The Effect of Trehalose and Poly(lactic-co-glycolic acid) (PLGA) Microparticles on the Release Kinetics of Hydrophobic Drugs in Polyurethane Scaffolds

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KEYWORDS. Polyurethane scaffold, trehalose, PLGA microparticles

BRIEF. This study looks at tailoring the release kinetics of hydrophobic drugs from polyurethane scaffolds.

ABSTRACT. There are 35 million cases of significant skin loss that need therapeutic interventions in a year. Autografts are standard treatments for substantial cutaneous injury but are not successful for chronic ulcers or infected wounds. Biodegradable polyurethane (PUR) scaffolds can support cellular proliferation and differentiation and are capable of releasing drugs to aid infected wounds. Some drugs used as treatments for the wounds are hydrophobic. To facilitate hydrophobic drug delivery using PUR scaffolds, the drug must be encapsulated in micelles, a spherical lipid aggregate. This study investigated the encapsulation method of the hydrophobic drugs in the micelles as well as trehalose and PLGA microparticles, which are mechanisms thought to respectively increase and decrease the release kinetics of the drug-loaded micelles when added to PUR scaffolds. The polymers containing a higher percentage of the hydrophobic block and molecular weight resulted in the optimal encapsulation efficiency. The addition of trehalose to the PUR composition did not have a significant impact on the drugs' release kinetics while the PLGA microparticles significantly decreased the release kinetics. The size of the PLGA microparticles was inversely related to the release kinetics. These results can aid in tailoring drugs release and optimizing wound-healing.

INTRODUCTION.

It was estimated in 1992 that there were 35.2 million cases of significant skin loss that need therapeutic interventions per year in the US and of those 7 million are chronic wounds [1 & 2]. Many skin wounds have chronic inflammation that can be treated with anti-inflammatory drugs. Drugs that accelerate new tissue growth and formation of blood vessels can also be delivered [3]. A compelling biomaterial that can deliver such drugs and promote skin regeneration is biodegradable polyurethanes (PURs).

There has been extensive research on biodegradable polyurethanes, which are scaffolds that can support the growth of new cells [4]. PURs are particularly favorable because their physical, mechanical, and chemical properties can be easily tailored for tensile strength, scaffold degradation rate, and other properties [4]. They can also potentially be used as injectable biomaterials for noninvasive therapies in tissue regeneration. PURs are injected to the wound site and adapt and mold to shape the area. [4]. Because of these unique properties, biodegradable PURs have been applied to an array of regenerative medicine and show a promising future [4].

One important factor of PUR scaffolds is their ability to incorporate drugs, especially hydrophobic drugs. Because hydrophobic drugs are not capable of dissolving in aqueous media, they must be encapsulated in micelles to solubilize the drug in physiologic conditions [5]. When these polymers are in an aqueous environment, the hydrophobic tail orients toward the center and the hydrophilic head orients outward; the hydrophobic drugs are encapsulated in the hydrophobic center, allowing the drug to be released when the micelle is dissolved (Figure S1). The micelles encapsulating the drugs are incorporated in the PUR scaffolds. *One of the goals of this study is to investigate the optimal efficiency of encapsulation of the hydrophobic drugs in the micelles.* It was hypothesized that polymers with higher molecular weight and hydrophobic content would yield better encapsulation of the drug in the micelle via formation of larger micelles and subsequently hold a larger quantity of these hydrophobic drugs.

