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Effects of surface area to volume ratio of PLGA scaffolds with different architectures on scaffold degradation characteristics and drug release kinetics

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Abstract: In this work, PLGA scaffolds with different architectures were fabricated to investigate the effects of surface area to volume ratio (SVR) (which resulted from the different architectures) on scaffold degradation characteristics and drug release kinetics with minocycline as the model drug. It was hypothesized that the thin strand scaffolds, which had the highest SVR, would degrade faster than the thick strand and globular scaffolds as the increase in surface area will allow more contact between water molecules and degradable ester groups in the polymer. However, it was found that globular scaffolds, which had the lowest SVR, resulted in the fastest degradation which demonstrated that the amount of degradation of the scaffolds does not only depend on the SVR but also on other factors such as the retention of acidic degradation byproducts in the scaffold and scaffold porosity. PLGA 50 : 50 globular scaffolds resulted in a biphasic release profile, with a burst release in the beginning and the middle of the release study which may be beneficial for some drug delivery applications. A clear correlation between SVR and release rates was not observed, indicating that besides the availability of more surface area for drug to diffuse out of the polymer matrix, other factors such as amount of scaffold degradation and scaffold porosity may play a role in determining drug release kinetics. Further studies, such as scanning electron microscopy, need to be performed in the future to further evaluate the porosity, morphology and structure of the scaffolds. © 2016 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 104A: 1202–1211, 2016.

Key Words: PLGA scaffolds, surface area to volume ratio, scaffold architecture, degradation characteristics, drug release kinetics

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INTRODUCTION

Biomaterial scaffolds have been extensively studied for the local and sustained delivery of therapeutic agents to overcome the many barriers associated with systemic delivery. Biomaterial properties such as their size, shape, charge, surface chemistry, morphology and physiochemical properties can be easily tailored to control the release of the molecules encapsulated in these scaffolds.[1] Poly(lactic-*co*-glycolic acid) (PLGA) is a copolymer that has been used in many FDA approved products for human applications[2] and has been extensively investigated for the delivery of different molecules, including DNA, RNA, proteins and drugs, due to its biocompatibility and biodegradable properties. PLGA has been used in many different forms, including micro-[3,4] or nano-particles,[5,6] and prefabricated scaffolds produced by solvent casting,[7,8] compression molding,[9,10] 3D printing,[11,12] soft lithography,[13,14] and electrospinning.[15,16] Although there has been success associated with the different fabrication techniques for prefabricated PLGA scaffolds (i.e., scaffolds that are fabricated prior to implantation *in vivo*), they may require different equipment and/or systems that can be costly and/or difficult to setup to successfully fabricate the scaffolds.

The early phase of the release of drugs from PLGA scaffolds is based on drug diffusion from the surface or in the polymer matrix which is followed by both drug diffusion and degradation of the polymer in the later phase of the release process.[17] Thus, degradation characteristics of the scaffolds play a significant role in determining the release of drugs entrapped in these scaffolds[18] and is an aspect that can be controlled to tailor the release kinetics of the drug. Theoretically, a higher amount of polymer degradation should lead to a faster release of drugs.[19] The drug release kinetics from PLGA scaffolds can be easily tailored from days to years by controlling the hydrolytic degradation of the ester groups in the polymer. The degradation

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characteristics can be altered by the molecular weight, lactic to glycolic acid ratio and end groups of the polymer.[20] The surface area to volume ratio (SVR) of a polymer matrix is also a factor that plays a role in determining the degradation characteristics of polymeric materials[19,21] which in turn affects the release kinetics of drugs.[18,19] This is because SVR is a key factor in diffusion-limited processes such as ester hydrolysis.[22] Faster degradation is expected for PLGA matrix with higher SVR[21] which should lead to an increased release of the loaded drug.[18]

The objective of this study was to investigate the effect of scaffold architecture and SVR on the degradation characteristics and drug release kinetics of PLGA scaffolds. It is hypothesized that a larger surface area will result in a higher amount of degradation as there will be higher buffer penetration through the polymer matrix[19] which will lead to an increase in hydrolysis as water molecules can more easily access the esters in the polymer matrix. Besides resulting in an increase in the amount of degradation, it is hypothesized that the drug release kinetics will also increase as a higher SVR polymeric matrix has more surface area for the encapsulated agents to diffuse out more rapidly.[19] To test these hypotheses, scaffolds with three different types of architectures (i.e., globular, thick strands, and thin strands), which resulted in different SVRs, were fabricated and the *in vitro* degradation characteristics and release kinetics of these scaffolds were investigated over a 28-day period. Minocycline, a tetracycline antibiotic, was used as a model drug for the release kinetic studies.

MATERIALS AND METHODS

Materials

PLGA 50 : 50 (LP-779, Internal Viscosity = 0.43 dL/g, MW = 61 kDa) and PLGA 75 : 25 (LP-632, Internal Visscosity = 0.49 dL/g, MW = 70 kDa) with an acid end group were obtained from Evonik Industries (Birmingham, AL). Minocycline, methylene chloride, methanol and ethanol were obtained from Sigma-Aldrich (St. Louis, MO). Ultra Pure Grade PBS was obtained from AMRESCO LLC (Solon, OH).

