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Perspective

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Nanobiotechnology for environment: innovative solutions for the management of harmful algal blooms

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Abstract:

Nanobiotechnology has played important roles in solving contemporary health problems including cancer and diabetes, but has not yet been widely exploited for problems in food security and environmental protection. Water scarcity is an emerging worldwide problem due to climate change and population increase. Current methods of managing water resources are not efficient or sustainable. In this perspective, we focus on harmful algal blooms to demonstrate how nanobiotechnology can be explored to understand microbe-environment interaction and allow for toxin/pollutant detection with significantly improved sensitivity. These capabilities hold potential for future development of sustainable solutions for drinking water management.

Key words: Harmful Algal Blooms (HABs), quorum sensing, biosensor, nanobiotechnology.

Introduction

Aquatic ecosystems are increasingly threatened by pollutants from human activities. One of the threats is the increasing occurrence of harmful algal blooms (HABs), which is directly caused by nutrient enrichment of waters by run-off from urban, agriculture, and industrial development, as well as climate warming [1]. HABs are caused by the sudden growth of particular species of microalgae and, in lakes, primarily cyanobacteria. These blooms increase the turbidity of the aquatic systems, suppress the growth of the underwater plants, can cause hypoxic zones, and disrupt the balance of all life forms within aquatic ecosystems. Some bloom species produce toxins that endanger fish habitats, as well as cause serious health problems in domestic and wild animals, and in humans [2]. For example, periodic blooms of the "Brown Tide" organism, *Aureococcus anophageefferens,* have devastated the local scallop fishery in Long Island Sound [3]. Nationwide, toxin-producing cyanobacteria, including *Microcystis aeruginosa* (*M. aeruginosa*), have been extensively reported in the Great Lakes [4] (See Fig. 1). Worldwide, HABs have been reported in Lake Victoria in Africa, Lake Taihu in China, and the Baltic Sea in Europe [1b]. HABs have repeatedly been in the news. Early 2016, Chilean salmon farmers were devastated by losses reported of almost one billion dollars in revenue due to HABs, which has had a lasting impact on the world fish market [5]. Just recently, Florida declared state of emergency because HABs have threatened drinking water resources in St Lucie and Martin Counties [6]. Other notable HAB crises include the complete drinking water shutdown in Toledo, Ohio, along Lake Erie [7].

HABs are known to be triggered by environmental cues including nutrient levels, temperature, light, and water currents [1b, 8]. There is a general agreement that nutrient enrichment is responsible for expansion and persistence of HABs. Nitrogen (N) and phosphorus (P) are the key nutrients for algal growth in aquatic ecosystems [8a, 9]; excess N and P is often blamed in lakes

for HABs [8a]. Laboratory studies also support findings that both N and P, as well as their ratio, affect the growth of *M. aeruginosa* [10]. Increasingly, experts have recognized that physical environmental factors such as temperature, light and water currents also play important roles in the onset of HABs [8a, 9, 11]. For example, it was previously reported that in Lake Taihu the growth of cyanobacteria (e.g. *M.* aeruginosa) was P-limited in spring and winter, while N-limited in summer [12]. Fluid flows or water currents were reported to influence the onset of HABs with wind towards the shoreline promoting HABs [8c].

Despite the urgency of the problem with respect to harmful algal blooms brought by climate change and population expansion, the exact cause for the onset of HABs is largely unknown[13]. As such, the management of HABs often involves expensive and large scale manipulation such as re-routing clean water from an adjacent river or lake to dilute the cyanobacteria concentration and their associated toxin concentration further disturbing the natural ecosystem. HABs are influenced by many environmental factors including nutrient concentration, temperature, water currents and light intensity [1b]. Current macro-scale technology, e.g. chemostat and pond assay, is not designed for high throughput screening for a large number of environmental conditions. The emerging nanobiotechnology overcomes the above limitations and has the potential to develop a mechanistic understanding of how multiple environmental factors impact the onset of HABs.

In this perspective, we will focus on how nanobiotechnology can be exploited to better understand single cell-environment interaction in the context of HABs. We propose that engineered nanoliter size habitats can provide well controlled environment for cells suitable for mechanistic understanding of cell-environment interaction. Such understanding along with emerging nanomaterials can be exploited for toxin detection in water at unprecedentedly high

sensitivity. We argue that nanotechnology will play critical roles in providing solutions for sustainable water resource management.

