# Research Proposal

Microfluidic Biosensors for the Detection of Organophosphates

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## I. Introduction & Background

Here we propose two types of microfluidic sensors for the detection of organophosphates (OP) neurotoxin. These devices use either organophosphorous hydrolase (OPH) enzyme or OPH-expressed *E. coli* to hydrolyze OP, thereby producing less toxic chemicals. For example, paraoxon, a model neurotoxin, is hydrolyzed by OPH to generate *p*-nitrophenol (PNP), diethyl phosphate, and protons (Figure 1). Thus, the main detection targets are pH change and PNP. The first approach proposed here relies

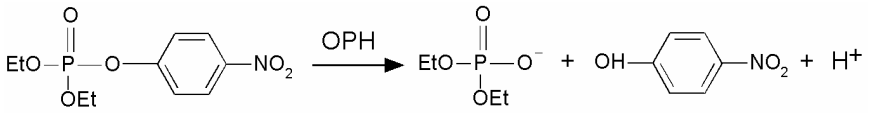



**Figure 1. Paraoxon hydrolysis by organophosphorous hydrolase (OPH).**

on an intracellular pH change in OPH-expressed *E. coli* cells (Figure 2). OPH-expressed cells loaded with a pH-sensitive fluorescence dye function as both a bioreactor and a biosensor. The continuous flow of paraoxon into the channel will change the intracellular pH of *E. coli* immobilized on the channel surface, leading to a change in fluorescence intensity.

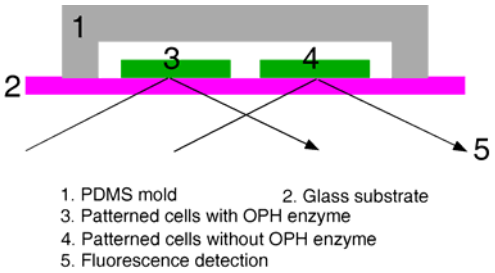



**Figure 2. A schematic diagram of microfluidic sensor for OP based on intracellular pH change in OPH-expressed *E. coli*.**

The other approach involves highly sensitive electrogenerated chemiluminescence (ECL) detection (Figure 3). An OP solution is pumped into the bottom channel and an ECL cocktail is pumped through the top channel. The two solutions do not mix because of laminar flow, but they are in electrical contact via the short crossover channel. Paraoxon is converted to electroactive PNP in the bottom channel when the solution passes through the microreactor, which is filled with beads having the OPH enzyme immobilized on their surface. The presence of PNP is detected by anodic oxidation at the electrode in the bottom channel of the microfluidic device. The electron resulting from this process is used at the cathode in the upper compartment to initiate an ECL reaction cascade. Because the photon flux is proportional to the rate of nitrophenol oxidation, the concentration of paraoxon can be qualitatively correlated with the ECL signal.

Recently, our group has been developing microfluidic devices that can perform multi-step reactions or multi-target sensing. We have demonstrated potential analytical applications of well-defined micro-reactor zones containing enzyme-[1,2], or DNA-immobilized beads[3] and hydrogel micropatches containing enzymes[1] and cells.[4] Furthermore, the beads have been shown to be very simple and effective for mixing solutions in microfluidic channels.[5] Our group has also reported that ECL can be a highly

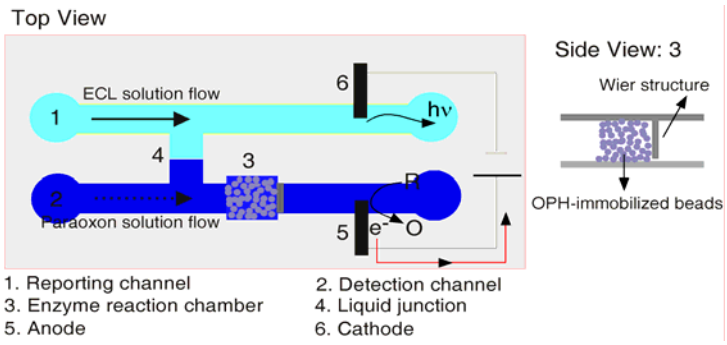



**Figure 3. Schematic diagram of a dual-channel microfluidic sensor for OP detection.**

sensitive detection module in microfluidic sensor applications.[6,7] The proposed project integrates these individual findings into a single, multifunctional sensor.

