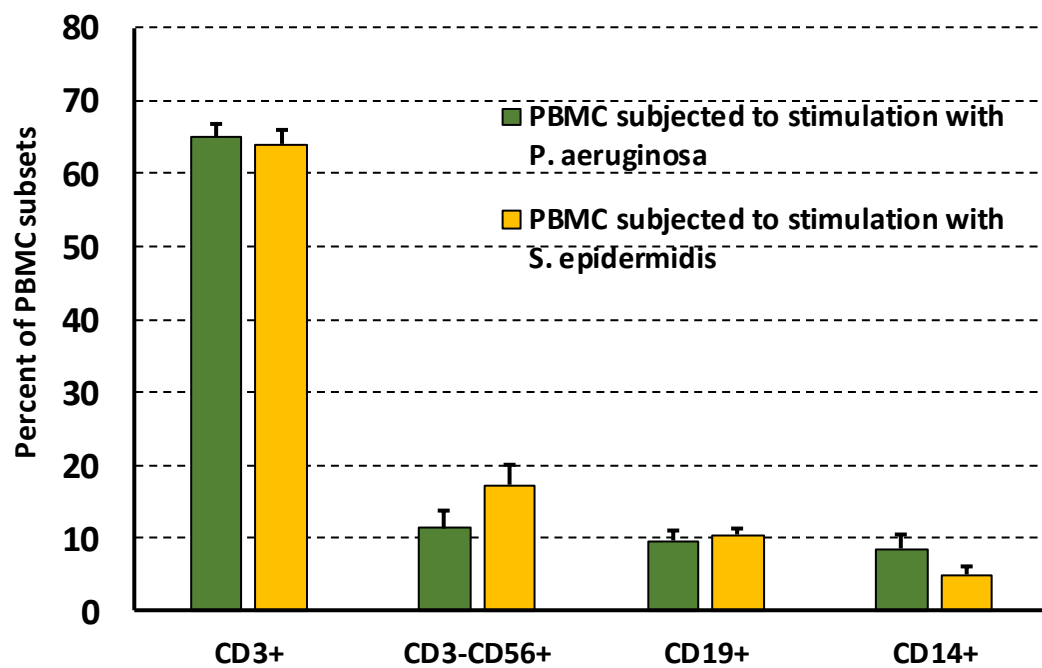
# Response of human peripheral blood mononuclear cells to *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* in planktonic and biofilm mode of growth

Esingul Kaya<sup>1†</sup>, Giovanna Batoni<sup>1†</sup>, Maria Grazia di Luca<sup>2</sup>, Eleonora Apolloni<sup>1</sup>, Alessandro Mazzoni<sup>3</sup>,  
Giuseppantonio Maisetta<sup>1</sup>, and Semih Esin<sup>1\*</sup>

