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Lab-on-a-disc device for screening of genetically engineered E.coli cells

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Introduction

Due to the limited natural availability and huge demand of plant secondary metabolites (e.g. p-Coumaric acid (pCA)) in the production of health care and nutritional products, E.coli system are often modified to construct strains containing artificial biosynthetic pathways for the production of these metabolites[1]. Usually, HPLC[2], TLC[3] and spectrophotometry[4] are the common approaches available to screen the modified strains by quantifying the produced secondary metabolites, which are expensive, tedious and time consuming. In this work, we propose electrochemical detection on a lab-on-a-disc (LOD) platform, as a low cost, fast, and easy-to-use approach with possibility of multiplexing as an alternative to traditionally used screening methods.

Fabrication

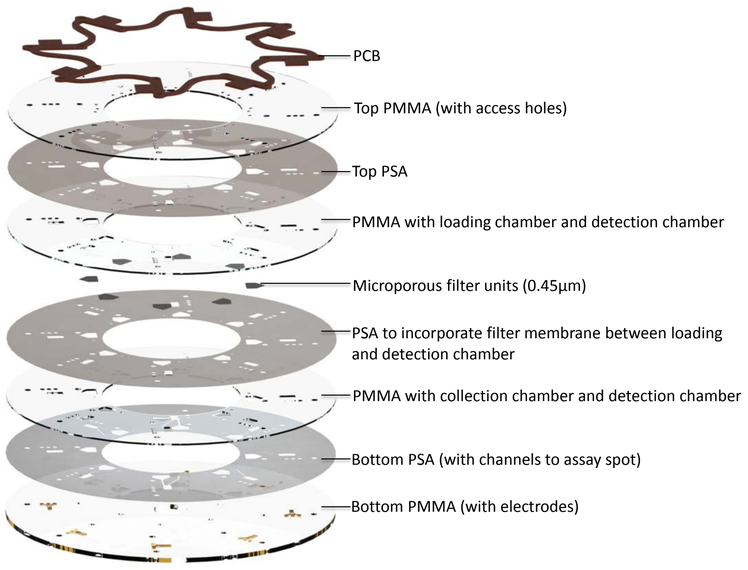

Figure 1. Exploded view of microfluidic assembly: 3-pressure sensitive adhesive (PSA) layers are interspread between 4-Poly methyl methacrylate (PMMA) substrates with portable circuit board (PCB) mounted at the top for interfacing the electrodes to the potentiostat.

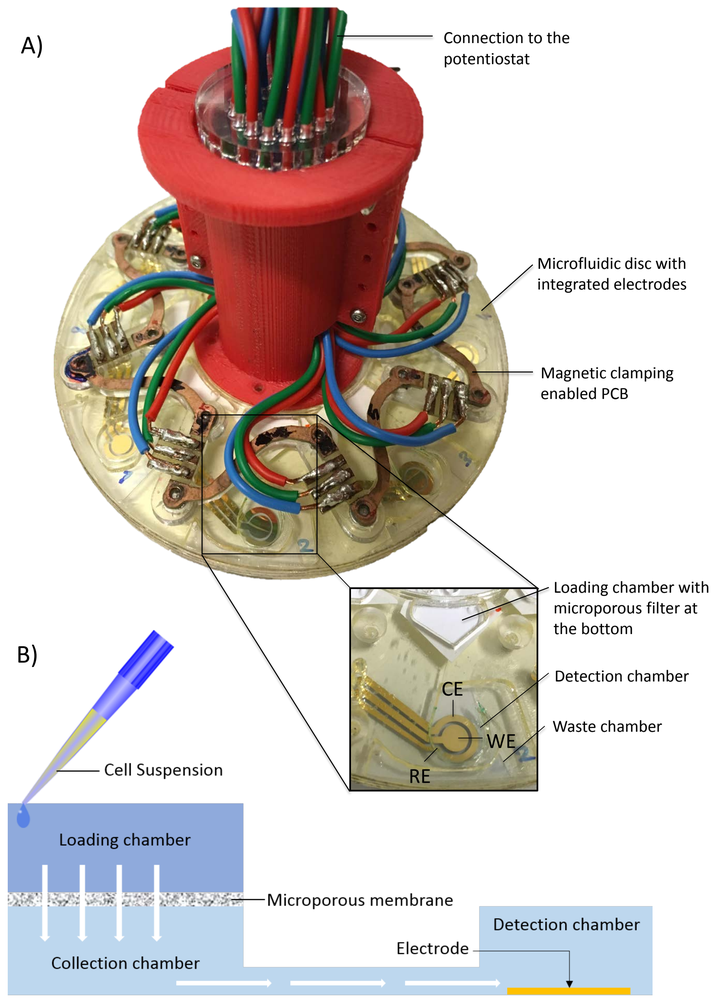

Figure2. A) Image of LOD assembly for electrochemical measurements; B) Schematic showing the Cross section of the microfluidic layout (arrows shows the direction of flow while spinning the disc).

