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Authors

Li, Zidong
Seker, Erkin

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Configurable Microfluidic Platform for Investigating Therapeutic Delivery from Biomedical Device Coatings

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DOI: 10.1039/C7LC00851AZidong Li¹, Erkin Seker^{2,*}Departments of ¹Biomedical Engineering and ²Electrical & Computer Engineering
University of California – Davis, Davis, CA 95616

ABSTRACT

Advanced biomedical device coatings have shown significant promise in delivery of therapeutics (e.g., small-molecule drugs, proteins) for a wide range of medical interventions ranging from targeted cancer therapy to management of atherosclerosis. In order to accelerate the development of such coatings, there is a need for tools to investigate the loading capacity and release kinetics with high temporal resolution and in a variety of physiological conditions. To address this need, we report a microfluidic platform, where the coating on a substrate can be mounted onto the microchannel and the device can be configured in two physiologically-relevant modes: (i) Flow-mode allows for monitoring the release from the coating in contact with a liquid flowing at a specific rate, modeling the case of a drug-eluting stent. (ii) Static-mode, where the channel is filled with a stationary gel, mimics the case of drug-eluting brain implant. We demonstrate the utility of the platform with a fluorescein-loaded nanoporous gold coating and monitor in real-time the release kinetics both under deionized water infusion and agarose gel-filled channel via fluorescence microscopy coupled to a LabVIEW-based interface.

KEYWORDS: drug delivery; mass transport; microfluidics; real-time microscopy

1. Introduction

Biomedical device coatings for controlled and in situ delivery of therapeutics have made a significant impact on the field of targeted cancer therapy^{1, 2}, neural interfaces^{3, 4}, orthopedic implants⁵, vascular stents⁶, and regenerative medicine⁷. The coatings typically consist of degrading materials (poly(lactic acid)⁸, poly (lactide-co-glycolide)⁹) or non-degrading materials (polydimethylsiloxane, polyurethanes¹⁰). For both cases, the *loading capacity* and *release kinetics* are the two important figures-of-merit. Nanostructured materials have particularly enhanced the capabilities of such coatings. Examples of such materials include porous anodic alumina¹¹, porous silicon¹², porous carbon¹³, carbon nanotubes^{14, 15}, and porous polymers¹⁶.

The conventional method to study drug release from these coating materials follows similar protocols. Briefly, the coating material is submerged in a concentrated drug solution in order to load the coating via passive diffusion of drug molecules. Alternatively, the coating can be synthesized while doping it with drug molecules¹⁷. The drug molecules are adsorbed on the material surface either physically through weak intermolecular interactions (e.g., electrostatic, van der Waals)^{18, 19} or via chemical linkers^{20, 21}. After the loading step, the drug-loaded coating is rinsed, and immersed in deionized water or a physiology-relevant buffer (typically referred to as the *elution medium*) to initiate the drug release. The elution medium is sampled periodically and analyzed by UV absorbance spectroscopy or fluorescence spectroscopy to determine the cumulative amount of drug molecules released. However, this conventional method has several shortcomings. Since the driving force for drug release is the concentration gradient between the coating and the elution medium, an equilibrium is reached before all the loaded molecules are released, thereby yielding an erroneous loading capacity. Another limitation is that the cumulative release profile (as opposed to an

instantaneous release profile) disguises the details of the release profile, such as minute non-monotonic changes in release rate.

In order to address these limitations, we developed a microfluidic platform that can accommodate any coating type (degrading or non-degrading) on a variety of substrates (e.g., glass, semiconductor, metal). By imposing convective flow inside the microchannel in contact, released molecules disperse into the mobile medium instead of accumulating as in the case of a stationary solution for the conventional method. Consequently, the microfluidic platform eliminates the influence of the premature leveling of the concentration gradient on loading capacity and release kinetics. By flowing liquid inside the device (*flow-mode*), the molecular release from the material in contact with flowing liquid can be monitored and it can mimic the case of a drug-eluting vascular stent. While by filling stationary agarose gel inside the device (*static-mode*), it can model the case of drug-eluting brain implant. For both cases, fluorescent tagging of the drug molecules or their surrogates allow for real-time monitoring of the release event with a fluorescence microscopy coupled with a LabVIEW interface for quantification. The microfluidic device and the schematics in two different modes are shown in Figure 1.