Scaffold fabrication

The scaffolds were prepared as done previously[23,24] with some modifications. This fabrication method was chosen because it is an easy and economical mold-less technique that has the versatility in producing scaffolds with different sizes and architectures. To prepare the polymer solution, 0.1 g of PLGA 50 : 50 or PLGA 75 : 25 was dissolved in 1 mL of tetraglycol (Sigma-Aldrich, St. Louis, MO) overnight for ~19.5 h, stirring at 60°C and 400 rpm. For scaffolds containing the model drug, minocycline, 0.01 g of drug was added to the PLGA solution 30 min before scaffold fabrication and stirred at 37°C and 400 rpm to allow dissolution. To produce scaffolds with different architectures, different delivery systems were used to inject 100 μL of PLGA solution or PLGA/drug solution into 20 mL PBS in a 100 mL beaker. For the formation of thin strand, thick strand and globular scaffolds, the solution was injected with a micropipette attached to a 10–200 μL tip, a micropipette attached to 100–1000 μL tip or a 1 mL plastic syringe (without a needle), respectively. The scaffolds were allowed to set for 2 min in PBS where it changed from a translucent to an opaque appearance. Scaffolds fabricated with this method, which is a similar method of fabrication to previous studies,[23,24] were denoted as "PBS only". To optimize the fabrication technique, an additional step was added to the protocol. After suspension in PBS at room temperature for 2 min, the scaffolds were transferred into 5 mL of PBS, methanol or ethanol and left in an incubator set at 37°C for 1 h without shaking (denoted as "PBS + PBS", "PBS + MeOH," and "PBS + EtOH," respectively). The PBS, methanol or ethanol was removed and the scaffolds were placed in a desiccator connected to a vacuum for 2 h to allow the remaining solvent to evaporate. The scaffolds were then immediately used for studies. It was determined that PBS + EtOH scaffolds had the highest drug loading and were able to prolong the release of the drug, and thus, this fabrication technique was used for all the other experiments. The optimization study for the fabrication technique was done in triplicate ($n = 3$) and with PLGA 50 : 50.

Scaffold dimensions and weight characterization

The diameters of the globular scaffold (all three axis, Diameter A, B, C which corresponds to the diameter with the longest, medium and shortest length, respectively) and the diameter and length of the thick and thin strands were determined using a digital caliper. The formulas for the surface area and volume of an ellipsoid (for the globular scaffold) and cylinder (for the thick and thin strand scaffolds) were used to obtain the surface area and volume of the scaffolds. The SVR was determined by dividing the surface area by the volume of each scaffold. The dimensions and weight of the scaffold after (i) 2 min in PBS and (ii) after 2 min in PBS, followed by 1 h in ethanol at 37°C were compared to determine the percent shrinkage and the percent weight loss of the scaffold. The characterization study was done in triplicate ($n = 3$). It is important to note that the scaffolds produced with this economical and easy fabrication technique were not perfect cylinders or ellipsoids as there were slight variation in their dimensions throughout the scaffold (such as the slight variation in the diameter of the thick and thin strand scaffolds along the scaffold length). By taking the average of three samples, a more accurate measurement could be presented.

Scaffold degradation

Scaffolds were placed in 1 mL of PBS and shaken at 37°C and 70 rpm. The solution was replaced with fresh PBS every 7 days. At each timepoint (0, 1, 7, 14, 28 days), the PBS solution was removed and the scaffolds were frozen down (−20°C) to avoid further degradation. At the end of the study, the scaffolds were lyophilized (Labconco, Kansas City, MO) and their weights were determined. The degradation study was conducted in triplicate ($n = 3$).

Drug release kinetics

Scaffolds containing the model drug, minocycline were prepared as stated above. The prepared scaffolds were placed in a scintillation vial with 1 mL of PBS. At each timepoint (0, 1, 3, 7, 10, 14, 21, 28 days), the amount of minocycline released into the solution was determined by measuring the absorbance at 350 nm[25] with a microplate reader (Tecan Infinite Pro 200, Switzerland) equipped with the Magellan data analysis software. The scaffolds were then replenished with 1 mL of fresh PBS. This study was conducted at $n = 4$. For the fabrication technique optimization study, the timepoints 0, 1, 3, 7, 10, and 14 days were used for the release study.

Drug loading and entrapment efficiency

The amount of drug remaining inside the scaffolds was determined using a previous protocol with some modification.[4] At the end of the release study, the scaffolds were completely dissolved in 1 mL of methylene chloride and then 15 mL of PBS was added to the solution. The mixture was then stirred vigorously on a magnetic stir plate to allow the methylene chloride to evaporate. The solution was then centrifuged to separate the precipitated polymer and the amount of drug in the solution (supernatant) was determined with a microplate reader as discussed above. The amount of total drug loaded in the scaffold was calculated by summing up the amount of drug released at each timepoint and the amount of drug remaining in the scaffold at the end of the study. This method was performed as we were unable to extract all of the drug that was entrapped in the scaffold via the standard method of determining drug loading immediately after fabrication of the scaffold as the lipophilic drug had a tendency to interact with the PLGA polymer instead of fully dissolving in the PBS. By the end of study, the amount of drug remaining in the scaffold could be determined, as the scaffolds were already partially degraded, allowing the minocycline to more readily dissolve in the PBS. The drug loading and entrapment efficiency analyses were done in triplicate (n = 3). The percent drug loaded and entrapment efficiency were determined with the equations below.

$$\%\ \text{Drug Loaded} = \frac{\text{Weight of drug in sample}}{\text{Total weight of sample}} * 100$$

$$\text{Entrapment efficiency}\ (\%) = \frac{\text{Actual amount of drug loaded}}{\text{Theorectical drug loading}} * 100$$

Statistical analysis

Multiple-factor analysis of variance (ANOVA) was conducted to identify if there were any significant differences among groups ($p < 0.05$) followed by the use of Tukey's Honestly Significantly Different (HSD) test to identify the specific groups that differed statistically significantly.

