Supporting Information

3D-Printed Drug Capture Materials Based on Genomic DNA Coatings

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Composition of Autodesk PR48

The composition of PR48 below is reproduced from the official technical document from Autodesk. All percentages are wt/wt.

Allnex Ebecryl 8210: 39.776 %

Sartomer SR 494: 39.776 %

Esstech TPO+: 0.400 %

Rahn Genomer 1122: 19.888 %

Mayzo OB+: 0.160 %

Mechanical properties of PR48

The mechanical properties of PR48 reported below are obtained from the technical data sheet provided by the manufacturer (Colorado Photopolymer Solutions):

Young's Modulus: 1400 MPa

Tensile Strength: 28 MPa

Elongation: 3%

Schematic of Digital Light Processing (DLP) Printing

In DLP printing, a build head is immersed in a bath of photoresist, where a digital micromirror device selectively projects an image to be cured in the photoresist onto the build head. The build head then moves a set amount up in the z-direction, where the process subsequently repeats itself again until the entire structure is printed, as seen in Figure S1.

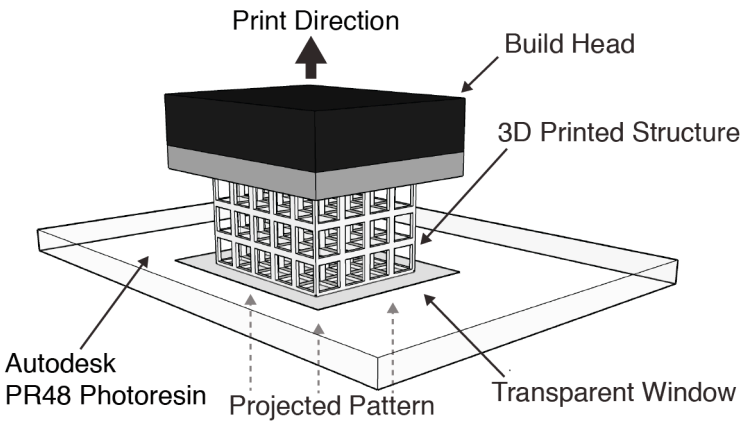

Figure S1. Schematic of DLP printing. The desired pattern is projected through the transparent window, inducing localized photopolymerization. When the pattern is complete, the build head moves a set distance up in the z-direction. This repeats until the structure is completed.

Successful vs. unsuccessful DNA coatings

Soaking of untreated lattices in neutral solutions of DNA resulted in a lower percentage of successful coats, with DNA sometimes precipitating in a fibrous mass within the lattice. This is depicted below in Figure S2a. The use of an acidic DNA solution prevented this from happening, as seen in Figure S2b.

