

SpiDentistry: The Intersection of Dentistry and Spider Silk

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Introduction

Spider Silk

Spider silks have caught the attention of researchers for many years due to their incredible strength, elasticity, and other mechanical properties. Other properties such as biocompatibility and biodegradability also make spider silks ideal biomaterials. Spiders produce six different types of silk as well as a glue (Figure 1). Each silk type possesses various properties that make them more ideal for specific applications.

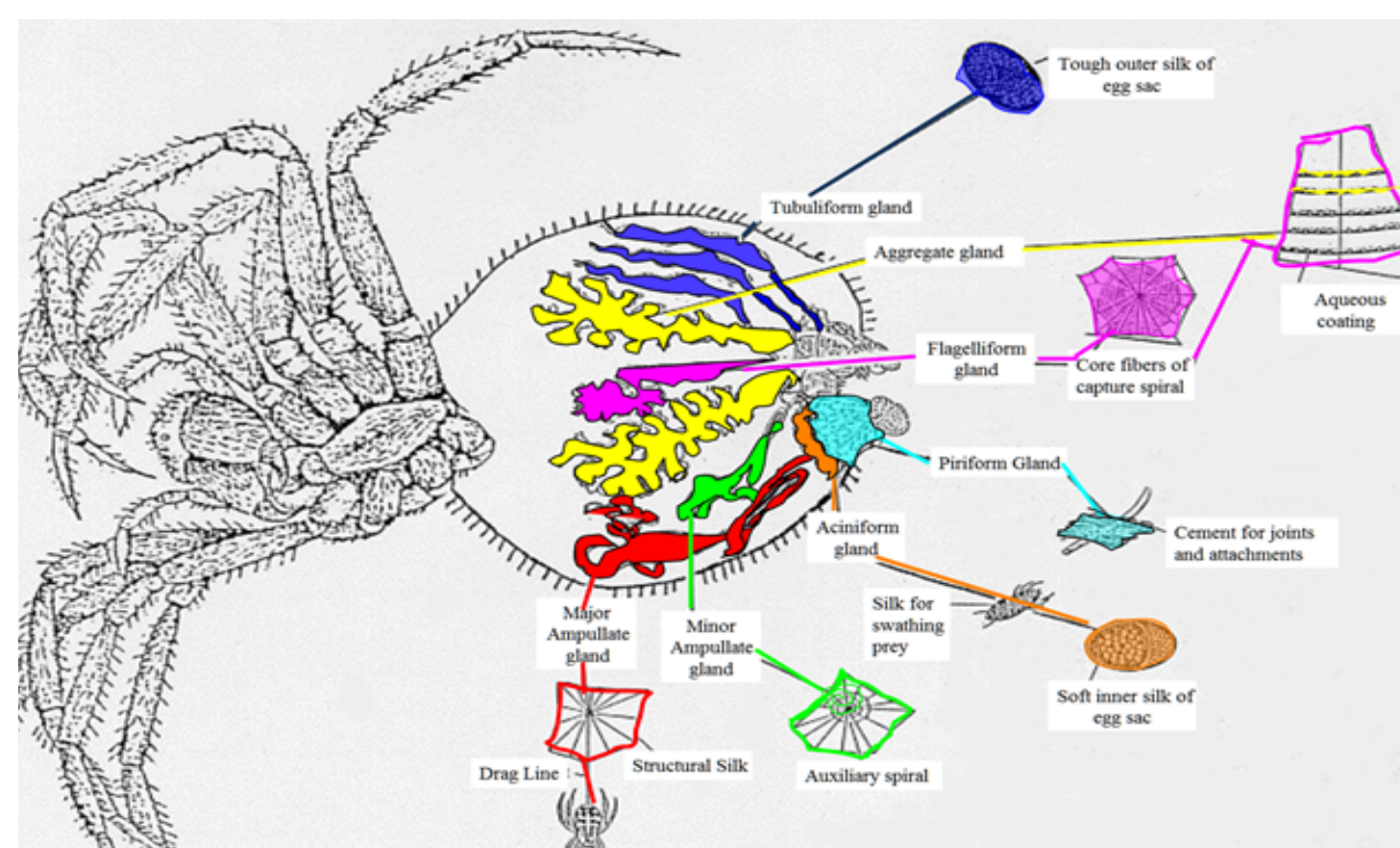


Figure 1. A diagram with the six silk glands and one silk glue gland from *Nephila clavipes*. Each type of silk has its function in the web and is correspondingly color-coded.