RESULTS

Optimization of scaffold fabrication

It was found that $92.9 \pm 22.6\%$ of the model drug was released after 1 day for the thin strand scaffolds made with a similar method applied by Kreb et al.[24] and Jeon et al.[23] (denoted as "PBS only") [Fig. 1(A)]. Scaffolds made with the additional step in the fabrication protocol (i.e. submergence into PBS, methanol or ethanol for 1 hr) were able to better sustain the release of the drug over the duration of the release study as seen in Figure 1(A). At the end of the study (14 days) PBS + PBS scaffolds released $46.6 \pm 9.8\%$ of the loaded drug compared to $65.2 \pm 5.9\%$ and $59.9 \pm 10.6\%$ for PBS + MeOH and PBS + EtOH scaffolds, respectively. PBS + EtOH scaffolds released significantly more drug (85.8 ± 31.3 μg) between day 0–1 compared to PBS + PBS (12.1 ± 4.9 μg) and PBS + MeOH (26.4 ± 7.0 μg) scaffolds as seen in Figure 1(B). For day 1–3, PBS + EtOH scaffolds released significantly more drug (31.2 ± 14.8 μg) compared to PBS only (3.5 ± 0.6 μg) and PBS + PBS (7.7 ± 0.9 μg) scaffolds. Although PBS only scaffolds, which were fabricated following a similar method by another group,[23,24] resulted in the highest amount of drug loaded (333.3 ± 9.6 μg) [Fig. 1(C)], they are not optimal scaffolds as almost all of the drug is released in 1 day [Fig. 1(A,B)]. The other fabrication techniques resulted in a lower amount of drug loaded as some drug is lost in the PBS, methanol or ethanol solution during the 1 hr incubation period in these solutions before the start of the release study. PBS + EtOH scaffolds successfully loaded 250.1 ± 31.2 μg drug compared to 53.9 ± 14.4 μg and 82.4 ± 17.1 μg for PBS + PBS and PBS + MeOH scaffolds, respectively [Fig. 1(C)]. At the end of the release study (after 14 days), PBS + EtOH scaffolds had significantly higher amount of drug remaining in the scaffolds (98.3 ± 15.2 μg) compared to the other scaffolds (22.3 ± 5.7 μg for PBS only, 29.1 ± 11.5 μg PBS + PBS and 29.3 ± 9.8 μg for PBS + MeOH scaffolds) [Fig. 1(D)].

Scaffold dimensions and weight characterization

Macroscopic images of the scaffolds with different architectures are shown in Figure 2. As expected, the polymer solution (PLGA in tetraglycol) was more viscous for PLGA 75 : 25 compared to PLGA 50 : 50 and thus, was not as easy to inject into PBS. PLGA 75 : 25 dispensed a finer stream of polymer solution, which resulted in a longer length (36.5 ± 4.9 mm vs. 23.8 ± 4.8 mm) and thinner diameter (1.7 ± 0.2 mm vs. 2.1 ± 0.3 mm) for the PLGA 75 : 25 thick strand scaffolds compared to those made of PLGA 50 : 50 (Table I). The thin strand scaffolds made of PLGA 75 : 25 also resulted in a longer length (162.7 ± 11.9 mm vs. 113.5 ± 17.0 mm) and thinner diameter (1.1 ± 0.1 mm vs. 1.3 ± 0.1 mm) compared to those made of PLGA 50 : 50. Globular scaffolds made of PLGA 50 : 50 resulted in larger diameters (6.0 ± 0.1, 6.1 ± 0.0, 6.3 ± 0.1 mm vs. 4.1 ± 0.4, 4.8 ± 0.3, 6.0 ± 0.1 mm) compared to scaffolds prepared with PLGA 75 : 25. When the polymer solution is less viscous (as in PLGA 50 : 50), the globular scaffolds are able to expand more as they are injected into the PBS and thus resulted in larger diameters. The globular PLGA 50 : 50

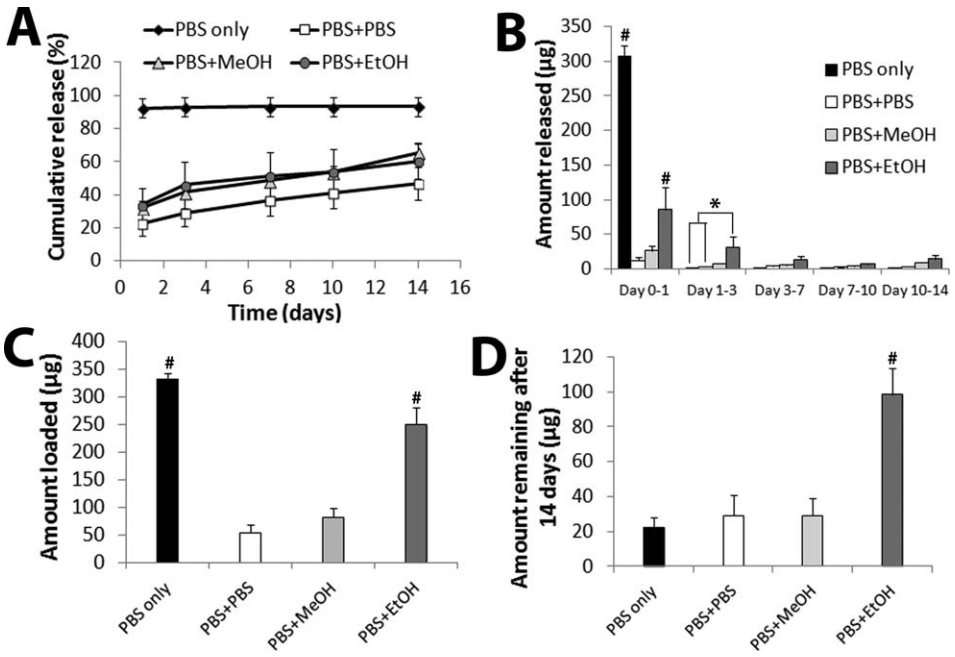

FIGURE 1. Optimization of scaffold fabrication technique: A: Cumulative release of drug from scaffolds fabricated with different techniques over 14 days. B: Amount of drug released during different time periods. # indicates statistically different ($p < 0.01$) compared to all other groups in the same timepoint. * indicates statistically different ($p < 0.05$) compared to the group indicated. C: Amount of drug loaded in the scaffolds. # indicates statistically different ($p < 0.01$) compared to all other groups. D: Amount of drug remaining in the scaffold at the end of the study (after 14 days). # indicates statistically different ($p < 0.01$) compared to all other groups. The results are expressed as mean ± standard deviation for $n = 3$.

scaffolds were heavier (97 ± 2 mg) compared to the globular scaffolds made of PLGA 75 : 25 (59 ± 17 mg) after 2 min in PBS. However, after 1 h in ethanol, their weights were quite comparable. The less viscous PLGA 50 : 50 which resulted in a loser polymer matrix may have allowed more water retention, however, after removing the scaffolds from the immersion in ethanol for 1 h, there was not much water retention in the scaffold as the PLGA precipitated and thus, the weights of scaffolds made with both types of PLGA were comparable (18 ± 0 mg for PLGA 50 : 50 vs.

