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Phagocytosis of *Escherichia coli* Biofilm Cells with Different Aspect Ratios: A Role of Substratum Material Stiffness

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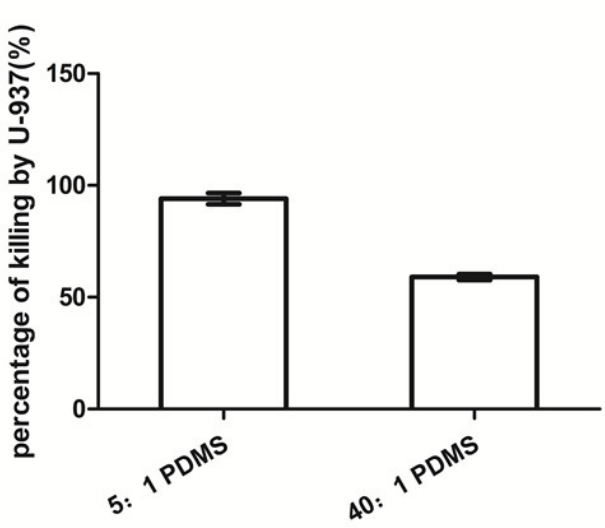

Fig. S1. Phagocytosis of 5-h biofilm cells of *E. coli* RP437/pGLO. The graph shows the percentage of killing after incubating with U-937 for 1 h in RPMI medium supplemented with 10% FBS at 37°C and 5% CO_2 on 5:1 or 40:1 PDMS surfaces (p=0.007, one-way ANOVA).

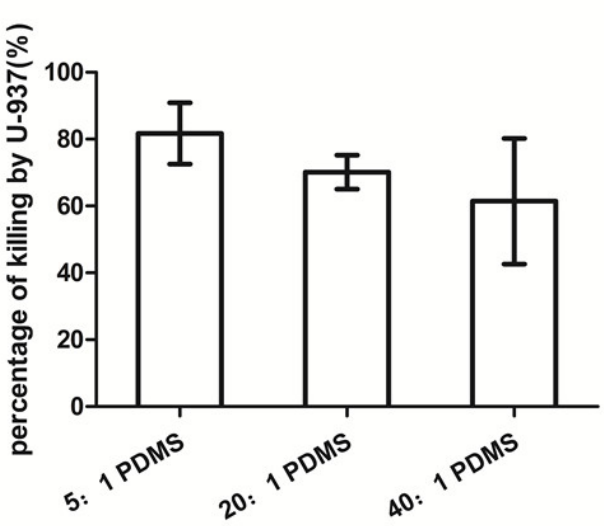

Fig. S2. Phagocytosis of 5-h biofilm cells of *E. coli* RP437/pBAD-mPlum. The graph shows the percentage of killing of bacterial cells after incubating with U-937 for 90 min in RPMI medium supplemented with 10% FBS at 37ºC and 5% CO_2 on 5:1, 20:1 or 40:1 PDMS surfaces (r=0.63, p=0.0019, Pearson correlation analysis).

