

## Supporting Information for

# Using a biocompatible diazide crosslinker to fabricate a robust polyelectrolyte multilayer film with enhanced effects on cell proliferation

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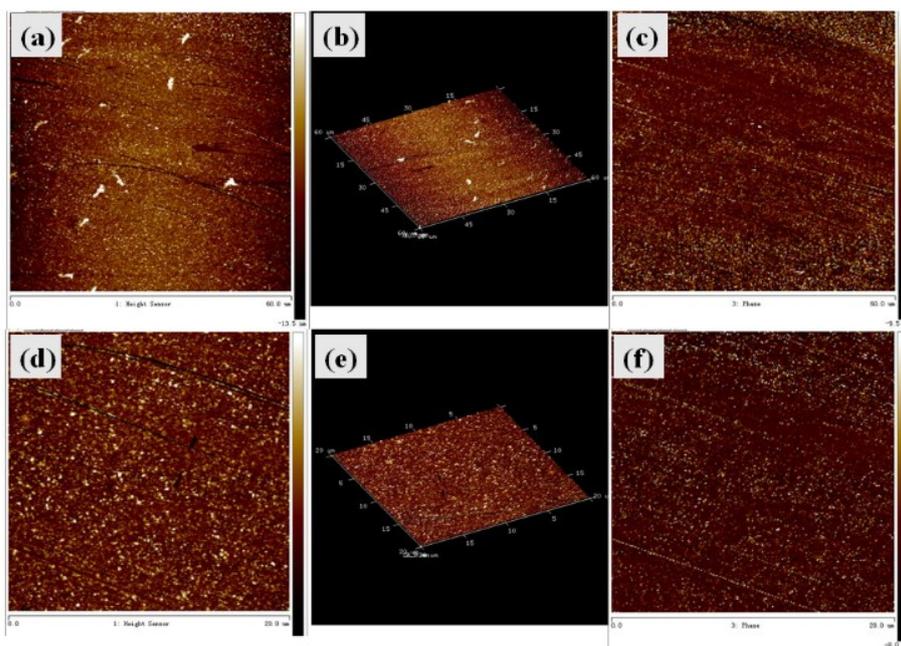
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## 1. Surface morphology of (PAH/CAT)<sub>5</sub>-PAH multilayer

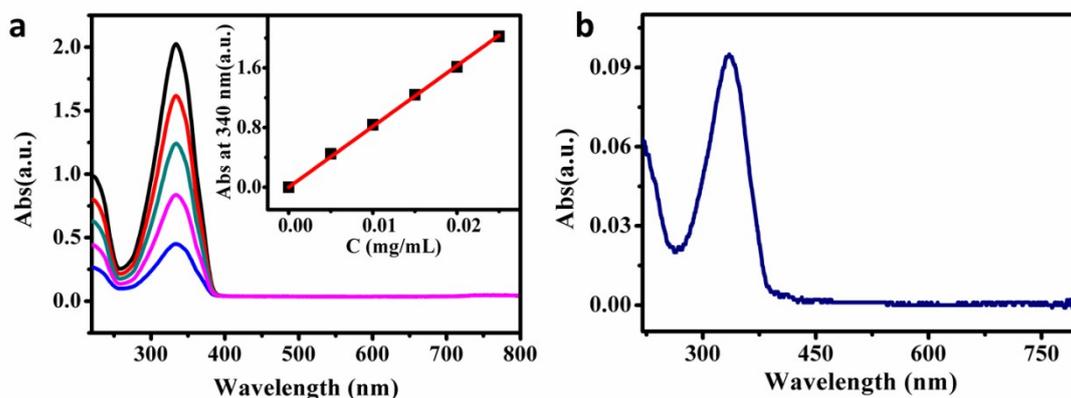
The surface morphology of (PAH/CAT)<sub>5</sub>-PAH multilayer was characterized through atomic force microscopy (AFM) images from a Dimension FastScan machine (Veeco, U. S. A.). The results were collected from at least three local spots on the substrate with the multilayer and displayed with typical images of 2D/3D height and phase morphology. From the 2D/3D height images with a large scanned size of both 20 x 20 μm<sup>2</sup> (**Figure S1a&b**) and 60 x 60 μm<sup>2</sup> (**Figure S1d&e**), we could observe that the substrates have been thoroughly covered by the multilayer as a continuous and compact film, which is further confirmed by the corresponding phase images (**Figure S1c&f**) with thoroughly similar components in the film.



**Figure S1.** AFM images of (a) 2D and (b) 3D height and (c) phase morphology with a scanned dimension of 60 x 60  $\mu\text{m}^2$ ; AFM images of (d) 2D and (e) 3D height and (f) phase morphology with a scanned dimension of 20 x 20  $\mu\text{m}^2$ .

