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Research paper

Water soluble polymer films for intravascular drug delivery of antithrombotic biomolecules

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ABSTRACT

Over the past 10 years, the number of percutaneous coronary intervention (PCI) procedures performed in the United States has increased by 33%; however, restenosis, which inhibits complete functional recovery of the vessel wall, remains a complication of this procedure. To traverse the complications associated with PCI, the investigation of therapeutic delivery has become an integral topic in modern research. One such therapeutic, a mimic of the proteoglycan decorin, termed DS-SILY, can mask exposed collagen and thereby effectively decrease platelet activation, has recently been developed by our lab. Drawing inspiration from coating technologies developed by the pharmaceutical industry, a fast-dissolving polymer film has been developed to deliver active therapeutic agents from a balloon catheter during PCI. This research investigates the release of DS-SILY from fast-dissolving polymer films composed of poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG). Thin, uniform polymer films were produced via spin coating technique. The dissolution speed of the polymer films was found to be dependent on the concentration of polymer solution, where at least 65% of the films were shown to dissolve into nanometer sized polymer fragments within 2 min. DS-SILY, up to 6.26 μg/cm^2, was loaded into the films and functional release of the mimic was demonstrated by its successful binding to collagen upon release. Furthermore, DS-SILY released from films resulted in increased platelet inhibition. These results indicate that use of fast-dissolving polymer films allow for the successful release of biomolecules and further investigation of their use for localized drug delivery during PCI procedures is warranted.

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1. Introduction

Due to improvements in the technology and healthcare fields, percutaneous coronary intervention (PCI), an invasive cardiovascular procedure performed to mechanically widen narrowed blood vessels, has become an effective method for the treatment of coronary artery disease. Over the past 10 years, the number of PCI procedures performed in the United States has increased by 33% [1], with millions of patients undergoing balloon angioplasty and the implantation of bare metal or drug-eluting stents [2]. While PCI successfully crushes fatty deposits in order to widen occluded vessel, this procedure is not without complications. The deployment of angioplasty balloons and stents results in the denudation of the endothelial cell layer lining the internal lumen of the vessel and subsequent exposure of the underlying collagenous connective tissue [3]. Platelets accumulate and activate on the exposed collagen [4], releasing various factors to recruit inflammatory cells and promote smooth muscle cell proliferation, migration, and extracellular matrix (ECM) production [5]. The result of this process is restenosis, a disease characterized by the re-occlusion of the vessel, ultimately inhibiting complete functional recovery of the vessel wall and prompting need for a second procedure.

To traverse the difficulties associated with the aftermath of PCI, the delivery of anti-restenotic compounds from both angioplasty balloons and stents has been investigated. Local delivery of therapeutics, such as paclitaxel and sirolimus, from drug-eluting balloons and stents has shown success with minimizing some of the key processes leading to restenosis [6]. However, these compounds demonstrate poor mechanisms for re-endothelialization of the injured vessel wall [7]. To promote complete functional recovery of blood vessels after PCI, the local delivery of a pro-healing therapeutic may be useful. Previously, our lab developed a mimic of the proteoglycan decorin, which has been shown to specifically bind to collagen, serving as a barrier to platelet adhesion and activation [8]. Furthermore, the peptidoglycan encouraged EC migration on collagen-coated substrates and delivery of the decorin mimic from porous balloons in porcine vessels resulted in significantly decreased vessel recoil compared to vessels treated with saline solution [8b]. While our previous work with the decorin mimic has

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shown promising results, delivery of this macromolecule via a porous balloon catheter is not ideal. Porous balloons have been shown to enhance vessel trauma due to the perfusion pressures required to push drug solution through pores and into the tissue and are associated with low transfer efficiency rates [9].

To eliminate the potential complications associated with therapeutic delivery via a porous balloon catheter, a fast-dissolving polymer coating can be used to deliver active therapeutic agents from the exterior surface of balloon catheter during PCI. Over the past few years, the pharmaceutical industry has been investigating the use of fast-dissolving films as a convenient means of delivering therapeutic agents orally [10]. These films typically employ a water-soluble polymer, which quickly hydrates, adheres, and dissolves, allowing for the quick administration of the active therapeutic compound. The deposition of drug formulations within thin films is ideal as the films provide a high amount of surface area for drug transfer and allow for homogeneous distribution of the active compound [10a,b].

The use of polymer coatings on balloon catheters has previously been investigated for the delivery of larger macromolecules [11]. DNA was successfully delivered from poly(ethylene imine)-coated balloons in rats; however, to ensure adequate delivery, coated balloons had to be expanded *in vivo* for 20 min, as DNA release was dependent upon polymer degradation [11]. However, to be efficacious in a clinical setting, therapeutic amounts of drug must be transferred from the balloon surface to the targeted site within 1–2 min, eliminating the possibility of extraneous damage to the tissue due to a lack of blood flow. Thus, the formulation used to coat the balloon is a critical factor for drug transfer during PCI and a fast-dissolving coating would allow for the quick delivery of active therapeutic agents.