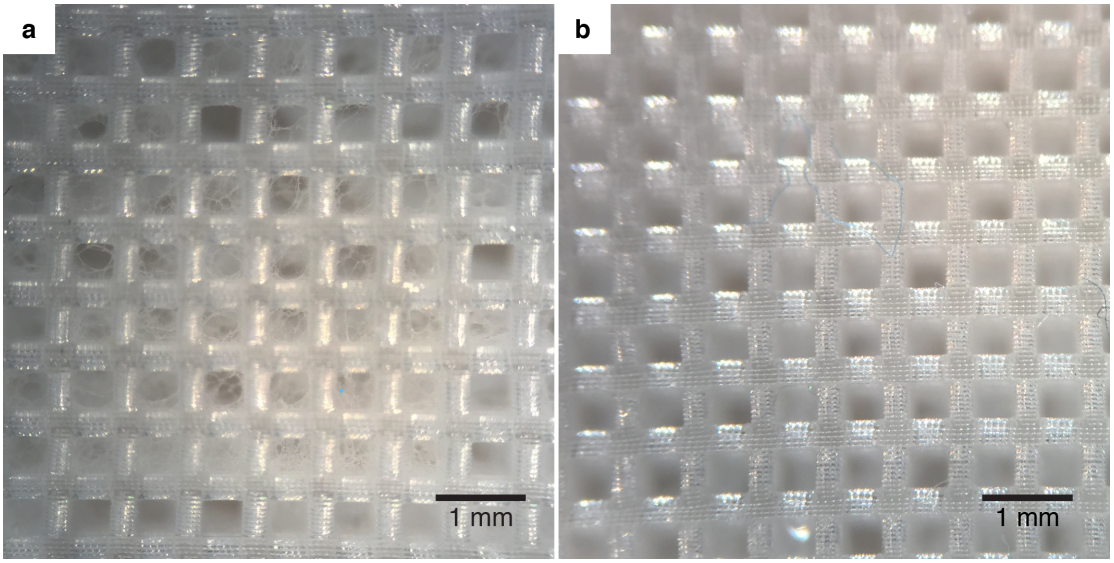

Figure S2. Differences in DNA coating with pH. a) Fibrous masses of DNA could sometimes be seen within the lattice when coating with a neutral solution of DNA. b) No precipitation could be seen when an acidic DNA solution was used instead.

Quantification of surface amines via Orange II assay

The surface amines on the aminated lattices were quantified using a colorimetric method based on the azo dye Orange II. The mechanism of the assay is outlined in Figure S3a: the aminated substrates are first placed in pH 3 Orange II solutions. The amines become protonated and are able to bind to the negatively charged Orange II molecule, turning the substrate orange. After washing off any unbound dye, the orange sample is then soaked in a 0.1 M NaOH solution to deprotonate the amine, freeing the Orange II molecule into solution. The substrate is removed, and the solution re-acidified to pH 3. The absorbance of the solution at 480nm is then measured and then correlated to its concentration in solution via the use of a calibration curve.

For simplicity, 3D printed PR48 plates with dimensions 5 mm (L) x 5 mm (W) x 0.5 mm (H) were used for the Orange II assay. The results of the Orange II assay for untreated and aminated plates are shown in Figure S3b. As expected, the untreated plates remained colorless after immersion in an aqueous Orange II solution (pH 3). Subsequent treatment of the plates in a 0.1 M NaOH solution also showed no color changes. In contrast, the aminated plates turned orange after soaking in an aqueous Orange II solution (pH 3). Incubation of the orange plates in a 0.1 M NaOH solution resulted in the plates reverting back to their original colorless forms. The results of the Orange II assay indicated that amination of the plates using neat ethylenediamine was successful.

We conducted the Orange II assay on plates that were soaked in ethylenediamine for 15, 30, 60, 120, and 180 minutes. The surface density of accessible amines as a function of ethylenediamine reaction time is shown in Figure S3c. It can be seen that the reaction tapers off after 60 minutes, at a value of 18.5 ± 0.5 nmol/mm^2.