TABLE I. Dimensions (Diameter and/or Length) and Weight for the Different PLGA Scaffolds in PBS for 2 min and in PBS for 2 min + Ethanol for 1 h

		Dimension (mm)			Weight (mg)		
Type of Scaffold		2 min in PBS	2 min in PBS + 1 hr in EtOH	% Shrinkage	2 min in PBS	2 min in PBS + 1 hr in EtOH	% Weight Loss
Globular	**PLGA 50:50**						
	Diameter A	6.0 ± 0.1	3.0 ± 0.3	49.6			
	Diameter B	6.1 ± 0.0	4.3 ± 0.3	30.1	97.4 ± 1.8	18.2 ± 1.4	81.3
	Diameter C	6.3 ± 0.1	4.5 ± 0.4	28.5			
	PLGA 75:25						
	Diameter A	4.1 ± 0.4	3.1 ± 0.4	24.6			
	Diameter B	4.8 ± 0.3	3.6 ± 0.4	23.8	58.7 ± 8.8	17.5 ± 2.5	70.3
	Diameter C	6.0 ± 0.1	5.4 ± 1.3	11.2			
Thick Strand	**PLGA 50:50**						
	Length	23.9 ± 4.8	16.4 ± 2.2	31.2	58.0 ± 2.4	11.7 ± 2.4	79.8
	Diameter	2.1 ± 0.3	1.3 ± 0.1	40.1			
	PLGA 75:25						
	Length	36.5 ± 4.9	16.8 ± 0.5	53.9	62.1 ± 3.9	13.5 ± 3.0	78.2
	Diameter	1.7 ± 0.2	1.4 ± 0.2	14.1			
Thin Strand	**PLGA 50:50**						
	Length	113.5 ± 17.0	N/A	N/A	80.4 ± 3.3	13.5 ± 0.5	83.2
	Diameter	1.3 ± 0.1	0.7 ± 0.0	31.2			
	PLGA 75:25						
	Length	162.7 ± 11.9	N/A	N/A	85.2 ± 1.3	12.7 ± 0.6	85.1
	Diameter	1.1 ± 0.1	0.8 ± 0.1	27.0			

The results are expressed as mean ± standard deviation for $n = 3$.

TABLE II. Surface Area, Volume, Surface Area to Volume Ratio (SVR), Percent Drug Loading and Entrapment Efficiency for PLGA scaffolds in PBS for 2 min

Type of Scaffold	Surface Area (mm^2)	Volume (mm^3)	Surface Area/ Volume Ratio (SVR)	% Drug Loaded	Entrapment Efficiency (%)
Globular					
PLGA 50:50	54.5 ± 4.8	35.4 ± 4.0	1.5 ± 0.0	4.1 ± 0.7	45.4 ± 8.0
PLGA 75:25	77.4 ± 7.1	62.3 ± 9.3	1.2 ± 0.1	2.4 ± 0.4	26.4 ± 4.1
Thick Strand					
PLGA 50:50	83.6 ± 9.0	83.6 ± 9.0	2.0 ± 0.3	3.2 ± 0.6	35.1 ± 6.1
PLGA 75:25	195.5 ± 2.4	80.3 ± 9.0	2.5 ± 0.3	2.1 ± 0.1	22.7 ± 1.5
Thin Strand					
PLGA 50:50	456.6 ± 35.7	145.3 ± 11.0	3.2 ± 0.3	2.2 ± 0.2	23.7 ± 2.1
PLGA 75:25	540.6 ± 85.5	142.8 ± 34.6	3.8 ± 0.4	1.3 ± 0.2	14.3 ± 2.7

The results are expressed as mean ± standard deviation for $n = 3$.

17 ± 0 mg for PLGA 75 : 25). Once placed in ethanol for 1 h after 2 min in PBS, all the scaffolds shrunk in their dimensions as well as lost weight (70–85% weight lost). This could be attributed to the loss of tetraglycol, polymer or water that was retained in the scaffold through diffusion. Furthermore, the placement of the scaffolds in ethanol resulted in polymer precipitation and shrinkage. Thin strand scaffolds were very tightly bound and thus, could not be pulled apart or untangled to measure the length accurately. Those dimensions were therefore not included in Table I. The scaffolds with different architectures resulted in different SVR as seen in Table II. For PLGA 50 : 50, the thin strand scaffolds had the highest SVR (3.2 ± 0.3 mm) followed by the thick strand scaffolds (2.0 ± 0.3 mm). The globular scaffolds had the lowest SVR (1.0 ± 0.0 mm) as expected. For PLGA 75 : 25, the same trend was observed. The thin strand, thick strand and globular scaffolds resulted in SVR of 3.8 ± 0.4 mm, 2.5 ± 0.3 mm and 1.2 ± 0.1 mm, respectively. Overall, PLGA 75 : 25 resulted in higher SVR compared to PLGA 50 : 50 for all the three types of scaffold.

