Living Microorganisms Entrapped in Nano-Structured Latex Formulations Piezoelectrically Printed onto Bioelectronic Devices

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Abstract:

Using piezoelectric deposition, living microorganisms in a latex formulation can be printed onto bioelectric devices for mercury (Hg^{+2}) detection. When *Escherichia coli* HB101 containing the *mer-lux* plasmid pRB28 is entrapped in a nano-porous latex ink, it survives piezoelectric printing, drying, rehydrating, and being induced with Hg^{+2} to luminescence through Lux synthesis. The reactivity of *E. coli mer-lux* when printed in dot arrays with two piezo tips (opening: 25 μm and 50 μm) was examined by inducing with 1 to 10,000 nM Hg^{+2}. The necessity of forming nano-pores in the dried latex ink and the ability of *E. coli* to survive piezoelectric deposition were investigated. Reactive microbial inks have many potential applications such as detecting mercury in the environment, in fish, or in metal recovery.

Introduction:

Organic mercury is a biologically active form of mercury that enters the environment as inorganic mercury through natural (volcanoes) and human (industrial plants) sources. Inorganic mercury easily oxidizes in the atmosphere and travels, as organic (methyl mercury) and ionic mercury (Hg^{+2}), through rainwater, through sediments, and through fish consumption, bringing it in contact with humans. When consumed, organic (methyl mercury) and ionic mercury (Hg^{+2}) can cause lose of nerve cells which leads to numbness, difficulty in speech, along with loss of coordination, sight, and hearing.

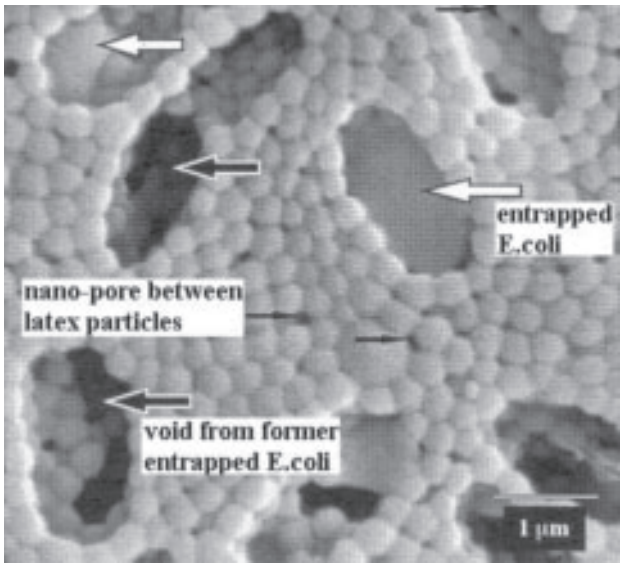

Figure 1: Nano-structured E. coli embedded acrylate/vinyl acetate latex composite.

Escherichia coli HB101 pRB28 *mer-lux* is capable of detecting biologically available Hg^{+2} and reacting through luminescence [1, 2]. *E. coli* is tough and can survive piezoelectric printing, drying, rehydrating, and being induced with Hg^{+2} and still express new gene products. The Hg^{+2} acts as a signal to the *E. coli* and the *E. coli* respond by luminescence, which can be detected by a scintillation counter. The *E. coli* are entrapped within a nano-structured material (Figure 1). Nano-pores are the spaces between the partially coalesced polymer particles. Nano-pores serve as a pathway for Hg^{+2} to get to the entrapped *E. coli*.

The *E. coli* latex polymer mixture which is reactive to Hg^{+2} when dried (printed) and rehydrated will be referred to as "smart" ink. "Smart" ink is similar to ink-jet ink because they both contain four basic properties: solvent, dye/pigment, binder, and additive. The piezoelectric printer (Nano Plotter GeSIM) is a highly accurate printer with the ability to print 10 x 10 dot arrays, with varying pitches and number of droplets per dot, with two different tips openings (nano = 50 μm and pico = 25 μm). Ultimately the piezoelectric printer could be used to print "smart" ink onto a bioluminescent bioreporter integrated circuit (BBIC)

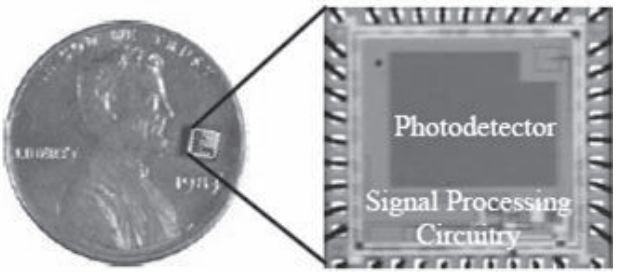

Figure 2: BBIC is 2 x 2 mm.