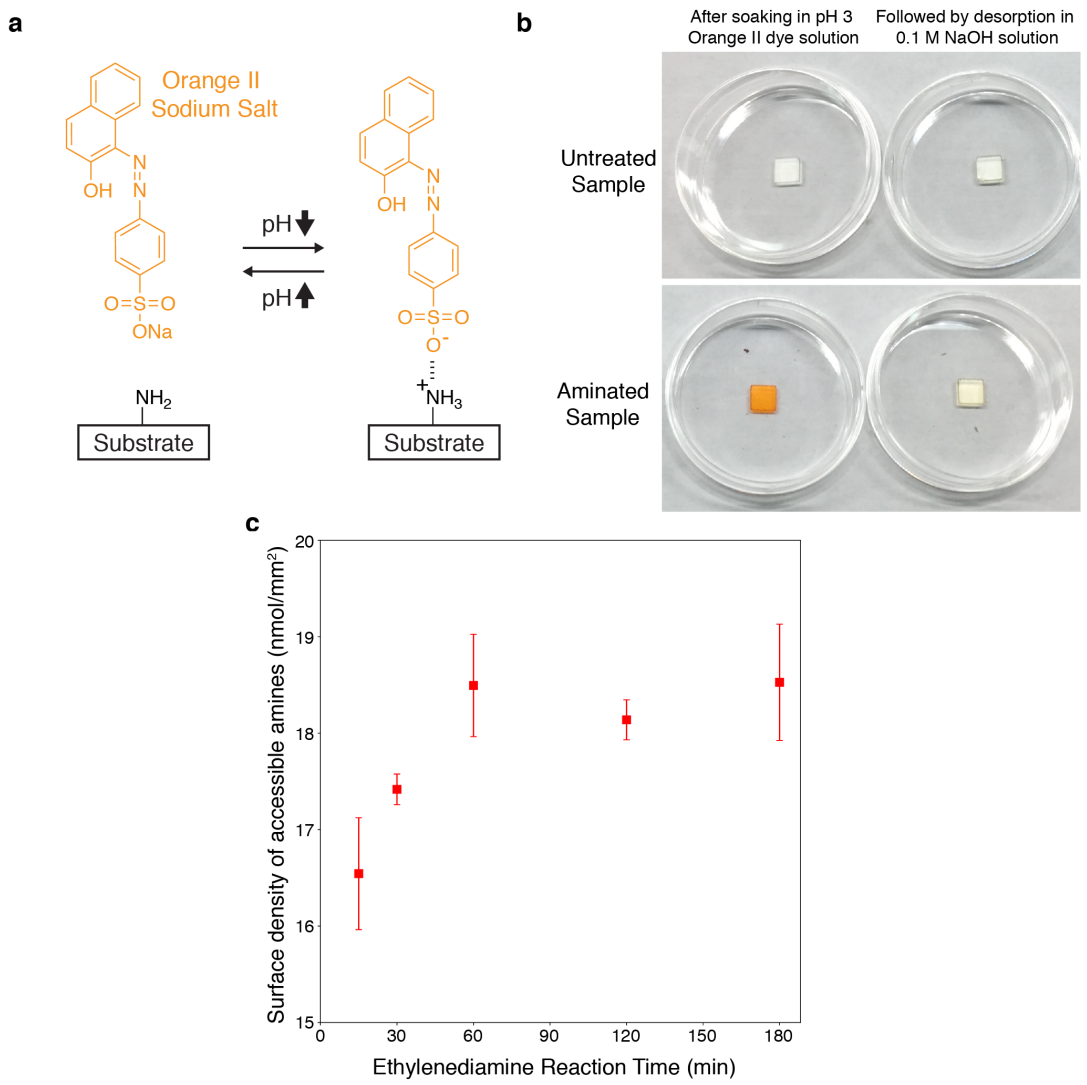

Figure S3. Orange II assay. a) Mechanism of Orange II assay. b) Comparison of untreated and aminated plates after Orange II assay. c) Surface density of accessible amines as a function of ethylenediamine reaction time.

Energy dispersive X-ray spectroscopy elemental mapping of untreated lattices

EDS elemental mapping of the untreated lattices indicate that the polymer substrate does not contain any phosphorous. Only carbon and oxygen were detected, but carbon was omitted in the analysis due to the conductive carbon coating used to enable imaging.