**OP detection methods.** OPs are toxic because they bind strongly to acetylcholinesterase (AchE), which is the enzyme responsible for breaking down the neurotransmitter acetylcholine. It is not surprising, therefore, that many of the first OP sensors were based on AchE inhibition.[8,9] The AchE family of sensors provided a wealth of information about how OP sensors could be optimized, but they generally suffered from low selectivity, long analysis time, and instability because of strong irreversible binding of certain inhibitors.[10] Several research groups have reported OPH-based sensors for the detection of OPs.[11-21] These OPH-based sensors have generally been more effective than the first-generation AchE sensors.[8,9] PNP has been detected by absorption spectroscopy[13] and amperometric methods.[15,18,22] pH changes have been probed using pH-sensitive fluorophores[16,23] and potentiometric methods.[11,14,15,24] Although fluorescence detection has shown the highest sensitivity, fluorescence sensors based on living cells immobilized within a microfluidic device has not been reported yet. Amperometric methods have typically demonstrated better sensitivity than other electrochemical and absorption methods, and they seem likely to be the method of choice for fabricating microsensor devices. For example, in a report that is closely related to the work proposed here, Wang and coworkers showed that it is possible to anodically oxidize PNP using screen-printed carbon electrodes coated with OPH enzymes and nafion.[15] Although our approach is based on the electrochemical approach demonstrated by Wang, the sensitivity can be greatly improved by coupling the oxidation of PNP to the ECL reaction.

**Cell immobilization methods.** Although cell-based sensing systems often exhibit a long response time, because of slow diffusion through the cell wall, as well as poor selectivity for substrates,[25] they have several advantages compared to enzyme based systems: (1) no enzyme extraction and purification is required; (2) depending on the environmental conditions cells may have a longer lifetime than purified enzymes;[26] and (3) cells provide a contained volume in the picoliter range that may reduce the necessity of microfabricating ultrasmall reaction vessels.[27,28] Cells can be immobilized onto surfaces by nonspecific adsorption, covalent bonding, biospecific affinity, or by entrapment with a polymer.[25] Methods for patterning cells onto surfaces include microcontact printing,[29-31] membrane-based patterning,[32] flow-based methods,[31,33] and hydrogel-based photolithography.[34] We and others have used photo-crosslinked hydrogel polymers for cell immobilization within microfluidic systems, because cells within hydrogels do not generally suffer a severe loss of cell viability.[35,36] Moreover, hydrogels

can be easily photopolymerized in a wide variety of geometries within channels after the microfluidic device is fully assembled. [1,37]

**Dual-channel microfluidic device.** For sensors based on ECL detection, a dual-channel microfluidic device will be used. Previously our group reported a two-channel microfluidic sensor based on the well-established anodic ECL reaction cascade involving oxidation of $Ru(bpy)_3^{2+}$ and tripropylamine (TPA).[6,7] The electroactive analytes detected included benzyl viologen ($BV^{2+}$), $Fe(CN_6)^{3-}$, and $Ru(NH_3)_6^{3+}$. The two-channel approach provides high sensitivity for detecting electroactive species by observing photons derived from the coupled ECL reaction. This dual-channel microfluidic sensor is composed of three key parts: (1) an H-shaped microfabricated channel; (2) a microchamber containing enzymes immobilized on beads; (3) a detector electrode and an ECL reporter electrode. From these results, we expect that a dual-channel microfluidic device based on a cathodic ECL reaction cascade involving $Ru(bpy)_3^{2+}$ and $S_2O_8^{2-}$ will work as well.

## II. Techniques

**Microchannel fabrication.** PDMS microfluidic device will be fabricated following a literature procedure.[38] Briefly, PDMS polymer molds are prepared by casting a PDMS prepolymer against a positive photo resist master and curing at 65°C for 2 h. The PDMS mold is irreversibly sealed to a clean microscope cover glass.

**Fluorescence spectroscopy.** Fluorescence spectroscopy will be used to study the fluorescence intensity change of *E. coli* cells caused by intracellular pH change. These experiments are undertaken using *E. coli* cells which are pre-loaded with pH-sensitive fluorescence dye and re-suspended in pH-controlled buffer solution.

**Fluorescence microscopy.** Fluorescence microscopy is extensively used to obtain images of biomaterials such as cells, DNA, proteins, and antibodies labeled with fluorophores. Microscopy will be used to probe fluorescence intensity changes caused by cytoplasmic pH changes within *E. coli* cells immobilized in the microfluidic channel.