Spider silk is the strongest fiber found in nature and one of the strongest materials known to man. Spider silk is tougher than Kevlar, more elastic than nylon, and stronger than steel by weight. These properties arise from specific structures in the protein that act like molecular building blocks or springs. Due to an inability to farm spiders, substantial work has been done to produce recombinant spider silk proteins (rSSPs) in transgenic hosts for large-scale production. Current hosts include bacteria, goats, alfalfa, and silkworms. All of these systems have pros and cons with regards to protein recovery and the final products.

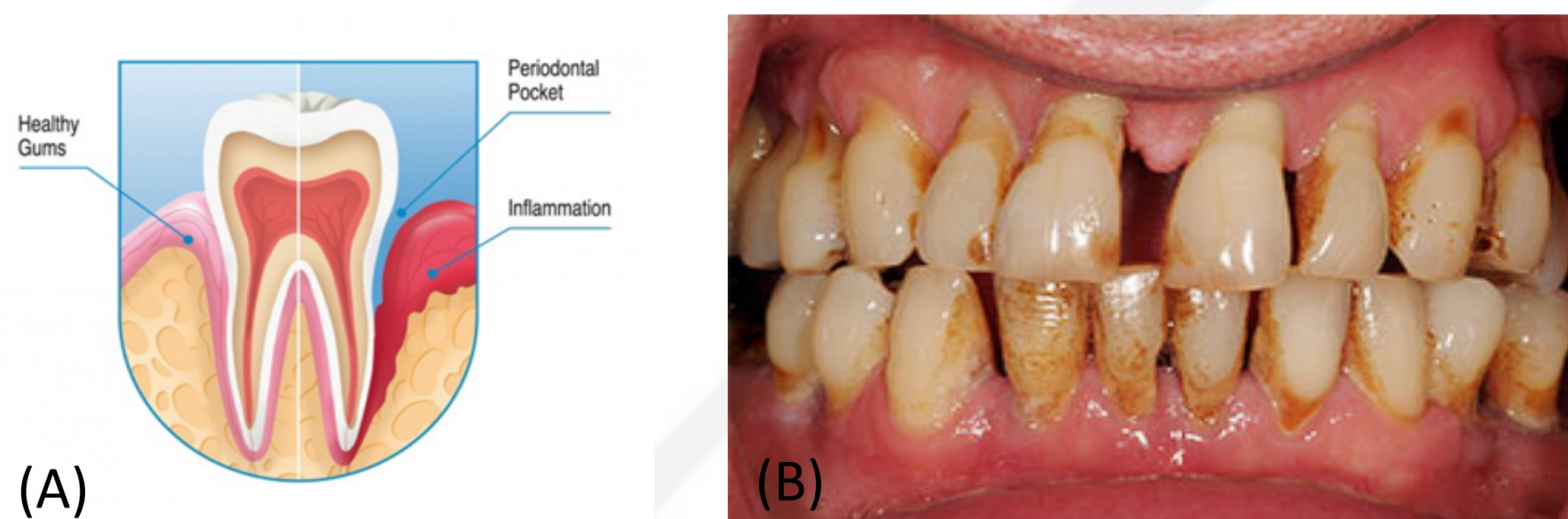


Figure 2. (A) Schematic of healthy gums vs. diseased gums. (B) Periodontitis with recession of gum and supporting tissue.