Nano-liter fluidic platform for understanding cell–environment interaction in the onset of Cyano Harmful Algal Blooms

A key component of understanding the onset of HABs is to know how single cyanobacterial cells grow under various environmental conditions. Micro-meter sized device is ideal for interrogating single cell-environment interactions because single cells and micro-meter device have similar size. This leads to two advantages: (i) cells within the micro-meter sized device can be easily imaged using a light microscope; (ii) cellular environmental conditions such as chemical gradients and temperature can be easily controlled within these devices. As a result, micro- nano- meter devices have been exploited extensively by the biomedical field for understanding contemporary diseases such as cancer and diabetes [14]. Currently, applications of micro- nano- meter sized device in environmental problems are limited. Many fundamental questions with respect to environmental microbes remain to be explored. Nanobiotechnology is in a unique position to enable a basic understanding of single microbe-environment interaction, as such, to understand the roles of multiple environmental cues in shaping the growth and spatial distribution of microbes within our aquatic ecosystems. In the following, we will limit our discussion to the studies of growth of cyanobacteria in fresh water in the context of harmful algal blooms. We note that these methods discussed can be easily extended to other microbes important for environment and food industry.

Microfluidic platforms have played important roles in revealing principles governing cell-environment interactions for both prokaryotic and eukaryotic cells [14b, 15]. Fig. 2 illustrates an

example where a eukaryotic green alga , *Chlamydomonas reinhardtii (C. reinhardtii)* growth was studied under well-controlled nitrogen gradients. The main feature of the design is an array of nanoliter habitats flanked on two sides by channels, patterned in a 1 mm thick agarose gel. Buffer and nutrients flow through the two side channels, and a nutrient gradient is established within the habitats via molecular diffusion. The advantage of this design is that the hydrogel-based habitats protect the cells from the exposure to the shear stress created by the control flows along the side channels, and at the same time, it allows for molecular transport from the control flows to the cells via diffusion. We also note that agarose gel walls surrounding the cell culture contain mostly water, thus the device supports long-term cell culture with minimal humidity issues, in contrast to the commonly used Polydimethylsiloxane (PDMS) device. Using this nanoliter fluidic device, we found that *C. reinhardtii* growth follows a Monod growth model equation that relates the soluble nutrient concentrations to algal growth rates, and obtained the half saturation constant of N. Additionally, this study reveal a unimodal distribution of growth, with both low and high N causing decreases in growth. This work demonstrates the enabling capability of quantitative measurements of microalgal growth kinetics using a nanoliter habitat platform.

Many groups of bacteria are known to use diffusible chemical signals to estimate their population densities and to coordinate and synchronize the physiology of individual cells. This phenomenon, sometimes referred to as quorum sensing, requires the synthesis of diffusible chemical signal molecules and their detection by sister cells [16]. Several recent studies suggest that cyanobacteria may utilize such chemical signals. *Microcystis aeruginosa* was reported to synthesize an acylhomoserine lactone, similar to pheromones that are synthesized by a wide range of bacteria[17]. This compound was purified by organic extraction of culture medium, and

when added to a culture of *M. aeruginosa,* stimulated the formation of an extracellular matrix and biofilm. In a separate report, *Gloeothece* sp was found to synthesize a similar pheromone[18]. Addition of this compound to a low-density culture stimulated at least two-fold the expression of 15 different proteins and downregulated expression of two proteins. It is tempting to speculate that the explosive growth and decline of harmful algal blooms could be due in part to chemical communication within these communities.

Using a similar microfluidic platform as shown in Fig. 2, our labs have begun to explore roles of cell-cell communication in the formation of algal blooms using *M. aeruginosa*. Experimental evidence has shown that quorum sensing signals played critical roles in the aggregation of *M. aeruginosa* [19], leading to possible roles in the formation of algal blooms. Here, *M. aeruginosa* were cultured in an array of nanoliter habitats in the presence of (QS) molecule, a gradient of 3-oxo-octanoyl homoserine lactone (OOHL), a molecule that resembles that produced by *M. aeruginosa*. Cells were seen to migrate toward the region containing the highest OOHL concentrations and to clump together there. This demonstrates that diffusible QS signals could influence the formation of aggregates of this bacterium. We hypothesize that the disruption of cell-cell communication can potentially be utilized for the disruption of HABs leading towards a sustainable management of HABs. Many laboratories have successfully sought strategies to disrupt cell-cell communication in other species of bacteria, especially human pathogens, so there is reason for optimism that quorum sensing antagonists could be developed.

