Single *E.coli* bacteria detection using a chemiluminescence digital microwell array chip

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INTRODUCTION

Bacterial contamination and infection is a major global health issue with the rising prevalence of antibiotic resistance, with *E. Coli* as an essential indicator for faecal contamination in drinking water. The current detection methods, such as cultures and immunolabeling, have various drawbacks, being unable to detect small amounts of *E. Coli* contamination, having a long processing time, and being unable to differentiate live from dead bacteria in water samples.

This study aims to investigate the chemiluminescence digital microwell array chip (chemLDMA chip) and its ability to quantify single *E. Coli* bacterium from various water samples under different environmental conditions.

METHODOLOGY

6-Chloro-4-Methylumbelliferyl-β-D-Glucuronide (6c- MUG) was used as the probe, which is cleaved by the commonly expressed enzyme β-D-glucuronidase (GUS) on *E. coli* to release fluorescein.

Three E.coli strains (ATCC 25922, ATCC 19020, EK-19) gave four samples (3 pure, 1 mixed in 1:1:1 ratio) tested in different turbidites (1, 5, 10 Nephelometric Turbidity Units) diluted to the same bacterial concentration. Samples were treated with temperatures from 4-45 °C and pH values from 5-9. Counting methods and plate counting were compared. Chlorination treatment was given to assess ability to differentiate live and dead *E. coli*.

Bright-field and fluorescent images were acquired along with real-time monitoring of E.coli detection via time-lapse with a Zeiss microscope, and ImageJ software was used for analysis. A one-way ANOVA with post hoc test and 0.05 significance was used to compare correlation and variance.

RESULTS

Single E.coli detection in microwell

The LDMA chip provided million-fold higher concentrations of bacteria compared to 96 well plate, thus increasing enzymatic reaction and detection speed significantly. As only viable E.coli emit bright blue fluorescence due to sufficient 6c-MUG hydrolysis, viability could be determined. The fluorescence detection of occupied microwells were correlated with the sample concentration calculated based on the Poisson distribution, with low and high magnification offering similar results.

Average generation time of *E.coli* from 1st to 2.5 was 36.2 ± 7.8 min. Due to the depletion of nutrition, bacteria grew slower at the later stage with a generation time between 2.5-4 h of 71.5 ± 28.4 min. The presence of *E. coli* could be detected in 2 h (qualitative), with accurate digital quantification accomplished in 4 h (quantitative).

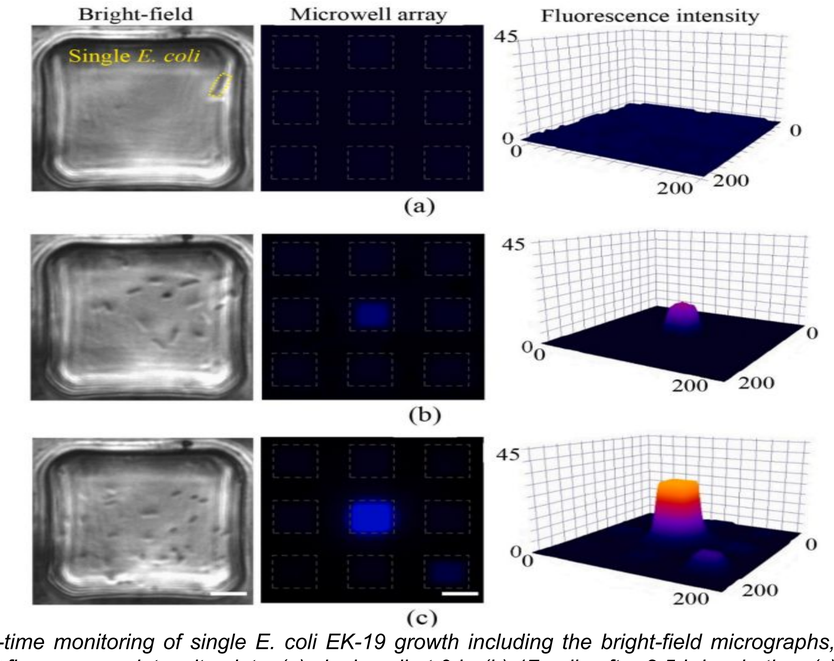

Fig. 1. Real-time monitoring of single E. coli EK-19 growth including the bright-field micrographs, fluorescence pictures and fluorescence intensity plots. (a) single cell at 0 h. (b) 17 cells after 2.5 h incubation. (c) 30 cells after 3.5 h incubation. The scale bars in bright-field and fluorescent images denote 5 and 30 μm, respectively.

E. coli detection in different environmental samples

Detection of *E.coli* was performed under mimicked environmental samples with changes to: TSS(total suspension solids), temperature, pH, and chlorination treatment. TSS, temperature and pH showed no significant difference in detection compared to conventional methods, and the LDMA chip was able to differentiate between live and dead E.coli through chlorination.

Hence, the chemLDMA chip could be easily employed for simultaneous sampling at multiple testing points (environmental or food samples) or longitudinal sampling at different time points (clinical samples from same patient) to obtain statistical data for analysis.