**Electrochemistry.** Three electrode cells, a potentiostat, and an X-Y recorder are used to obtain cyclic voltammograms of PNP and paraoxon. This data will determine the potential region that should be used to couple PNP to the ECL reporter reaction.

## III. Preliminary Results

This section consists of three parts. The first part describes the fabrication of hydrogel-entrapped *E.coli*, one of the methods used for immobilizing cells in a microfluidic channel. The micro-bioreactor functionality of hydrogel-entrapped *E. coli* will also be described. The second part discusses preliminary results of a microfluidic sensor for OP based on intracellular pH changes in OPH-expressed *E. coli*. The third part describes preliminary results from a study of a dual-channel microfluidic sensor for OP that employs an ECL reporting system.

**A Microfluidic Bioreactor Based on Hydrogel- Entrapped *E. coli*: Cell Viability, Lysis, and Intracellular Enzyme Reactions.[4]** Hydrogel immobilization of biological materials is effective because pores within the gel are on the order of 1 – 10 nm in diameter.[39-41] This permits ingress of small molecules, such as enzyme substrates,

but it prevents egress of relatively large entities such as enzymes and cells. Mass transport of small molecules into hydrogels can occur by diffusion or migration but not by convection.[3] We fabricated hydrogel micropatches containing *E. coli* cells by *in-situ* UV-photopolymerization in microfluidic devices composed of PDMS molds and glass channel covers. Figure 4A shows an optical micrograph of an *E. coli* cells-containing hydrogel micropatch that is photolithographically defined within a microfluidic channel. The micropatch extends from the top to the bottom of the channel, but there is space on either side for fluid to flow. Small molecules are able to penetrate the hydrogel by diffusion but the much large cells are trapped within the hydrogel. A high resolution optical micrograph of the same hydrogel micropatch after staining the entrapped cells with crystal violet and KI is shown in Fig 4B.

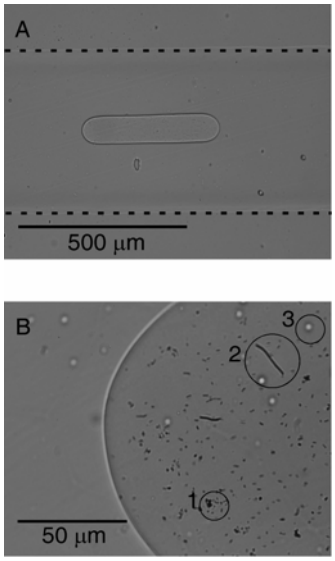



**Figure 4. (A) Optical micrograph of a hydrogel micropatch containing bacteria that is photolithographically defined within a microfluidic channel. (B) A high-resolution optical micrograph of the same hydrogel micropatch after staining the entrapped cells with crystal violet and KI.**

It is important to know whether the hydrogel-entrapped cells are viable, because cell membranes can be damaged during UV photo-polymerization. Propidium iodide (PI) alone can be used to test the viability of hydrogel-entrapped *E. coli* cells: live cells show no fluorescence in the presence of PI, while dead cells show red fluorescence. Figures 5A and 5B are optical and fluorescence micrographs, respectively, of hydrogel-entrapped *E. coli* cells. The optical micrograph reveals that many *E. coli* cells are entrapped in the hydrogel micropatch (Figure 5A), but except for very weak background fluorescence they do not exhibit the characteristic red color of dead cells (Figure 5B). We conclude that photopolymerization does not cause serious defects in the cell membranes. This observation is consistent with previous reports for hydrogel-entrapped mammalian cells.[39] Indeed, we observed that *E. coli* cell membranes were intact even after several

