Synthesis and Evaluation of Polyaspartate Peptoid

Polymers URECA Proposal Submitted by: (Student Name)

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Introduction

Peptoid polymers are a class of synthetic polymers that mimic the structure and properties of peptides and proteins. Due to their unique properties and design flexibility, peptoid polymers have found applications in drug discovery, materials science, biotechnology, and other fields.[1] Peptoid polymers are being explored for drug development due to their ability to mimic biological molecules. They can be designed to interact with specific biological targets, potentially leading to the development of novel therapeutic agents.[2] Peptoid polymers can also be employed in biological assays and diagnostics due to their ability to bind to target molecules selectively.[3] This makes them useful in the development of biosensors and diagnostic tools. Researchers continue to explore and develop new peptoid-based materials for various applications. Polyaspartate peptoid polymers are a yet unexplored class of peptoid polymers derived from polyaspartic acid. Polyaspartic acid is a biodegradable polymer composed of repeating aspartic acid units, and when it is modified into a peptoid form, it becomes polyaspartate peptoid polymer. One of the key features of polyaspartate peptoid polymers is their stability, particularly in combating bacterial biofilms. Unlike some synthetic polymers that degrade easily, peptoids are known for their resilience and resistance to breakdown. This stability is crucial for mitigating the formation of bacterial biofilms, which is a primary focus of this project. By remaining intact over time, polyaspartate peptoid polymers can effectively reduce the environmental impact associated with biofilm formation. Polyaspartate peptoid polymers are often water-soluble, making them suitable for various applications where solubility in aqueous solutions is essential.[4] This characteristic is particularly valuable in fields such as biomedicine, where water solubility is often a critical factor. Like other peptoid polymers, polyaspartate peptoids can be designed with diverse side chains, allowing for the introduction of various functional groups. This modifiability provides researchers with the ability to tailor the

properties of polyaspartate peptoids for specific applications, such as drug delivery and biomaterials. Polyaspartate peptoid polymers exhibit structural similarity to polyaspartate peptides but offer improved stability against proteases. This enhanced stability makes them advantageous in various applications. With their unique combination of properties, polyaspartate peptoid polymers find utility across diverse fields. They are explored for use in drug delivery systems, biomaterials, and environmentally friendly coatings.[5]

Purpose

The proposed research aims to investigate the potential of never before made polyaspartate peptoid polymers in combating *Pseudomonas aeruginosa* biofilms.[6] These biofilms are tough communities of bacteria that can cause persistent infections and are particularly challenging to treat. Our study focuses on understanding whether these polymers can disrupt or prevent the formation of biofilms and how they might change the structure of existing biofilms. We're interested in exploring how these special polymers interact with biofilms because we believe they could offer new ways to manage and prevent biofilm-related infections. Specifically, we're looking at how polyaspartate peptoids compare to traditional peptides in terms of their ability to resist breakdown by proteases, enzymes that can break down proteins. Understanding this difference is important because it could help us develop materials that are more resilient against bacterial enzymes. By investigating these properties, we aim to uncover insights that could lead to the development of better antimicrobial materials. This has important implications for various fields, including healthcare, where new materials are needed to create drugs and medical devices that can effectively combat bacterial infections. Additionally, some peptoid polymers with a specific sidechain have shown antibiofilm properties, unlike their peptide counterparts which are susceptible to breakdown. This suggests that peptoid polymers could offer unique advantages in

fighting biofilm-related infections. Lastly, polyaspartate peptoid polymers have never been synthesized, so determining the best conditions for polymer synthesis will be valuable contribution to the field of peptoid polymers.

