Electronic Supplementary Material (ESI) for Lab on a Chip

An integrated microfluidic platform for evaluating *in-vivo* antimicrobial activity of natural compounds using a whole-animal infection model

SUPPLEMENTARY INFORMATION

Jianping Yang, Zuanguang Chen*, Poying Ching, Qiujia Shi and Xinchun Li

(School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China)

* e-mail: chenzg@mail.sysu.edu.cn

Electronic Supplementary Material (ESI) for Lab on a Chip

Experimental

Device fabrication

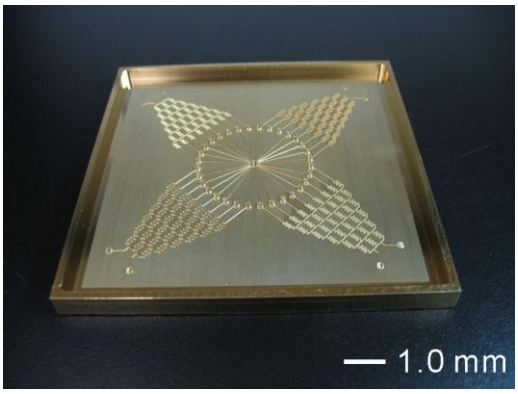

Fig. s1 Photograph of the master mold of the microfluidic chip. It is a plate copper alloy (10 cm × 10 cm) patterned by computer numeric control milling technique.

Microfluidic device operation

The detailed process of loading relatively uniform worms into each chamber:

The inlet tube is a pipette tip, and the worms are loaded from the open tube. In order to avoid accumulation of worms at the inputs of the channels, we applied a repeated dropping way to load the animals, and the suspension of worms is dilute (about 1.0-1.5 worm μl^{-1}). At first, 100μl suspension was added into the inlet tube drop by drop. Then, 100 μl M9 buffer was dropped in the same way to put the worms into the channel. Average 3-4 worms can be loaded into each channel per round. All worms could be introduced into the channel by repeating the same procedure several times. Occasionally, some worms would accumulate at the inlet ports. However, this accumulation could be untied by adding more M9 solution to suspend the worms again, or pulling out the inlet tube and picking out the

mess. In addition, another measure was used to speed up the loading. Here, a micro pump was used to assist introduction. The experimental setup is shown as Fig. s6. The worms would easily enter the channel by traction force which is generated through the pump extraction. We also characterized the effect of extraction velocity on the uniformity of nematodes in the chambers. High velocity will promote the pace of loading but the difference of worm number become greater among the chambers because it will induce strong traction force to pull the worms through the gate sill into the chambers. Considering the loading speed and the uniformity number of worms, the fluid velocity is optimized as 10 μl/min.

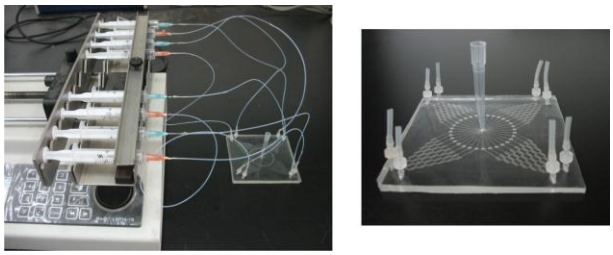

Fig. s2. The photographs of the experimental setup.

Characterization of the key components of the device

The changes of the channel and the entrance:

When M9 buffer solution is rapidly pushed into the device by a transferpettor at a rate of 30 μl/s, pressure increase in the device expands the channel and the entrance. Here, we add another group of micrographs to further demonstrate the changes of the channel (Fig. s3 in ESI). The channel is 60 μm wide under normal condition, whereas it expands to 90 μm width under the pressure. We infer that the size of the channel increases 1.5 times, and the entrance may also expand about 1.5 times on the same principle. Therefore, the entrance becomes large enough for a worm to pass.