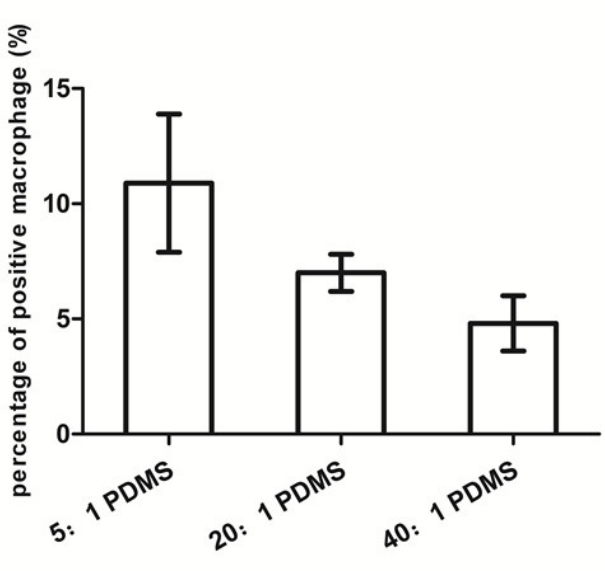

Fig. S3. Percentage of positive macrophage cells (associated with *E. coli* RP437/pBAD-mPlum cells) under microscopy. The graph shows the percentage of positive U-937 cells after incubating with 5-h *E. coli* RP437/pBAD-mPlum biofilms for 90 min in RPMI medium supplemented with 10% FBS at 37ºC and 5% CO_2 (r=0.83, p<0.001, Pearson correlation analysis).

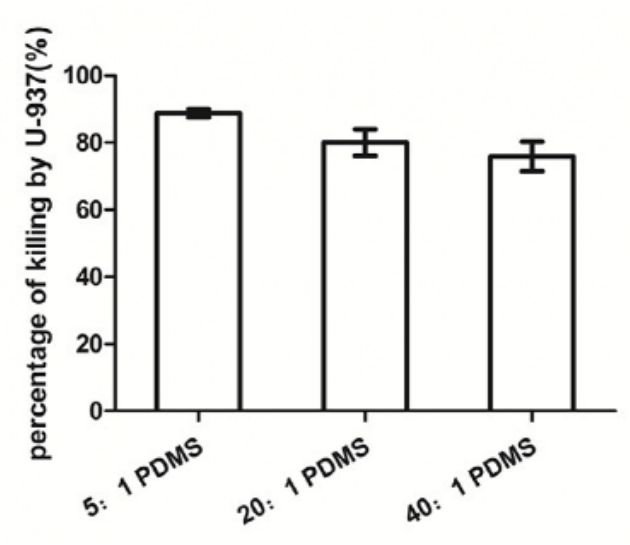

Fig. S4. Phagocytosis of 5-h *E. coli* RP437/pBAD-mPlum biofilm cells with U937 cells attached first. The graph shows the killing of bacterial cells detached from 5:1, 20:1 or 40:1 PDMS surfaces, which were incubated with U-937 cells (attached on the same surfaces of petri dish) for 90 min in RPMI medium supplemented with 10% FBS at 37°C and 5% CO_2 (r=0.80, p=0.0095, Pearson correlation analysis).

Table S1: Raw data of related figures.

Figures	CFU or Imaging Analysis								
	5:1 PDMS			20:1 PDMS			40:1 PDMS		
	BT	**AT**	**KR/RP**	**BT**	**AT**	**KR/RP**	**BT**	**AT**	**KR/RP**
1	200000	40000	80%	130000	40000	69%	180000	60000	67%
	150000	20000	87%	200000	50000	75%	200000	70000	65%
	130000	20000	85%	200000	60000	70%	120000	50000	58%
2	22	2	9.1%	63	4	6.3%	33	2	6.1%
	26	3	11.5%	81	6	7.4%	36	2	5.6%
	23	2	8.7%	63	5	7.9%	45	2	4.4%
	25	3	12.0%				20	1	5.0%
	28	3	10.7%				35	2	5.7%
5	40000	11000	72%	40000	11000	72%	30000	10000	67%
	40000	10000	75%	40000	14000	65%	30000	11000	63%
	40000	9000	78%	40000	13000	68%	30000	11000	63%
6	30	4	12.9%	56	6	10.7%	46	3	6.5%
	41	5	12.2%	57	5	7.0%	46	4	8.7%
	50	6	13.3%	66	6	9.1%	40	3	7.5%
	41	6	14.6%				42	3	7.1%
	35	5	14.3%				26	2	7.7%
7	110000	20000	82%	90000	30000	67%	100000	32000	68%
	80000	21000	74%	80000	24000	70%	80000	30000	62%
	70000	12000	83%	40000	11000	72%	80000	33000	59%

CFU: colony forming unit

BT: cell number before treatment

AT: cell number after treatment

KR: killing ratio

RP: ratio of positive macrophages (associated with bacteria).