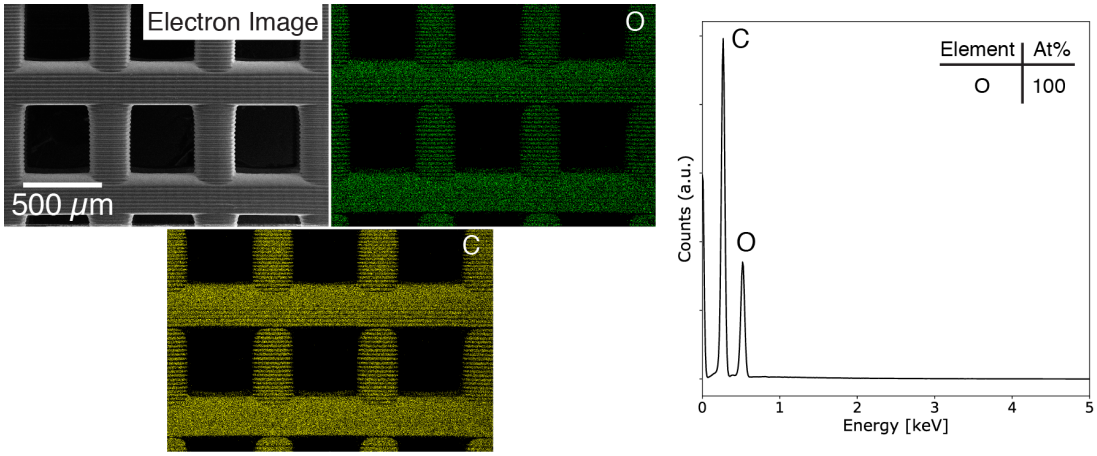

Figure S4. EDS Elemental map of the untreated lattices indicate that the underlying polymer substrate does not contain any phosphorous.

Energy dispersive X-ray spectroscopy elemental mapping of DNA films

An acidic solution of DNA was first prepared by dissolving 2.5 mg of DNA in 1 mL of acidified pH 3 water (deionized water adjusted to pH 3 with 37% hydrochloric acid). 300 µL of the DNA solution was then cast onto a silicon wafer, and then dried under vacuum at room temperature for 180 minutes. The DNA film was then coated with 10 nm of carbon to enable SEM/EDS characterization. An SEM image of the film, and its corresponding phosphorous, nitrogen, and sodium map is shown below in Figure S5. The EDS spectrum of the area within the orange box in the SEM image is also shown. The percentage of phosphorous in a pure DNA film is about 8.6 at%.

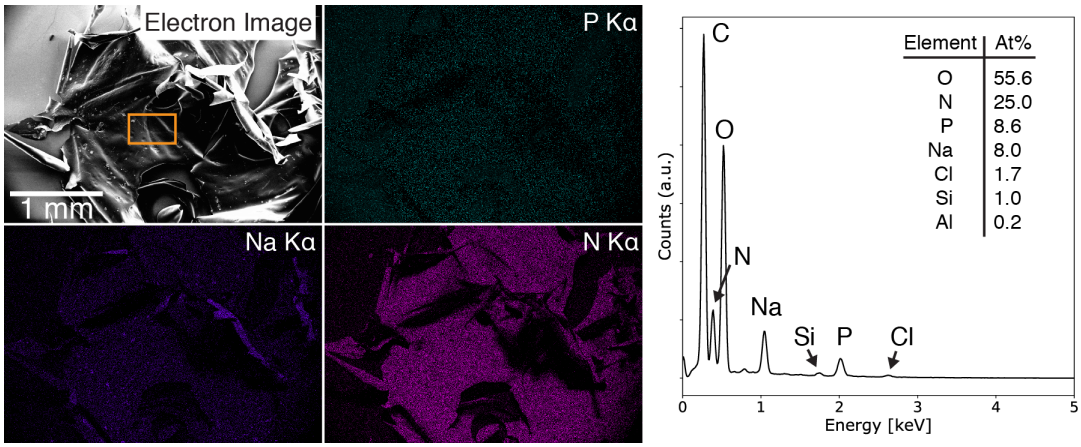

Figure S5. EDS Elemental map of DNA films cast on a silicon wafer. Nitrogen, sodium, and phosphorous can be seen throughout the film. The EDS spectrum of the area within the orange box is also shown on the right.

Representative UV-Vis spectra from leaching study

Representative absorption spectra from the DNA-PBS solutions obtained from the leaching studies for the different types of lattices are shown below in Figure S6. The DNA-PBS solutions from the DNA-UVC and eDNA-UVC lattices absorb so little that their spectra have to be rescaled to visualize them. The rescaled spectra are showed in panel e and f. The dotted line at 260 nm provides a guide to the eye as to what the A_{260} values are. It is interesting to note that unlike the other spectra, for the eDNA-UVC spectra, the 260 nm point is not at a local maxima, which would be expected for DNA. Despite this, it was assumed that the absorption at 260 nm was still due to DNA. This would give us an upper bound as to the DNA leached.