(Figure 2) [3]. This would allow the BBIC to detect Hg^{+2} through the luminescence of the *E. coli* entrapped in the nano-structured latex formulation.

This project had three goals. The first goal was to determine if the "smart" ink would be reactive to various Hg^{+2} concentrations when piezoelectrically printed with the two different tip openings. The second was to determine if nano-pores were a necessary component within the dried "smart" ink. Finally, the third goal was to determine the reactivity change, if any, between a manually pipetted macrodot (not printed, not subjected to strong sheer stress) of similar volume to a piezoelectrically printed array of "smart" ink.

Procedure:

Grow an overnight *E. coli mer-lux* culture. The following morning, start a new culture at an OD_{600}=0.1 and let it grow until OD_{600}=1.0. Centrifuge and re-suspend the *E. coli* multiple times. Obtain the weight of the wet cell pellet. Prepare the "smart" ink recipe following Lyngberg et al. [4] but with a dilution with water to 25% of the original concentration. Glycerol and sucrose were only used when nano-pores were needed in the "smart" ink [1]. Print the "smart" ink with the piezoelectric printer (Nano Plotter GeSIM) in 10 x 10 dot array with five drops/dot with either the nano (50 μm opening) or pico (25 μm opening) tip onto polyester. When making macrodots, an estimated volume was used for the 10 x 10 piezoelectrically printed array. Pipette 250 nl (nano tip array) and 100 nl (pico tip array) onto polyester and let dry for an hour. Rehydrate and induce the arrays or macrodots with Hg^{+2} concentrations of 0 nM, 1 nM, 10 nM, 100 nM, 1,000 nM, and 10,000 nM. Place into a liquid scintillation counter and record the luminescence for 720 minutes.

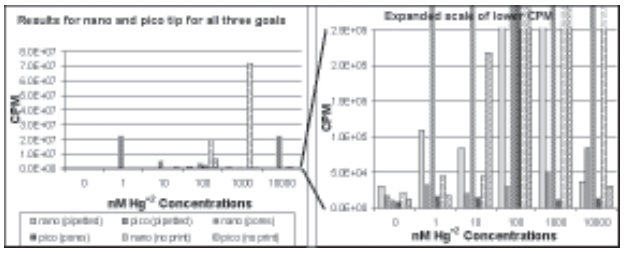

Figure 3: All E. coli mer-lux Hg^{+2} induction results.

Results (Figure 3):

Goal 1: "Smart" ink can be piezoelectrically printed through the nano and pico tips and still be able to react when induced with Hg^{+2}. Goal 2: When nano-pores are created by the addition of glycerol and sucrose strong luminescence does occur from the nano and pico tips. There is an average of over 200-fold increase, with a maxiumum of 2000-fold increase, when there are nano-pores versus when there are not nano-pores. Goal 3: Some vitality is lost when the "smart" ink is piezoelectrically printed versus manually pipetted in a macrodot.

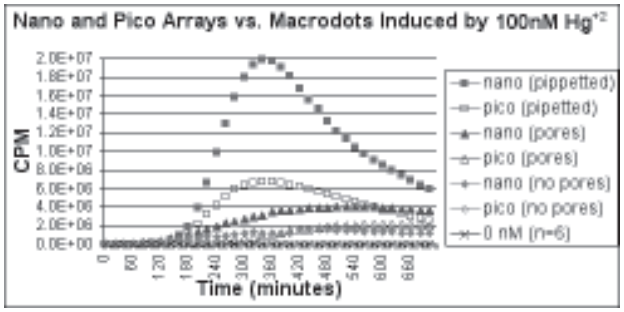

Figure 4: All E. coli mer-lux induction results at 100 nM Hg^{+2}.

Conclusions (Figure 4):

The "smart" ink can be printed with the nano and pico tips and still detectably react to Hg^{+2}. Nano-pores are necessary for detectable luminescence. *E. coli* suffer a lose of vitality but are still reactive when piezoelectrically printed as an array versus pipetted by hand as a macrodot.

Future Work:

Manipulate the "smart" ink recipe to get smaller dot and pitch size as well as to detect lower Hg^{+2} concentrations. Determine an improved "smart" ink recipe to increase *E. coli* vitality and reactivity when piezoelectrically printed.

Acknowledgments:

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Figure 1 and 2: Courtesy of Professor M. C. Flickinger, University of Minnesota, Nanotechnology Day, May 2004.

References:

[1] Lyngberg, O.K. et al. Biotechnol. Bioeng. 62: 44-55 (1999).
[2] Lyngberg, O.K. et al. J.Ind. Microbiol. Biotechnol. 23: 668-676 (1999).
[3] Nivens, D.E. et al. Journal of Applied Microbiology. 96: 33-46 (2004).
[4] Lyngberg, O.K. et al. Biotechnol. Prog. 17: 1169-79 (2001).