Nanomaterial based biosensors for detecting toxins from HABs

The main threat HABs pose to human health is the toxin produced by the cyanobacteria, referred to as cyanotoxin. Microcystins are a family of cyanotoxins with approximately 90

known variants, among which Microcystin-LR (MC-LR) is the most toxic [20]. These hepatotoxins are cyclic heptapeptides produced through nonribosomal peptide synthases and cause damage to the liver. They are thought to interfere with DNA damage repair pathways and also increase expression of the proto-oncogenes, genes involved in the response to DNA damage, cell cycle arrest, and apoptosis. Exposure to MC-LR in drinking water or consumption of fish can lead to liver failure [21]. Standard methods of detecting cyanotoxins are liquid chromatography with mass spectrometry (LC-MS) and enzyme linked immunosorbent assay (ELISA) [22 23]. LC-MS has been the golden standard for toxin detection from HABs because it is highly repeatable with low detection limits, and allows for detection of multiple toxins. However, LC-MS analysis requires expensive instruments that are not well suited for *in situ* testing. Samples typically need to be sent to a centralized facility, delaying the turnaround time for the test results. ELISA, in contrast, is easy to set up, readily available in most biological labs. The limitation of ELISA is its specificity, and often results in false readouts. Real time PCR has been used to detect the DNA copies of microsystin synthetase. This method is straightforward to implement, however, the relation between the gene copies of microsytin synthetase and its activity is still under debate [24].

The frequent occurrence of HABs has led to the urgent need of *in situ* toxin detection methods, most notably, biosensors [25 25b]. A biosensor requires a biorecognition component, an amplification mechanism, and a transducer for signal readout [26]. Biosensors can be made compact and handheld, suitable for field work. Traditional biosensors for toxin detection in water are electrochemical biosensors [27]. In this platform, electroactive product is generated through enzymatic inhibition by microcystin-LR, which is subsequently measured using bioelectrochemical method. The detection limit can reach to 37 μg/L, which is sufficient for

158 screening samples from field. A more recent and robust biosensor for detecting MC-LR uses the
159 mechanism of surface plasmon resonance (SPR) [28]. In this platform, MC-LR is covalently
160 bounded to the substrate, and the flow in of the MC-LR antibody results in a SPR signal. This
161 method has proved to be sensitive, and has a low detection limit of 73 ng/L.

162 An important component of a biosensor is the biorecognition element [20]. Immunosensors
163 depend critically on the presentation of antibodies to the MC-LR. The rapid development of
164 nanomaterial field has led to materials that facilitate sensitive and specific binding between the
165 MC-LR and its biorecognition molecule. An illustrative example is given in Fig. 3 where
166 plasmonic nanoparticle complex is developed for detecting low concentration of microcystin-
167 LR [29]. Here, a nanoparticle complex is formed by a gold nanoparticle immobilized with an anti
168 MC-LR antibody (AuNP+anti MC-LR Abs) and a silver nanoparticle immobilized with BSA-
169 MCLR conjugate (AgNP+BSA-MCLR). The silver nanoparticle serves as competitive inhibitor
170 for the binding between anti MC-LR Abs on AuNPs and MC-LR in the sample. Interestingly, the
171 nanoparticle complex exhibits strong chiral dichroism (CD or differential absorption of left- and
172 right-handed circularly polarized light which can be measured accurately. In the presence of
173 MC-LR, the nanoparticle complex dissociates, leading to a decrease in CD readout (Fig 3B). A
174 low detection limit of 0.8 ng/L has been reported using this method.
175
176 More recently, an anti MC-LR aptamer in conjunction with a photoelectrochemical (PEC)
177 technique is used to detect low concentration of MC-LR toxins [30]. The PEC system consists of
178 an ITO electrode (immobilized with MC-LR aptamers), N-doped graphene cathode and a
179 seminconductor BiOBr. When the BiOBr is subjected to UV light, electric current will be
180 generated within the PEC system. This electric current is increased in the presence of MC-LR,

and this increase has been reported to be proportional to the logarithm of MC-LR concentration[30]. Because of the ability to differentiate the activating signal (light) with the readout (current), this aptamer based biosensor is by far the most sensitive sensor, and reaches the detection limit of 0.03 ng/L. This is 1000-fold below the World Health Organization (WHO) guideline of 1 μg/L. Here, we note that PEC technique allows for an easy integration with the development of hand-held biosensors (Fig. 3C), as well as microfluidic platform in the future.

