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Quantifying Mafenide Release from 3D Printed Phenylalanine-Coumarin Copolyester Scaffolds

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Abstract

This project addressed burn wound healing through controlled release of the antibacterial drug mafenide encapsulated by a copolyster into 3D printed scaffolds. Scaffolds were printed at 25^{o}C and 0.77-1 bar in a cross-hatch pattern with uniform thickness, distance between parallel layers, and diameter then photo-crosslinked. The number of scaffolds available for testing was limited by the amount of polymer that could be synthesized. A high-performance liquid chromatography method was developed specifically for this experiment and used to determine daily release of mafenide from the scaffold into a 2-mL phosphate buffer solution. During the first 24 hours a large burst release of 299.9 μg or 7.9% of the initial drug amount was observed for the 2-layer scaffolds and 403.1 μg or 8.7% of its initial drug amount for the 3-layer scaffolds. Within 120 hours, the release rate had dropped to less than 100 μg per day for both scaffold types. The cumulative average release for the 2-layer and 3-layer scaffolds was determined to be 1449.0 μg (38.8% of the initial total) and 1469.5 μg (31.8% of the initial total) respectively. The results suggest that these scaffolds have the potential to become a viable treatment option in the future.

Executive Summary

During the burn wound healing process, bacterial infection is a significant issue that can complicate treatment and patient recovery. This project aimed to address burn wound healing and bacterial contamination through controlled release of antibacterial drugs encapsulated by a polymer into 3D printed scaffolds. Mafenide, typically as mafenide acetate, is one such drug used to prevent infection in burn wounds. The drug is commonly applied as a topical cream but induces some negative side effects such as pain on contact and the presence of residue on the skin (Falcone et. al.). Because of these side effects, the use of 3D printed scaffolds containing mafenide may be a better treatment method, as it also increases the potential to optimize recovery and reduce costs.

Scaffolds of two and three layers containing 10% mafenide by weight and a phenylalanine-coumarin copolyester were printed. A phosphate buffer solution and incubator were used to simulate conditions within the body. Daily release of mafenide was quantified by collecting supernatant samples and analyzing them using high performance liquid chromatography (HPLC) in order to determine if the drug could be delivered in suitable quantities to provide potentially viable treatment.

The experiment ran for 384 hours, and it was determined that mafenide could be released in quantifiable amounts. The average amount of mafenide released daily and the cumulative average amount of mafenide released over the entire period were determined for the 2-layer and 3-layer scaffolds. An initial burst release of mafenide was observed in this experiment. During the first 24 hours, the 2-layer scaffolds released roughly 299.9 μg or 7.9% of the initial total drug on average whereas the 3-layer scaffold released 403.1 μg or 8.7% of the initial total drug. By 120 hours, both the 2-layer and 3-layer scaffolds were releasing less than 100 μg per day. Despite initially releasing different amounts of drug, the 2-layer and 3-layer scaffolds delivered almost

identical total amounts of mafenide during the 384-hour period. The cumulative average release for the 2-layer scaffolds was determined to be 1449.0 μg while the cumulative release amount for the 3-layer scaffold was 1469.5 μg. These release amounts correspond to roughly 38.8% of the initial total mafenide for the 2-layer scaffolds and 31.8% of the initial total mafenide for the 3-layer scaffolds.

From these results it can be concluded that mafenide can successfully be encapsulated by a polymer into a matrix that can be used to 3D print scaffolds for drug delivery. The release data shows that mafenide may be released from these structures for an extended period of time. HPLC analysis of irradiated mafenide indicates that the drug does not break down as a result of exposure to UV light. As a result, the photo-crosslinking process is not harmful to the mafenide and can be implemented in order to stabilize the scaffolds. The initial surge release of mafenide from the scaffold suggests that the polymer-drug matrix contained greater amounts of drug at the surface. Because small percentages of the initial total amount of mafenide can be released even after a prolonged duration, this treatment method may be viable if the initial amount of mafenide present in the scaffold enables the appropriate dosage to be delivered.