Drug loading and entrapment efficiency

Globular scaffolds resulted in the highest % drug loaded (4.1 ± 0.7% and 2.4 ± 0.4% for PLGA 50 : 50 and 75 : 25, respectively), followed by the thick (3.2 ± 0.6% and 2.1 ± 0.1%) and thin (2.2 ± 0.2% and 1.3 ± 0.2%) strand scaffolds (Table II). The globular scaffolds also had the highest entrapment efficiency (45.4 ± 8.0% and 26.4 ± 4.1% for PLGA 50 : 50 and 75 : 25, respectively) compared to the thick (35.1 ± 6.1% and 22.7 ± 1.5%) and thin (23.7 ± 2.1 and 14.3 ± 2.7%) strand scaffolds. This most probably resulted from the lower SVR in the globular scaffolds which results in fewer surfaces for the drug to diffuse out as the scaffold hardens.

Scaffold degradation

Globular scaffolds had the most degradation for both PLGA 50 : 50 [Fig. 3(A)] and PLGA 75 : 25 [Fig. 3(B)]. For PLGA 50 : 50, this was followed by the thick strand and then the thin strand scaffolds and for PLGA 75 : 25, the amounts of degradation of the thin and thick strand scaffolds were similar. At the last timepoint tested (28 days), there was an 83.2 ± 4.9% weight loss for the globular scaffolds followed by 70.0 ± 4.3% and 60.7 ± 4.9% for the thick and thin strand scaffolds, respectively for PLGA 50 : 50. For PLGA 75 : 25, there was a 53.0 ± 1.8% weight loss of the globular scaffolds compared to 24.1 ± 0.8% and 26.3 ± 1.8% for the thick and thin strand scaffolds, respectively. Overall, scaffolds made with PLGA 50 : 50 degraded more than scaffolds made with PLGA 75 : 25 as expected. PLGA 75 : 25 has more lactic acid compared to PLGA 50 : 50 and thus, its ester linkages are not as easily degraded as those in PLGA 50 : 50.

Drug release kinetics

The drug release kinetics from the scaffolds made with different architectures are shown in Figure 4(A) (PLGA 50 : 50) and 4B (PLGA 75 : 25). To facilitate the comparison of the release rates of the different scaffolds, the amount of drug released was divided into four time segments as done previously[26–28] (Table III). For PLGA 50 : 50, the globular

TABLE III. Release Kinetics of Drug from Scaffolds in Four Different Phases

Group	Phase 1 (%/day) First 24 h	Phase 2 (%/day) Days 1-3	Phase 3 (%/day) Days 3-14	Phase 4 (%/day) Days 14-28
PLGA 50:50 Globular	21.6 ± 1.4#*	4.5 ±1.3	1.8 ± 0.2	1.9 ±1.0
Thick	39.8 ± 6.8*	4.7 ± 0.7	1.7 ± 0.1	1.3 ± 0.2
Thin	42.5 ± 3.5*	5.5 ± 0.3	1.6 ±0.1	0.8 ± 0.1
PLGA 75:25 Globular	39.4 ± 6.9*	7.0 ± 1.6+	1.4 ± 0.2	0.7 ± 0.3
Thick	25.3 ± 3.7#*	4.4 ± 0.7	1.3 ± 0.1	0.8 ± 0.0
Thin	43.0 ± 4.0*	4.9 ± 1.2	1.0 ± 0.1	0.7 ± 0.1

The results are expressed as mean ± standard deviation (% release per day) for $n = 4$. # indicates statistically significant difference ($p < 0.01$) between one group from all other groups. * indicates statistically significant difference ($p < 0.01$) between one phase from all other phases within the same group. + indicates statistically significant difference ($p < 0.05$) between one phase from all other phases within the same group.

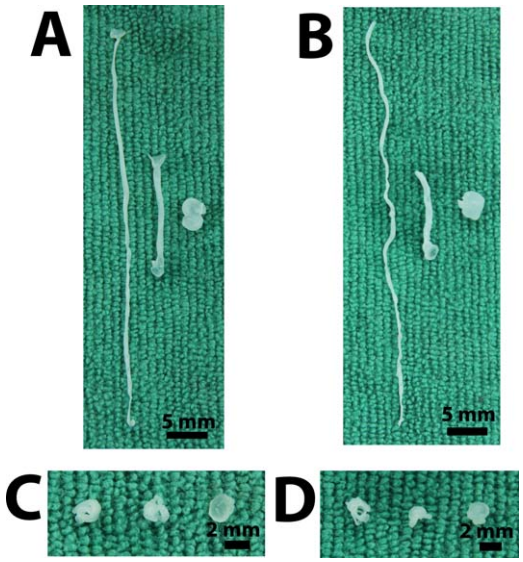

FIGURE 2. Macroscopic images of scaffolds with different architecture: A: PLGA 50 : 50 scaffolds after 2 min in PBS. B: PLGA 75 : 25 scaffolds after 2 min in PBS. C: PLGA 50 : 50 scaffolds after 2 min in PBS and 1 h in ethanol. D: PLGA 75 : 25 scaffolds after 2 min in PBS and 1 h in ethanol. From left to right: thin strand, thick strand and globular scaffolds.

scaffolds had a significantly lower release in the first 24 h (Phase 1) (21.6 ± 1.4%) compared to the thin and thick strand scaffolds (42.5 ± 3.5% and 39.8 ± 6.8%, respectively). For PLGA 75 : 25, the thick strand scaffolds had a significantly lower release in the first 24 h (25.3 ± 3.7%) compared to the thin strand and globular scaffolds (43.0 ± 4.0% and 39.4 ± 6.9%, respectively). For the PLGA 75 : 25 globular scaffolds, there was significantly more drug released/day between Days 1–3 (7.0 ± 1.6%) compared to Days 3–14 and 14–28 (1.4 ± 0.2% and 0.7 ±0.3%, respectively). For all the scaffolds, there was a significantly higher amount of drug released in Phase 1 compared to the other phases. Overall there was a higher amount of drug released from the globular scaffolds for the 14, 17, and 21 day timepoints and for the 3 h, 1, 3, and 5 day timepoints for PLGA 50 : 50 [Fig. 4(C)] and 75 : 25 [Fig. 4(D)], respectively. For PLGA 50 : 50, there was significantly higher amount of drug loaded in the globular scaffolds (470.4 ± 82.3 μg) compared to the thin (174.6 ± 15.5 μg) and thick (191.1 ± 33.4 μg) strand scaffolds (data not shown). For the PLGA 75 : 25, there was also significantly higher amount of drug loaded in the globular scaffolds (290.1 ± 44.8 μg) compared to the thin (106.5 ± 20.1 μg) and thick (152.7 ± 10.0 μg) strand scaffolds (data not shown).