Future perspective on sustainable water management

Looking forward, nanobiotechnology can be exploited to better understand cell-environment interaction at a fundamental level, and identify environmental conditions under which HABs occur. We emphasize here roles of multiple environmental cues in the formation of HABs, in particular cell-cell communication. In parallel, the rapid development of nanomaterials can be capitalized to make better and cheaper nanosensors for *in situ* toxin detection. We note that nanosensors can be adapted for use with ubiquitous mobile "smart phones". In the near future, it is conceivable that mobile phone -based devices will detect toxins in water, analyse the environmental conditions, and come up with solutions for managing HABs in a sustainable way.

Abbreviations Used

ELISA	Enzyme Linked Immunosorbent assay
HABs	Harmful Algal Blooms
MC-LR	Microcystin-LR
N	Nitrogen
P	Phosphorus

PDMS	Polydimethylsiloxane
SPR	Surface Plasmon Resonance
QS	Quorum Sensing

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Figure Captions

Figure 1. Harmful algal blooms in Lake Erie. The lake is covered by a thick layer of algal blooms, depleting drinking water resources and threatening aquatic life forms. HABs in lake Erie caused water shutdown in Toledo Ohio in 2014. This lake was hit by an even larger magnitude of HAB in 2015 [4b]**.** Photo credit: Tom Archer

Figure 2. A hydrogel-based array nanoliter habitat system for studies of microalgal growth kinetics. A. A photograph of the device on a microscope stage. **B.** Each device consists of a group of 64 nanoliter habitats, each having 100 μm x 100 μm x 100 μm or 10^{-3} nanoliter in volume, flanked by two side channels. Nutrient and buffer flow through source and sink channel respectively to provide a linear concentration gradient. The distance between the two side channels is 2 mm. **C.** Micrographs of one row of nanoliter habitats with *Chlamydomonas reinhardtii* (*C. reinhardtii*) in the presence of ammonium (NH_4Cl or N) gradient at different time points. The N concentration in source and sink channel is 15 μM and 0 respectively and the gradient is 7.5 μM/mm. **D.** Specific growth rate of *C. reinhardtii* as a function of ammonium concentration. The fit of this curve to Monod equation provides the first quantitative measurement of the half saturation constant of *C. reinhardtii* in NH_4Cl substrate to be 1.2 ± 0.3 μM. This graph is adapted from Kim et al, Lab Chip, 2015 [31].

Figure 3. Nanomaterial-based biosensor (A-B) The chiral property of a nanoparticle complex is used to detect low concentration of toxin MC-LR. **(A)** The nanoparticle complex is formed between a MC-LR antibody immobilized on a gold nanoparticle (AuNP) and a MC-LR-BSA conjugate immobilized on a silver nanoparticle (AgNP). The presence of MC-LR leads to dissociation of Au-NP and AgNP complex due to the high affinity of MC-LR to AuNP Abs than MC-LR-BSA to AuNP+Abs. **(B)** The circular dichroism signal (ΔCD) decreases linearly with

359 the increase of MC-LR concentration. Here, the AgNP with MC-LR-BSA serves as a
360 competitive inhibitor. Graphs (A,B) are adapted from Ref. [29] with permission from the
361 American Chemical Society. **(C)** Illustration of future integration of biosensor with smart phone.

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Figure 1.