Figure 1. The microfluidic device and the schematics in flow-mode (modeling a drug-eluting vascular stent) and static-mode (modeling a drug-eluting neural interface).

The operation of the microfluidic platform is demonstrated with nanoporous gold (np-Au) as a model drug delivery coating, since it allows for a wide range of release kinetics due to its tunable morphology and surface chemistry²¹⁻²⁴ – two important factors that influence loading capacity and release kinetics.

2. Experimental

2.1 Chemicals and Materials

Nitric acid (70%) and fluorescein sodium were purchased from Sigma-Aldrich. Sulfuric acid (96%) and hydrogen peroxide (30%) were obtained from J. T. Baker. Polydimethylsiloxane (PDMS) was purchased from Dow Corning. Thin glass coverslips (0.15 mm-thick) and glass slides (1 mm-thick) were purchased from Electron Microscopy Sciences. Gold, silver, and chrome targets (99.95% pure) were obtained from Kurt J. Lesker. Agarose powder was obtained from Apex. Epoxy adhesive was obtained from DEVCON.

2.2 Nanoporous Gold Fabrication and Fluorescein Loading

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Np-Au films were patterned on coverslips by sequentially sputter-depositing a 160 nm-thick chrome adhesive layer, an 80 nm-thick gold seed layer, and a 600 nm-thick co-sputtered silver-gold alloy layer. After deposition, the samples were immersed into 70% nitric acid at 55°C to produce the np-Au films via a process known as *dealloying*. A portion of the fabricated chips were annealed on the hot plate at 250°C and 400°C for 90 seconds to produce coarser morphologies^{22, 23, 25}. The np-Au chips were immersed in 10 mM fluorescein solution prepared in deionized (DI) water at room temperature for 16 hours. The loaded np-Au chips were rinsed in DI water and dried under nitrogen gas flow.

2.3 Microfluidic Platform Fabrication

The microfluidic features were created in glass slide by 355 nm UV laser ablation and the laser-patterned structure was bonded to a thin glass coverslip using oxygen plasma-activated PDMS (10 μm) layer as the intermediate adhesive. The detailed fabrication flow for the microfluidic device is outlined in Figure 2. First, the glass slide and coverslip were cleaned in piranha solution, consisting of 1:4 ratio (by volume) of hydrogen peroxide and sulfuric acid. Then the glass slide was spin-coated with pre-mixed PDMS (1:10 w/w curing agent to base) on a spinner to produce a uniform 10 μm-thick PDMS layer. Polyimide tape was placed on the PDMS surface to protect the surface from fine debris generated during UV laser cutting. After laser machining, the polyimide tape was removed. The glass slide was bonded to the laser-machined glass coverslip assisted by alignment marks following oxygen plasma treatment. After bonding, the device was kept at 85°C on a hot plate for 30 minutes to promote a stronger bond between glass slide and coverslip. A glass reservoir was epoxied on the glass slide as an inlet and PDMS tubing connector was plasma bonded to the glass as an outlet. The plasma treatment was performed in a quartz chamber (Techniques RIE) at 30 W power under 0.5 Torr chamber pressure for 12 seconds with an oxygen flow rate of 20 sccm.

Figure 2. The fabrication process steps for the microfluidic device.

The fluorescein-loaded np-Au chips were mounted onto the microfluidic platform over the fluid access window at the channel floor to allow released molecules to disperse into the flowing medium in the microfluidic channel. In order to ensure a leak-free coupling between the chip and microfluidic platform, epoxy was applied along the perimeter of the chip. The elution medium was then introduced from the inlet glass reservoir filled with DI water to the outlet that was connected to a syringe pump. The flow rate inside the channel was

maintained with a syringe pump at 1 $\mu\text{L}/\text{min}$. The schematic of the test system can be seen in Figure S1.