In this study, the release kinetics, or release rate, of the hydrophobic drug-loaded micelles from the PUR scaffolds is explored. This is an important study because different drugs need to be released in different manners. Some drugs need to be released in a slow and sustained period of time while others need to be released quickly due to short half-lives of the drugs. There are a multitude of intermediates that can be used to tune release kinetics. In our study, two methods were used to alter the release kinetics. Trehalose was added to the PUR scaffold in an attempt to increase the release kinetics of the hydrophobic drugs. In previous studies, trehalose was shown to stabilize the drug during the lyophilization process, which removes any liquids and prevents aggregation [6]. In addition, trehalose dissolves quickly in water, within a few hours, while PURs degrade slowly, in weeks or months. A second method of altering the release kinetics is incorporating the hydrophobic drugs within poly(lactic-co-glycolic acid) (PLGA) microparticles. Previous studies showed that PLGA microparticles slowed the release kinetics due to the additional layer of PLGA the drug has to diffuse through [7]. *Aim two of this study is to examine the effects of trehalose and microparticles on the release kinetics of a typical drug.*

MATERIALS AND METHODS.

Micelle Development.

The polymer that will be used in this study to construct micelles is poly(EG-b-(DMAEMA-co-BMA)). Poly(ethylene glycol) (PEG) is the hydrophilic head of the polymer, and DMAEMA-co-BMA is the hydrophobic tail. The hydrophobic content of the polymer was varied by changing the amount of BMA. The polymers were placed in H_2O to form micelles. Polymers with varying molecular weights measured in kilo Daltons (kDa) were constructed by incorporating sufficient amounts of PEG and BMA into an aqueous solution such that the resulting polymers were 25%, 50%, 60%, and 75% BMA. Polymers were formed with varying weights of 13 kDa (Short), 18 kDa (Medium), and 23 kDa (Long). Polymers are abbreviated with their BMA content and weights. For example, 60% BMA with 23kDa weight is 60L.

Nile Red Encapsulation in Polymeric Micelles.

Nile red, a fluorescent dye, was used to simulate hydrophobic drugs because solubilized Nile red has strong fluorescence that can easily be measured [5]. When encapsulated in intact micelles, a strong fluorescence is shown. The amount of polymer solution, Nile red, and trehalose needed for a 50:1 trehalose-to-polymer ratio and 25–1000:1 polymer-to-Nile red ratio were calculated, mixed together, and plated in a well plate. The trehalose was added simply to stabilize the micelles and prevent aggregation. Nile red fluorescence was measured using a FL600 Microplate Fluorscence Reader at an excitation wavelength of 530 nm and an emission wavelength of 590 nm. The procedure was repeated with polymers of varying molecular weight and hydrophobic content to see if polymer type affected the release kinetics.

Addition of Trehalose to Polyurethane Scaffolds.

The polymer solution, Nile red, and trehalose needed for a 50:1 trehalose-to-polymer ratio, 100:1 polymer-to-Nile red ratio, and 10% or 20% trehalose relative to PUR scaffold mass were added together. The percentage of trehalose was changed to test the effect of it on the release kinetics. This was repeated for the following polymers: 50L, 60L, and 75L. To make a standard curve, the procedure was repeated using 10% of the volumes previously used. The solutions

were then lyophilized to remove water by reducing both the temperature and pressure in order to keep the amount of water constant. The standards were reconstituted with phosphate buffered saline (PBS), and serial dilutions were prepared and placed into a well plate, which was measured for fluorescence. After the polymer solution with trehalose was lyophilized, a spatula was used to crush the solid into a powder. To form the polyurethane scaffold, a polyol mix was first prepared by mixing T6C3G1L900 polyol, water, and catalyst triethylene diamine (TEDA). The polyol mix was added to the crushed polymer and trehalose solution. The isocyanate LTIPEG was then added to the mixture. The polyol and isocyanate combine to form the polyurethane scaffold [4]. Water forms the pores of the scaffold, and TEDA speeds up the reaction [4].

Preparing Poly (lactic-co-glycolic acid) Microparticles.