## Supplementary materials



**Scheme S1.** Main steps of the experimental procedure adopted to setup the bacteria:PBMC co-cultures.

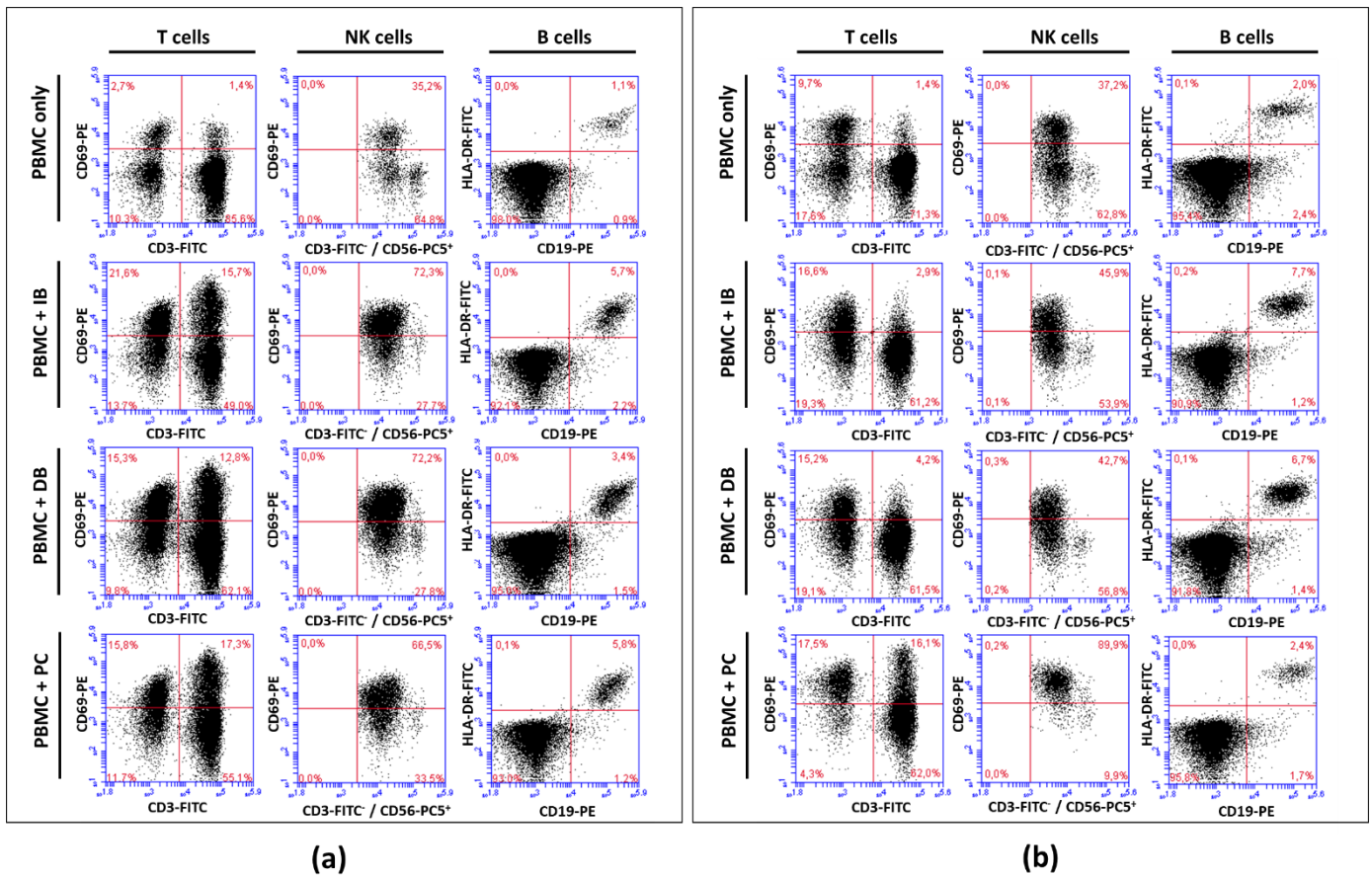


**Figure S1.** Percentages of different cell subsets in PBMC subjected to stimulation with *P. aeruginosa* or *S. epidermidis* (biofilms, disrupted biofilms or planktonic bacteria) at time 0. The figure shows the mean values  $\pm$  SEM of the 8 donors tested for *P. aeruginosa* and of the 7 donors tested for *S. epidermidis*. Two donors were tested with both species.

**Table S1.** Absolute cell numbers of PBMC subsets after 24h culture with or without bacteria.

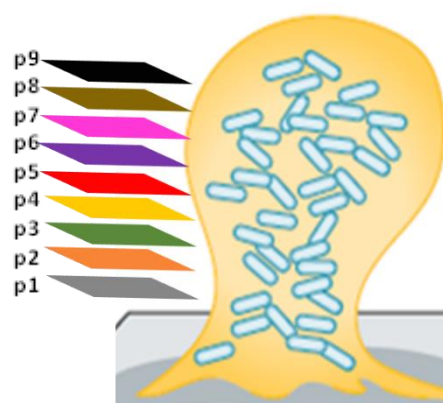
	<i>P. aeruginosa</i>			<i>S. epidermidis</i>		
	T cells	NK cells	B cells	T cells	NK cells	B cells
PBMC	252909±44803	17234±3688	8915±2148	184647±12394	34822±13440	9354±1865
IB	190964±33139	15490±2581	11532±2029	180516±29854	50134±23259	23144±4647
DB	202051±38697	13895±1933	11640±2266	176903±28885	50737±19656	20983±3847
PC	177837±40275	11386±1967	11039±2353	176907±25588	45224±18546	13849±1989

Mean values ± SEM from n=7 (*P. aeruginosa*) and n=8 (*S. epidermidis*) independent experiments are shown. Numbers of monocytes were not shown as they were less than 0.5 % in all conditions.

