Title: Capture and Detection of Botulinum Neurotoxin (BoNT) in Complex Food Matrices using Novel Biosensor Platforms

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Center of Excellence: Food Protection and Defense Institute (FPDI) (Emeritus)
COE Lead/Co-Lead Institution: University of Minnesota

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Abstract: In an effort to improve early BoNT/A detection, we have developed four different sensors to capture and detect botulinum neurotoxin. 1. toxin-responsive hydrogel sensors; 2. self-assembled monolayers (SAMs), SAMs consisting of an immobilized synthetic peptide that mimicked the toxin's in vivo SNAP-25 protein substrate were formed on Au and interfaced with arrayed microfluidic channels; 3. We also have devised a microfluidic platform that incorporates substrate-laden silica beads for sensing the proteolytic activity of botulinum neurotoxin type A (BoNT/A). 4. We developed an enrichment platform for botulinum neurotoxin type B (BoNT/B) that has been realized through the fusion of bioconjugation chemistry and microfluidics. Micrometer-sized magnetic beads were conjugated to a 22 mer peptide derived from the neuronal synaptotagmin II protein that is specific for BoNT/B heavy chain binding. To further improve performances of microfluidics and lab on chips in sensing of biological/chemical to electrical/optical signal transduction, we successfully developed polydimethylsiloxane (PDMS) microlens arrays fabricated through liquid-phase photopolymerization and molding. The gist of this fabrication process is to form liquid menisci of variable radii of curvature at an array of apertures through pneumatic control, followed by photopolymerization under ultraviolet radiance.

End User Engagement:
- Academic Community
- DHS Labs
- Federal Bureau of Investigation
- Food and Agriculture Industries
- Food and Drug Administration