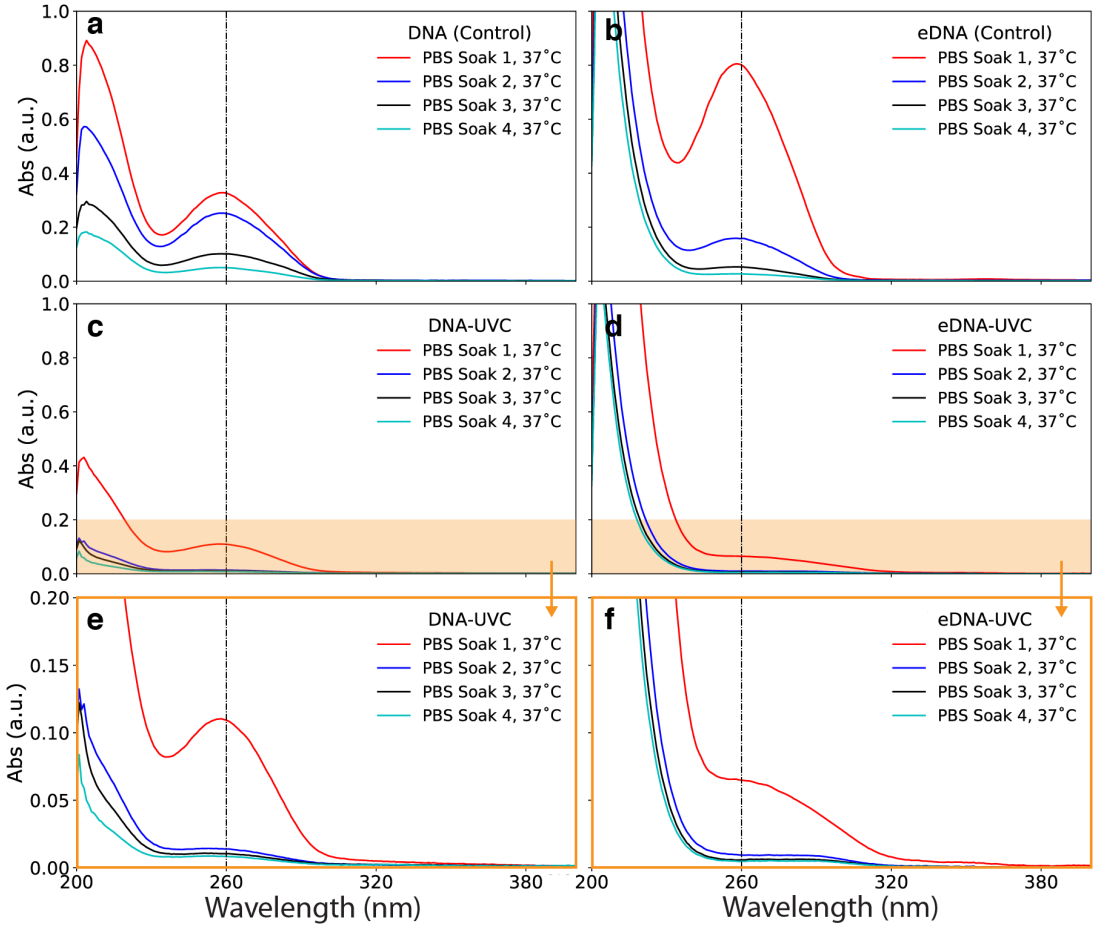

Figure S6. Representative absorption spectra from the DNA-PBS solutions obtained from the leaching studies for the a) DNA (Control), b) eDNA (Control), c) DNA-UVC, d) eDNA-UVC lattices. e) DNA-UVC spectra, but with y-axis rescaled to 0.2. f) eDNA-UVC spectra, but with y-axis rescaled to 0.2.