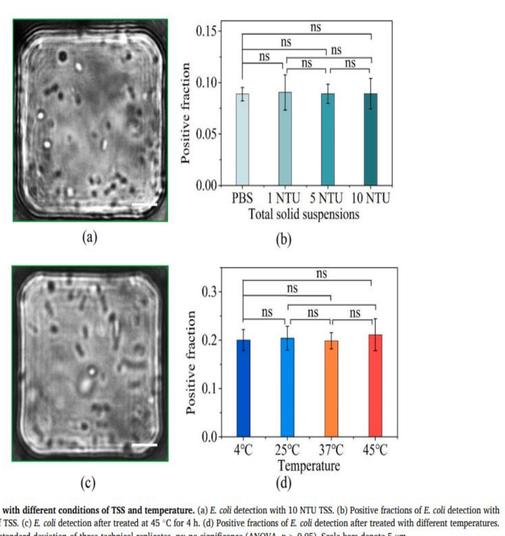

Fig. 3. *E. coli* detection with different conditions of TSS and temperature. (a) *E. coli* detection with 10 NTU TSS. (b) Positive fractions of *E. coli* detection with various concentrations of TSS. (c) *E. coli* detection after treated at 45 °C for 4 h. (d) Positive fractions of *E. coli* detection after treated with different temperatures. Error bars represent the standard deviation of three technical replicates. ns: no significance (ANOVA, $p > 0.05$). Scale bars denote 5 μm.

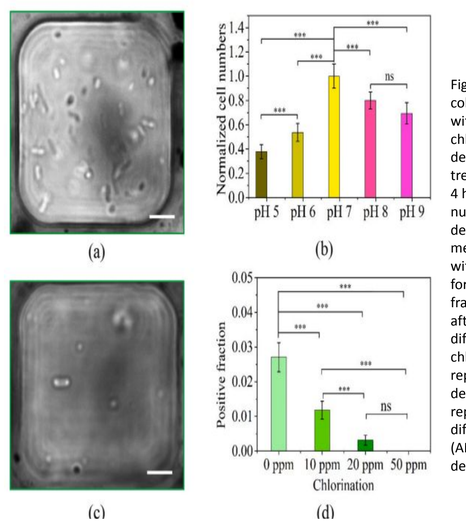

Fig. 4. Detection of treated E. coli in the solutions with different pH and chlorination. (a) EK-19 colony derived from live E. coli treated with pH 5 solution for 4 h. (b) Normalized cell numbers of E. coli detection using proposed method. (c) E. coli treated with chlorination of 50 ppm for 4 h. (d) Positive fractions of E. coli detection after treated with different concentrations of chlorination. Error bars represent the standard deviation of three technical replicates. ***: Significance difference, ns: no significance (ANOVA, p > 0.05). Scale bars denote 5 μm.

DISCUSSION

The chemLDMA chip was able to quantify single *E. coli* within 2-4h, which is significantly less than 24h from conventional culture methods. Due to the strong correlation between the micro-colonies and bright fluorescence intensity, the chemLDMA chip can be used for direct colony observation for *E. coli* quantification without sophisticated instruments, reducing imaging time. the chemLDMA chip is not significantly affected by variations in temperature, TSS, pH, and chlorination, and is able to differentiate viable from dead *E. coli* bacteria.

Compared to specific existing methods, the chemLDMA chip has:

1. Higher throughput and faster screening time than digital dipstick
2. Lower throughput but faster screening time and less prone to contamination & human error than agar-based microwell-array chip
3. Higher throughput, less complex mechanism and less prone to contamination than microwell-SERS system

However, some limitations of the chemLDMA chip include how it relies on the presence of the GUS enzyme; this can result in false negatives from E.coli strains that do not carry the enzyme. The accuracy of the chemLDMA chip can also be affected by delays in time to analysis due to transport or other reasons, as it relies on the presence of viable *E.coli* to propagate from the sample.

The chemLDMA chip has much potential to be improved. The current detection limit of the chemLDMA chip is 560 CFU/mL using a 1.8μL sample volume; this can be improved to 56 CFU/mL if the sample is concentrated 10-fold using centrifugation or using multiple chips simultaneously. Moreover, a mobile imaging analysis platform (Chen et al., 2021) could be integrated with the chemLDMA chip to allow for portable, point-of-care testing. There is also much heterogeneity of single *E. Coli*, with different doubling times and metabolic activities (Wu et al., 2018), highlighting the potential of the chemLDMA chip in single bacteria research.

CONCLUSION

The chemLDMA chip is effective for rapid detection of viable E. coli at the single-bacterium level. It is quicker than conventional methods at 2-4 hours, can detect different E. coli strains, and differentiate live and dead bacteria. Additionally, the chemLDMA chip has tolerance to TSS and temperature, and the ability to detect viable E. coli after pH and chlorination treatments. Hence, chemLDMA could be a useful alternative to conventional plate counting, and potentially in related fields such as food monitoring and bacterial infection diagnosis clinically.