Executive Summary (2009): In an effort to improve early BoNT/A detection, we have developed four different sensors to capture and detect botulinum neurotoxin. 1. toxin-responsive hydrogel sensors; 2. self-assembled monolayers (SAMs), SAMs consisting of an immobilized synthetic peptide that mimicked the toxin's in vivo SNAP-25 protein substrate were formed on Au and interfaced with arrayed microfluidic channels; 3. We also have devised a microfluidic platform that incorporates substrate-laden silica beads for sensing the proteolytic activity of botulinum neurotoxin type A (BoNT/A). 4. We developed an enrichment platform for botulinum neurotoxin type B (BoNT/B) that has been realized through the fusion of bioconjugation chemistry and microfluidics. Micrometer-sized magnetic beads were conjugated to a 22 mer peptide derived from the neuronal synaptotagmin II protein that is specific for BoNT/B heavy chain binding. To further improve performances of microfluidics and lab on chips in sensing of biological/chemical to electrical/optical signal transduction, we successfully developed
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1. Microfluidic sensing of botulinum toxin 1.1: Substrate-Modified Hydrogels for Autonomous Sensing of Botulinum Neurotoxin Type A. In an effort to improve early BoNT/A detection, we have developed toxin-responsive hydrogel sensors. The responsiveness of the hydrogels relies on toxin enzymatic activity and is therefore specific, as BoNT/A has a substrate cleavage site unique to its type. The autonomous BoNT/A sensor was generated by housing toxin-sensitive hydrogels within microfluidic channels, requiring less than 20 μl of contaminated fluid for visual output indicating the presence of BoNT/A. We reported peptide-modified hydrogels for sensing enzymatic activity of BoNT/A which has been published as “Substrate-Modified Hydrogels for Autonomous Sensing of Botulinum Neurotoxin Type A.” Megan L. Frisk, William H. Tepp, Guangyun Lin, Eric A Johnson and David J. Beebe. 2007. Chem. Mater. 19, 5842-5844. 1.2: Self-Assembled Peptide Monolayers as a Toxin Sensing Mechanism within Arrayed Microchannels. A sensor for the lethal bacterial enzyme, botulinum neurotoxin type A (BoNT/A), was developed using self-assembled monolayers (SAMs). SAMs consisting of an immobilized synthetic peptide that mimicked the toxin’s in vivo SNAP-25 protein substrate were formed on Au and interfaced with arrayed microfluidic channels. Efforts to optimize SAM composition and assay conditions for greatest reaction efficiency and sensitivity are described in detail in the published paper. Channel design provided facile fluid manipulation, sample incubation, analyze concentration, and fluorescence detection all within a single microfluidic channel, thus avoiding sample transfer and loss. Peptide SAMs were exposed to varying concentrations of BoNT/A or its catalytic light chain (ALC), resulting in enzymatic cleavage of the peptide substrate from the surface. Fluorescence detection was achieved down to 20 pg/mL ALC and 3 pg/mL BoNT/A in 3 h. Toxin sensing was also accomplished in vegetable soup, demonstrating practicality of the method. The modular design of this microfluidic SAM platform allows for extension to sensing other toxins that operate via enzymatic cleavage, such as the remaining BoNT serotypes B-G, anthrax, and tetanus toxin. 1.3: Bead-based microfluidic toxin sensor integrating evaporative signal amplification We have devised a microfluidic platform that incorporates substrate-laden silica beads for sensing the proteolytic activity of botulinum neurotoxin type A (BoNT/A)-one of the most poisonous substances known and a significant biological threat. The sensor relies on toxin-mediated cleavage of a fluorophore-tagged peptide substrate specific for only BoNT/A. Peptide immobilized on beads is recognized and cleaved by the toxin, releasing fluorescent fragments into solution that can be concentrated at an isolated port via evaporation and detected using microscopy. Evaporative concentration in combination with a specific channel geometry provides up to a 3-fold signal amplification in 35 min, allowing for detection of low levels of fluorophore-labeled peptide-a task not easily accomplished using traditional channel designs. Our bead-based microfluidic platform can sense BoNT/A down to 10 pg of toxin per mL buffer solution in 3.5 h and can be adapted to sensing other toxins that operate via enzymatic cleavage of a known substrate. 1.4: Synaptotagmin II Peptide-Bead Conjugated for Botulinum Toxin Enrichment and Detection in Microchannels. We report an enrichment platform for botulinum neurotoxin type B (BoNT/B) that has been realized through the fusion of bioconjugation chemistry and microfluidics. Micrometer-sized magnetic beads were conjugated to a 22 mer peptide derived from the neuronal synaptotagmin II protein that is specific for BoNT/B heavy chain binding. Bead-peptide conjugates were integrated into arrayed, polymeric microfluidic channels. Exposure to picogram quantities of the type B toxin produced visible signals using near-infrared immunofluorescence. Our sensitive microscale approach require only 5 μl of adulterated sample without any preprocessing (dilution, centrifugation, filtering etc.) with a “hands-on” time of only 1 hour. All assay steps-from capture to detection- were performed directly in microchannels using passive pumping for fluid manipulation, thereby increasing through relative to existing detection methodologies and simplifying assay utility. 2.Design and optimization of tunable microlens and microlens arrays 2.1 To further improve performances of microfluidics and lab on chips in sensing of biological/chemical to electrical/optical signal transducing, we successfully developed polydimethylsiloxane (PDMS) microlens arrays fabricated through liquid-phase photopolymerization and
molding. The gist of this fabrication process is to form liquid menisci of variable radii of curvature at an array of apertures through pneumatic control, followed by photopolymerization under ultraviolet radiance. 2.2 To provide a better sensing of biological/chemical to electrical/optical signal transducing in the horizontal direction, we extended our previous research of parallel liquid microlenses within microfluidics. In additional to the single tunable liquid microlens, we successfully demonstrated the formation and tuning of a microlens array in a single microchannel device.

Peer-reviewed journal articles produced from this project

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<td>Synaptotagmin II Peptide-Bead Conjugate for Botulinum Toxin Enrichment and Detection in</td>
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<td>Dong, L., and H. Jiang.</td>
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<td>Frisk, Megan L., Erwin Berthier, William H. Tepp, Eric A. Johnson, and</td>
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