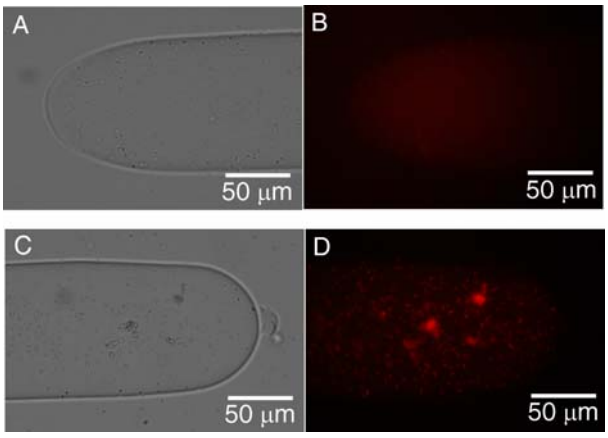
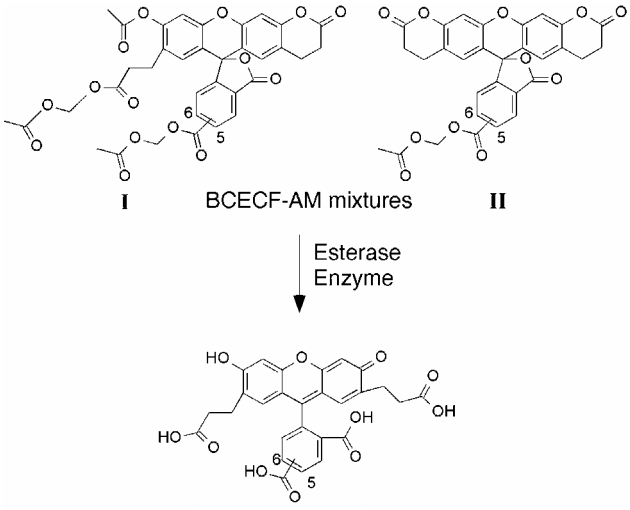



**Figure 5. (A) Optical micrograph of a hydrogel micropatch containing bacteria that is photolithographically defined within a microfluidic channel. (B) Fluorescence micrograph of the same micropatch after incubating with PI. (C) Optical micrograph of a hydrogel micropatch analogous to the one shown in (A), but after cell lysis with 1 % (w/v) SDS solution. (D) Fluorescence micrograph of the micropatch containing the lysed cells after incubating with PI.**

minutes of UV exposure**.**

Lysis agents create pores in cellular membranes, and this in turn should permit dyes such as PI to permeate into the cell interior. Therefore, to confirm that the hydrogel-entrapped cells do not have compromised membranes, they were lysed with an SDS solution and the resulting micrographs were compared with those just discussed. The optical micrograph in Figure 5C and the corresponding strong red fluorescence observed in Figure 5D indicate that SDS lysis occurs within the hydrogel. This finding is useful for two reasons. First, it shows that agents present within a surrounding solution can penetrate the hydrogel and perform a chemical operation on the cells residing therein. Second, if lysis agents that do not cause severe loss of enzyme activity are selected,[42] the resulting cell poration will enhance the rate of mass transfer of small molecules across the cellular membrane. This means that the contents of lysed hydrogel-entrapped *E. coli* cells can be used as biocatalysts or sensing agents.




**Scheme 1**

Although enzyme activity in membrane-compromised cells can be significant,[26] typically cellular membranes protect enzymes, thus allowing higher activity. To test the enzyme activity of live, hydrogel-entrapped *E. coli* cells, we relied on an esterase-catalyzed hydrolysis reaction. The dye BCECF-AM (**I** and **II** in Scheme 1) exhibits fluorescence when hydrolyzed to BCECF (**III** in Scheme 1) by intracellular esterase.[43 44] Conveniently, hydrophobic BCECF-AM substrates easily penetrate the cell membrane, but the hydrolysis product, BCECF, is retained within the cells. The fluorescence micrograph shown in Figure 6A was obtained after encapsulating *E. coli* cells within a hydrogel micropatch and then flowing BCECF-AM through the microfluidic channel surrounding the micropatch. As indicated by the intense green fluorescence, BCECF-AM is able to diffuse through 1 - 5 nm hydrogel pores and the cell membranes, where it encounters intracellular esterase enzymes that convert it to the highly fluorescent BCECF form. The weak green fluorescence of the hydrogel and the solution inside the microfluidic channel arises