DISCUSSION

In this work, prefabricated PLGA scaffolds were developed using an economical and easy technique (does not require any molds, equipment or setups). Kreb et al. previously developed an injectable PLGA scaffold which transitions into a solid once added into an aqueous environment.[24] Jeon et al.[23]investigated the delivery of plasmid DNA (pDNA) from these globular scaffolds and found that to achieve a sustain release; the pDNA had to be first incorporated within PLGA microspheres which were then loaded into the scaffolds. It was determined that 98.2 ± 11.7% of the pDNA was released within the first 7 days from the globular scaffolds without a microsphere component. When the pDNA is encapsulated within microspheres before loading into the scaffold, there was a prolonged release with a cumulative release of 43.8 ± 6.8% after 70 days. Following a similar fabrication method, we found that 96.3 ± 0.8% of our model drug minocycline, which was incorporated in the scaffold without a microsphere component, was released after 1 day and 98.1 ± 0.7% was released by the end of study (14 days) from globular scaffolds. Compared to the LacZ pDNA used by Kreb et al. which may be around 7–8 kb in size or $4.55 - 5.20 \times 10^6$ g/mol, minocycline has a molecular weight of 493.94 g/mol which is much smaller and thus able to easily diffuse out of the polymer matrix. Besides the size of the payload, the charge of the payload may also play a role in facilitating its release from PLGA scaffolds. PLGA nanoparticles have been shown by Panyam et al. to have a negative charge at physiological and alkaline pH and a positive charge in acidic pH.[29] As the PLGA degrades, an acidic environment is created and thus, the highly negatively charged pDNA will interact more closely with the positively charged polymer compared to minocycline (which is a zwitterion at pH 7.4),[30] which may hinder the release of the pDNA. To increase the drug loading and prolong the release

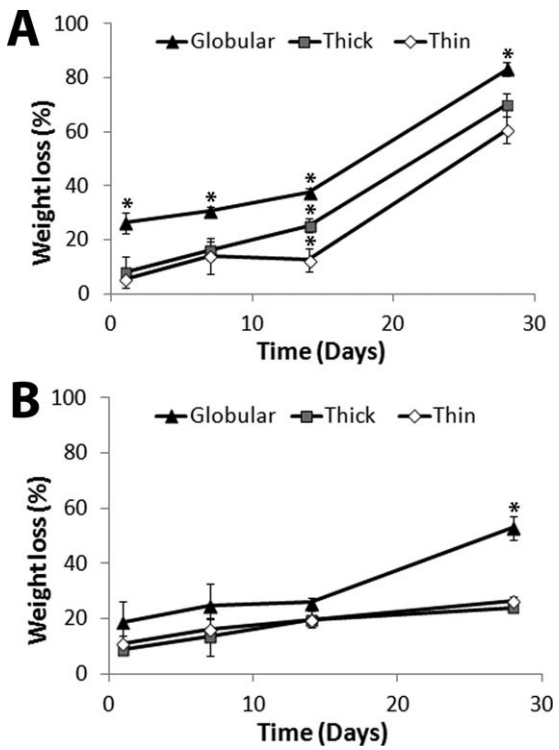

FIGURE 3. Degradation of scaffolds with different architecture: % Weight loss of scaffolds made of (A) PLGA 50 : 50 and (B) PLGA 75:25 over 28 days. * indicates statistically different ($p < 0.05$) compared to all other groups in the same timepoint. The results are expressed as mean ± standard deviation for $n = 3$.

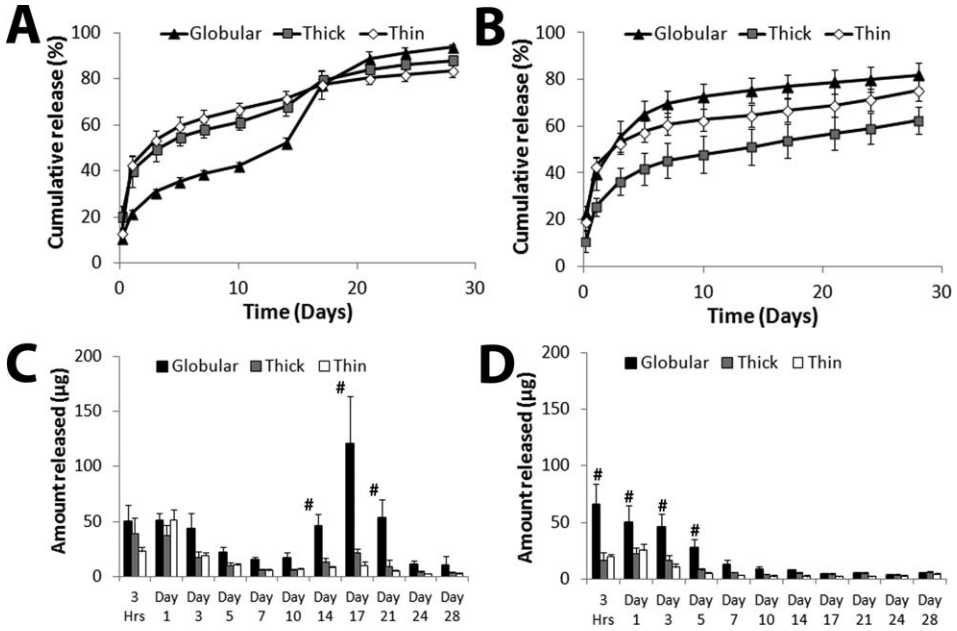

FIGURE 4. Release kinetics of scaffolds with different architecture. Cumulative release of drug from scaffolds made of (A) PLGA 50:50 and (B) PLGA 75:25 over 28 days. Amount of drug released during different time periods for scaffolds made of (C) PLGA 50:50 and (D) PLGA 75:25.). #indicates statistically different ($p < 0.05$) compared to all other groups in the same timepoint. The results are expressed as mean ± standard deviation for $n = 4$.