Periodontal Disease

Periodontal disease (Figure 2) is an inflammatory disease that affects both soft and hard structures that support the teeth. In the more severe form of periodontal disease called periodontitis, the gums pull away from the tooth and supporting gum tissues are destroyed. Bone can be lost, and the teeth may loosen or eventually fall out. According to recent findings from the Centers for Disease Control and Prevention (CDC), half of Americans aged 30 or older have periodontitis. Reducing pocket depth and eliminating existing bacteria are important to prevent the progression of periodontal disease. Deeper pockets are more difficult for patients and dental care professionals to clean. In periodontal treatment antimicrobial chips, spheres, or gels are placed into the periodontal pocket in order to eliminate bacteria allowing the supporting gum tissue to reattach to the tooth.

Introduction Continued

Spider Silk Use in Periodontal Disease

Spider silks are an ideal material for periodontal chips/gels because they are biocompatible, biodegradable, lightweight, strong, and create no immune response. Furthermore, the various silks types and forms allow for tunable materials and properties. Spider silk materials may have an advantage over other products because of the ability to release medication more consistently over a longer period of time and be adjusted as needed. Unlike products currently on the market, our chip/gels are biocompatible, which aids in reducing the bodies immune response to periodontal treatment. The ability to incorporate multiple bioactive compounds into the spider silk materials allows them to become multifunctional treatment devices against inflammatory response, infection, and other related complications that come from periodontitis.



Figure 3. Comparison of periodontal chips from the left to right: Cross-linked spider silk chip, normal spider silk chip, and PerioChip.

The purpose of this research project is to develop and test spider silk chips/gels and compare them with products currently on the market to determine the feasibility and potential of spider silk materials. To characterize these silk and conventional materials the release, activity, robustness, geometry, and longevity will be analyzed.

Materials and Methods

Spider silk proteins were obtained through the purification of milk from transgenic goats expressing the spider silk proteins MaSp1 (M4) and MaSp2 (M5). Tested in this experiment were preparations of 25% (w/v), 12.5% (w/v), and 6.25% (w/v) concentrations of spider silk with ratios of 80:20 M4:M5 and 100% M4. The rSSPs are first solubilized in deionized water with mild heat (<130 °C) and pressure. These conditions mildly denature the proteins and force them into the aqueous solution. Once the rSSPs are in solution chlorhexidine gluconate (CHG) was added to the solubilized dope in a 1:1 mixture. This addition brought the final concentrations of silk to 12.5% (w/v), 6.25% (w/v), and 3.125% (w/v). This solution is then used to form the chips by pipetting 100 μm of the solution onto a polydimethylsiloxane (PDMS) mold and allowed to form and cure overnight.

These periodontal chips were then placed into 5 mL of phosphate buffered saline (PBS) and allowed to release the medication. In order to track the amount of CHG released, samples were taken each day, and a new PBS was added. This process was repeated for fourteen days. In order to test the amount of CHG released we used the process of reverse phase ultra pressure liquid chromatography (RP-UPLC). Using known standards, the amount of CHG released could be calculated based upon the corresponding absorbance amount and retention time. All of the samples, both spider silk and PerioChips were analyzed with this method.

Antimicrobial activity was tested and observed during this project. The chips were placed on a plate of *E. coli* after spreading the cells and placed in a 37 °C incubator and observed over fourteen days.

Results

Through our research we have been able to produce products that are similar to those currently used in dentistry. Our release profiles shown in (Figure 4) illustrate the robustness of spider silk, allowing a more consistent release of CHG over a longer period of time with more burst phases. These profile translate to more consistent and sustained inhibition of the microbes and other factors present in periodontal disease.