This work explores an alternative treatment method to those commonly used for mafenide that, if successful, may help patients avoid negative side effects while still receiving the necessary treatment for their burn wounds. This research provides the foundation for additional investigation into mafenide encapsulation and delivery. Because the results show that this method of drug delivery may be viable for mafenide, it opens the possibility to explore other antibacterial drugs for burn wounds using this process.

Future work on this topic should be done to investigate the efficacy of this treatment option for mafenide. In future experimentation, obtaining multiple scaffolds with more uniform weights

for each number of layers being tested should be prioritized to ensure greater accuracy in the results. The goal of future work should be to modify different experimental variables in order to achieve a consistent daily delivery of the drug. Changes can be made to mafenide concentration, scaffold geometry, scaffold dimensions, number of scaffold layers, and encapsulating polymer in order to achieve this goal.

Introduction

This project sought to address burn-wound healing and principles of tissue engineering via the controlled release of antibacterial therapeutics incorporated into 3D printed scaffolds. Burns are typically acute injuries that fully heal within a short period of time with limited scarring (Nun et. al.). More severe burns, such as third-degree burns, may take significantly longer time to heal while requiring skin grafts and other treatment. During the wound healing process, it is essential that bacterial contamination be eliminated or minimized in order to prevent infection of the injured tissue. Topical creams are typically used to address this issue, but due to possible negative side effects and the need to continually apply the ointment to the affected area, the use of 3D printed scaffolds containing antibacterial drugs may be a more favorable alternative. This method of controlled release offers the potential to optimize drug concentration, reduce treatment cost, and improve patient compliance (Nun et al.).

The focus of this project was on the bacteriostatic agent mafenide. This research builds off previous work completed in Dr. Joy's laboratory related to quantifying release rates for drugs incorporated into 3D printed scaffolds, e.g., the anti-inflammatory drug dexamethasone. Scaffolds containing 10% mafenide by weight and a phenylalanine-coumarin copolyester were printed with either 2 or 3 layers. The scaffolds were placed in a phosphate buffer solution (PBS) which was collected and replaced with fresh solution every 24 hours. Drug delivery was quantified using high performance liquid chromatography (HPLC). Detailed experimental methods, results, and analysis are contained within this report.

Background

Mafenide, typically as mafenide acetate, is a sulfa antibiotic drug that is often delivered in a topical cream or aqueous solution (Falcone et. al.). The drug is highly soluble in water, has low affinity for plasma proteins, and inhibits bacterial growth with minimal to no onset of resistance (Falcone et. al.). As mafenide acetate the drug has a molecular wight of 246.3 g/mol and is soluble in water up to a level of 250 mg/mL (Elsner and Zilberman). Mafenide is often used to inhibit bacterial growth in burn wounds to decrease the risk of infection and promote healing. Elsner and Zilberman note that the drug is effective on second and even third-degree burns. According to Falcone et. al., mafenide has been successful in reducing infections and increasing the survivability of burn victims. Despite its efficacy, negative side effects of topical mafenide creams exist, such as pain on application and the formation of a gummy layer upon drying that must be removed with water (Falcone et. al.). Other treatment options utilizing mafenide may be more desirable to avoid these adverse effects.

Falcone et. al. investigated wound dressings containing mafenide and determined that continual saturation of the dressing is the most effective method of ensuring optimal drug delivery. Elsner and Zilberman also studied mafenide wound dressings, among others, by creating a structure containing a polyglyconate core and a porous poly(DL-lactic-co-glycolic acid) shell then adding the drug. They found that desirable release profiles for mafenide could be obtained from these structures and that factors could be controlled to lower the burst release that is common in these scenarios (Elsner and Zilberman). Previous work in Dr. Joy's laboratory, such as the experiment described in Jain et. al. in which dexamethasone was used, has shown that scaffolds containing drugs encapsulated in a polymer can be 3D printed and used as means of drug delivery.