Methods

The study's plan involves synthesizing different kinds of polyaspartate peptoid polymers to see how well they can stop bacteria from growing and forming biofilms. By synthesizing polymers of different lengths, we will determine how polymer size relates to antibacterial activity. To synthesize these polymers, we followed specific steps outlined in previous research. For synthesis of smaller peptoids (10-20 monomers) we will use a technique well established in our lab called the submonomer method, which involves putting together small building blocks one at a time on a solid support to make the polymer.[7] For synthesis of larger polymers (20+ monomers) N-carboxyanhydride (NCA) polymerization will be done following the methodology detailed by Luxenhofer *et al*.[8] This method will involve the synthesis of NCA building blocks that will then be polymerized into the desired length of polyaspartate peptoid polymer. To test how effective these polymers are against *P. aeruginosa*, we will use two kinds of tests. One is called the Minimum Inhibitory Concentration (MIC) test, where we will measure the smallest amount of the polymer needed to stop bacteria from growing.[9]The other test is called Minimum Biofilm Inhibitory Concentration (MBIC) assay, where we determine how well the polymers prevent bacteria from forming biofilms. We will follow standard protocols for these tests as we have done previously, collecting all data in technical and biological triplicate.[10]

Preliminary Results

Our efforts to synthesize a 10-mer polyaspartate peptoid polymer using the submonomer method are already underway. Synthesis proceeded smoothly for this well-established technique,

though purification of this highly hydrophilic polymer required some optimization. However, this was successful and pure 10-mer polyaspartate peptoid polymer was confirmed by mass spectrometry. Afterward, we tested the 10-mer polyaspartate peptoid polymer against bacterial biofilms, focusing on *P. aeruginosa*. After evaluating its effectiveness over several days, we found that the polyaspartate peptoid did not noticeably affect the *P. aeruginosa* growth in the MIC assay. In response to these results and in consultation with collaborator Dr. Justin Miller, we refined our approach by modifying the synthesis of the polyaspartate peptoid. This time, we added oleic acid, a long fatty acid chain, to the N-terminus of the peptoid. This modification aims to make the molecule more hydrophobic, potentially enhancing its effectiveness against bacteria. Synthesis of this derivative is now complete, and we are working to purify and characterize this molecule.

Proposed research

With the initial creation of the smaller polyaspartate polymer completed, our next step is to see how adding the fatty acid chain affects bacterial biofilms. This is important because it will show us if the modified polymer is better at fighting bacteria. We are going to carefully look at how these changes impact the polymer's ability to work against *P. aeruginosa* biofilms. To do this, we'll start by making something called NCA (N-carboxyanhydride) monomers. These are highly reactive monomer building blocks that will be used to synthesize larger polypeptoids as done previously.[11] Initial efforts will focus on replicating previous work to learn how to synthesize and characterize polymers using this new technique. Next, we'll synthesize the NCA called N-(O-t-butoxy) carboxymethylglycine, which will be used to synthesize larger polyaspartate peptoid polymers. Success in this step represents a significant advancement in our research trajectory, enabling the exploration of novel polymer compositions with diverse physicochemical properties. To see how effective our polymers are against bacteria, we will carry out MIC and MBIC assays

comparing polymer length to antimicrobial activity. In short, these assays will involve adding varying concentrations of polymer to new or well-established bacterial inoculants in growth media, incubating for 24 hours, and measuring bacterial viability. These tests will help us understand if our polymers are good at fighting bacteria.

Conclusion

In conclusion, our research endeavors to advance the field of antibacterial polymers by synthesizing novel polyaspartate peptoids and exploring their efficacy against *P. aeruginosa* biofilms. Through meticulous experimentation and strategic modifications, we aim to enhance the antibacterial properties of these polymers, ultimately contributing to the development of innovative solutions for combating bacterial infections. The proposed timeline outlines a systematic approach to our research, from initial synthesis to comprehensive testing and refinement. With the necessary resources, we can continue to push the boundaries of polymer science, unlocking new avenues for addressing antibiotic resistance and improving healthcare outcomes.

Timeline:

- **Fall 2023**: Synthesize the 10-mer polyaspartate peptoid polymer and conduct testing against *Pseudomonas aeruginosa* bacterial biofilms.
- **Spring 2024:** Modify the 10-mer polyaspartate peptoid by incorporating a fatty acid chain and proceed with the synthesis of the NCA building block.
- **Fall 2024:** Synthesize the poly NCAs, polymerize N-(O-t-butoxy)carboxymethylglycine) NCA, and synthesize poly(N-carboxymethylglycine)$_{25}$. Additionally, perform MIC and MBIC assays.

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