Drawing inspiration from the rapidly-dissolving polymer film technologies developed by the pharmaceutical industry [10], this work investigated the development of fast-dissolving polymer films composed of poly(vinyl alcohol) (PVA) and poly(ethylene glycol) to release our decorin mimic. PVA has previously been utilized in a variety of tissue engineering and drug delivery applications due to the biocompatible and non-immunogenic nature of the polymer [12]. Due to its water-soluble nature, PVA has been investigated for use in fast-dissolving film formulations in many studies [10b]. However, research has previously indicated that films composed purely of PVA may be brittle, difficult to handle, and do not possess the fast-dissolving characteristics needed for this application [13]. The addition of low molecular weight PEG has been shown to effectively decrease the glass transition temperature and crystallinity of polymer chains [14]. Previous research has demonstrated that films composed of PVA and PEG form smooth, almost transparent films with good flexibility [10a]. Therefore, to enhance film flexibility and dissolution of films produced in this work, the low molecular weight PEG was added to the PVA solution to serve as a plasticizer.

In this paper, we report a first step towards the development of a fast-dissolving polymer film for potential use as a local delivery system during balloon angioplasty. We first characterized both the film fabrication process and the properties of the resulting polymer films. In addition, this work examined the release of our decorin mimic from the polymer films, investigating its functionality upon release via its ability to bind to collagen and inhibit platelet activation.

2. Experimental section

2.1. Materials

Poly(vinyl alcohol) (PVA, MW 11,000–31,000 Da, 98–99% hydrolyzed) was purchased from Alpha Aesar. Poly(ethylene glycol) (PEG-400, MW 400 Da), boric acid, and theophylline were obtained from Sigma. The peptide RRANAALKAGELYKSILYGC (noted as SILY) and a biotin labeled version of the SILY peptide were purchased from Genscript. Dermatan sulfate (DS, MW 46275 Da) was obtained from Celsus Laboratories. Sodium meta-periodate, *N*-[b-maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH), and *o*-phthalaldehyde were purchased from Thermo Fisher Scientific. Fibrillar equine collagen was purchased from Chronolog; streptavidin–HRP and ELISA kits were obtained from R&D systems. All other reagents and supplies were purchased from VWR unless otherwise noted.

2.2. Peptidoglycan synthesis

The decorin mimic (DS-SILY) was fabricated as previously described [8b]. Briefly, vicinal hydroxyl groups present on the backbone of dermatan sulfate (DS) were oxidized via standard periodate oxidation to form aldehyde moieties. The number of aldehyde groups produced per DS molecule was controlled by varying the sodium meta-periodate concentration. Oxidized DS was then covalently coupled to the heterobifunctional crosslinker *N*-[β-maleimidopropionic acid] hydrazide, trifluoroacetic acid salt (BMPH) in 1× PBS. The collagen-binding peptide sequence RRANAALKAGELYKSILYGC (noted as SILY), derived from the platelet receptor to type I collagen, was conjugated to the DS-BMPH compound; specifically, the thiol group on the cysteine amino acid reacted with the maleimide group of BMPH to form a thioether bond. Purifications were performed at each step by size exclusion chromatography and the number of attached peptides was determined by the consumption of BMPH in the second reaction step. For visualization purposes in this study, the peptidoglycan was biotin labeled by reacting 1 mol of $SILY_{biotin}$ per mole of DS-BMPH for 1 h, followed by the addition of unlabeled SILY to complete the reaction. The final product DS-$SILY_n$, where n indicates the number of attached SILY peptides, was purified in ultrapure H_2O, lyophilized, and stored at −20 °C until use.

2.3. Preparation of PVA/PEG-400/DS-$SILY_n$ thin films

A 20% (w/v) aqueous PVA solution was prepared with distilled H_2O, by mixing and heating at 90 °C for 6 h. Solutions of 10% (w/v) PVA were formed by diluting the 20% PVA solution with distilled H_2O. Low molecular weight PEG-400 was added to the PVA solutions to obtain a PVA/PEG-400 M ratio of 75:25, where the final concentration was 6.6% (w/v) and 3.3% (w/v) PEG-400 in 20% and 10% PVA solutions, respectively. DS-$SILY_{10}$ or DS-$SILY_{20}$ was added to 10% PVA-3.3% PEG-400 solutions with a resulting concentration of 1, 2, or 4 mg/mL DS-$SILY_n$ in the final film precursor solution. Due to the highly viscous nature of the 20% PVA solution, all film precursor solutions were prepared at 50 °C. To produce homogenous thin films, a spin coating process was utilized. Film precursor solution was pipetted onto a pre-weighed glass coverslip and spun for 60 s at 2000 rpm on a spin coater (VTC-100, MTI Corporation) to produce thin films. Films were allowed to dry for 24 h at room temperature prior to use. To determine film weight, films on coverslips were weighed and the mass of the pre-weighed coverslip was subtracted.