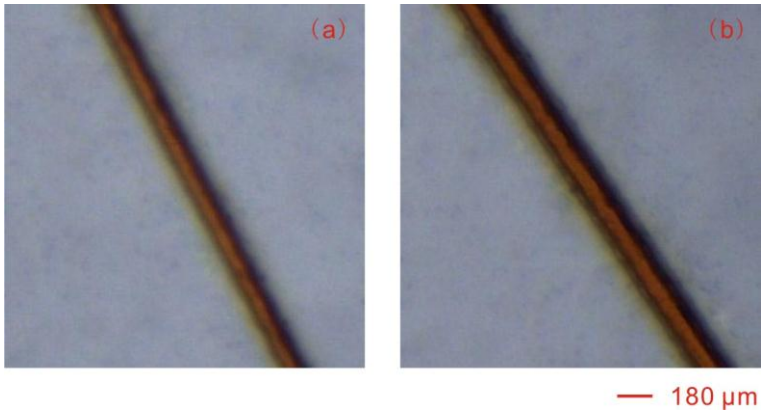

Fig. s3. Micrographs of the channel before and after pressure. (a) The channel under normal condition (10 μl/min). (b) The channel expands under pressure by a transferpettor to push M9 buffer at a rate of 30 μl/s.

Characterization of multiple gradients generation

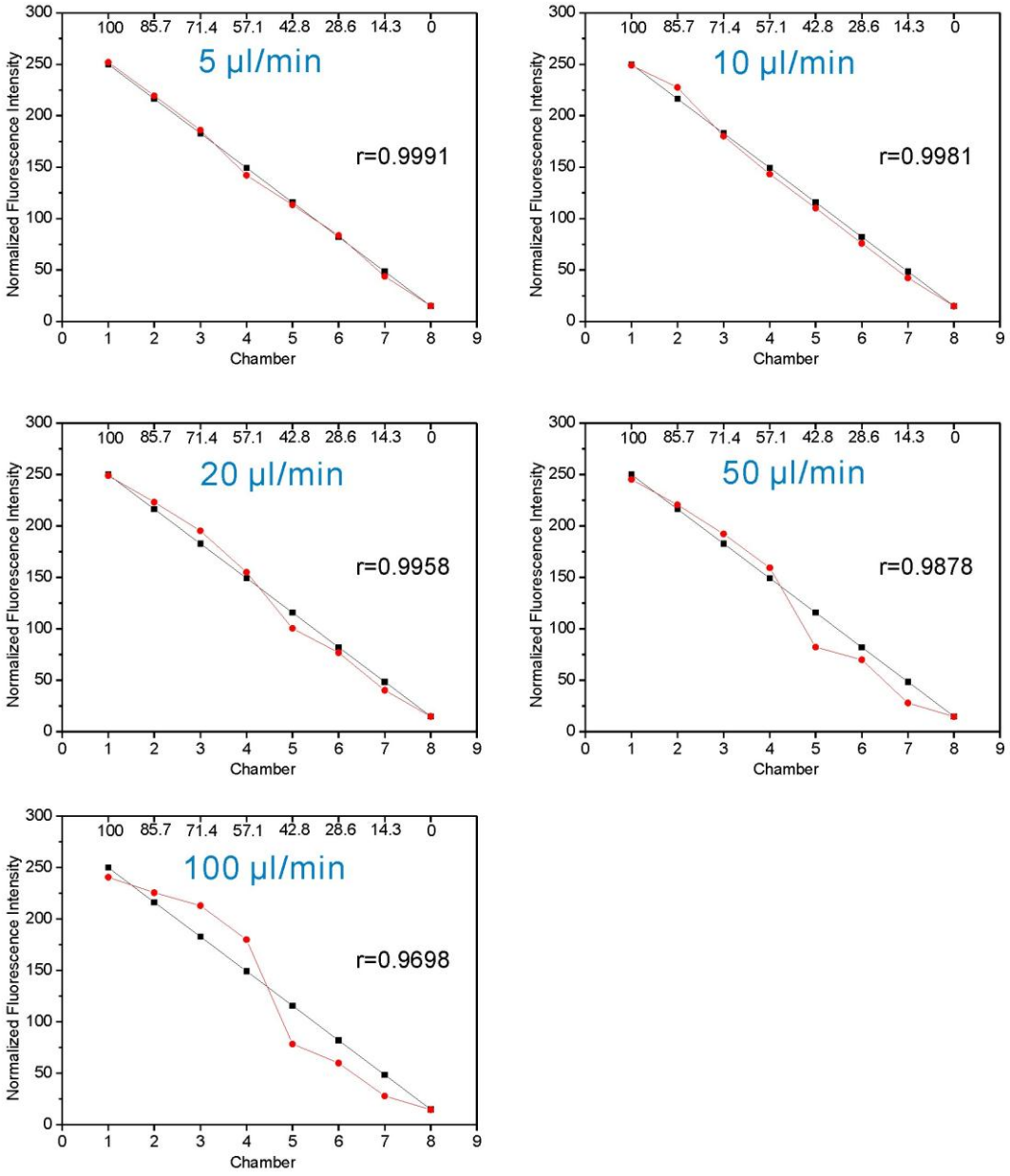

Fig. s4 The comparison between the experimental data and the theoretical estimations on the fluorescence intensity