Estimation of DNA adsorbed on the surface

The amount of DNA on the surface of the lattices *after* the leaching experiments can be estimated by finding the difference between the total amount of DNA leached from the Control sets of lattices and that from the irradiated lattices. This includes the amount of DNA that was leached during the PBS soak step between UVC irradiations or ambient light exposures. The assumption here is that the amount of DNA leached from the Control lattices represents the total amount of DNA that was initially adsorbed onto the surface.

This assumption is valid for the eDNA (Control) lattices since EDS analysis of the eDNA (Control) lattices post leach showed that only a small amount of phosphorous could be detected on the edges of some horizontal beams (Figure 3c). Thus, the amount of DNA leached from the eDNA (Control) lattices is close to the amount of DNA that was initially adsorbed. In contrast, for the DNA (Control) lattices, there is still a moderate amount of phosphorous left after the leaching experiments (Figure 3b). The total amount of DNA leached from them is thus only a large fraction of the initial DNA adsorbed on the surface. The implication of this is that our estimation will only provide a lower bound for the true amount of DNA left on the DNA-UVC lattices. We can use the EDS images to estimate the amount of DNA left on the surface by determining the ratio of the groove areas that contain phosphorous to the areas that do not. Using this analysis, we determined that ~25% of the grooves have DNA on them. This value represents an upper-bound since a key assumption here is that the thickness of the coating does not change.

Thus, at least 75% of the DNA was leached from the DNA (Control) lattices during the leaching study.

eDNA-UVC

Including the amount of DNA leached in between the UVC irradiation steps, the eDNA (Control) lattices leached a total of 186.5 ± 32.7 ng of DNA/mm^2. On the other hand, the eDNA-UVC lattices leached a total of 10.6 ± 2.0 ng/mm^2. By taking the difference between the two values, we can approximate the amount of DNA on the eDNA-UVC lattices at the end of the leaching experiments to be 175.9 ± 32.8 ng/mm^2. As discussed above, this value should be close to the true amount on the surface. In this case, ~6% of the initial adsorbed DNA was leached during the leaching studies. Assuming that subsequent 30-minute soaks in PBS would result in similar DNA leaching values of ~0.1 ng/mm^2, this would correspond to a loss of ~0.1% of the DNA present on the surface per 30 minutes.

DNA-UVC

Similarly, the total amount of DNA leached by the DNA (Control) and DNA-UVC lattices were determined to be 141.7 ± 15.9 and 24.8 ± 1.4 ng/mm^2. By taking the difference between the values, the amount of DNA on the surface of the DNA-UVC lattices after the leaching experiments was approximately 116.8 ± 16.0 ng/mm^2. It is important to emphasize that this value *underestimates* the true value of DNA on the surface of these lattices and should be taken as a lower bound. Using this value, ~18% of the initially adsorbed DNA was leached from the DNA-UVC lattices. Assuming that

subsequent 30-minute soaks in PBS would result in similar leaching values of ~0.5 ng/mm^2, this would represent a loss of ~0.4% of the DNA present on the surface per 30 minutes. In this case, this ~0.4% loss represents an upper-bound for amount of DNA leached in subsequent exposures.

DNA Calibration Curve

The calibration curve was constructed by determining the Abs_{260} of PBS solutions with known concentrations of DNA.