2.4. Investigation of film thickness

To determine film thickness, 1 mg/mL FITC was added to the polymer solutions and films were formed using spin coating technique, as described previously. Film thickness was visualized using an Olympus FV1000 confocal microscope with 20× objective. Scans were completed with an area of 512 mm^2 and one stack, 75 μm (1 μm per step) beginning at the film-glass interface, was

imaged at five separate locations in each film. Each image was taken at the same exposure settings to ensure similar darkness values; films lacking FITC were utilized as controls. FV10-ASW software was used to determine the average thickness of the films based of FITC fluorescence.

2.5. Film dissolution

2.5.1. Dissolution rate of thin films

To examine the amount of film dissolved over time, films on coverslips were placed in 12-well plates and 1 mL of H_2O, heated to 37 °C, was added to the top of the film. After 1 min, H_2O was removed and fresh H_2O added to the film; the water exchange process was repeated again at 2, 5, 10, and 20 min after the initial film dissolution. All samples were frozen, lyophilized to dryness, and weighed to determine the mass of polymer dissolved at each particular time point.

2.5.2. Size of dissolved polymer fragments

Films on coverslips were placed in 12-well plates and 2 mL of 1× PBS, heated to 37 °C, was added to the top of the film; films were allowed to dissolve in PBS at 37 °C for 5 min, while shaking at 200 rpm. Upon dissolution, the size of the polymer fragments was measured using dynamic light scattering (DLS; Zetasizer Nano ZS-90, Malvern) at a scattering angle of 90° and wavelength of 658 nm. Disposable polystyrene cuvettes were cleaned once with 95% ethanol and three times with DI H_2O prior to use. The dissolved film solution was then transferred to the cuvette and analyzed at 37 °C. Malvern software was utilized to calculate the mean effective diameter of the polymer fragments via the Stoke–Einstein equation.

To detect for the presence of larger particles, films were dissolved in PBS and the absorbance spectrum (wavelengths 390–750 nm) of the dissolved film solutions was measured. The wavelength spectrum of the dissolved film solutions was then compared to that of the original polymer solution to detect any particles that may have formed during film dissolution.

2.6. Examination of PVA content in thin films

The amount of PVA within the films was determined using a colorimetric method based on the formation of a colored complex between two adjacent hydroxyl groups of PVA and an iodine molecule [15]. Films were dissolved in 1 mL H_2O at 37 °C for 5 min. To determine PVA content in the dissolved film solution, 30 μL of 0.65 M boric acid solution, 5 μL of 0.05 M I_2/0.15 M KI solution, and 65 μL of ultrapure H_2O were added to 100 μL of dissolved film solution. Samples were incubated at room temperature for 15 min, and the absorbance of the samples was measured at 690 nm. A standard curve of PVA, prepared under identical conditions, was utilized to determine the amount of PVA in the film solution.

2.7. Peptidoglycan content within thin films

To determine the amount of DS-SILY within the films, *o*-phthalaldehyde (fluoraldehyde), a primary amine-reactive fluorescent detection reagent, was used to detect the primary amines on the N-terminus of the SILY peptides bound to the dermatan sulfate backbone of the peptidoglycan. Films, fabricated as described previously, were dissolved in 1 mL H_2O at 37 °C for 5 min. To detect the amine groups present on the peptidoglycans, samples of the dissolved film solutions were added to the fluoraldehyde reagent at a ratio of 1:5. A standard curve, containing known amounts of either DS-$SILY_{10}$ or DS-$SILY_{20}$, was used to determine the amount of DS-SILY within the solution. The fluorescence was measured at an excitation of 340 nm and emission of 460 nm.

2.8. Peptidoglycan binding to collagen upon release from thin films

Fibrillar collagen, at a concentration of 50 μg/mL diluted in isotonic glucose, was coated onto the surface of a 96-well high-bind plate and incubated over night at 4 °C. Unbound collagen was removed by rinsing three times with 1× PBS. Plates were then blocked with 1% BSA in 1× PBS for 2 h at room temperature. Films containing DS-$SILY_{10}$ or DS-$SILY_{20}$ were dissolved in 1× PBS containing 1% BSA and immediately added to the collagen-coated surfaces. Samples were allowed to incubate at 37 °C, with shaking at 200 rpm, for 15 min. Plates were then rinsed three times with 1× PBS. To detect DS-$SILY_n$-biotin bound to the collagen-coated surfaces, samples were then incubated with streptavidin–HRP, diluted 1:200 in 1% BSA in 1× PBS, for 20 min at room temperature with shaking. Plates were then rinsed three times with 1× PBS to remove any unbound streptavidin–HRP prior to the addition of 1:1 hydrogen peroxide:tetramethylbenzidine, inducing a colorimetric change. Upon 20 min of incubation, the reaction was stopped via the addition of 2 N sulfuric acid and absorbance was read at 540 nm.