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2.4 Molecular Release Quantification

In the *flow-mode*, released fluorescein molecules were carried with the flowing medium in the microchannels towards the outlet port. The amount of released fluorescein molecules was quantified from the fluorescence intensity by epi-fluorescence microscopy at the downstream of the channel. Four different morphologies of gold thin films were tested, including planar gold (pl-Au), standard np-Au, np-Au annealed at 250 $^{\circ}\text{C}$ and 400 $^{\circ}\text{C}$ respectively. In this configuration, the mobile medium transports the released fluorescein molecules downstream with a volumetric flow rate of 1 $\mu\text{L}/\text{min}$. A short time-lapse video (20 frames that lasts for 6 seconds) was recorded with fluorescence microscope with decreasing acquisition frequency (i.e., every 1 minute for the first 40 minutes, every 5 minutes for the following hour, and finally every 10 minutes until the end of release experiment). In this manner, the dynamic release profiles, especially at the early timepoints, were successfully captured. Each video sample with 20 frames was then uploaded to ImageJ²⁶ and the intensity of every frame inside the video sample was extracted into a grey value with a timestamp. The intensity value was finally converted to fluorescein concentration using a calibration curve (Figure S3).

In the *static-mode*, agarose-PBS gel was filled inside the channel, where the fluorescein molecules diffused from the standard np-Au film into the microfluidic channel through the gel. Fluorescence images were taken sequentially at multiple locations along the channel to obtain the fluorescein diffusion profiles inside the gel-filled microchannel.

In both modes, the fluorescence intensity was extracted into a grey scale by uploading fluorescent images to ImageJ. A calibration curve was used to convert the intensity of the fluorescence readout to the actual concentration of the fluorescein solution.

3. Results and Discussion

3.1 Molecular Release Coating

The np-Au thin film coating is produced by a self-assembly dealloying process, where silver from a silver-rich gold-silver alloy in nitric acid and gold atoms surface-diffuse to create a bicontinuous open-pore structure²⁷. The morphology of np-Au is typically described by features such as ligaments and pores as described in Figure S2. The pore size was typically between 20 and 120 nm and the pore density was uniform across the entire np-Au sample²¹⁻²⁴. The pore morphology of np-Au can be easily modified by thermal treatment, where enhanced gold surface diffusion leads to coarser ligaments yet reduced pore density²². Specifically, the average pore sizes (extracted by digital image processing as described previously²¹⁻²⁴) for the np-Au samples displayed in Figure 3 are 65 nm, 70 nm, and 125 nm respectively for standard np-Au to that treated at 400 $^{\circ}\text{C}$. The pores follow an interconnected tortuous path into thickness of the porous film (~600 nm).

3.2 Microfluidic Platform

In order to reduce non-specific fluorescein adsorption inside the channel, the amount of exposed PDMS was minimized²⁸. Therefore, the majority of the microfluidic device was made of glass with only 10 μm -thin PDMS layer used as an interface to bond the two glass layers. High-pressure flow was infused into the device to verify that the device was sealed completely. The microfluidic channel was 200 μm wide and 100 μm deep. The chip mounting scheme can easily be extended to different materials. While different thin films coatings (e.g., porous anodic alumina or carbon nanotubes) can be deposited on hard

carrier substrates, thick materials (e.g., porous glasses) can be directly mounted over the fluid access window. Soft drug-eluting coatings (e.g., hydrogels) can be casted onto hard carrier substrates for coupling them to the microfluidic platform.