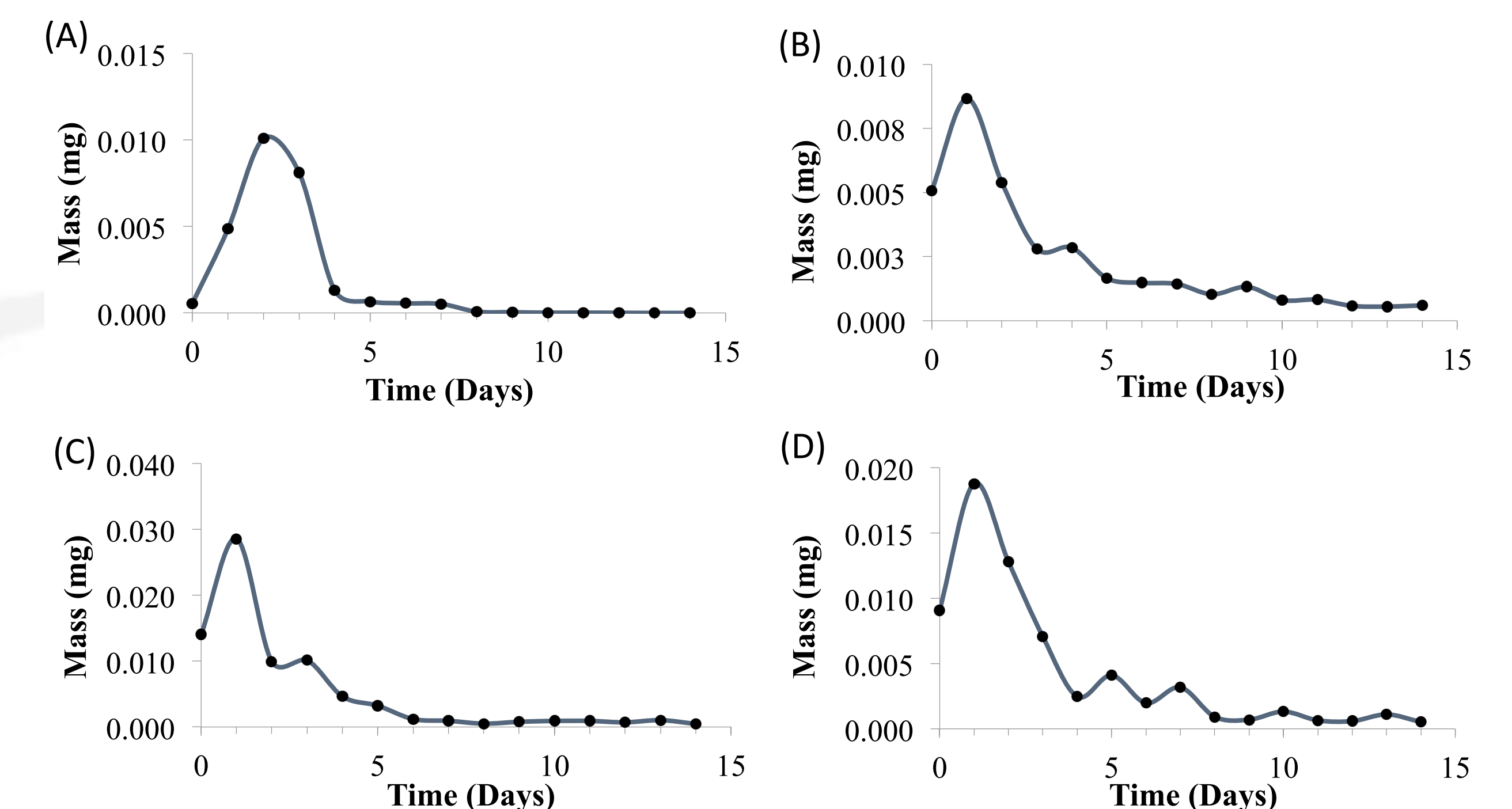


Figure 4. Representative release profiles of PerioChips and rSSPs chips. (A) Release curve for conventional PerioChip device. (B) Release curve for 12.5% (w/v) 80:20 (M4:M5) spider silk chip. (C) Release profile for 6.25% (w/v) 80:20 (M4:M5) spider silk chip. (D) Release profile for 6.25% (w/v) M4 spider silk chip.