2.9. Inhibition of platelet binding and activation

Microplates were coated with fibrillar collagen, as described for the binding affinity assays, without blocking with BSA. Films containing DS-$SILY_{10}$ or DS-$SILY_{20}$ were dissolved in 1× PBS and added to the collagen-coated wells. Controls included films composed only of PVA and PEG-400 or films containing DS. Treatments were incubated at 37 °C with shaking at 200 rpm for 15 min. Wells were then rinsed of unbound treatment by removing the treatment solution, adding 1× PBS, and shaking the wells for 24 h. During the 24 h, the PBS treatment was changed three times.

Human whole blood was collected from healthy volunteers by venipuncture following the approved Purdue IRB protocol (IRB Protocol #0901007687) and with informed consent. The first 5 mL of blood was discarded and approximately 10 mL was then collected into citrated glass vacutainers. Blood was centrifuged in the glass tube for 20 min at 200*g* at 25 °C. The top layer of the centrifuged blood, the platelet rich plasma (PRP), was used for platelet studies.

PRP (50 mL/well) was added to the microplate for 1 h at room temperature without shaking. After 1 h of incubation, 45 μL of PRP was removed from each well and added to a microcentrifuge tube containing 5 μL of ETP solution (107 mM EDTA, 12 mM theophylline, and 2.8 mM prostaglandin E_1) to inhibit further platelet activation. Tubes were centrifuged at 4 °C for 30 min at 2000*g* to pellet platelets. The supernatant was collected for ELISA studies to test for the presence of the platelet activation markers: platelet factor 4 (PF-4) and b-thromboglobulin (NAP-2). Sandwich ELISAs were performed following the manufacturer's protocols. It was necessary to dilute the platelet serum 10,000fold in 1% BSA in 1× PBS in order for the values to fall within the linear range.

2.10. Statistical analysis

Results are expressed as means ± standard error of the mean. Statistical analysis was performed using SAS software (SAS Institute). All results were analyzed using ANOVA with Tukey's HSD post hoc test. The threshold for statistical significance was set at $p < 0.05$.

3. Results and discussion

3.1. Mass and thickness of polymer thin films

In this present work, we examine the development and characterization of a fast-dissolving polymer film to deliver therapeutics

upon film dissolution. The fast-dissolving thin films in this study were fabricated from PVA and PEG-400 using a spin coating technique. Examination of films via phase microscopy indicated that the thin polymer films did not contain cracks (data not shown). Films formed from solutions of 20%PVA + 6.6%PEG-400 exhibited masses of 8.9 ± 0.4 μg/mm^2. As expected, the weight of 20%PVA + 6.6%PEG-400 films was significantly increased compared to films spun from 10%PVA + 3.3%PEG-400 solutions; films formed from solutions of lower polymer concentration were found to weigh 2.7 ± 0.2 μg/mm^2. The addition of the peptidoglycan to 10%PVA + 3.3%PEG-400 solutions did not result in a significant change in film weight, even at the highest concentrations of DS-SILY$_{10}$ or DS-SILY$_{20}$ added. The water content of the films was examined via vapor sorption techniques [16]. Upon drying films to 0% room humidity, it was determined that only 0.5% of the weight of films is due to unbound water.

To ensure that the films produced for this study exhibited homogenous thickness, confocal microscopy was utilized to detect FITC dye incorporated within the fast-dissolving polymer films. As the amount of polymer in the initial solutions decreased, a corresponding decrease in film thickness was observed; films formed from 20%PVA + 6.6%PEG-400 and 10%PVA + 3.3%PEG-400 solutions demonstrated thicknesses of 53.3 ± 2.5 μm and 19.8 ± 1.4 μm, respectively (Table 1). No significant change in thickness was exhibited in films fabricated from 10%PVA + 3.3%PEG-400 solutions with 1, 2, or 4 mg/mL DS-SILY$_{10}$ compared polymer films produced without the addition of DS-SILY$_{10}$. For all films examined, the thickness of the films was found to be similar at each of the five locations measured.

Examination of the mass and thickness of the plain polymer films demonstrated that 20%PVA + 6.6%PEG-400 films weigh ~3.1 times more and are ~2.8 times thicker than 10%PVA + 3.3%PEG-400 films. These results are not unexpected as the increase in polymer concentration allows for higher solution viscosity, resulting in less excess polymer solution being ejected off the edge of the glass substrate during the spin coating process [17]. However, for either of the polymer solutions utilized in this study, the efficiency of film formation using spin coating technique was low, as only 12.7% and 8.2% of the original polymer in 20%PVA + 6.6%PEG-400 and 10%PVA + 3.3%PEG-400 solutions was incorporated into the films, respectively. While the efficiency of film formation in this work remains low, fast-dissolving films, such as the ones produced in this work, are likely to be coated onto angioplasty balloons using either a spray or dip-coat technique [18]. Thus, the amount of polymer solution required for production should be minimized and efficiency better controlled via an alternative process.