3.3 Molecular Release in Flow-Mode

In order to assess the microfluidic platform’s ability to differentiate between molecular release profiles from different coatings, np-Au thin films with various coarseness on glass coverslips were used. Since the np-Au sample can be easily detached from the microfluidic device, it was possible to use the same microfluidic platform to study release kinetics from gold coatings with different morphologies, assisting in reducing experiment-to-experiment variability. The SEM images of the different gold thin films are shown in Figure 3B. The pl-Au has a smooth surface without porous structure, while, standard (un-annealed) np-Au has the highest pore coverage. With increased temperature during the thermal treatment, the pore size increases and pore surface coverage decreases. The corresponding molecular release profiles for the four different morphologies are illustrated as instantaneous release profile (Figure 3A inset) and the corresponding time-integral cumulative release profile (Figure 3A). It is evident that the instantaneous profile captures the nuances of the release rate across time, while these details are lost in the conventional cumulative presentation. Even though pl-Au has flat surface, there were residual fluorescein molecules non-specifically adsorbed on the gold surface, which led to the release of a small amount of fluorescein. The pore density decreased for standard np-Au, thermally-treated at 250 °C compared to that at 400 °C. Consequently, the effective surface area and, hand in hand, the loading capacity also decreased.

Figure 3. A) Cumulative release profiles from different np-Au morphologies (standard np-Au, thermally-treated at 250 °C, thermally-treated np-Au at 400 °C, and pl-Au). Inset shows the corresponding instantaneous release profiles. B) SEM images of the four gold thin film morphologies.

We also investigated the effect of pore morphology on the release kinetics. The cumulative release profiles of each np-Au sample were normalized to the corresponding total amount release in order to obtain the fractional release profiles (Figure 4). The fractional release profiles were analyzed and fitted to theoretical models to understand the underlying release mechanisms.

Previous studies have shown that Weibull function is useful in interpreting molecular release profiles for complex geometries^{23, 29}. Briefly, fractional release profiles are fitted to the Weibull function (Equation 1), where M_t is the fractional release at an arbitrary time point, t ,

and M_{∞} is the loading capacity, to extract coefficients of a and b .

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$$\frac{M_t}{M_{\infty}} = 1 - \exp(-at^b) \quad (1)$$

The exponent b provides insight into the transport mechanism for the molecular release as a function of the coating geometry. Specifically, when $b < 0.75$, the release follows a Fickian diffusion; when b is in the range between 0.75 and 1.0, it indicates a combined mechanism of diffusion in normal Euclidian substrate with contribution of another release mechanism; when $b > 1.0$, the Weibull function has the sigmoid shape and it indicates a complex transport mechanism that governs the release²⁹.

As shown in the insets in Figure 5, Weibull function fitted all the release profiles of np-Au very well with $R^2 = 0.99$. The extracted b values of all np-Au morphologies ranged between 0.78 and 0.96, which suggests that the underlying transport is a combination of Fickian diffusion and another release mechanism that is plausibly dictated by frequent surface-molecule interactions in the high-aspect ratio np-Au³⁰.

Figure 4. Fractional release profiles for different np-Au morphologies. The profile is fitted to Weibull distribution function to provide insight into the transport mechanisms of the molecular release, where the fit parameter, b , serves a predictor of the mechanism.

3.4 Molecular Release in Static-Mode

For this configuration, we filled the microchannel with 0.6% agarose-PBS gel, which mimics diffusive and mechanical properties of brain tissue^{31, 32}. The fluorescein molecules migrated outside the np-Au thin film and diffused into the agarose gel embedded in the microchannel. In a time-lapse manner, fluorescence images were captured along the channel for up to 8 mm away from the chip to observe the diffused fluorescein profile. The corresponding spatial distribution of fluorescein at advancing timepoints is summarized in Figure 5 inset, where the x-axis origin is the edge of the np-Au film and y-axis is the fluorescein concentration. The main plot in Figure 5 shows the peak fluorescein concentrations (approximately at $x = 0$) for different time points during the release. Two distinct regions, which we refer to as *burst release* and *pure diffusion*, are immediately noticeable. The initial burst release is due to rapid displacement of physio-adsorbed fluorescein molecules on gold with chloride ions in the agarose-PBS gel. As previously reported²⁴, this leads to the immediate release of