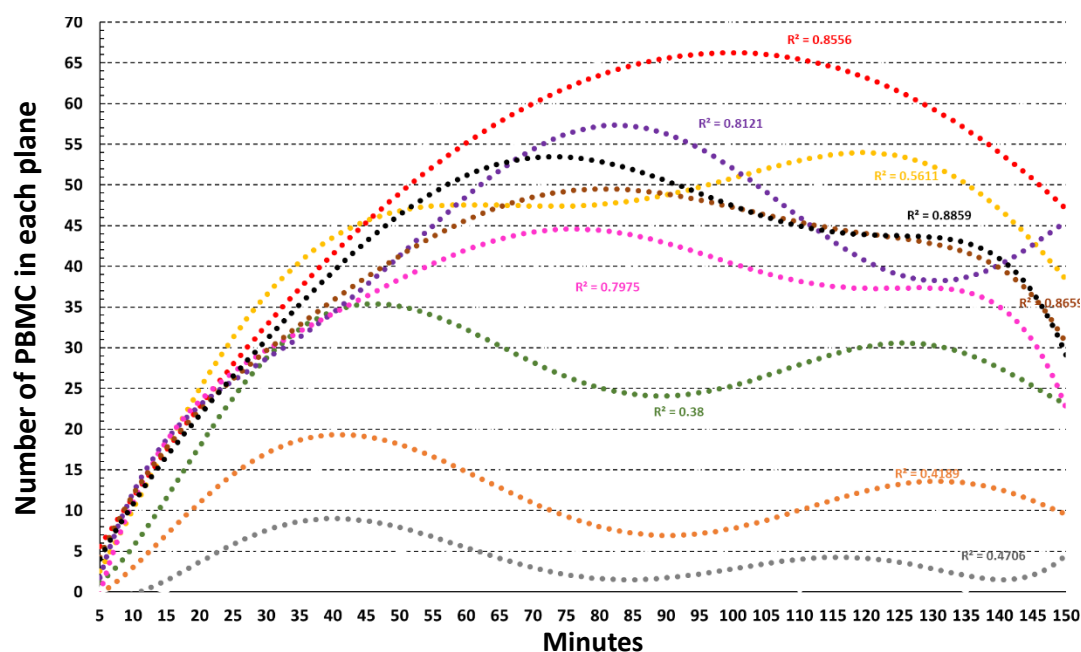


**Figure S2.** Percentages of activated cell subsets (T cells, NK cells, and B cells) in PBMC subjected to stimulation with *P. aeruginosa* or *S. epidermidis*. The figure shows data (dot plots) of PBMC from a representative donor tested for *P. aeruginosa* (a) and for *S. epidermidis* (b). IB: intact biofilms; DB: disrupted biofilms; PC: planktonic bacteria. For NK cells CD69 staining on CD56<sup>+</sup>CD3<sup>-</sup> cells gated from CD3 versus CD56 plots are shown.

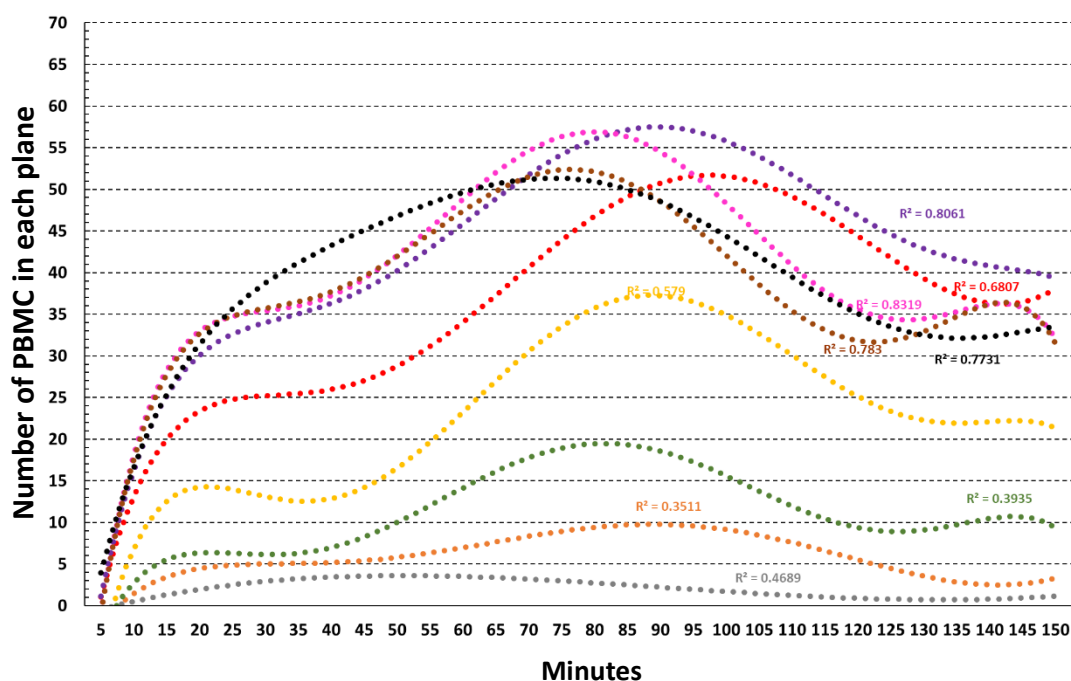
(a)



(b)



(c)



**Figure S3.** Computer assisted quantification of immune cell (PBMC) numbers interacting at different times and at different depths with the biofilm of *P. aeruginosa* and *S. epidermidis*. Images from nine different planes, from p1 (the deepest) to p9 (the outermost) were acquired for biofilms of both species (a). Immune cell numbers were evaluated in each plane via Harmony software (Perkin Elmer Inc.) for *P. aeruginosa* (b) and *S. epidermidis* (c). Graphics depict the trend-lines elaborated according to polynomial equation by Microsoft Excel. In the case of *P. aeruginosa*, most of the cells were localized in the middle section of the biofilm (i.e., plane 5) after 60-90 min. of co-incubation, whereas in the case of *S. epidermidis*, the majority of the cells were found in superficial planes (i.e., 6, 7, 8, and 9). Few cells were able to reach the deepest layers of the biofilms. R<sup>2</sup>: R-squared value for reliability of each trend-line.