3.2. Dissolution of polymer thin films

As a potential application of these films is use for quick release of therapeutic from angioplasty balloons during PCI, the time required for dissolution is an important parameter that was investigated. Films fabricated using 20%PVA + 6.6%PEG-400 solutions were found to dissolve more slowly than films formed from 10%PVA + 3.3%PEG-400 (Table 2). After 1 min, ~40% of 20%PVA + 6.6%PEG-400 films had dissolved, while more than 71% of 10%PVA + 3.3%PEG-400 films had dissolved into solution. Furthermore, results indicated that 10%PVA + 3.3%PEG-400 films dissolved completely within 2 min in solution, whereas approximately 5 min were required for the 20%PVA + 6.6%PEG-400 films to completely dissolve. As our results demonstrated that the time required for 20%PVA + 6.6%PEG-400 films to completely dissolve was longer than films composed of 10%PVA + 3.3%PEG-400, we have shown that the amount of time required for the polymer film to dissolve can be altered by increasing or decreasing the thickness of the film. This result is not unexpected as the 20%PVA + 6.6%PEG-400 films exhibited increased thickness due an increased amount of polymer within the film. Thus, the length of time required for water to penetrate into the polymer chains located through the depth of the film increases [19]. Regardless, over 65% of films formed from 20%PVA + 6.6%PEG-400 or 10%PVA + 3.3%PEG-400 solutions dissolved after 2 min. Furthermore, the addition of either DS-SILY$_{10}$ or DS-SILY$_{20}$ to 10%PVA + 3.3%PEG-400 films was not found to significantly alter dissolution rate of the films. This result is important as it indicates that these films are fast-dissolving, which is necessary for drug delivery from an angioplasty balloon as balloon expansion should only take 1–2 min to ensure that blood flow to the surrounding tissues is minimally obstructed.

In order to detect the size of the polymer fragments produced via film dissolution, DLS was utilized to analyze fragment size in films dissolved in PBS at 37 °C. A general trend was seen such that for the films tested, two populations of polymeric fragments were evident in the dissolved film solutions; the size difference between the two populations was approximately one order of magnitude different (Table 1). Films formed from 20%PVA + 6.6%PEG-400 solutions resulted in fragments with an average hydrodynamic diameter of 108.1 ± 17.4 nm and 12.3 ± 1.4 nm. Similarly, 10%PVA + 3.3%PEG-400 films were also found to dissolve into fragments with two different size populations, each found to be of a similar size as those produced from 20%PVA + 6.6%PEG-400 films. Examination of the size of PVA molecules in solution revealed average hydrodynamic diameters of 112.3 ± 5.2 and 11.9 ± 0.6 nm, values similar to previously reported results [20]. Literature has previously demonstrated that low molecular weight PEG-400 exhibits a hydrodynamic diameter of 0.5–1 nm, which was below the detection threshold of the DLS system utilized in this study [21]. Therefore, it is likely that both populations observed in this study are composed of PVA.

The addition of 4 mg/mL DS-SILY$_{10}$ to 10%PVA + 3.3%PEG-400 solutions did not result in a significant change in fragment size, nor was the appearance of a third fragment population observed.

Table 1
Summary of film thickness and hydrodynamic diameter of polymer fragments upon film dissolution.

DS-SILY$_{10}$ (mg/mL)	Thickness (μm)	Fragment size (nm)	
		Peak 1	Peak 2
20% PVA + 6.6% PEG-400			
–	53.3 ± 1.6*	12.29 ± 1.40	108.08 ± 17.37
10% PVA + 3.3% PEG-400			
–	19.9 ± 1.4	11.73 ± 1.54	104.53 ± 17.23
1	18.1 ± 1.0	–	–
2	20.4 ± 1.2	–	–
4	19.0 ± 1.1	11.89 ± 1.56	110.54 ± 21.47

* Represents statistical difference in thickness from 10%PVA + 3.3%PEG-400 films.

Table 2
Summary of the percentage of PVA/PEG-400 films dissolved at varying time points (%).

DS-SILY$_{10}$ (mg/mL)	DS-SILY$_{20}$ (mg/mL)	1 min	2 min	5 min
20% PVA + 6.6% PEG-400				
–	–	39.7 ± 1.9	65.2 ± 1.7	102.4 ± 0.8
10% PVA + 3.3% PEG-400				
–	–	71.2 ± 3.4	96.8 ± 1.4	–
1	–	67.3 ± 4.9	101.2 ± 3.1	–
2	–	68.2 ± 2.3	98.4 ± 2.5	–
4	–	69.1 ± 2.7	98.7 ± 3.6	–
–	1	72.6 ± 4.4	100.3 ± 4.1	–
–	2	68.5 ± 2.1	97.4 ± 3.6	–
–	4	71.9 ± 1.2	98.1 ± 4.2	–

In solution, DS-SILY$_{10}$ was found to have a hydrodynamic diameter of 13.5 ± 1.4 nm similar to previous reports for DS of similar molecular weight [22]. Due to the similarities between molecular weight and linear structure shared by PVA and the DS backbone of the peptidoglycan, the fragment populations observed in this study likely contain both PVA and DS-SILY$_n$. We speculate that the occurrence of a second population of larger polymer fragments is due to the aggregation of smaller polymer chains into larger aggregates, a phenomenon that has been previously demonstrated in literature [23].