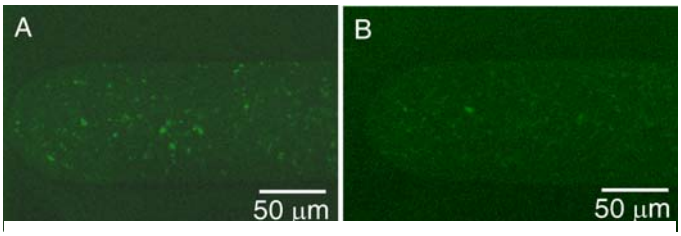



**Figure 6. Fluorescence micrographs of hydrogel micropatches containing bacteria and photolithographically defined within a microfluidic channel. (A) After incubating with BCECF-AM for 1 h. (B) Same experiment as in (A), but after cell-lysis with SDS**

from slow BCECF-AM hydrolysis by the phosphate buffered saline (PBS) solution of pH 7.2. When *E. coli* cells suspended in PBS or entrapped in hydrogel were incubated with 10 μM of BCECF for 1 h, the cells showed very weak fluorescence. This result indicates that the fluorescence of *E. coli* cells mainly arises from the enzyme hydrolysis reaction occurring within the cells, rather than from diffusion of BCECF (derived from slow solution hydrolysis of BCECF-AM) from the solution into the cells. Lysed hydrogel-entrapped *E. coli* cells were also incubated with BCECF-AM. Because SDS usually severely damages enzymes, only very weak intracellular fluorescence is observed after lysis (Figure 6B). In this case, fluorescence probably does result from transport of BCECF from solution directly into the membrane-compromised cells.

**Intracellular pH-calibration using BCECF fluorescence dye.** The pH dependence of BCECF fluorescence intensity can be used to calibrate aqueous solution or intracellular pH as shown in Figure 7. The fluorescence emission intensity was observed at fixed wavelength of 535 nm. Two different excitation wavelengths (490 nm and 439 nm) were chosen to obtain the fluorescence intensity ratio. This ratiometric method can minimize fluorescence measurement artifacts, such as photo-bleaching and instrumental instability. The internal pH calibration curve for *E. coli* was obtained by titrating the cell solution in the presence of the ionophore and $K^+$ present at the intracellular concentration of 200 mM. The *in-situ* calibration curve of BCECF in *E. coli* is a little different from the BCECF calibration in solution because of the effect of the two different molecular environments. However, both curves exhibit a very drastic change in the fluorescence intensity ratio and high linearity in the pH 6 – 8 region. These calibration curves can be used to measure solution and intracellular pH. It is known that *E. coli* cells try to maintain their internal pH regardless of the pH of the external solution. This is termed pH homeostasis. For example, the internal pH change is maintained at 7.6 ± 0.2 over an external pH range of about 5.5 to 9.[45] This pH homeostasis will make it difficult to

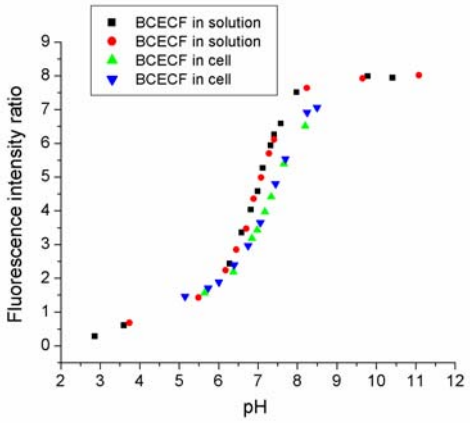



**Figure 7. Fluorescence intensity ratio curve of BCECF versus pH.**

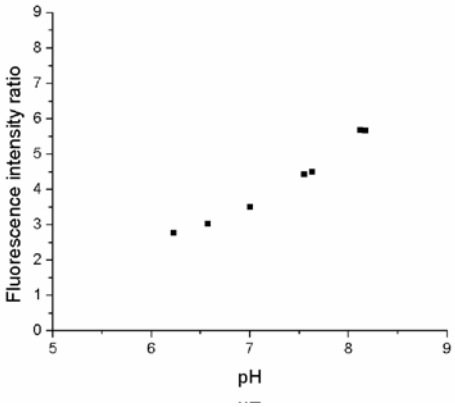



**Figure 8. External pH effect on the fluorescence intensity of BCECF loaded in *E. coli*.**

observe fluorescence intensity changes within the cell. However, as shown in Figure 8 the external pH change does result in a measurable intracellular pH change. This is probably because pH homeostasis is not highly efficient under our experimental conditions. Thus, we expect that the intracellular pH change will also be measurable when protons are generated by the OPH enzyme reaction.

**Electrochemistry of nitrophenol and paraoxon**. Figures 9 and 10 show cyclic voltammograms of paraoxon and PNP in the reduction and oxidation potential regions. We used carbon electrodes for these experiments, because they can be easily fabricated, provide a wide potential window, and allow for simple surface modifications. Preliminary results using carbon electrodes indicate that paraoxon and PNP have nearly identical electrochemistry at negative potentials, and therefore this part of the electrochemical window will not be useful for our experiments. However, only PNP is oxidized at positive potentials, and therefore PNP can be detected by coupling its oxidation to the cathodic ECL system described previously.