Electrode Characterization and pCA detection

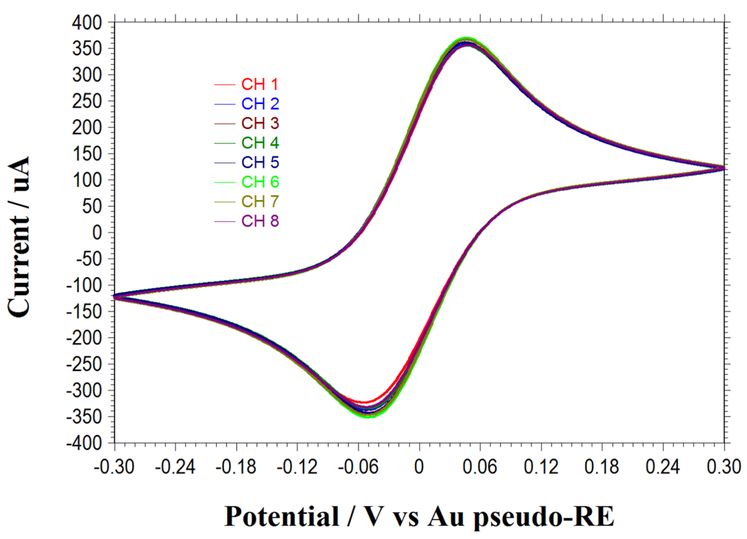

Figure3. Cyclic voltammograms recorded simultaneously on an array of 8-electrodes on the disc in the presence of 10mM ferro/ferricyanide in PBS supporting electrolyte; scan rate 50mV/s.

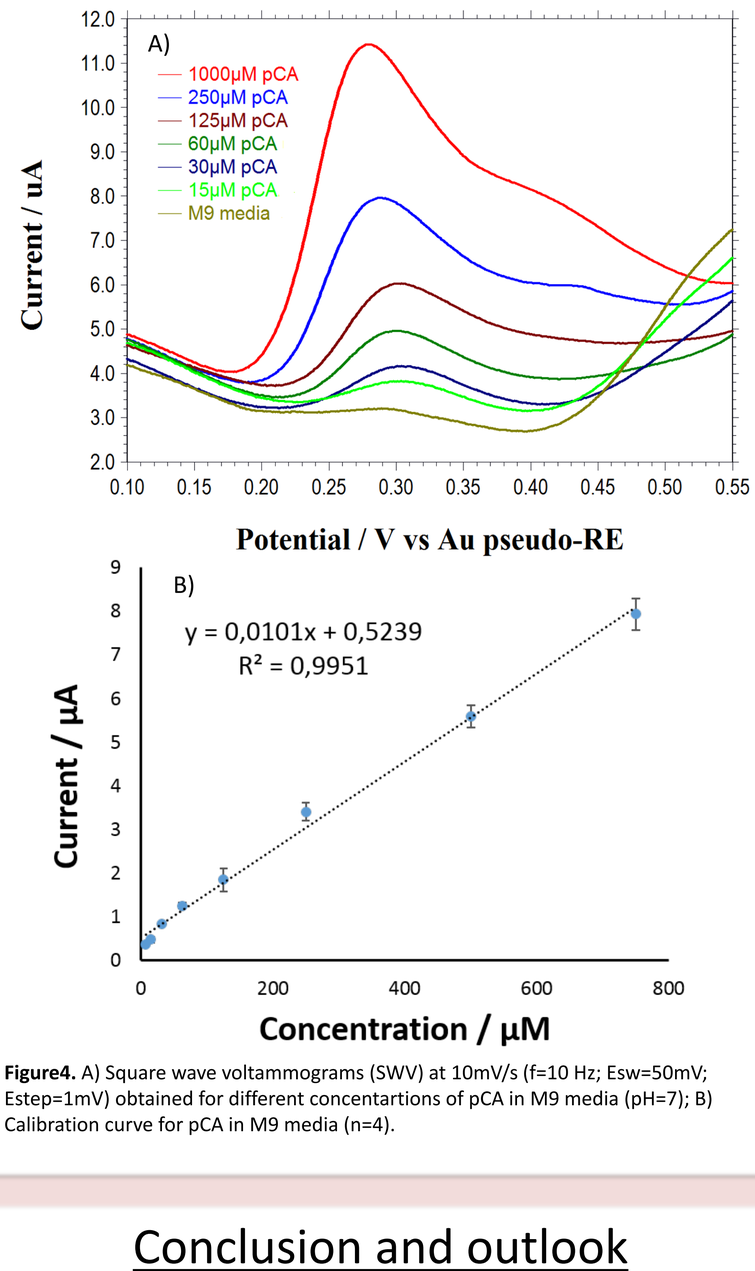

Figure4. A) Square wave voltammograms (SWV) at 10mV/s (f=10 Hz; Esw=50mV; Estep=1mV) obtained for different concentartions of pCA in M9 media (pH=7); B) Calibration curve for pCA in M9 media (n=4).