While the sizes of the PVA and DS-SILY molecules are similar to values reported in literature, there are several limitations of DLS technology for our application. Namely, DLS is model-dependent and is typically accurate only for spherical particles. It is unlikely that the polymer fragments in this study are purely spherical; thus, the DLS results may be slightly skewed. However, any particles arising from the dissolution of these fast-dissolving films are likely on the nanometer scale, as the formation of micron-sized or larger particles was not detected in dissolved film solutions, as indicated using UV Vis Spectrophotometry (results not shown). It is encouraging that the polymer fragments remain on the nanometer scale as it is necessary for the fast-dissolving films to dissolve into fragments that are small enough to pass through the minutest capillaries without getting stuck and blocking blood flow to the surrounding tissue. In addition, the nanometer size of the polymer fragments should allow for their removal from the blood stream via non-specific clearance of the reticuloendothelial system, a phenomenon that it often seen by intravenously injected nanoparticles [24].

3.3. Evaluation of PVA amount in films

To better characterize the composition of the films, a colorimetric assay was utilized to evaluate the amount of PVA within the thin films. As expected, as the amount of PVA in polymer solution used to fabricate the films decreased, a corresponding decrease in the amount of PVA within the films was observed. On average, films fabricated from 20%PVA + 6.6%PEG-400 solutions contained 5.6 ± 0.4 μg/mm^2 PVA, whereas films produced using 10%PVA + 3.3%PEG-400 solutions contained 1.8 ± 0.1 μg/mm^2 PVA. As ~3.1 times more PVA was found in 20%PVA + 6.6%PEG-400 films compared to 10%PVA + 3.3%PEG-400 films, this result further confirms our previous findings comparing the weight and thickness of 20%PVA + 6.6%PEG-400 films to 10%PVA + 3.3%PEG-400 films. Interestingly, comparison of the amount of PVA to the total weight of the film indicated that PVA accounted for ~61–65% of the total film weight. This result was observed regardless of whether the films were formed using 20%PVA + 6.6%PEG-400 or 10%PVA + 3.3%PEG-400 solution. Thus, it can be assumed that the ratio of PVA/PEG-400 within the films remains relatively constant during film fabrication. Furthermore, the addition of 1, 2, or 4 mg/mL of DS-SILY$_{10}$ or DS-SILY$_{20}$ to 10%PVA + 3.3%PEG-400 solutions did not result in significant changes to the PVA content of the films.

3.4. Release of DS-SILY$_n$ from films

The amount of peptidoglycan within 10%PVA + 3.3%PEG-400 polymeric films was quantified using a fluoraldehyde assay, as described above. A general trend was observed such that as the amount of DS-SILY$_n$ incorporated within the polymer solutions increased, the amount of DS-SILY$_n$ incorporated within the films also increased (Fig. 1). Films fabricated from 10%PVA + 3.3%PEG-400 solutions with 1, 2, or 4 mg/mL DS-SILY$_{10}$ released 1.6 ± 0.1, 3.4 ± 0.4, or 6.6 ± 0.5 μg/cm^2 of DS-SILY$_{10}$, respectively. The amount of DS-SILY$_{20}$ released from the films was similar to the amount of DS-SILY$_{10}$ added for all DS-SILY$_n$ concentrations. This result is encouraging as it shows that therapeutics with analogous physicochemical properties can be loaded with similar efficiency.

Based on film weight and composition, it can be estimated that only ~75–85% of the original DS-SILY$_n$ incorporated into the films was released. While the amount of DS-SILY$_n$ detected from the films is lower than expected, it may be attributed to the entanglement of the DS-SILY molecules with PVA molecules, forming larger aggregates as previously mentioned above, inhibiting their detection. Regardless, our results indicate that by increasing the initial concentration of DS-SILY$_n$ in the 10%PVA + 3.3%PEG-400 solution, the amount of therapeutic loaded within the films can also be increased. Furthermore, increased quantities of therapeutics within the films were achieved without compromising the physical properties of the polymer films, indicating that varying amounts of therapeutics can be loaded into these fast-dissolving films, allowing for drug-loading flexibility within the system without disrupting the intrinsic properties of the film.