The experimental methods described below were largely based off the design set forth in Jain et. al. with some modifications due to the difference in drugs being used.

Experimental Methods

Experimentation began with the synthesis of the phenylalanine monomer. A stir bar and 3-phenylpropanoic acid were added to a round bottom flask. An addition funnel was attached and 2 cycles of vacuum and nitrogen purging were done to the flask. Dry methanol was added to the flask which was placed in an ice bath to control the temperature and reaction. After 5 minutes of stirring, thionyl chloride was added dropwise to the flask. The contents were allowed to react for 24 hours, then sodium sulfate was added to control the pH of the mixture. Water containing a couple drops of hydrogen chloride was added to dissolve the sodium sulfate. The mixture was dried, and the mass of the intermediate product of 3-phenylpropionate was obtained. The final monomer product was obtained by adding diethanolamine to the intermediate product and allowing the two to react.

Small amounts of unreacted materials and intermediate product remained present in the mixture, meaning separation of the desired phenylalanine polyester monomer was necessary. The contents of the mixture were run through a column packed with a large layer of silica as the stationary phase and a smaller layer of sand on top. The sand ensured the mobile phase would be uniform when entering the silica. Because the chemicals move through the stationary phase at different rates, separation of the contents could be achieved. Thin layer chromatography (TLC) was performed as liquid drained from the column to determine the contents of the exiting fluid. When only the desired product showed up in the TLC tests, the exiting fluid was collected. The collected product was placed in a rotovap to provide separation.

The phenylalanine monomer was copolymerized with a long-chain coumarin polyester containing a seven-carbon tail. The two monomers were added to a flask with succinic acid and 1,4-Dimethylpyridinium p-toluenesulfonate (DPTS). Three cycles of vacuum and nitrogen were done, then dichloromethane (DCM) was added. After the contents dissolved, N,N'-Diisopropylcarbodiimide (DIC) was added dropwise to the flask, and the reaction was allowed to proceed for 48 hours. The solution was filtered with DCM and placed in a rotovap. The contents were then placed in dialysis bags and left in methanol overnight. The polymer was precipitated using methanol then dried via a centrifuge and then vacuum.

Mafenide and polymer were combined by dissolution to create a mixture containing 10% drug. Due to solubility issues, the mixture was precipitated in ether and dried using high vacuum overnight. Drug-polymer scaffolds of two and three layers with a diameter of 12 mm were created using a 3D printer set to 25^{o}C and within a pump pressure range of 0.77 to 1 bar. The scaffolds were printed in a cross-hatch pattern with uniform dimensions, including layer thickness and distance between parallel layers. After printing, the scaffolds were photo-crosslinked using UV light at an intensity range of 87-95 mW/cm^2. The 2-layer scaffolds were exposed to the UV for 300 seconds on each face. The 3-layer scaffolds were exposed to the UV for 300 seconds on each face and then an additional 300 seconds on the top side. Four 2-layer scaffolds and one 3-layer scaffold remained intact for experimentation after printing and crosslinking. A control group of scaffolds containing only polymer were also printed. The weight of each scaffold was obtained as well as the weight of each 20 mL vial used to hold each scaffold for the duration of the experiment.

A phosphate buffer solution (PBS) with a pH of 2.5 was used to facilitate the release of mafenide from the 3D printed scaffolds. The solution was prepared using ultrapure water, sodium dihydrogen phosphate monohydrate, and 85% phosphoric acid. Scaffolds were submerged in 2 mL

of PBS and then placed in an incubator set to 37^{o}C and 60 RPM. The supernatant was collected every 24 hours and replaced with 2 mL of fresh PBS. For the control scaffolds, this was done weekly instead.