fluorescein molecules from np-Au thin films. In the pure diffusion region, this burst-released fluorescein bolus should follow a classical Fickian diffusion into the hydrogel, as the diffusive path for fluorescein is mostly water in the 0.6% agarose gel³³⁻³⁷. This is in contrast with the molecular transport in np-Au, where frequent surface-molecule interactions dictate the mass transport²³. The pure diffusion region can be studied using a limited-source diffusion model, commonly used by the semiconductor industry to model diffusion of a certain dose of implanted impurities (analogous to the fluorescein bolus) within a semiconductor substrate (analogous the agarose gel)³⁸. The limited-source diffusion equation is shown in Equation 2:

$$N(x, t) = \frac{Q}{\sqrt{\pi Dt}} \exp\left(-\left(\frac{x}{2\sqrt{Dt}}\right)^2\right) \quad (2)$$

where Q is the total dose of diffusing molecules, x is the distance from the diffusion source, D is the diffusion coefficient. The extracted diffusion coefficient from fitting the data in pure diffusion region to Equation 2 yields $3.85 \times 10^{-6} \text{ cm}^2/\text{s}$, which is sufficiently close to the reported values (ranging between $4.25 \times 10^{-6} \text{ cm}^2/\text{s}$ and $6.4 \times 10^{-6} \text{ cm}^2/\text{s}$) for fluorescein in water and agarose gels of similar water-to-agarose ratio³³⁻³⁷.

Figure 5. The peak fluorescein concentration in the microfluidic channel as a function of release duration and corresponding spatial distribution of fluorescein along the channel away from the np-Au source at different time points (Inset). The red dotted-line is for visual aid only and the blue solid-line is a curve-fit to the limited-source diffusion equation.

3.5 Real-Time Monitoring of Molecular Release with LabVIEW Interface

In the studies described until now, the fluorescein release profile was determined by extracting fluorescein concentration from time-lapse fluorescence images processed by ImageJ. While this method is powerful for release studies, in order to observe the release kinetics in real-time, for example for closed-loop control of release kinetics by imposing certain stimulus, there is a need for online measurements. To that end, we created a LabVIEW interface that continuously captures the time-varying fluorescence intensity and converts it to a concentration value (based on an initial calibration curve) for a user-defined region-of-interest in the channel. The concentration of released molecules with the corresponding timestamp and the real-time fluorescein levels can be saved as a spreadsheet for additional data analysis. The LabVIEW front panel is shown in Figure 6. The key features are the simultaneous display of both instantaneous and cumulative release profiles, as well as the remaining fluorescein amount in the coating of interest (based on

loading capacity determined from previous experiments). In addition, it is shown the LabVIEW interface successfully captures the PBS-triggered burst release (video in Electronic Supporting Information), highlighting the ability to use this platform for characterizing stimulus-responsive drug delivery systems.

Figure 6. The front panel of LabVIEW control system: A) Intensity from a user-defined region-of-interest; B) Fluorescein level; C) Instantaneous release rate; D) Instantaneous release profile; E) Cumulative release profile

4. Conclusion

We reported a versatile microfluidic platform that can be configured in two physiologically-relevant modes. In the flow-mode (mimicking a drug-eluting vascular stent), real-time monitoring of molecular release from coatings with different morphologies was achieved. In the static-mode, fluorescein transport within a static medium (mimicking a drug-eluting brain implant) was investigated. For both cases, fitting the data to established mathematical models, provided insight into the properties of the coating as well as the drug molecule surrogates. We expect this platform to assist future studies on development of controlled release coatings. The microfluidic platform can be expanded for more sophisticated physiological cases. For example, by flowing and seeding cells inside the device³⁹, it can represent a more realistic physiological model. The device can also be used to investigate stimulus-responsive materials by imposing external triggers (e.g., optical, magnetic, electrical, thermal) and capturing rapidly-varying molecular concentrations⁴⁰. Finally, it is possible to further enhance the versatility of the platform by integrating other quantification techniques on the microfluidic device, such as embedding electrodes for downstream electrochemical analysis, daisy-chaining with mass-spectroscopy or high-performance liquid chromatography instruments.

Conflict of interest

The authors declare no competing financial interests.

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