3.5. Extent DS-SILY$_n$ binding to collagen

Upon release from the thin polymeric films, DS-SILY$_n$ was demonstrated to remain functional, as demonstrated by its ability to bind to collagen (Fig. 2). A general trend was observed such that as the amount of DS-SILY$_{10}$ or DS-SILY$_{20}$ increased within films, a corresponding increase in absorbance, due to the reaction of bound HRP with tetramethylbenzidine, was exhibited. An increase in absorbance indicates that more DS-SILY$_n$ is bound to the surface of the collagen-coated substrate. However, inherent differences in the amount of DS-SILY$_n$ able to bind to collagen were observed. For all films examined, significantly increased amounts of DS-SILY$_{20}$ were bound to collagen compared to DS-SILY$_{10}$; DS-SILY$_{20}$ exhibited absorbance values that were 3.2, 2.9, and 2.8 times larger than the absorbance values elicited by DS-SILY$_{10}$ at 1, 2, and 4 mg/mL, respectively. As DS-SILY$_{20}$ contains approximately 20 collagen-binding peptides covalently bound to a dermatan sulfate backbone, whereas only 10 peptides are covalently attached to create DS-SILY$_{10}$, this result is not unexpected. Previously, we have shown that peptidoglycans exhibiting larger numbers of ECM-binding

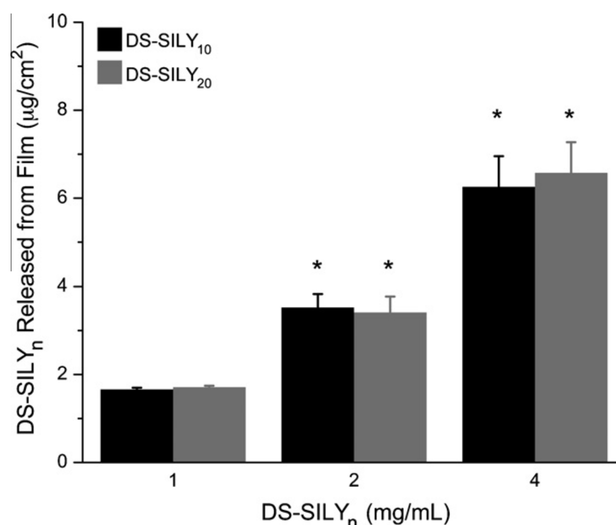


Fig. 1. Examination of peptidoglycan released from films indicated that as the amount of DS-SILY$_n$ within the polymeric solutions increased, the amount of DS-SILY$_n$ released from the films significantly increased. Similar amounts of DS-SILY$_{10}$ and DS-SILY$_{20}$ were released from films composed of 10%PVA + 3.3%PEG-400 with 1, 2, or 4 mg/mL DS-SILY$_n$. Error bars represent standard error; * represents significance from 10%PVA + 3.3%PEG-400 films with 1 mg/mL DS-SILY$_n$. $N > 5$ films.

peptides result in increased binding due to the larger number of available interactions between the peptide and the ECM molecule [8b,25]. Furthermore, in comparison with a standard absorbance curve with known DS-SILY$_n$ amounts, it was estimated that approximately 90–100% of the DS-SILY$_n$ released from films, as well as in polymer precursor solutions, remained active and was able to bind to collagen.

3.6. Inhibition of collagen-induced platelet binding and activation

To further measure the functionality of DS-SILY$_n$ upon release from fast-dissolving films, the release of platelet activation factors PF-4 and NAP-2 from human platelets on collagen surfaces alone, or on collagen surfaces exposed to DS-SILY$_n$-loaded fast-dissolving films was examined. As the amount of DS-SILY$_{10}$ or DS-SILY$_{20}$ within the films increased, a corresponding increase in platelet inhibition was observed; this general trend was observed for both PF-4 (Fig. 3A) and NAP-2 (Fig. 3B). Maximal inhibition was observed when films containing 4 mg/mL DS-SILY$_{20}$ were used, where ~65% of PF-4 and ~25% of NAP-2 were inhibited. This result is not unexpected as more DS-SILY$_n$ is available to bind to the collagen, effectively masking the protein from platelets and preventing activation [8b]. For films fabricated with lower concentrations of peptidoglycan, DS-SILY$_{20}$ was found to more effectively inhibit PF-4 and NAP-2 release from platelets, compared to films containing DS-SILY$_{10}$. However, for films fabricated from solutions containing 4 mg/mL of peptidoglycan, similar levels of PF-4 and NAP-2 inhibition were observed between films containing 4 mg/mL DS-SILY$_{10}$ and DS-SILY$_{20}$. This result is interesting as films formed with 4 mg/mL DS-SILY$_{10}$ demonstrated significantly decreased amounts of peptidoglycan bound to collagen compared to DS-SILY$_{20}$-incorporated films. This data suggest that there may be some threshold for DS-SILY coverage that is needed to maximally inhibit activation; however, additional studies are required to better understand this phenomenon. Regardless, our results demonstrate that the release of DS-SILY$_n$ from 10%PVA + 3.3%-PEG-400 films significantly decreases the platelet activation factors released.

