**Research Plan** **Student Name:** Katherine Zhang

**Project Title:** Dialdehyde Cellulose Nanocrystal Hydrogel Synthesis for Antibiotic Remediation

## A. Rationale

Globally, antibiotic usage exceeds 100,000 tons around the world annually[1–4]. In addition, incomplete metabolism of antibiotics prompts up to 90% of consumed antibiotics to be excreted and perpetuated within wastewater, surface water, and groundwater[5–9]. Accumulation of trace amounts of antibiotics in humans can cause detrimental health impacts such as the impairment of the gut microbiota which increases risks for chronic diseases such as diabetes, obesity, and asthma.[10–13]. Furthermore, the presence of antibiotics within the environment accelerates the development of antibiotic resistant bacteria[8,13–15]. Doxycycline hydrochloride (Doxy), a broad-spectrum tetracycline antibiotic, was examined herein due to its widespread usage, environmental toxicity, low biodegradability, and complex molecular structure[16]. Doxy, among other antibiotics, are ineffectively removed by oxidizing agents used in municipal wastewater treatments resulting in its presence in released effluent. The discharge of treated wastewater contaminated with antibiotics instigates the necessity to develop an effective method to remediate antibiotics[17]. Adsorption among remediation technologies , shows superior potential due to its simple design, ease of operation[18], and relative low-cost[19,20]. Dialdehyde cellulose nanocrystals (DCNC) offer additional advantages of facilitating the Schiff Base reaction as a binding mechanism of Doxy's distinct amino groups to DCNC's aldehyde groups[21,22]. However, DCNC is structurally limited in practical applications due to its difficulty to be recovered leading to secondary pollution[23–25]. DCNC can be combined into a hydrogel network to synthesize a sustainable and recoverable adsorbent. Synthesis of a sustainable DCNC hydrogel can be achieved via a sodium alginate (SA) platform, commonly used in hydrogel formation because it is naturally occurring[26], low-cost, and abundance with carboxylate groups that allows for hydrogel composite formation with other materials to enhance its adsorption performance[27–29]. Thus, the synthesis of a DCNC-SA hydrogel may be an ideal candidate to address recovery limitations of DCNC water treatment applications while effectively remediating antibiotic contaminated water.

## B. Research Questions, Hypotheses, Engineering Goals, Expected Outcomes

### *I. Research Questions*

a. How can a DCNC-SA be successfully synthesized?

b. How can DCNC-SA remediate Doxy contaminated water?

c. How can DCNC-SA be reused?

### *II. Hypotheses*

a. If DCNC and SA are crosslinked in $CaCl_2$ solution, then a hydrogel will successfully be synthesized

b. If DCNC-SA is put in Doxy solution, then Doxy will be effectively remediated.

c. If DCNC-SA is put in multiple adsorption-desorption cycles, then Doxy remediation will still be effective.

### *III. Engineering Goals*

The goal of this project is to synthesize a DCNC-SA hydrogel and determine its adsorption and reusability properties in Doxy solutions.

### *IV. Expected Outcomes*

a. After combining DCNC and SA, the mixture can be crosslinked with calcium ions and can form hydrogel beads.

b. DCNC-SA will have a negative surface charge and porous structure that contributes to its adsorption properties.

c. DCNC-SA will have a high regeneration efficiency after multiple adsorption/desorption cycles.

## C. Procedures, Risk and Safety, Data Analysis

### <u>I. Procedures</u>

**Note: Data to determine DCNC-SA's adsorption capacities will be measured using a UV-vis spectrometer (GENYSYS 10S, Thermo Fisher, USA, 840-208100) at a wavelength of 272 nm to determine concentrations of each solution.*