of 100 µM FITC using the microfluidic CGG at flow rates of 5, 10, 20, 50, 100 µl/min. The correlation factor is higher than 0.99 when the flow velocity is lower than 20.0 µl/min.

Effect of liquid culture media on nematode killing

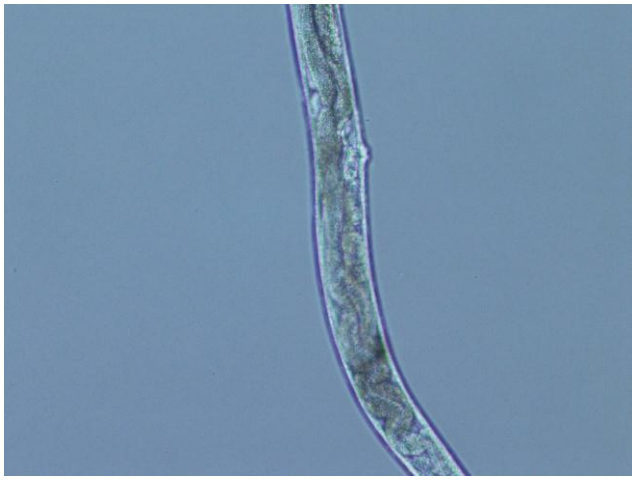

Fig. s5 Bright field photograph of the worm after feeding for 6 h on DiI-labelled *S. aureus* ATCC 25923. The intestinal lumen of the infected worm was significantly distended with bacteria.