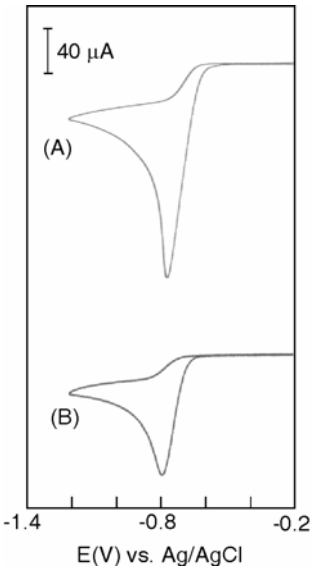



**Figure 9. Cyclic voltammograms of (a) paraoxon and (b)*p*-nitrophenol in pH 7.0 phosphate buffer at reducing potentials.**

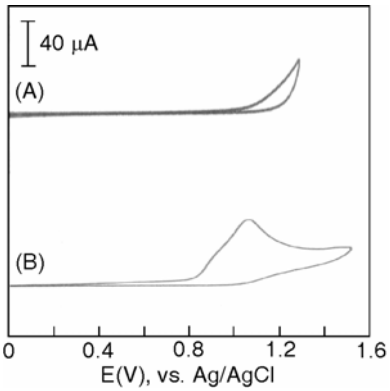



**Figure 10. Cyclic voltammograms of (a) paraoxon and (b) p-nitrophenol in pH 7.0 phosphate buffer at oxidation potentials.**

## IV. Proposed Research

**Fluorescence spectroscopy study of BCECF-loaded *E. coli* cells that are suspended in pH-controlled buffer solution.** Here, BCECF-loaded cell solutions will be directly used to test the presence of organophosphates in water. These experiments will be useful to optimize experimental conditions for fluorescence-based microfluidic OP sensors. Fluorescence spectroscopy is the main detection tool for these experiments. The fluorescence intensity ratio can be obtained as described in Section III. For this experiment we should observe the following. First, the fluorescence intensity ratio of BCECF-loaded cells should be independent of cell concentration at a given pH if the

inner filter effect (nonlinear dependence of fluorescence intensity on the analyte concentration resulting from light absorption by the analyte at high concentrations) is negligible. Second, the fluorescence intensity ratio should be highly dependent on the pH of the buffer medium when BCECF is loaded into the cells, because the pH of buffer medium affects the intracellular pH. Third, the change in the fluorescence intensity ratio when adding paraoxon should depend on the concentration of the buffer medium, because higher buffer concentrations should result in smaller observed changes in the intracellular pH. Fourth, from a plot of fluorescence intensity ratio change versus paraoxon concentration, the detection limit (S/N =3) and sensitivity (slope) can be estimated. Fifth, non-specific cellular responses must be considered, because cells contain muliple-enzymes that may interfere with the OPH-specific responses. For example, sugars such as glucose, fructose, and sucrose[11] will be added to a solution suspended with BCECF-loaded cells to observe whether a fluorescence intensity change occurs. Wild-type *E. coli* cells (not having OPH enzymes) will be used as a control experiment. OPH-expressed and wild type *E. col* cells were kindly provided by Dr. Frank Raushel and his group (Department of chemistry, Texas A&M Univ.). Sixth, the effect of BCECF leakage from *E. coli* cells on the fluorescence intensity change should be also considered.

**A microfluidic sensor for OP based on intracellular pH changes of *E. coli*.** It will be convenient to make cell patterns in a microfluidic device to use them as sensor units. However, unlike mammalian cells, patterning of *E. coli* cells is not widely studied.[46] The simplest approach for patterning cells is to use hydrogels of the type discussed in Section III. Because we expect the fluorescence intensity change in the OPH-expressed *E. coli* caused by the OPH enzyme reaction to be very small, wild-type *E. coli* cells will be immobilized in the same channel and used as an internal reference. The schematic diagram for this method is shown in Figure 11. Two solutions will be pumped into one channel to form a laminar flow. The two solutions will be photopolymerizable hydrogel precursor solutions suspended with OPH-expressed *E. coli* cells and wild-type *E. coli* cells (not having OPH enzyme), respectively. Both *E. coli* cells will be preloaded with BCECF, the pH-sensitive fluorescence dye, before suspending them in the hydrogel precursor solution. After shining UV-light on the microfluidic channel through a slit-type photomask, unreacted hydrogel precursor solutions will be washed away with deionized water. Hydrogel micropatches can be fabricated with critical dimensions as small as 10 μm size using this approach. Cell viability will be determined using the method described in Section III. The attributes of the hydrogel cell-