### *1. Synthesis of DCNC-SA*

*1.1. Preparation of DCNC*

a. Measure 92.9 g of cellulose nanocrystals (CNC; Process Development at the University of Maine, USA) into a capped bottle

b. In a separate container, measure 21.389 g of sodium periodate (> 99.8%) and mix with 178.6 g of distilled water in a dark room to prevent photocatalytic decomposition of sodium periodate

c. Cover CNC bottle with aluminum foil and stir at 150 RPM in an oil bath at 54 °C

d. Add sodium periodate solution into CNC bottle in a dark room

e. After 6 hours, transfer solution into dialysis membranes until conductivity of the distilled water dialysis bath is below 5 µS/cm for 12 hours

f. Centrifuge final product at 6000 RPM for 10 minutes

*1.2. Preparation of DCNC-SA Hydrogel*

a. Mix DCNC (0.799 wt%) and SA (2 wt%) in water at 400 RPM for 30 minutes in DCNC:SA ratios of 1.25:2, 1.25:1.25, and 1.25:0.5 as well as 2 wt% SA

b. Load mixture into syringe and attach plastic tubing (inner diameter of 0.2 mm)

c. Place syringe into mechanical syringe pump with ejection speed of 30 mL/hr into $CaCl_2$ (150 mL; 1M) with a stir speed of 50 RPM, drop height of 10 cm from the surface of $CaCl_2$ solution, and 30 min crosslinking time.

*1.3. DCNC-SA Hydrogel Characterization*

a. Press air-dried DCNC-SA sample into solid sample pellet

b. Place sample into FTIR instrument securely

c. Gather background spectra to subtract from result spectra to determine spectra of sample

d. Transfer analysis to library program to indicate what materials are present in the composition of the DCNC-SA

e. Repeat to obtain data for SA and DCNC

***2. Adsorption Performance of DCNC-SA Hydrogels***

*2.1. Adsorption Isotherm*

a. Place 0.2 ± 0.05 g of DCNC-SA in Doxy solutions (pH 7) that ranged from 0 to 4000 ppm in a shaker (180 RPM) for 24 hours at room temperature.

b. After 24 hours, measure adsorption capacities

*2.2. Adsorption Kinetics*

a. 0.2 ± 0.05 g of DCNC-SA in Doxy solutions (pH 7, 400 ppm) were placed in a shaker (180 RPM)

b. Adsorption capacities were measured after 5 to 400 minutes

*2.3. pH Study*

a. Create Doxy solutions at pH 3, 7, 9, and 11 by adjusting with 0.1 M HCl or NaOH and monitor via a pH meter (FE20, Mettler Toledo, USA).

b. Place 0.2 ± 0.05 g of DCNC-SA hydrogel in Doxy solutions (100 ppm) in a shaker (180 RPM)

c. After 24 hours, measure adsorption capacities

*2.4. Dosage Study*

a. Add 0.02 g, 0.05 g, 0.1 g, 0.2 g, and 0.5 g of DCNC-SA into Doxy solution (pH 7, 900 ppm)

b. After 24 hours, measure adsorption capacities

***3. Regeneration Study***

a. Add 1.00 g of DCNC-SA into 25 mL of 400 ppm Doxy solution

b. After 30 minutes, measure adsorption capacity and filter out DCNC-SA using a 20-mesh sieve

c. Immerse adsorbate into 40 mL of 1:1 ratio of HCl (2M):Ethanol (100%) for desorption and stir vigorously

d. Put regenerated sample into 400 ppm Doxy solution and repeat until data is obtained for 3 regeneration cycles

## II. Risk and Safety

For this experiment, Doxy will be treated as a hazardous chemical. All experiments handling Doxy will be conducted with goggles, gloves, lab coat, and disposable face mask for personal protection. Doxy is harmful if swallowed, causes skin irritation, causes serious eye irritation, and may cause respiratory irritation. Wash hands before breaks and at the end of work.

HCl, NaOH, sodium periodate, and sodium alginate will be handled when wearing gloves, a lab coat, and safety glasses at all times. HCl, NaOH, sodium periodate, and sodium alginate can cause eye irritation, skin irritation, kidney damage, irritation of digestive tract, burns, and damage to tissues it comes into contact with. Wear disposable face mask when handling solid chemicals.

## III. Data Analysis

A computer with software that is capable of organizing quantitative data into graphs and charts, such as Microsoft Excel (Version 16.15) will be used. The synthesized graphs will show absorption capacities. It is necessary to study the trends within adsorption isotherm and kinetics by using Excel's built-in functions to find the best curve fitting and equation for that curve.