Samples were analyzed using high-performance liquid chromatography (HPLC). A method was prepared specifically for this experiment. The temperature of the column was controlled at 20^{o}C, and a wavelength of 235 nm was chosen for the UV lamp. The mobile phase consisted of PBS and acetonitrile flowing at 1 mL/min for 10 minutes. From 0 to 3 minutes, the mixture consisted of 90% PBS (10% acetonitrile). From 3 minutes to 6 minutes the PBS content dropped to 75%. From 6 minutes to 9 minutes the PBS concentration dropped again to 60%. For the final minute, the mixture was set to 45% PBS. A calibration curve was prepared by using this HPLC method to obtain the peak area of various known concentrations of mafenide in PBS. The concentration of each sample was then determined from this calibration curve. The amount of drug released could then be determined. Additionally, in order to monitor drug sensitivity and degradation resulting from UV light, a 1 mg sample of mafenide was irradiated with 87-95 mW/cm^2 for two cycles of 300 seconds then placed in 1 mL PBS and analyzed using this HPLC method.

After the duration of the experiment, the scaffolds were dried using liquid nitrogen then left under vacuum overnight. The mass of the vial and scaffold together was measured after removing solid residue that remained in the vials. The final mass of the scaffold could be determined by subtracting the previously obtained vial weight from the combined weight with the scaffold.

Discussions/Analysis

The number of scaffolds that could be printed was limited by the amount of polymer that could be synthesized. Because of this, there was insufficient material to obtain multiple 3-layer scaffolds. Since average values were reported for the amount of mafenide released, this may have affected the results for the 3-layer scaffold. Additionally, the first 2-layer scaffold contained significantly more mass than the other 2-layer scaffolds as well as the 3-layer scaffold. Because it was printed first, excess material may have accumulated in the 3D printing needle head, leading to its larger size. This outlier may have affected the release results since so few scaffolds could be tested. In future experiments, the initial mass of each scaffold and the amount of drug present should be more uniform to obtain a more representative average.

The polymer degradation study provided evidence that there is no interference with the mafenide peak, indicating accurate results for mafenide release were obtained from the HPLC testing. Also, the analysis of the irradiated mafenide indicates that the drug did not denature as a result of the crosslinking method performed in this experiment. This means that the drug would still be viable after incorporation into the scaffold and has the potential to provide treatment in this form.

The release data shows that mafenide can successfully be encapsulated by a polymer into a 3D printed scaffold which may then be used to provide transdermal or subdermal delivery of the drug. The trends observed in the release data regarding an initial burst release of a large amount of drug followed by a sharp drop off in daily release matches closely with other experiments of a similar nature as revealed with the dexamethasone encapsulations described in Jain et. al. This is the result of large amounts of drug being present at the surface of the 3D printed matrix instead of being uniformly dispersed (Jain et. al.). Scanning electron microscope (SEM) imaging can be used

in the future to further analyze the scaffolds and the pores within to determine whether or not this was the case.

The similarity between the cumulative amount of mafenide released for the 2-layer and 3-layer scaffolds is surprising. Because more drug was present on average in the 3-layer scaffold, it was expected to have a higher cumulative average release. This disparity can also be observed with the cumulative average percentage of drug released, since the 3-layer scaffold released roughly 7% less of the initial amount of drug than the 2-layer scaffolds did after 384 hours. The aforementioned issues regarding the limited number of scaffolds available conflated these discrepancies between expected and observed results, since only one 3-layer scaffold could be tested.

Additional work on this topic should be done to investigate the viability of this treatment option for mafenide. In future experimentation, obtaining enough polymer to print multiple scaffolds for each number of layers being tested is essential to obtain representative results. Developing a method to achieve a consistent daily delivery of the drug should be the goal of future work on this topic. Changes to mafenide concentration, scaffold geometry, scaffold dimensions, number of scaffold layers, and encapsulating polymer are all variables with the potential to be explored to achieve this outcome.

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