Effect of infection time on worm survival

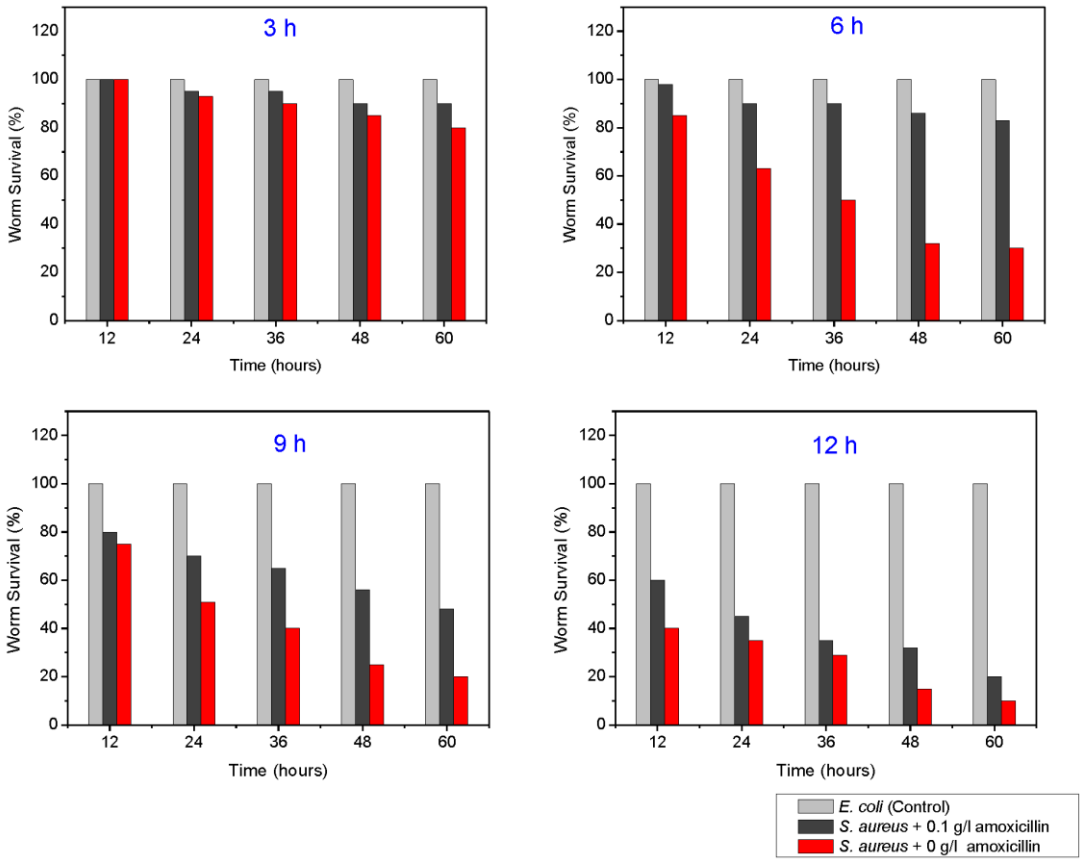

Fig. s6 The effect of infection time on worm survival in 10% BHI in M9 buffer with *S. aureus* ATCC 25923. The worms can be rescued to varying degrees by100 µg/ml amoxicillin after various lengths of exposure time as: 3 h (a), 6 h (b), 9 h (c) and 12 h (d). *E. coli* op50 as control, 110-120 *glp-4; sek-1 C. elegans* used in each tests and 3 independent assays for each exposure time.

Effect of treatment time on worm survival

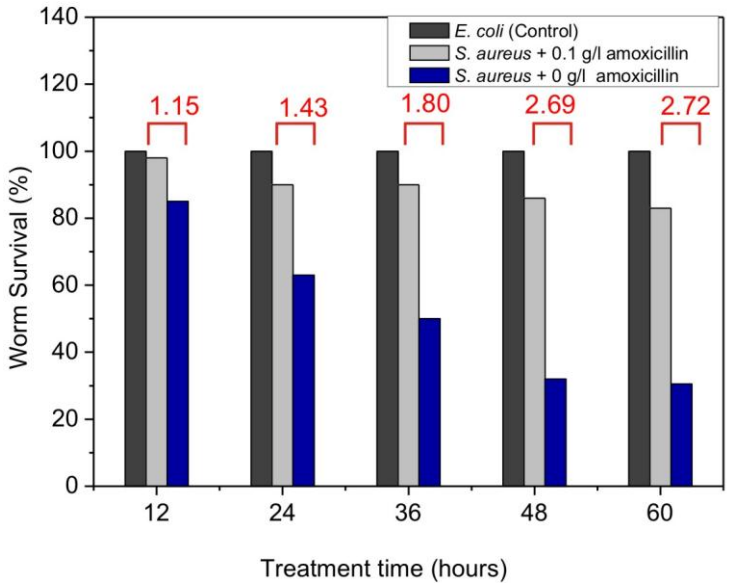

Fig. s7 The effect of treatment time with 100.0 μg/ml amoxicillin on the survival of the infected worms by *S. aureus* ATCC 25923 in 10% BHI-M9 medium. *E. coli* op50 as control, 110-120 *glp-4; sek-1 C. elegans* used in each tests and 3 independent assays for each exposure time.