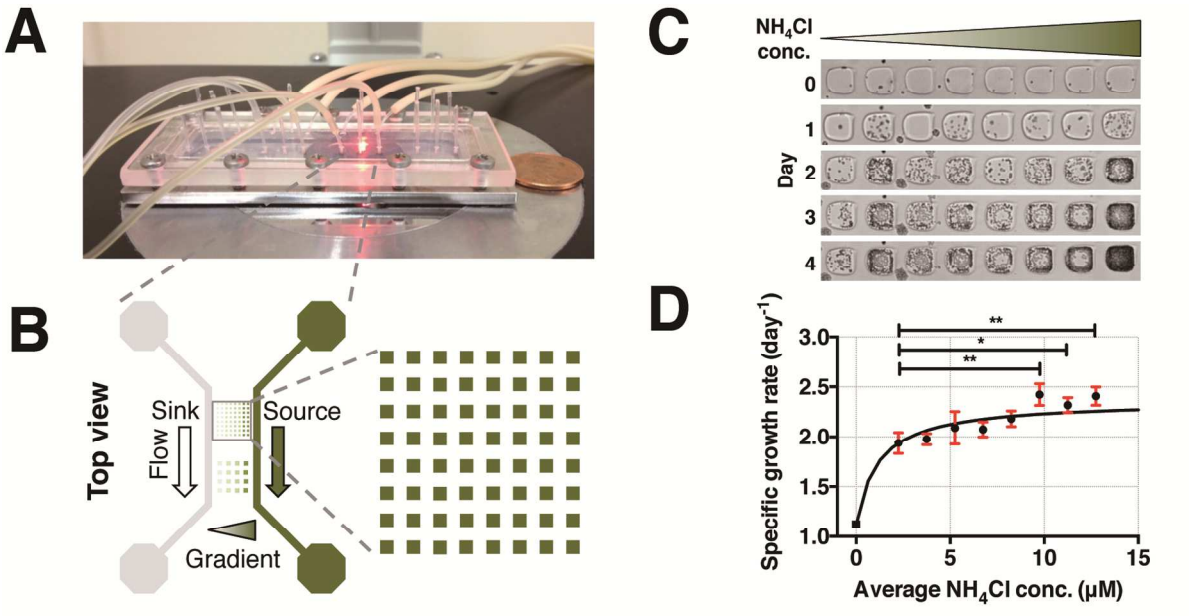
A
B
Top view
Sink
Flow
Source
Gradient
C
NH4Cl conc.
Day
0
1
2
3
4
D
Specific growth rate (day-1)
3.0
2.5
2.0
1.5
1.0
0
5
10
15
Average NH4Cl conc. (μM)
**
*
**

Figure 2.

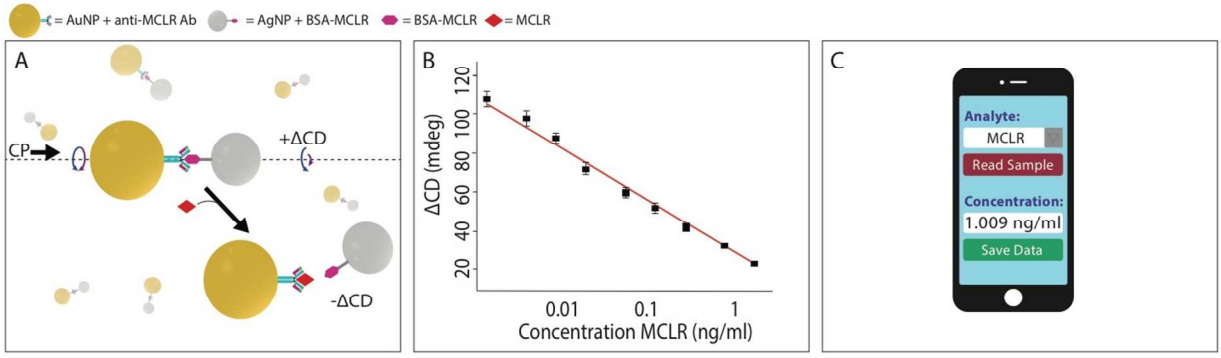
= AuNP + anti-MCLR Ab
= AgNP + BSA-MCLR
= BSA-MCLR
= MCLR
A
CP
+ΔCD
-ΔCD
B
ΔCD (mdeg)
120
100
80
60
40
20
0.01
0.1
1
Concentration MCLR (ng/ml)
C
Analyte:
MCLR
Read Sample
Concentration:
1.009 ng/ml
Save Data

Figure 3.

TOC graphics