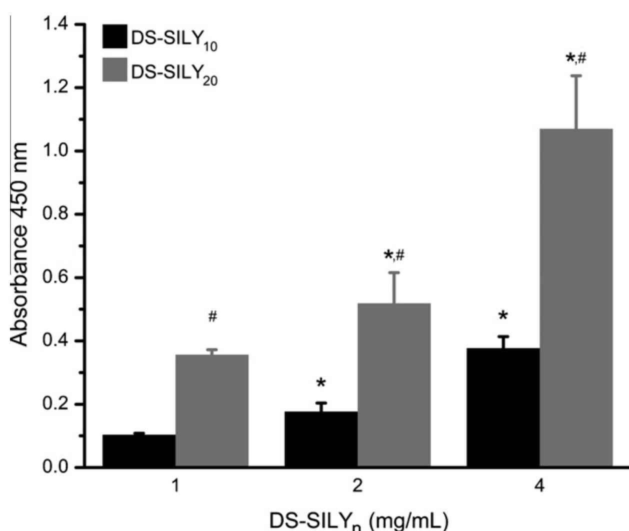
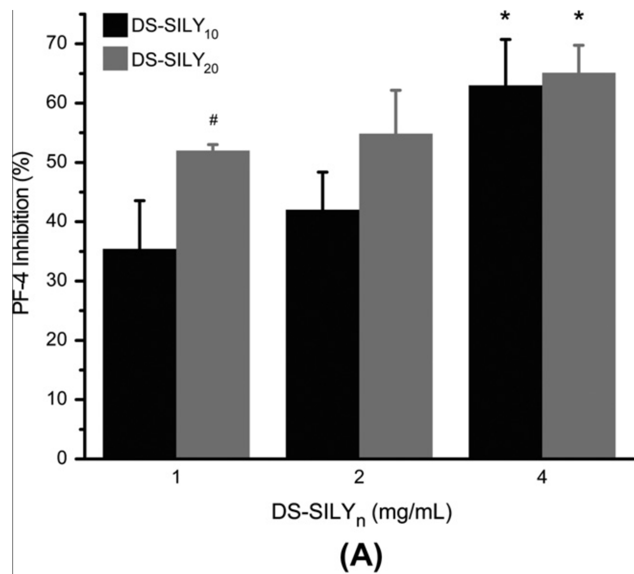


Fig. 2. Examination of the amount of DS-SILY$_n$ bound to collagen-coated substrates demonstrated that as the concentration of either DS-SILY$_{10}$ or DS-SILY$_{20}$ in the polymeric solution increased, the amount of peptidoglycan bound to collagen increased. DS-SILY$_{20}$ exhibited increased binding compared to DS-SILY$_{10}$ at all concentrations examined. Error bars represent standard error. * represents significance from 10%PVA + 3.3%PEG-400 films with 1 mg/mL DS-SILY$_n$; # represents significance from DS-SILY$_{10}$ at a similar concentration. $N > 5$ films.



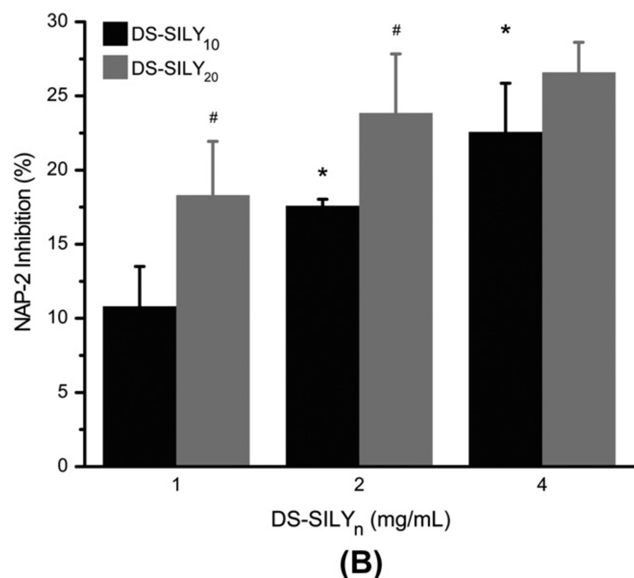


Fig. 3. Examination of the effects of DS-SILY$_{10}$ and DS-SILY$_{20}$ released from 10%PVA + 3.3%PEG-400 films on the inhibition of collagen-induced platelet activation factors (A) PF-4 and (B) NAP-2. Inhibition of both PF-4 and NAP-2 increased with a corresponding increase DS-SILY$_n$ in films. Error bars represent standard error. * represents significance from 10%PVA + 3.3%PEG-400 films with 1 mg/mL DS-SILY$_n$; # represents significance from DS-SILY$_{10}$ at a similar concentration. $N > 5$ films.

4. Conclusion

The use of fast-dissolving polymer films for drug-delivery applications during PCI is very exciting. The polymer films examined in this study are unique as they allow for the release of functional therapeutics within 1–2 min after exposure to solvent, where the speed at which the films dissolve can be controlled by the amount of polymer incorporated into, that is, thickness of, the film. Furthermore, dissolution of the polymer film results in polymer fragments with sizes that should not inhibit blood flow in the minutest of capillaries. We have demonstrated that therapeutics can be added to the polymer solution, allowing for their incorporation in and subsequent release from the films upon dissolution. The therapeutic utilized in this study, DS-SILY, was found to bind to collagen and inhibit platelet activation upon release, validating the ability of these fast-dissolving films to deliver functional biopoly-

mer therapeutics. These results further encourage the exploration of fast-dissolving polymer films for drug delivery applications.

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