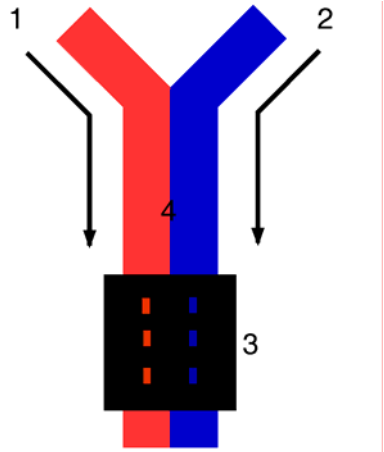



**Figure 11. A schematic diagram of making cell patterns using hydrogel precursor solutions.**

immobilization method can be summarized as follows: (1) hydrogel-entrapped cells should have intact membranes as discussed in previous sections; (2) control experiments will be simplified because OPH-expressed and wild-type *E. coli* cells will be immobilized in the same channel; (3) to enhance the mass-transfer rate of analytes into the cells, cell membranes can be porated using mild lysis agents without having a severe loss of enzyme activity. The fluorescence image and intensity data can be collected using epi-fluorescence microscopy. Paraoxon solution will be continuously pumped into the channel until a steady-state fluorescence intensity is obtained. Wild type *E. coli* cells will not exhibit fluorescence intensity change, while OPH-expressed *E. coli* will show decreased fluorescence intensity. The fluorescence intensity of patterned cells can be averaged using data-acquisition software. This will be helpful to obtain the relationship between the fluorescence intensity change and the paraoxon concentration.

The second cell patterning method is a modification of previously reported methods.[33,46] Poly-L-lysine (PLL) has been used to enhance cell adhesion to surfaces via nonspecific electrostatic interactions between the positively charged PLL surface and negatively charged cells. Recently, it was reported that PLL also enhances microbial adhesion of some types of *E. coli* to surfaces.[46] The method that we are going to use is described in Figure 12. First, a clean glass substrate will be precoated with PLL. Then, OPH-expressed *E. coli*, albumin, and wild-type *E. coli* solutions will be pumped into one channel to form a laminar flow. Because PLL is also effective for adhering albumin, which is a protein used for preventing cell adhesion on surfaces, the two *E. coli* cell areas can be separated from each other. The albumin layer will prevent different cell types from encroaching each other during cell-growth. After cell-growth, the cells can be loaded with pH-sensitive BCECF fluorescence dye. This patterned layer of cells can be used as a sensor unit. This method has the following advantages, compared with the first method: (1) the patterning procedure will not affect cell viability; (2) the cell patterns can be changed by using diverse shapes of fluidic channels; (3) the mass-transfer rate of organophosphates across the cell membrane will be faster, because OP has to diffuse through the hydrogel pores in the first method. However, some cells adhered onto the surface may be lost when the solution flow rate is high.

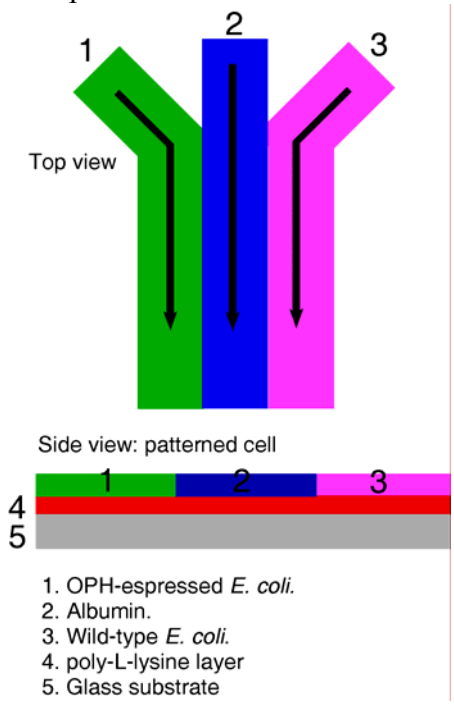



**Figure 12. A schematic diagram of making cell patterns on poly-L-lysine coated glass.**

**A dual-channel microfluidic sensor for OP using ECL reporting system.** A two-channel microfluidic device will be fabricated as previously reported.[6,7] My study will differ from those previously reported in that enzymes will be used to provide selectivity. Additionally, a different type of electrode will be required. The enzyme