Micelles were incorporated in PLGA microparticles using double emulsion [8]. PLGA microparticles were prepared in three sizes: 1 μm, 20 μm, and 100 μm. A mixture of the 75L polymer, Nile red, and trehalose were added to a solution of PLGA dissolved in dichloromethane (DCM). To prepare 100 μm microparticles, the mixture was emulsified by sonification for 30 seconds at a speed of 3. For 20 μm microparticles, the mixtures were sonicated for 60 seconds at a speed of 10. After sonification, the mixture for 20 μm and 100 μm were pipetted into beakers, which stirred with *polyvinyl alcohol* (PVA) for 2 hours at speed 10. For 1 μm microparticles, the mixture was sonicated for 30 seconds at a speed of 3. It was added to PVA and homogenized for 30 seconds. The solution was pipetted into a beaker and stirred for 2 hours at speed 10. The solutions were then centrifuged (100 μm microparticles: 2500 rpm for 1.5 minutes, 20 μm and 1 μm microparticles: 5000 rpm for 10 minutes). After centrifuging, the supernatant was poured off and the microparticles were washed with water three times. The microparticles were incorporated in polyurethane scaffolds to form 10% and 20% of the scaffold mass.

Measuring Nile red release from Polyurethane Scaffolds.

The polyurethane scaffolds were weighed and divided into three equal sections. Each section was put in a tube filled with PBS and placed on a shaker in an incubator at 37°C. After 24 hours, the PUR and PBS solution was pipetted into a well plate, and the fluorescence was measured. Fresh PBS was re-added and placed in the incubator. Measurements were collected for a week. The amount of Nile red released was calculated using the standard curve. Assuming the Nile red was evenly distributed, the amount of Nile red in each section was calculated. The fraction of Nile red released from each polyurethane scaffold was calculated by dividing the total accumulated Nile red released by the total Nile red encapsulated in the micelles. The experiment was performed twice to confirm results.

Power Law Model.

A power law curve was fit for each trial for both the trehalose and microparticle experiments. The equation of the power line, $y = ax^b$ was calculated using Excel (Microsoft, Redmond, WA). The values of the exponents of *b* in these equations were compared, because the exponent of power curves indicate whether or not the Nile red released from the polyurethane scaffolds was diffusion controlled. If the power equation line has an exponent of 0.5, the Nile red was released through controlled diffusion. If the exponent isn't 0.5, Nile red was released through a combination of diffusion and degradation mechanisms. The closer to 1.0 the exponent is, the more amount of Nile red released was caused from degradation mechanisms [9].

RESULTS.

Encapsulation of Nile Red in Micelles.

The encapsulation efficiency of the Nile red in the micelles was measured using the fluorescence of the Nile red. Higher fluorescence indicates higher encapsulation of Nile red in the micelles. The optimal encapsulation efficiency of Nile red was measured from the 60L polymer with a 100:1 polymer to Nile red ratio (Figure 1). Generally, as the polymer-to-Nile red ratio decreased, the encapsulation efficiency increased. For most polymer types, the 100:1 polymer-to-Nile red ratio was shown to have the optimal encapsulation efficiency (Figure 1). A general trend was shown that the polymers with higher molecular weight and hydrophobic content (BMA) encapsulated higher amounts of the Nile red (Figure 1). A one way ANOVA analysis on the polymer types with 100:1 polymer-to-Nile red ratio determined the 60L polymer had significantly higher encapsulation than all the other polymer types with the exception of the 75L polymer. The 75L polymer had significantly higher encapsulation than the 50S, 25S, and 25L polymer types. Overall, the polymers with hydrophobic contents ranging 50-75% had significantly higher encapsulation efficiencies than those with 25%.

Effect of Trehalose on Release Kinetics of Nile red.

Polyurethane scaffolds were constructed using 50L, 60L, and 75L polymers since those were found to have the optimal encapsulation efficiencies. The scaffolds with 50L polymer and 20% trehalose had significantly faster release kinetics of the Nile red than the 10% trehalose scaffolds (p values < 0.05) in the first experiment, but did not have a significant impact in the second experiment (Figure 2). The 50L polymer scaffold released the highest percentage of Nile red within the week, ~40% for experiment 1 and ~30% for experiment 2 (Figure 2). The 60L polymer showed that the trehalose significantly slowed the release kinetics (p value < 0.05) in the first experiment, but did not have a significant impact in the second experiment (Figure S2). The 75L polymer showed that trehalose did not have a significant impact in both experiments. The 60L polymer scaffold released ~20% of the Nile red within the week, and the 75L polymer scaffold released ~30% for both experiments (Figure S2). Overall, the 50L and 60L polymer data were inconclusive regarding the release kinetics while the 75L data showed trehalose had no significant impact. Both experiments showed that the 50L polymer released the highest percentage of the Nile red encapsulated within a week.

