

## NWRI-BIOLARGO GRADUATE FELLOW SEMI-ANNUAL PROGRESS REPORT

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Project Title: In-pipe Electroporation Disinfection Cell (EDC) Enabling High-Efficiency Secondary Disinfection for Drinking Water

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### Background and Introduction

Disinfection is an essential step to protect people from pathogenic infection, and can be classified as primary and secondary disinfection in drinking water treatment. Primary disinfection inactivates or removes the pathogens in a centralized unit such as a drinking water treatment plant. Secondary disinfection provides long-lasting disinfection effects in the water distribution systems, such as pipelines.<sup>1</sup>

The most widely used primary disinfection approach is chlorination because of its low cost, high efficiency, and abundant engineered experience. More importantly, the remaining free chlorine and/or chloramines can provide long-lasting disinfection through distribution pipes without extra treatment steps.

The formation of disinfection-byproducts (DBPs) during chlorination has been identified as a threat to human health because of their carcinogenicity. This problem can be solved by using alternative primary disinfection techniques, such as ultraviolet (UV), ozonation, and membrane filtration. However, none of the above three methods provide residual antimicrobial power; these techniques pose additional problems such as bacterial regrowth, high energy consumption, and high maintenance cost. A reliable treatment technique is urgently needed for secondary disinfection without forming DBPs.

Low-voltage electroporation inactivation has emerged as a promising water disinfection technique. When exposing biological cells to a high-strength electric field, the permeability of the cell membrane dramatically increases, and electroporation occurs. As the external electric field reaches a sufficiently high level (1-10 kV/cm), the cell membrane is damaged due to irreversible electroporation, which results in inactivation.<sup>2</sup>

Traditionally, a high voltage (>1 kV) has to be applied, and the energy consumption is intensive. This problem can be overcome by adopting nanowire-modified electrodes. The nanowire structure can enhance the local electric field near the tips by three to four orders of magnitude and, thus, cause inactivation with much lower voltage. To take advantage of this phenomenon, a series of low-voltage powered electroporation-disinfection cells (EDCs) have been developed.<sup>3</sup> A recently introduced EDC was equipped with two CuO nanowire modified Cu foam electrodes. Promisingly high cell inactivation efficiencies (>6 log) have been achieved with short hydraulic retention times (HRTs) of a few seconds.<sup>4</sup>

In addition to high efficiency and low energy use, low-voltage electroporation disinfection has the following advantages compared with traditional disinfection methods: a. no chemicals are added and DBPs are not generated; b. it offers universally effective inactivation to most pathogens; and c. pathogens do not develop tolerance to electroporation treatment.

Like other non-chlorine-based approaches, disinfection using low-voltage electroporation also does not produce residual antimicrobial power. The strategy we propose to address this issue is to develop a coaxial-electrode electroporation-disinfection cell (CEEDC) that can be implemented in pipelines of the water distribution system.

### *Hypothesis*

- The CEEDC can achieve high and universal inactivation efficiency against the model bacteria.
- The main inactivation mechanism involved in the CEEDC is irreversible electroporation, which will not cause generate DBPs.
- The CEEDC disinfection is easy to configure, consumes less energy, and is highly scalable.

### *Objectives*

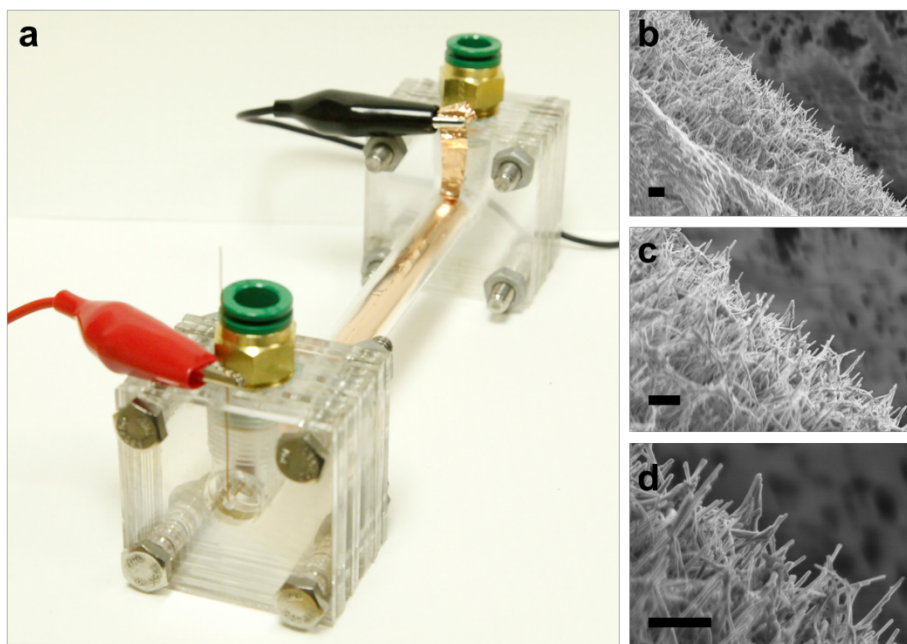
The objectives of this project are to: a. investigate the influence of the operation parameters (applied voltage, HRT, etc.,) and the reactor design on disinfection performance; b. improve disinfection efficiency and treatment capacity by fabricating highly stable, nanowire-assisted electrode materials; and c. find alternative energy sources for the in-pipe application of the CEEDC.