of drug, we investigated the incorporation of an additional step to the fabrication protocol. After allowing the scaffolds to set in PBS for 2 min, the scaffolds were placed in PBS, methanol or ethanol for 1 hr. It was found that scaffolds fabricated with ethanol had a higher amount of drug loaded and a higher amount of drug remaining in the scaffolds at the end of the study (Fig. 1) compared to those obtained with the other fabrication techniques tested. PLGA precipitates in alcohol and thus, results in entrapment of the drug in the scaffolds. The ability of ethanol to better precipitate PLGA compared to methanol[31] may have resulted in the increase in drug entrapment efficiency with ethanol.

Scaffolds with different architectures were successfully fabricated with the optimized technique (that is, 2 min in PBS and 1 h in ethanol) and resulted in different SVR (Table II). It was hypothesized that scaffold degradation and release kinetics would increase with higher SVRs. The different viscosity of the PLGA 75 : 25 and PLGA 50 : 50 polymer solutions resulted in a difference in the extrusion of the polymer from the delivery device which affected the dimensions of the scaffolds. Although fabricated as elongated scaffolds when the polymer solution is injected into PBS [Fig. 2(A,B)], the thick and thin strand scaffolds also resulted in a globular shape [Fig. 2(C,D)] as the strands collapse into a ball when placed in ethanol for 1 h in a 15 mL tube. This creates scaffolds with similar overall shape, however, these scaffolds may have different porosity (which was not evaluated in this study), which will allow circulation of nutrients and metabolic wastes as well as cell attachment and tissue ingrowth. This facilitates vascularization and a firm attachment to surrounding tissue if used for tissue engineering applications[32,33] such as for cartilage and bone regeneration.

Biodegradable polymers can be classified as surface eroding or bulk eroding polymers.[34] For polyesters such as PLGA, bulk degradation occurs faster than surface degradation.[21] For surface eroding materials, there is a constant velocity for the erosion throughout the degradation process.[35] However, for bulk erosion polymers, the erosion velocity is not constant.[36] There is usually no erosion of the polymer for a long period of time and then the erosion will occur spontaneously which was seen in the PLGA scaffolds fabricated in this study. For PLGA 50 : 50, there was 7–17% weight loss between 1–14 days, and a markedly higher degradation (44–48% in weight loss) between 14–28 days. For PLGA 75 : 25, there was a 7–11% weight loss between 1–14 days and 4–27% weight loss between 14–28 days. Since PLGA 75 : 25 is more hydrophobic compared to PLGA 50 : 50, at the end of the study (28 days), the polymer may still be in a phase where not much erosion is occurring as the point where spontaneous rapid erosion occurs may not have been achieved. Globular scaffolds made from PLGA 50 : 50 and PLGA 75 : 25 lost 26.3 ± 3.9% and 19.0 ± 7.4% of its weight between 0–1 day, respectively, compared to only around 5–11% for thin and thick strands. The loss in mass in the first day of the degradation study did not result from degradation but from the further loss of entrapped tetraglycol. The results indicated that globular scaffolds had a higher tendency to entrap tetraglycol in the scaffolds compared to the thick and thin strand scaffolds, which was also observed visually. Globular scaffolds have a lower SVR and thus have less surface area for the solvent to escape. Dunne et al. have shown that larger microparticles which result in lower SVR usually have limited diffusion and results in higher accumulation of substances inside the particles

compared to smaller microparticles.[37] The tetraglycol remaining in the scaffolds is not a cytotoxic concern as it is a biocompatible, FDA approved solvent[24] which is often used for injectable biomaterial systems.[23,24,38]

In this work, it was hypothesized that the thin strand scaffolds, which had the highest SVR, would have a higher amount of degradation than the thick strand and globular scaffolds as the availability of more surface area will allow more contact between water molecules and the degradable ester groups in the polymer. However, it was found that the globular scaffolds, which had the lowest SVR, resulted in the highest amount of degradation. The higher amount of tetraglycol entrapped and lost by globular scaffolds the first day explains why there is a higher loss of mass for these scaffolds in the first timepoint (day 0-1). For the other timepoints, there are a few hypotheses on why there was a higher amount of degradation observed for the globular scaffolds, compared to the other two types of scaffolds. It has been shown in the fabrication of PLGA microparticles that porosity can be created as an artifact of the fabrication technique, such as the loss of absorbed solvent from the oil phase that leaks out of particles after hardening[39] or water during the freeze drying step at the end of the fabrication process.[40] We hypothesize that the same effect may be occurring in our PLGA scaffolds. Due to its low SVR, globular scaffolds tend to have more space in the center of the scaffold as the entrapped tetraglycol slowly diffuses out of the harden scaffold leaving pores or channels. This resulted in the scaffolds being less dense and more porous compared to the thick and thin strand scaffolds where the tetraglycol escaped more rapidly during the fabrication process, allowing the polymer matrix to harden without leaving pores in the polymer matrix. The hollowness in the polymer matrix of the globular scaffolds results in increased contact of the polymer matrix with water molecules, and thus, more degradation compared to the thick and thin strand scaffolds. It is important to note that the method used to determine the surface area and volume does not take into account the pores that could be introduced by the loss of solvent and thus, the SVRs of the globular scaffolds may have been underestimated. The increase in surface area introduced by the pores may explain why higher degradation was observed with the globular scaffolds. Furthermore, it is important to take into account that ester hydrolysis of PLGA results in acidic monomers (lactic and glycolic acid) and oligomers that further autocatalyze the degradation of the parent polymer by decreasing the pH of the polymer matrix environment.[18] Although SVR may play a significant role in determining the degradation of polymer matrix by providing more surfaces for water molecules to interact and penetrate the scaffold,[23,25] factors that affect retention of the acidic degradation products in the polymeric matrix also play a part in how fast the scaffold degrades. It is expected that larger surface area will results in easier release of the degradation products.[19] Dunne et al. found a linear relationship between PLGA particle size and degradation rate.[37] Larger PLGA particles, which have a lower SVR, degrade faster as the acidic byproducts are retained in the particles which lead to higher autocatalytic degradation of the remaining material. Thus, in this study, globular scaffolds, which have lower SVR will more likely retain the acidic degradation product which will lead to higher autocatalytic degradation compared to the thick and thin strand scaffolds.