reaction zone will be filled with OPH-immobilized beads. Biotinylated OPH enzymes will be immobilized onto commercially available stereptavidin-coated beads (~ 15 μm) via streptavidin-biotin conjugations. The activity and kinetics of the enzymes immobilized on the beads can be compared to the free enzymes by using absorption or potentiometric methods. Enzyme-immobilized beads will be packed into the microreactor with a syringe and retained by a 7 – 12 μm deep wier (Figure 3). Instead of ITO (Indium Tin Oxide) electrodes, carbon electrodes will be used in this study, because these provide a wide potential window that will allow the electrochemical reaction of PNP to be observed as shown in Section III. Carbon electrodes will be fabricated using screen-printing or other methods.

The relationship between ECL intensity and PNP concentration will be determined using the microfluidic sensor in the absence of the OPH enzyme. PNP-containing solutions and the ECL cocktail will be pumped into the detection and reporting channels, respectively. At the ECL-onset voltage, the photon signal will be measured and correlated to the PNP concentration. Because the solutions will be continuously flowing into the channel, a steady-state ECL signal should be observed. The resulting calibration curve will be useful for quantifying the results of the enzyme-based experiment and for determining the detection limit of PNP. The long-term stability of the electrodes will be also investigated. Because the test solution flow rate, the quantity and size of beads packed in the channel, and the channel dimensions are important factors for high conversion efficiency of paraoxon to PNP, these factors should be optimized for OP sensing. The flow rate can be controlled using a precision syringe pump and the amount of beads can be controlled by varying the volume of the reaction chamber. We will use these parameters to optimize the sensor, determine the detection limit, determine the linear range of the sensor, and check for selectivity in the presence of interferences such as diisopropylmethyl phosphonate (DIMP) and dimethyl methane phosphonate (DMMP), which have similar structures to the organophosphate neurotoxins.

**Prospects for real applications**

In this research we plan to use paraoxon as a model organophosphate, because it is less toxic than real nerve agents and because it has a very similar structure to parathion (a widely used pesticide known as one of the most toxic compounds certified by the EPA). Because parathion generates nitrophenol and protons upon exposure to OPH, the approach proposed here can be applied for the detection of parathion. Besides parathion, our proposed sensor will be able to detect many organophosphates used for pesticides such as coumaphos, NPEPP, fensulfothion, and diazinon, all of which will produce electroactive species or protons.

As a nerve-agent sensor the approach described here has two drawbacks. First, microfluidic sensors are normally (but not always) limited to detection of organophosphaes dissolved in liquid solvents. Second, most warfare agents, such as Sarin and Tabun, are hydrolyzed by OPH into protons and non-electroactive compononets. It may be possible for us to address both of these issues. For example, hydrolysis of all organophosphate nerve agents results in generation of protons and these are electroactive and detectable in non-aqueous solvents. Similarly, the intracellular approach to sensing, based on pH-sensitive dyes, should be useful for detecting protons even in aqueous

environments. To accommodate the need for sensing vapors, air can be bubbled through solvents and the resulting solution can be analyzed.

## V. Summary

Two types of microfluidic devices for sensing organophosphates have been proposed. These will provide new types of portable, cost-effective, and highly sensitive microfluidic sensors for organophosphates (OP). Both methods rely on organophosphorous hydrolase (OPH) enzyme reaction, which hydrolyze OP into detection target products. The first approach is based on the detection of intracellular pH change in OPH-expressed *E. coli* by using pH-sensitive fluorescence dye. Two inherent advantages of this proposed design are the use of sensitive fluorescence detection and differential measurement enabled by the use of an *in-situ* control. The second sensor is a dual-channel microfluidic device using electrogenerated chemiluminescence (ECL). This microfluidic sensor derives its excellent sensitivity from photonic reporting of electrochemical reactions, and its selectivity results from the enzyme microreactor and the adjustable potential of the sensing electrode.

## VI. References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