## **Progress to Date**

### *Experimental Design*

A bench-scale prototype of the tubular CEEDCs was fabricated (Figure 1a), which consisted of one cylindrical treatment chamber in the middle and two tube-fitting modules on the sides that serve as the inlet and outlet. The treatment chamber consisted of a round, acrylic tube with 0.95 cm inner diameter, 13.8 cm long. A cylindrical copper shim was used to cover the whole internal surface of the tube, serving as the outer negative electrode. A nanowire-modified copper wire was hung in the center along the tube, serving as the center positive electrode. The growth of the copper oxide nanowire structure was realized through a one-step vapor-phase approach. To enhance the stability of the nanowires, the nanowire-modified electrodes were coated with a thin layer of polydopamine by being immersed in a dopamine solution (2.0 g/L) buffered with Tris (pH 8.5) and kept at 40°C for 4 hours in the open air for each coating cycle.

The model bacteria, *E. coli*, were cultured aerobically at 35°C to log phase (6 to 12 hours) in a shaker at 200 rpm. Then, the bacteria solution was harvested by centrifugation at 4000 rpm for 5 minutes and washed three times with deionized water. Then the bacteria solution was diluted using deionized water to approximately  $1 \times 10^7$  colony-forming units (CFU)/mL. The prepared bacteria solution was pumped by a peristaltic pump into the CECIC with a fixed flow rate. Considering the chamber volume of 10 mL, flow rates were selected in the range of 0.7 to 10.0 mL/min, corresponding to HRTs of 14.3 minutes to 1 minute. At a specific HRT, a direct-current voltage (0-2 V) was applied between the two electrodes by a power source. Treated samples were collected after four times the effective volume (40 mL) flowed through the cell.



**Figure 1.** (a) Image of the coaxial-electrode electroporation disinfection cell. (b-d) Scanning electron microscope images of copper oxide nanowires coated with a polydopamine protection layer. The scale bar in each figure is 1  $\mu\text{m}$ .

### Data Collection and Analysis

The bacterial concentrations of both influent ( $C_{in}$ ) and effluent ( $C_{eff}$ ) samples were recorded by standard spread plating techniques. The inactivation efficiency was calculated using the following equation:

$$\text{Log removal efficiency} = -\log_{10} (C_{eff}/C_{in})$$

The copper concentrations in the influent and effluent bacterial solution will be measured using a Copper Test Kit (HACH, porphyrin method 8143). The operating current was measured by the power source and recorded at the sampling point.

### Discussion of Results

The electrode material described above was fabricated and the morphology was characterized by scanning electron microscope images. Nanowires 1 to 3  $\mu\text{m}$  long were observed growing perpendicular to the surface of the electrode (Figure 1b-1d). The aggregation and agglomeration effects are observed with an increase of the coating time.

Then the fabricated electrodes were deployed into the CEEDC for disinfection experiments. Experimental parameters studied include hydraulic retention time, applied voltage, and the coating time of the electrodes. Generally, the inactivation efficiency increases with increasing voltage and hydraulic retention time. The coating time of the polydopamine protection layer does not affect the disinfection performance within a certain range (< 16 hours). After being operated at 1V and 10 minutes hydraulic retention time, the CEEDC achieved total removal of *E. coli*. No bacteria were detected in the effluent.

## Conclusions

Based on the preliminary results, CEEDC is able to inactivate the model bacteria, *E. coli*, with a high efficiency. This study increases knowledge of how the polydopamine coating affects the morphology of the nanowire structure, the disinfection efficiency, and the electrochemically released copper in the effluent. Furthermore, the CEEDC offers a new reactor configuration considering its rational design, high scalability, and easy configuration.

This study will provide the foundation of the mechanisms and methods to scale up for the in-pipe CEEDC. Full-scale CEEDCs can potentially replace traditional residual chlorine methods to enable continuous municipal disinfection. The in-pipe electroporation-disinfection can be incorporated with other non-chlorine-based disinfection methods, such as UV and ozone, to provide residual antimicrobial power, if they are more readily applicable in full-scale application at water treatment facilities.

## Next Steps

Detailed disinfection experiments will be performed to better understand electroporation disinfection. The following study will focus on the stability of the electrode material, the scalability of the reactor configuration, as well as the universal disinfection behavior. The bacterial inactivation mechanisms will be studied, and direct and indirect evidence will be collected to study the existence of irreversible electroporation. The most exciting and difficult part of this study is to visualize irreversible electroporation on the cell membranes. The observation method will be developed as the project proceeds.

## References

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