For the PLGA 50 : 50 scaffold, globular scaffolds had a significantly lower burst release (Phase 1, first 24 h) compared to the thin and thick strand scaffolds. The higher SVR of the thin and thick strand scaffolds could have accounted for the higher percent of burst release compared to globular scaffolds where the drug will need to travel a longer distance to diffuse out. After Phase 1, the three different architectures had comparable release rates for Phase 2, 3, and 4. For PLGA 75 : 25, the thick strand scaffolds had a significantly lower percent of burst release (Phase 1, first 24 h) compared to the thin strand and globular scaffolds. The globular scaffolds released a higher percent of drugs/day in Phase 2 compared to in Phase 3 and 4 but for the thin and thick strand scaffolds, a comparable percent released/day was observed for Phase 2, 3, and 4. The high burst release for the PLGA 75 : 25 globular scaffolds could have resulted from the slower precipitation of the scaffolds compared to the PLGA 50 : 50 globular scaffolds. PLGA 75 : 25 polymer solution is more viscous, and thus, it may take longer for the tetraglycol to diffuse out of the globular scaffold, allowing more drug to be release during the process.

PLGA 50 : 50 globular scaffolds released a great amount of drug between day 14 and 21. This drastic change in percent released could have resulted from the release of the drug due to the bulk erosion of the polymer matrix at the middle of the study. This unique profile for the globular scaffolds may be an advantage for some applications where a burst release in the beginning and in the middle of the treatment is needed. This biphasic profile was not as prevalent for thin and thick strands scaffolds, which indicates that the release of drug from these scaffolds may be more dependent on diffusion and not so much on the erosion of the matrix as observed in globular scaffolds.

Overall, the globular scaffolds released a higher amount of drug for certain timepoints for PLGA 50 : 50 [Fig. 4(C)] and PLGA 75 : 25 [Fig. 4(D)]. This was expected, as the globular scaffolds were able to successfully load more drugs compared to the other two types of scaffolds. The globular scaffolds had a higher entrapment efficiency and % drug loading, and thus, can deliver more drug at each timepoint compared to the other scaffolds. The lower SVR for the globular scaffolds may have resulted in less loss of drug through diffusion as the scaffolds harden. The PLGA 50 : 50 scaffolds (80–95%) released more drugs in the end of the study compared to PLGA 75 : 25 (~60–80%). This was expected as more degradation was observed for PLGA 50 : 50 scaffolds compared to PLGA 75 : 25 which would lead to more loss of drug from the eroded matrix. A clear trend between SVR and drug release rates were not observed which indicates that besides the availability of more surface area for drugs to diffuse out of the polymer matrix, other factors such as amount of scaffold degradation and scaffold porosity play a role in determining the release of entrapped drug.

CONCLUSION

Scaffolds with different architectures (i.e., globular, thick strand, and thin strand) were fabricated through an easy and economical mold-less technique for controlled release of drugs. This fabrication method is versatile as the size and architecture of the scaffold can be easily tailored by the volume of material that is dispensed and method it is injected into an aqueous solution, respectively. These scaffolds had different SVR and resulted in different degradation characteristics and release kinetics rates. Although it was hypothesized that an increase in SVR will result in enhanced degradation which would lead to an increase in release of the encapsulated drug, the contrary was observed. This could have resulted from the increase in accumulation of acidic byproducts in scaffolds with lower SVR which led to an increase in autocatalytic degradation. The presence of a more porous and less dense polymer matrix for the globular scaffolds due to the slower loss of tetraglycol during the fabrication process could also play a role in increasing the amount of degradation of the globular scaffolds as water molecules can more easily access the ester sites. Further studies, such as scanning electron microscopy, need to be performed in the future to further evaluate the porosity, morphology and structure of the scaffolds. At the end of the studies, scaffolds made with PLGA 50 : 50 resulted in more degradation and drug release compared to those made with PLGA 75 : 25, as expected with the higher lactic to glycolic acid ratio. PLGA 50 : 50 globular scaffolds resulted in a biphasic release profile with a burst release in the beginning and in the middle of the release study which may be beneficial for certain drug delivery applications. To further increase entrapment efficiency and prolong drug release, these scaffolds may benefit from preloading the drug in PLGA microparticles before incorporation into the scaffolds as seen previously by Jeon et al.[23] and thus future studies are being focus on applying this method. It was concluded that there is a complex relationship between SVR, and the degradation characteristics and drug release kinetics of PLGA polymeric scaffolds investigated in this study as many other factors can play a role in determining the relationship between these factors. In this work, we showed that scaffolds with different architectures can be produced with an easy and economical fabrication technique, which result in different SVRs, degradation characteristics and drug release kinetics. The ease and versatility of this fabrication method, which are important factors to consider in scaffold design and fabrication, makes this a promising approach for the development of drug delivery scaffolds or composites for different applications including tissue engineering and cancer therapy.

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