Pathogenic microbes continually evolve to acquire resistance to antibiotic treatments, creating an enormous global health crisis. The consequences are extreme—a recent report projected the human cost of antimicrobial resistance to be $700 trillion cumulative premature deaths by 2050 with a loss of up to $100 trillion to the global economy. Despite the prevalence of antibiotic resistance rising rapidly, the rate of development of novel antibiotics substantially lags behind the rate at which pathogens evolve resistance to them. A strategy to maintain the efficacy of existing and future antibiotics is to continue the push towards pathogen-potentialtizing knowledge of genetics, ecological conditions, evolutionary landscapes, and mechanisms of drug action.

I propose to create a high-throughput system to evaluate sensitivity of microbes to various antibiotics, engineer a novel biocompatible device for implementation of large-dose antibiotic delivery, and compute an inverse model to explore the temporal antibiotic resistance of bacteria during treatment with antibiotics.

**Intelectual Merit** Bacteria resist antibiotics by modifying their cell wall proteins or target of the drug, modifying the antibiotic, or overexpressing their efflux pumps proteins to expel the antibiotic and reduce its efficacy. Engineering strategies to develop antibiotic resistance and eventually overcome it are critical now. Currently, researchers are attempting to do this by studying drug interactions, with regards to synergy and antagonism, to optimize killing of microbes. The current systems are time-consuming and drug-intensive. Furthermore, progress towards overcoming antibiotic resistance has been encumbered by the lack of a high-throughput system to study antibiotic susceptibility and knowledge of compatible biomarkers for large-dose antibiotic delivery.

If successful, this project will understand how collateral sensitivity—which is an organism resistant to one drug displays increasing sensitivity to a second drug—helps clear bacterial population relative to treatment either with one drug or a combination of drugs simultaneously (Fig. 1A). Exploring collateral sensitivity will lead to the evolution of resistance and increase the lateral clearance of bacterial pathogens in my interdisciplinary project, which integrates microbiology, mechanical engineering, materials science, and mathematical biology, as part of the IM call-out. I intend to create a microfluidic system to study the effectiveness of collateral sensitivity, develop a large-scale drug delivery device to be tested in vitro, and provide a mathematical basis for understanding the dynamics of bacterial treatment and resistance.

**Approach** With guidance from Dr. [Name], I will leverage the resources of the Laboratory and the Center for undergraduate students to work on Aim 1 and Aim 2 of this project.

**Aim 1:** Evaluate different antibiotic regimens with bacteria using microfluidics. To circumvent the time and financial constraints on antibiotic susceptibility testing, I recently worked to fabricate a multiplexed microfluidic device with support from [funding agency]. This continuous-flow system (Fig. 1B) can enable parallel monitoring of mutations and changes in cell morphology in real time and measure phenotypic heterogeneity at a single-cell level in genetically uniform microbial populations.

**Aim 2:** Develop biomaterials for gauge resistant delivery device with a holding capacity of 50 μg of drug. Figure 2 illustrates the device that can be used to inject, release time-controlled drug release increases robust mechanical properties over one month (using the tissue machine). The device is designed to have an optimal period of cycling drug delivery for collateral sensitivity and determine when this approach is a better treatment option.

**Broader Impacts** A high-throughput platform for rapid antibiotic susceptibility testing combined with a predictive mathematical model represents a significant step forward in developing new treatments to quickly eradicate bacterial infections while minimizing resistance to current and future drugs. I will partner with industries through a research program and translate results of my research to the clinic for developing large-dose antibiotic delivery devices. Moreover, to respond to the growing need to generate highly educated and exposing them to a variety of research topics, I am organizing bootcamps workshops through the College Connection program in my capacity as a Society of Women Engineers graduate student leader. These will incorporate interdisciplinary techniques used in my project, such as microfluidics, biotechnologies, and mathematical modeling. This model can serve as a platform for a general and systematic approach for understanding the interactions of antibiotics and resistance.