Effect of PLGA Microparticles on Release Kinetics of Nile red.

Polyurethane scaffolds were constructed with small, medium, and large sized microparticles encapsulating Nile red particles. In both experiments, all results showed that the 20% microparticles had slower release kinetics than that of the 10% microparticles. For the scaffold with small microparticles, the microparticles did not significantly slow the release kinetics (p-value > 0.05) while the medium and large microparticles did significantly impact it (p-value < 0.05) (Figure S3 & S4). The effect of microparticle size on the release kinetics of Nile red was also analyzed. Overall, as the size of the microparticles decreased, the amount of Nile red released increased (Figure 3). The only significant difference of Nile red released was between the small and large microparticles (p value <0.05) (Figure 3). In relation to the total Nile red released within the 7 day period, scaffolds with varying microparticle sizes released similar amounts, ~12-14% for experiment 1 and ~20% for experiment 2 (Figure 3).

Diffusion Control.

A power law model of drug release was fitted for every trial for both the trehalose and microparticle experiments. The 75L polymer scaffold with 10% trehalose was the only scaffold that exhibited stable diffusion, with an exponent of 0.5414 ± 0.0863. The exponents of the other polymers with trehalose were close to 0.5, ranging from 0.4371 to 0.7590 (Table 1). The exponents from the power curve for the microparticle curve ranged from 0.6687 to 0.8735 (Table 1).

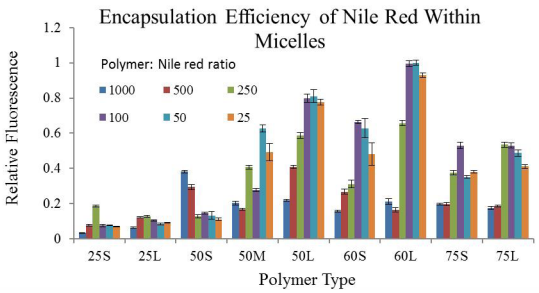


Figure 1. Polymers with higher molecular weight and BMA content yielded higher encapsulation efficiency for each polymer type.

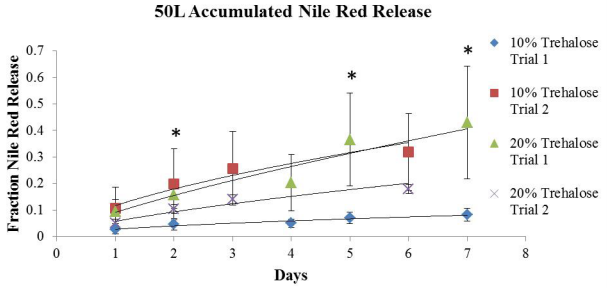


Figure 2. 50L scaffolds show trehalose significantly increased release kinetics of Nile red for Trial 1 but decreased release kinetics for Trial 2.

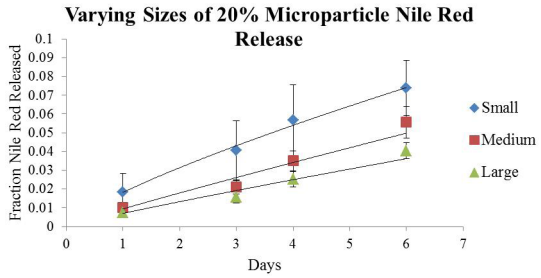
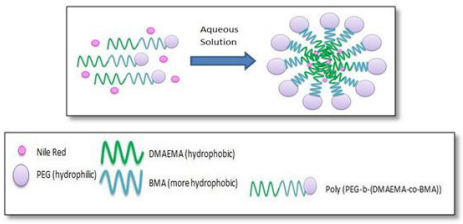


Figure 3. As the size of the microparticles decrease, the amount of Nile red released increases.

