Supplementary Information

Randomly Distributed Arrays of Optically Coded Functional Microbeads for Toxicity Screening and Monitoring

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+ Equal contribution

Supplementary contents

Supplementary note.

Size optimization of optically coded functional microbeads

The electrostatic system optimization was conducted on the effects of electrode distance from the hardening solution to needle, infuse rate, and applied potential in order to find a proper size of microbeads for the fixed cell concentrations at optical density of 0.7 at 600 nm (7×10^8 cells/ml) with 2% sodium-alginate solution. We observed the decreased bead size as the electrode distance from the hardening solution was decreased. As increasing infuse rate from 2 ml/hr to 10 ml/hr, the size of microbeads was also increased. The bead diameters decrease significantly with the increase in the applied voltages (**Fig. A**). The amount of fluorescent microspheres was also optimized through volume and emission intensity analysis (**Fig. B**). The microbeads diameter of 0.75 mm was found as an optimal for the number of bacterial cells (~7.0 $x10^6$), the number of fluorescent microspheres ($5.3x10^5$) for the distinct identification of the embedded strains in individual bead, and the infusion rate in terms of reliable and prompt bioluminescence emission in response to the tested chemicals.

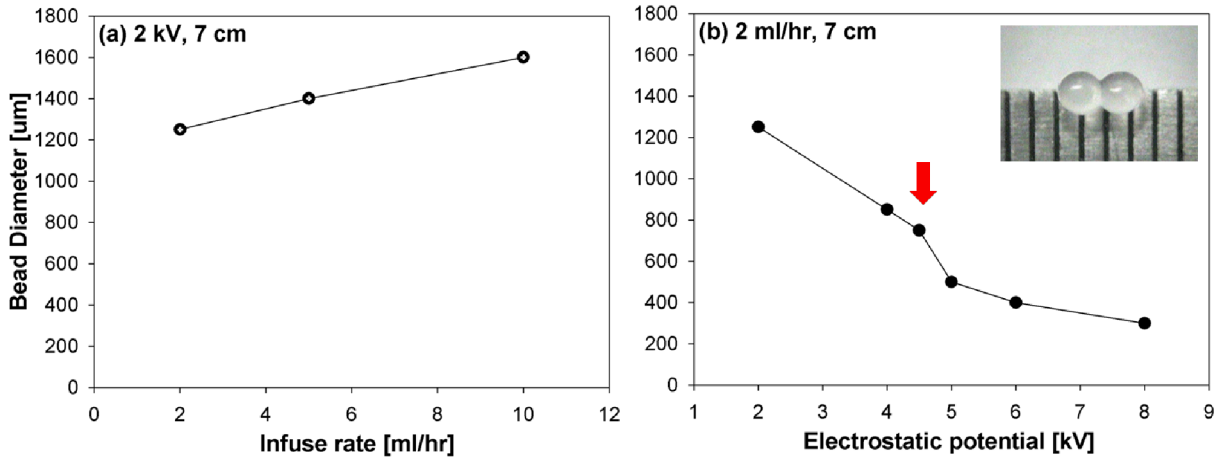
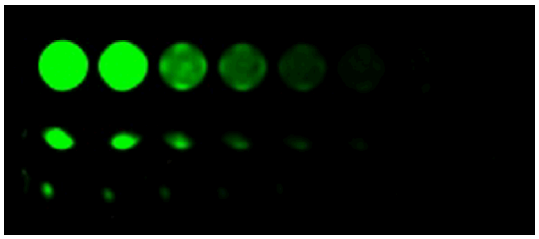


Figure A. Effects of the infuse rate and applied potential on the microbead diameters: 7 cm of the electrode distance from the hardening solution and 30.5 gauge needle were used. The inset in the left panel shows the photograph of the microbead placed on 0.5 mm scaled ruler.

a



b

Volume	Number of Beads							
100 μl	$1x10^7$	$5x10^6$	$2.5x10^6$	$1.25x10^6$	$6.25x10^5$	$3.13x10^5$	$1.56x10^5$	$7.81x10^4$
10 μl	$1x10^6$	$5x10^5$	$2.5x10^5$	$1.25x10^5$	$6.25x10^4$	$3.13x10^4$	$1.56x10^4$	$7.81x10^3$
1 μl	$1x10^5$	$5x10^4$	$2.5x10^4$	$1.25x10^4$	$6.25x10^3$	$3.13x10^3$	$1.56x10^3$	$7.81x10^2$

c

Volume	Total Signal (Photon counts)				
100 μl	32.3628	29.1863	9.97003	3.00667	-
10 μl	6.31	6.01374	3.72444	1.50382	-
1 μl	0.68335	0.54486	-	-	-

Figure B. Optimization of the number of fluorescent microspheres in a single microbead in terms of the fluorescence intensity. (a) A fluorescence image taken from a 96-well plate. This image was taken by Maestro 2™ which is the same instrument used to obtain the image of optically coded microbead chips in this study. (b) The number of fluorescent microspheres which were loaded in the 96 wells plate with different liquid volumes. (c) Total fluorescence photo counts obtained from the wells where only green fluorescence microspheres placed in (a) After auto intensity alien protocol is applied. Only if the optically coded functional microbead contains over $1x10^5$ fluorescent microspheres in 1 μl, the fluorescence can be detectable by Maestro 2™ instrument.