## Subject-Specific Guidelines

1. Human participants research: N/A
2. Vertebrate animal research: N/A
3. Potentially hazardous biological agents research: N/A
4. ***Hazardous chemicals, activities & devices:***

## I. Risk Assessment Process

*Doxycycline hydrochloride (Doxy; > 95%):*

- Toxicity: 2
- Reactivity: 1
- Flammability: 1
- Corrosive: N/A
- Health Hazard: 2
- Flammability Hazard: 1
- Instability: 0
- Swallowed: Accidental ingestion of the material may be harmful; animal experiments indicate that ingestion of less than 150 gram may be fatal or may produce serious damage to the health of the individual.
- Eye: This material can cause eye irritation and damage.

- Inhaled: The material can cause respiratory irritation in some persons. The body's response to such irritation can cause further lung damage.
- Chronic Health Effects: Long-term exposure to respiratory irritants may result in disease of the airways involving difficult breathing and related systemic problems. There is some evidence that human exposure to the material may result in developmental toxicity. This evidence is based on animal studies where effects have been observed in the absence of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not secondary non-specific consequences of the other toxic effects.
- Handling & storage: Store in cool place. Keep container tightly closed in a dry and well-ventilated place (recommended storage temperature -20 °C). Avoid all personal contact, including inhalation.

*CNC:*

- Toxicity: N/A

- Flammability: 0
- Corrosive: N/A
- Health Hazard: 1 – Irritant
- Reactivity Hazard: 0
- Contact Rating: 0
- Inhalation: No adverse health effected expected. Treat similarly to a nuisance dust.
- Eye contact: No adverse effects expected but may cause irritation.
- Skin contact: No adverse effects expected.
- Ingestion: Large doses may cause gastro-intestinal upset.
- Handling & storage: Avoid mist formation and control electrical equipment and ignition sources. Employ grounding, venting, and explosion relief provisions in accord with accepted engineering. Containers of this material

may be hazardous when empty since they retain product residues (dust, solids); observe all warnings and precautions listed for the product.

*Sodium Periodate (>99.8%):*

- Specific target organ toxicity – (repeated exposure): 1
- Reactivity (Reactive Hazard): Yes
- Flammability: 3
- Corrosive: 1

- Health Hazard: 3
- Physical Hazard: OX
- Handling & storage: Wear personal protective equipment. Do not get in eyes, on skin, or on clothing. Do not ingest. Do not breath vapors/dust. Avoid dust formation. Keep away from clothing and other combustible materials. Keep containers tightly closed in a dry, cool, and well-ventilated place. Corrosives area. Do not store near combustible materials.

*Sodium Alginate (*2 wt%*):*

- Toxicity: N/A
- Reactivity: N/A
- Flammability: 0
- Corrosive: N/A
- Health Hazard: 0
- Chronic Health Hazard: 0
- Physical Hazard: 0
- Handling & storage: Avoid contact with skin and eyes. Provide appropriate exhaust ventilation at places where dust is formed. Keep container tightly closed in a dry and well-ventilated place.

*Hydrochloric Acid (0.1 M):*

- Toxicity: N/A
- Reactivity: N/A
- Flammability: N/A
- Corrosive: 1
- Health Hazard: 3
- Flammability Hazard: 0
- Physical Hazard: 0
- Eye contact: Serious eye damage
- Skin contact: Skin corrosion
- Handling & storage: Avoid contact with skin and eyes. Avoid inhalation of vapor or mist. Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

## II. Supervision

A designated supervisor trained in the techniques to be used will provide direct supervision at all times.

## III. Safety Precautions

*For ALL Chemicals*

- Eye/face Protection: Wear appropriate protective eyeglasses or chemical safety goggles.
- Skin and body protection: Wear appropriate protective gloves and clothing to prevent skin exposure.
- Respiratory Protection: Handle in accordance with good industrial hygiene and safety practice.

## IV. Methods of Disposal

All chemical wastes will be disposed in labeled containers to a designated location in the lab as hazardous waste. Hazardous waste pickups will be requested by emailing HazWaste@stonybrook.edu.

All regulated medical and chemical wastes generated in university laboratories and other facilities must be disposed of in accordance to the guidelines and regulations mandated by the New York State Department of Environmental Conservation.

**NO ADDENDUMS EXIST.**

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