Table 1. Table of exponents of power curve showing that for the trehalose experiment, the Nile red was released through mainly diffusion control and for microparticle experiment, the Nile red was released through diffusion control and scaffold degradation



DISCUSSION.

The first goal of the study was to optimize the encapsulation efficiency of hydrophobic drugs in micelles. As anticipated, a trend was shown that polymers with higher molecular weight, which create larger micelles due to longer polymers, and higher hydrophobic content, which can attract more hydrophobic drugs, better encapsulated the drugs. These results indicate that the polymers with higher hydrophobic content and higher molecular weight should be used to create micelles for encapsulating hydrophobic drugs. Therefore, these polymers were used when incorporating the hydrophobic drugs into polyurethane scaffolds.

The second goal was to study the effect of trehalose on the release kinetics of the drug. Most experiments showed that trehalose had an insignificant impact on the release kinetics. Two experiments were inconclusive, showing a significant increase in the release kinetics for the 50L polymer and a significant decrease for the 60L polymer. However, trehalose should still be added to the polyurethane scaffolds to protect the micelles during freezing and lyophilization [6]. For both experiments, the 50L polymer released the highest amount of the drug within 7 days, because it was the least hydrophobic and the micelle was more soluble in aqueous solutions. These results show that polymer type may affect the fraction of hydrophobic drug released. It is possible that polymers with lower hydrophobic content could release more of the drug encapsulated in the scaffold within a period of time. However, future studies will need to be conducted to confirm this hypothesis.

The effect of PLGA microparticles on the release kinetics of hydrophobic drugs was studied as well. Both experiments indicated that the medium and large PLGA microparticles significantly slowed the release kinetics of the hydrophobic drugs, which was shown in a previous study by Lin [7]. However, the small PLGA microparticles did not have a significant impact on the drug release. The experiments showed that the size of microparticle was inversely related to the release kinetics. This was due to the layer of PLGA the hydrophobic drug had to diffuse out of in addition to the polyurethane scaffold. The larger the microparticle, the larger the PLGA layer and thus the slower it took the drug to diffuse and be released. The small PLGA microparticles had a PLGA layer so thin that it had a negligible effect on the release kinetics of the drugs. These results show that hydrophobic drugs should be incorporated into medium and large PLGA microparticles if the drug needs to be released in a sustained manner. The varying sizes of microparticles released similar amounts of the drug within a week and did not affect the fraction of drug released.

The power law model showed inconclusive data for the hydrophobic drug release with trehalose. Although the exponents ranged past the 0.5 threshold, they were closer to 0.5 then 1. Thus, it was hypothesized the drug was released from the scaffold due primarily to diffusion of the drug. It was also shown that the hydrophobic drug released from polyurethane scaffolds with microparticles was released from both diffusion control and scaffold degradation since the exponents ranged closer to 1 than 0.5.

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SUPPORTING INFORMATION.

Supplemental Methods.

Figure S1. Diagram of formation of micelles from Poly(EG-b-(DMAEMA-co-BMA)) unimers.

Figure S2. 60L scaffolds show trehalose significantly decreased release kinetics of Nile red for Trial 1 and decreased released kinetics for Trial 2.

Figure S3. Medium microparticles significantly decreased release kinetics of Nile red for Trial 1 and 2.

Figure S4. Large microparticles significantly decreased release kinetics of Nile red for Trial 1 and 2.

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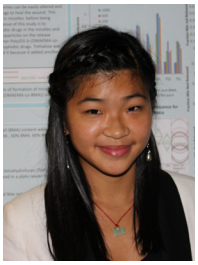

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