Although the release profiles were characterized for all chip devices, more practical applications were also demonstrated by the spider silk chips through inhibition studies against various microbe species

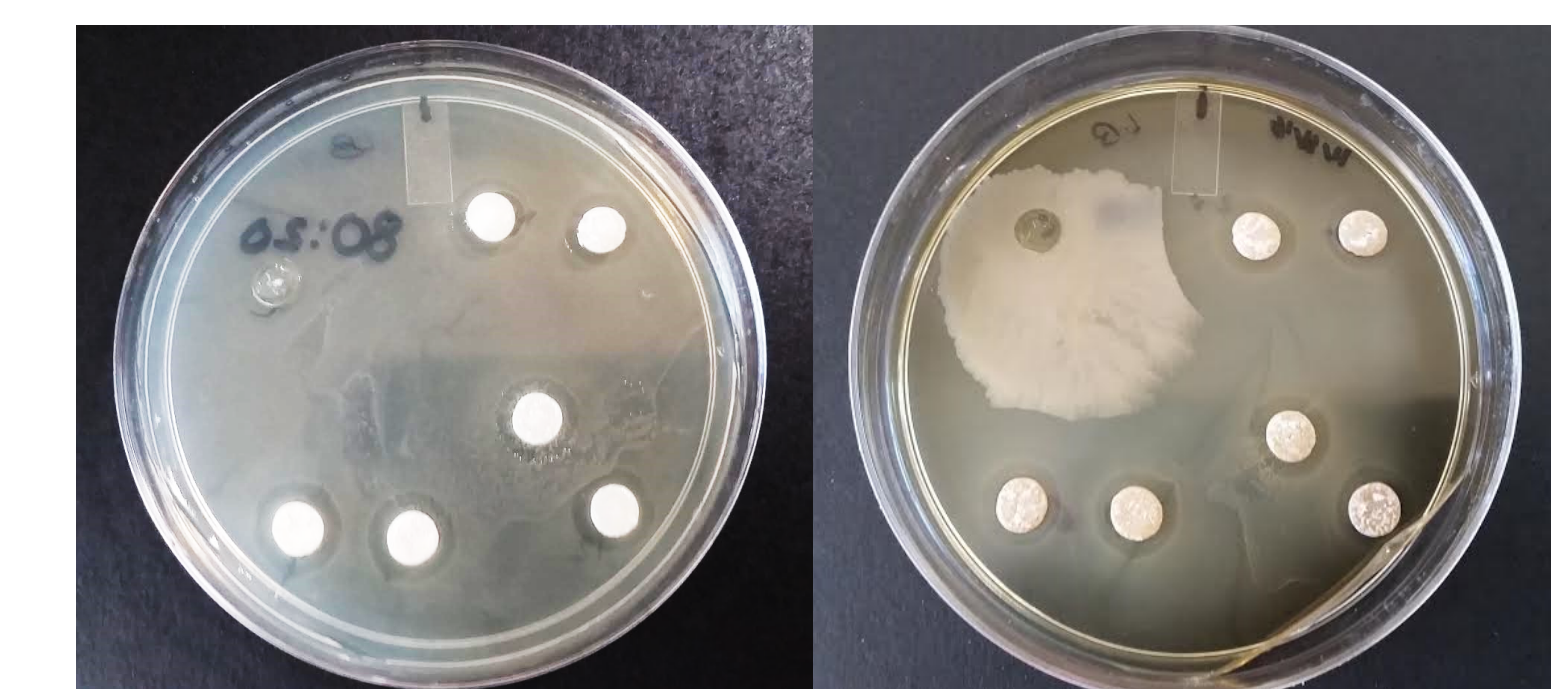


Figure 5. Zone of inhibition studied for spider silk chips. Top left chip: control (no chlorhexidine). (A) Growth after 48 hours with zones of inhibition was present. (B) One week after initial plating: a definitive zone is still observed. Unplanned mold contaminants were present on the control but inhibited by functionalized spider silk chips.

Conclusions and Future Work

After an analysis of our results we are confident that the ability to tailor or tune the final product to perform specific tasks is possible for the treatment of periodontal disease. This customization is accomplished the simple alterations of concentrations and types of silk and can be greatly expanded upon for further diversity.

Currently our future plans with spider silk and dentistry are leading us to a massive group collaboration with Roseman University. Using spider silk as a drug delivery system for MMP inhibitors, growth factors, and antimicrobial additives, to treat chronic periodontal disease. We look forward to this collaboration and see a bright future in this research. More in depth testing and characterization will also be conducted as we look towards the opportunities to test our silk as an antimicrobial root canal sealer or other dental treatment.