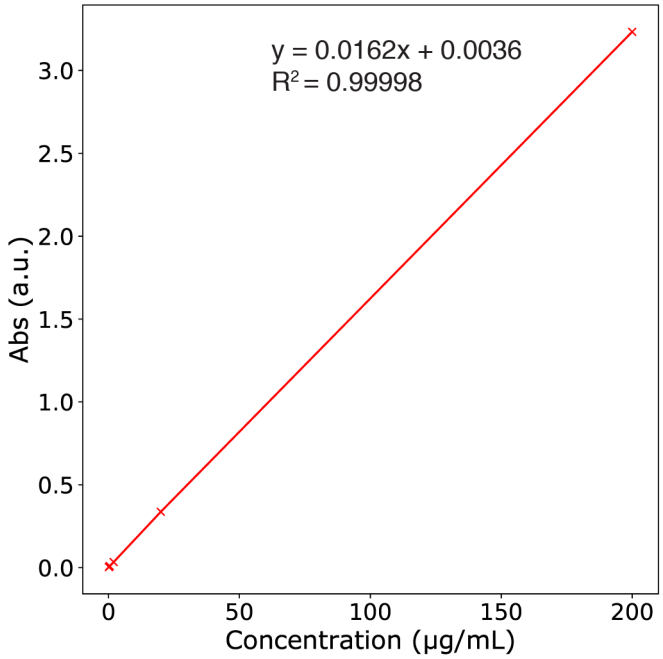

Figure S7. DNA Calibration curve. Concentrations of 200, 20, 2, 0.5, 0.4 µg/mL were used to construct this curve.

Representative absorption spectra for UVC only and Amine-UVC lattices

Representative absorption spectra for the UVC only and amine-UVC lattices are shown below in Figure S8. For the UVC only lattices, virtually no signal can be seen, even after rescaling the y-axis to 0.2. For the amine-UVC lattices, a small signal can be seen after rescaling the y-axis. The A_{260} values of these spectra were determined and converted to background "DNA" via the calibration curve in Figure S9. These background "DNA" values were then subtracted from the measured DNA determined from the A_{260} values from the DNA (Control), DNA-UVC, eDNA (Control), and eDNA-UVC lattices. It is interesting to note that the amine-UVC spectra look similar to that of the eDNA-UVC spectra.

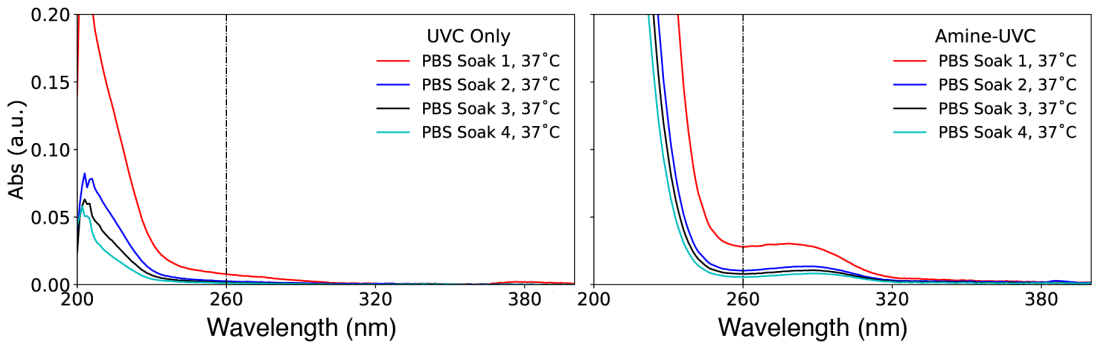

Figure S8. Representative UV-Vis absorption spectra for the UVC only (left) and amine-UVC lattices (right). Note that the y-axes have been set to 0.2.

Doxorubicin calibration curve (PBS)

The calibration curve was constructed by determining the fluorescence intensity at 590 nm of PBS solutions with known concentrations of doxorubicin. 3 x 100 µL of each of these solutions were placed in a 96-well microplate. The concentrations of doxorubicin in each well were then measured by way of fluorescence on a microplate reader (Molecular Devices Flexstation 3). The measurement parameters were as follows: λ_{ex} = 480 nm, λ_{ex} = 550 - 590 nm, $\lambda_{cut\text{-}off}$ = 530 nm, sensitivity = 100. The average of the three measurements was calculated and the standard deviation determined. Note: the standard deviation here represents the standard deviation of the measurement. A 4th order polynomial fit was used to construct the calibration curve. To determine the concentration of doxorubicin from a known count value, the polynomial was solved using Python (numpy.roots function).