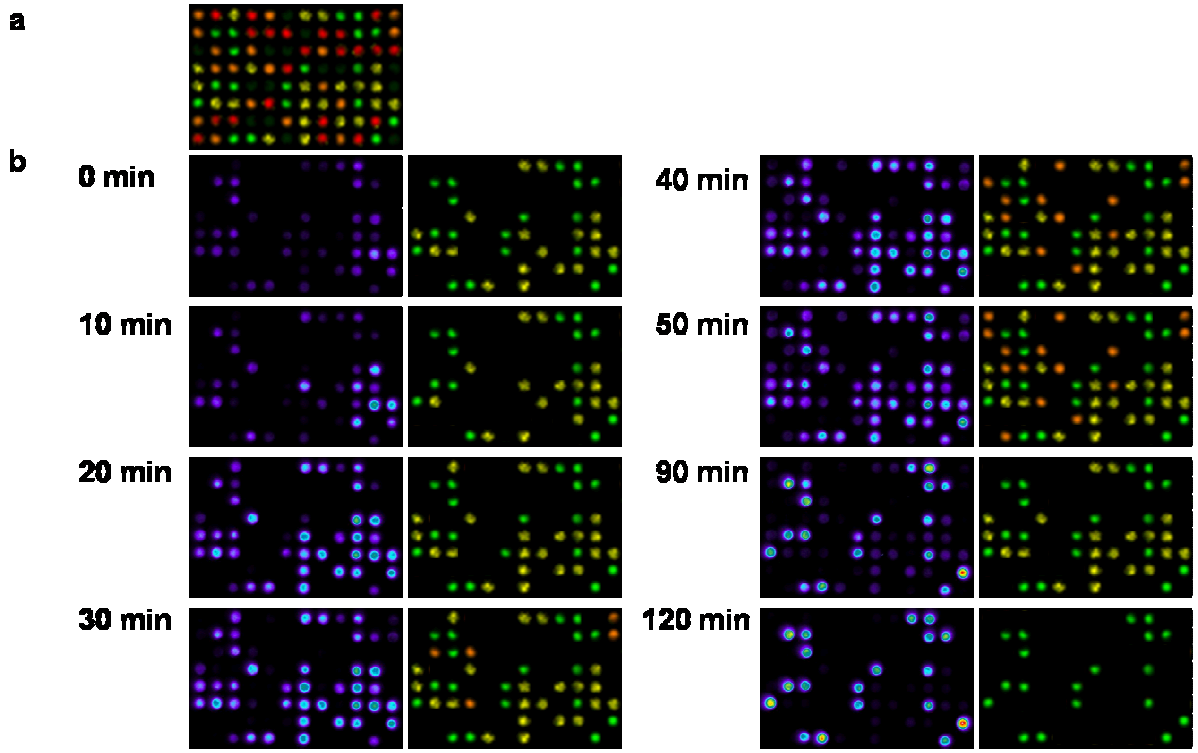


Fig. S1. The changes in the images of fluorescence and bioluminescence emission of the randomly scattered array chips of fluorescent coded functional microbeads for 45 ppm of H_2O_2 within 2 hours. (a) The fluorescence emission from randomly scattered individual microbead with its self-identification fluorescence code. (b) The analysis of the time-lapse images of bioluminescence and fluorescence of the randomly scattered microbead array chip at 0 min, 10 min, 30 min, 40 min, 50 min, 90 min and 120 min after exposure. The first column showed the time course images of the bioluminescence, and the second column presented it corresponding fluorescence image map showing the identification of each microbead. Initially DPD2794 and DPD2540 showed weak bioluminescence as a basal level. At 30 min exposure, DPD2511 started to emit recognizable bioluminescence until 50 min after exposure, when H_2O_2 degradation occurs due to an oxidative defense mechanism such as *katG* in *E. coli*, which is completed within an hour [1]. This oxidative stress trigger an indirect DNA damage which makes induction of DPD2794 coded with green fluorescence that is continued to be remained until 120 min after exposure.

Reference

1. B. C. Kim and M. B. Gu, *Process Biochem.*, 2007, **42**, 392-400.