SINGLE-CELL IMAGING FOR BACTERIA ENUMERATION UNDER LOW OPTICAL RESOLUTION CONDITION BASED ON CELL INDUCED NANOPROBES AGGREGATION IN DROPLETS

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This paper presents a novel method for directly imaging and counting bacteria at single cell level under low optical resolution condition by inducing nanoprobes aggregation on the cell surface and forming cell-core shell particles. Two kinds of gold nanoprobes with polyethyleneimine (PEI-AuNPs) and citrate (SC-AuNPs) modified respectively have been fabricated and could form quick multi-layer adsorption on the bacteria cells. The adsorption leads to a visible precipitate particle which could be automatically imaged and counted in the microdroplet. To validate the method, *E.coli* samples has been quantified in less than 0.5 hour. Compared with traditional plate-spread counting and PCR, no time-consuming procedures of culture and high cost biochemical reagent needed in the developed method.

It has been reported that the live bacteria usually got a negative charged surface and could conjugates of cationic, hydrophobic, monolayer-protected nanoparticles [1]. Based on this principle, two nanoparticles with opposite charging have been designed and fabricated, which can form multi-layer cell-core shell particle (tens of μm) and enables single cell imaging and counting without high resolution optical equipment. As shown in **Fig. 1**, the PEI-AuNPs was firstly mixed with the bacteria samples outside the chip. The PEI-AuNPs could be conjugated on the surface of bacteria membrane to form the first adsorption layer. The microfluidic chip featured with sample mixing microchannels, droplets generation part and droplets storage chambers. The chip has 3 inlets for the mixture of PEI-AuNPs and *E. coli*, SC-AuNPs and oil respectively. After the inlets of aqueous phase, a flexion channel part was designed for the intensive mixing of *E. coli* and nanoprobes suspension. The droplets were generated from the continuous phase shearing on dispersed phase at the T-junction part. A chamber array was designed for droplets storage and imaging.

As shown in the **Fig. 2A**, the PEI-AuNPs showed a diameter about 10~25 nm. In **Fig. 2B**, the PEI-AuNPs was evenly distributed on the surface of *E. coli* cells. After the multi-layer adsorption of gold nanoparticle on the bacteria membrane, the precipitate particle was visible under optical microscope, as shown in **Fig. 2C**. When under 100X magnification, the cell was still not obvious, as shown in **Fig. 3A**. Then the SC-AuNPs was added to the mixture, the precipitate induced from the gold nanoparticle multi-layer adsorption was obvious visible under 100X magnification as shown in **Fig. 3B**. The droplets with precipitation particle were distinguishable compared with other empty droplets under magnification of 100X. In order to insure the precipitation particle was induced by the bacteria cells, the **Fig. 3C** gave a blank control test that replaced the bacteria sample with pure water. There was no aggregation happened inside droplets without bacteria. The images of the droplets was automatically processed though image J as shown in **Fig. 4A-C**. In the range of 10^3~10^5 cfu mL^{-1}, as shown in **Fig. 4D**, the calibration plots displayed a good nonlinear curve relationship between with the droplets image counting and the concentration of *E. coli*.

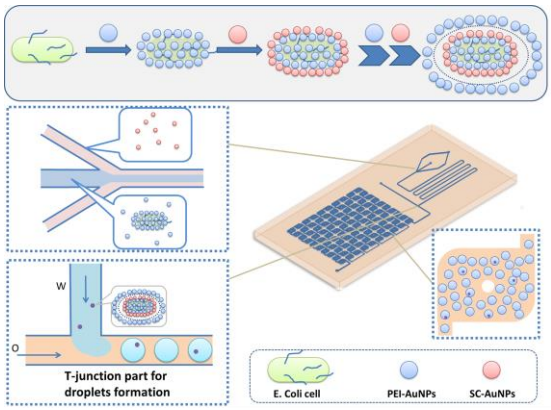

Fig.1 Preparation and schematic illustration of the bioassay protocol.

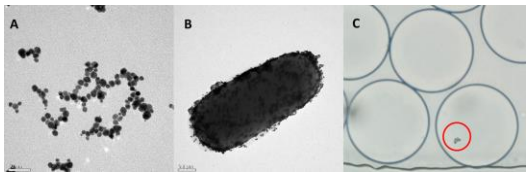

Fig. 2 TEM images of prepared PEI-AuNPs (A); E. coli conjugated with PEI-AuNPs (B) ; E. coli conjugated with multi-layer gold nanoparticles under 400X optical microscope (C).

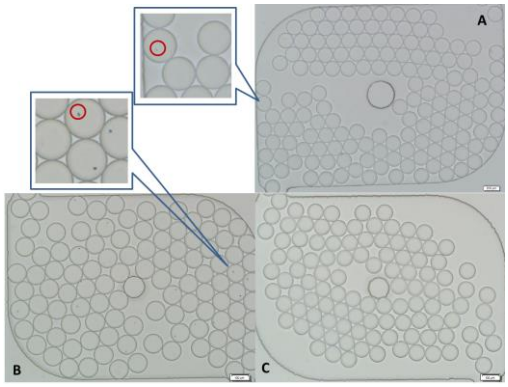

Fig. 3 Droplets generated with bacteria encapsulated pictures under magnification of 100X (A); bacteria and nanoprobes encapsulated (B); and nanoprobes with no bacteria encapsulated (C).

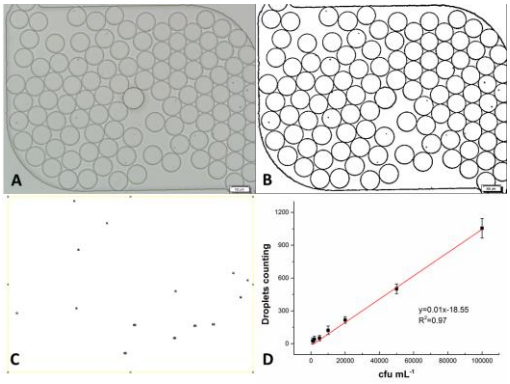

Fig. 4 The picture processing and bacteria counting with imageJ (A, B, C); and calibration curves of the bioassay toward E. coli (D).

Table 1. Comparison of different methods for the detection of bacteria.

Method	Analysis time	LOD	Working range	Ref.
Non functionalized AuNPs (PCR)	3-4 h	2.6×10^4 copies/μL	2.6×10^4-2.6×10^{11}	[2]
Functionalized AuNPs (PCR)	<8 h	2×10^9 copies/μL	2×10^9-2×10^{11}	[3]
HRP-amplification DNA sensor strips (Elctrochemical)	3-5 h	$<10^3$ cfu mL^{-1}	-	[4]
Magnetic Nanoparticles-Aptamer (Colorimetric)	~1 h	7.5×10^5 cfu mL^{-1}	-	[5]
Double probe amplification (Microfluidics)	~1 h	1×10^3 cfu mL^{-1}	1×10^3-1×10^5 cfu mL^{-1}	This work

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