pCA detection from supernatant

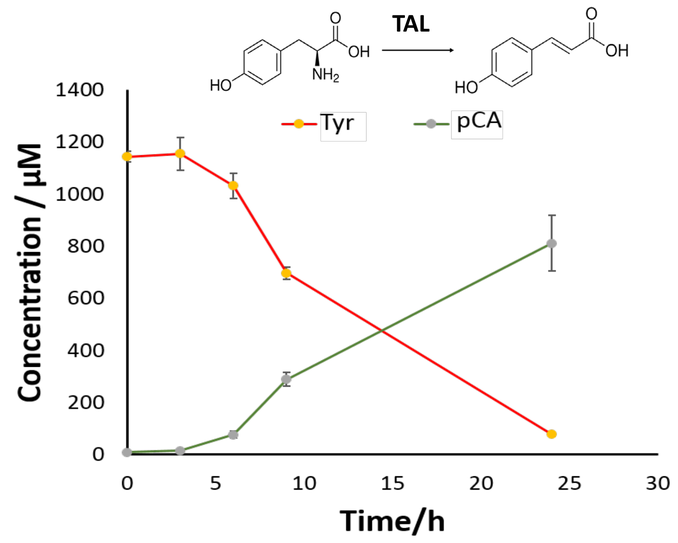

Figure5. Concentration of Tyrosine and pCA in supernatant of TAL(+) E.coli strain from time 0 to 24 hours obtained by HPLC; the inset shows the Conversion of Tyrosine to pCA by Tyrosine Ammonia lyase gene (TAL)

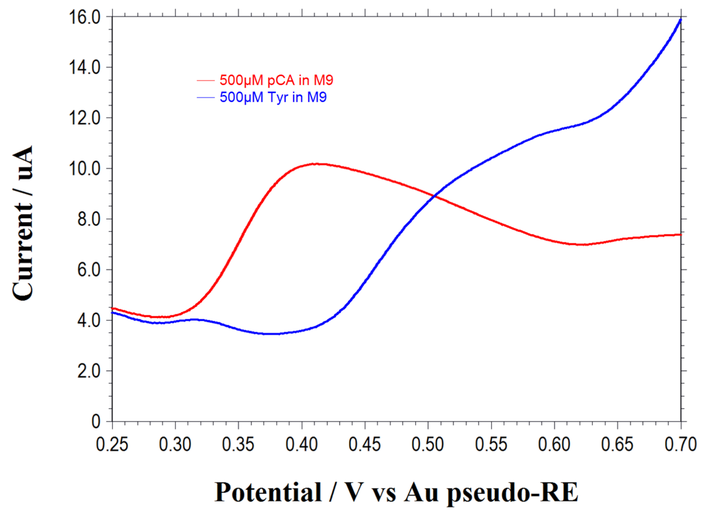

Figure6. SWV at 10mV/s obtained for pCA and Tyrosine in M9 media (pH=6); pH matched with the physiological pH of the supernatant of TAL(+) E.coli strain after 24 hours of cell culture.

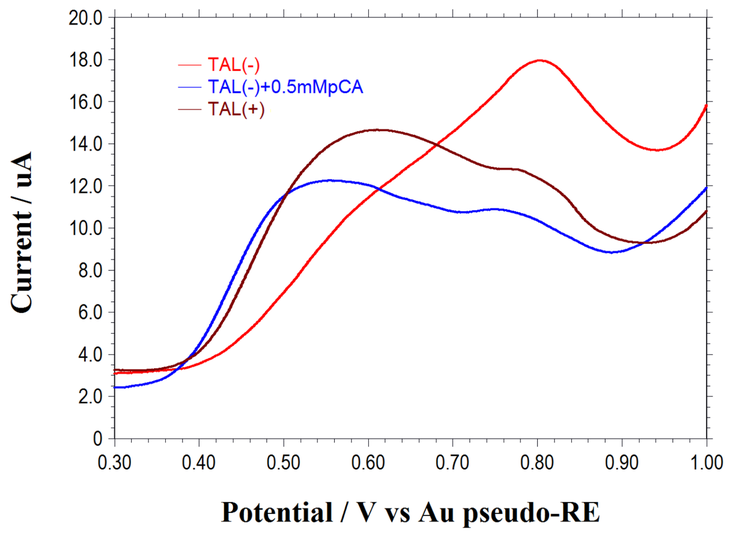

Figure7. SWV at 10mV/s obtained for supernatant from TAL negative strain, TAL positive strain and pCA spiked in TAL negative strain.

Conclusion and outlook

The Lab-on-a-disc system with integrated reproducible electrodes is suitable for reliable detection of pCA from culture medium as well as from the bacterial supernatant. Integrated microporous filter membrane allows the cell free detection directly on to the platform without any prior sample pretreatment. Genetically engineered E.coli cells can easily be distinguished and screened for the successful integration of TAL (Tyrosine Ammonia Lyase) gene in bacterial genome by detecting the presence of pCA in supernatant after 24 hours of culture. The developed LOD platform will be used to screen genetically modified bacterial strains to evaluate the pCA production rate during the culture.

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