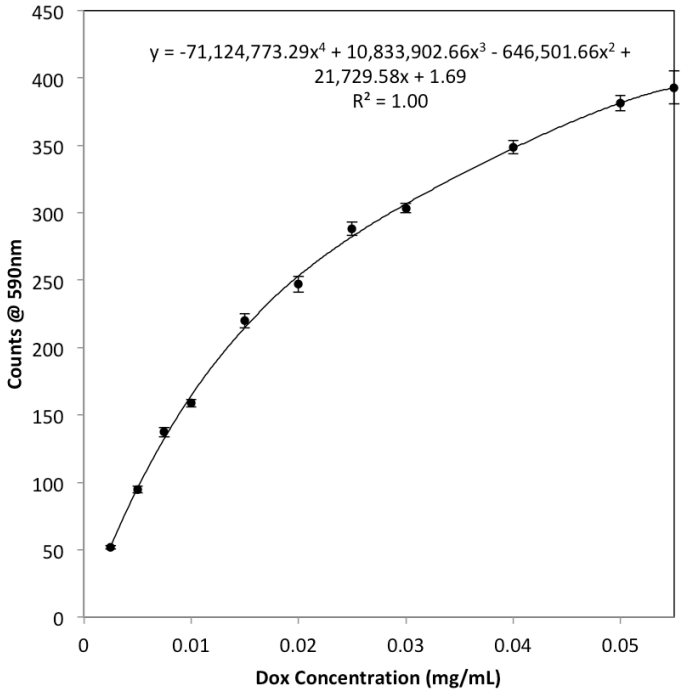

Figure S9. Doxorubicin calibration curve in PBS. Points represent the mean of the three measurements. Error bars here represent the standard error of measurement (n = 3).

Doxorubicin calibration curve (HS)

The calibration curve was constructed by determining the fluorescence intensity at 590 nm of HS solutions with known concentrations of doxorubicin. 3 x 100 µL of each of these solutions were placed in a 96-well microplate. The concentrations of doxorubicin in each well were then measured by way of fluorescence on a microplate reader (Molecular Devices Flexstation 3). The measurement parameters were as follows: λ_{ex} = 480 nm, λ_{ex} = 550 - 590 nm, $\lambda_{cut\text{-}off}$ = 530 nm, sensitivity = 100. The average of the three measurements was calculated and the standard deviation determined. Note: the standard deviation here represents the standard deviation of the measurement. A 2nd order polynomial fit was used to construct the calibration curve. To determine the concentration of doxorubicin from a known count value, the polynomial was solved using Python (numpy.roots function).

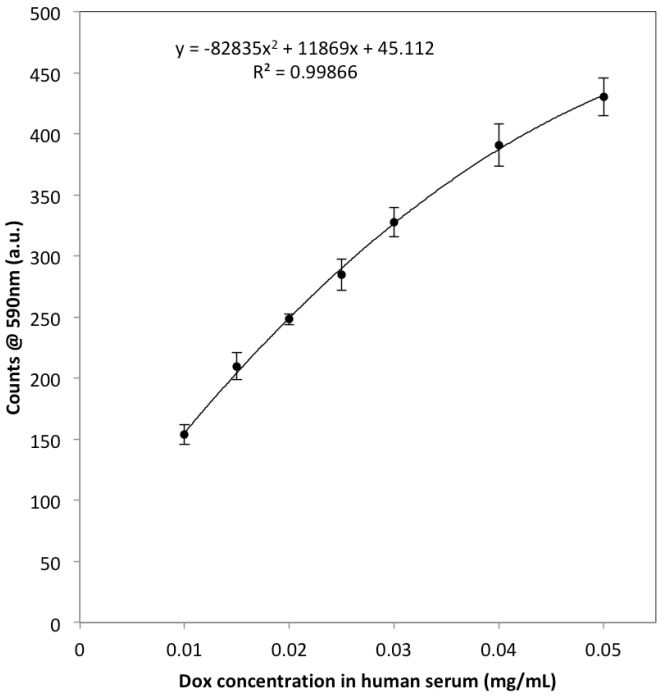

Figure S10. Doxorubicin calibration curve in HS. Points represent the mean of the three measurements. Error bars here represent the standard error of measurement (n = 3).