## 2. Determination of DAS content in the PAH/CAT multilayer.

The saturated amount of DAS absorbed in the (PAH/CAT)<sub>5</sub>-PAH multilayered film on the quartz substrates was determined with a calibration curve. First of all we obtained a calibration curve (**Figure S2a**) with UV-visible spectra of DAS at different concentrations, which gives a molar extinction coefficient of  $4.39 \times 10^4 \text{ L} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ . Secondly we treated the quartz substrate bearing the (PAH/CAT)<sub>5</sub>-PAH multilayered film with NaOH (aq, pH=10) under ultrasonic condition to total remove the multilayer. The alkaline solution with disassembled polyelectrolytes and DAS was detected with UV-visible spectra (**Figure S2b**), and the concentration of this solution was determined through the above calibration curve. Finally the washed DAS amount was calculated to be  $0.21 \mu\text{g} \cdot \text{cm}^{-2} \cdot \text{bilayer}^{-1}$  when taking the quartz substrate size of  $3 \times 1.3 \times 0.1 \text{ cm}^3$ .

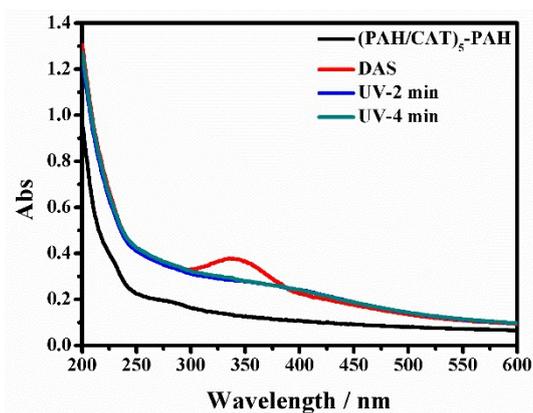


**Figure S2.** (a) The calibration curve with UV-visible spectra of DAS at different concentrations; (b) the UV-visible spectra of removed DAS in alkaline solution.

### 3. Calculation of the reaction kinetics of DAS.

The kinetics of the photo reaction of DAS was calculated through the UV-visible spectra in **Figure 1c**. The absorbance of the featured absorption of DAS at 340 nm was used for calculation by  $\ln[(A_0 - A_{min}) / (A_t - A_{min})]$ , where  $A_0$  is the absorbance before photo irradiation,  $A_{min}$  is the absorbance after complete photo-reaction of DAS and  $A_t$  is the absorbance at any reaction time,  $t$ . We plotted the data between  $\ln[(A_0 - A_{min}) / (A_t - A_{min})]$  and  $t$  in **Figure 1c**, which presented a fitted linear correlation and indicated the feature of the photo reaction to be first order. The slope of the correlation in the inset in **Figure 1c** determines the reaction rate constant as  $0.107 \text{ s}^{-1}$ .

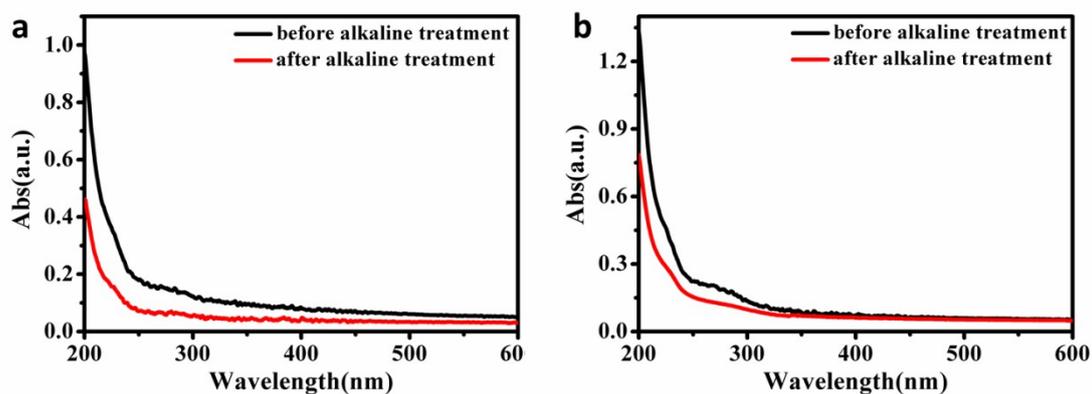
We irradiated the substrates with both sides in a sequential way and the UV-visible spectra in **Figure 1c** was obtained afterwards. We have measured the stepwise UV-visible spectra in this sequential irradiation to further clarify the kinetics. The results are summarized in **Figure S3**, which showed that the UV-visible spectra were similar after irradiation of single side (2 min) or both sides (2 min for the top side and another 2 min for the bottom side). This may be attributed to the high transparency of quartz substrate in the range of 300 nm to 350 nm, within which the featured absorption of DAS is located. The transmitted UV energy is sufficient to induced similar crosslinking of the back side film to that of the front side film. The purpose of such sequential irradiation of both sides is to make sure complete crosslinking.



**Figure S3.** UV-visible spectra of the as-prepared (PAH/CAT)<sub>5</sub>-PAH multilayer (black line) and after it is loaded with DAS for 30 min (red line), irradiated the top side of the substrate with UV for 2 min (blue line) and subsequently the bottom side for another 2 min (green line), which leads to totally 4-min exposure to UV light.

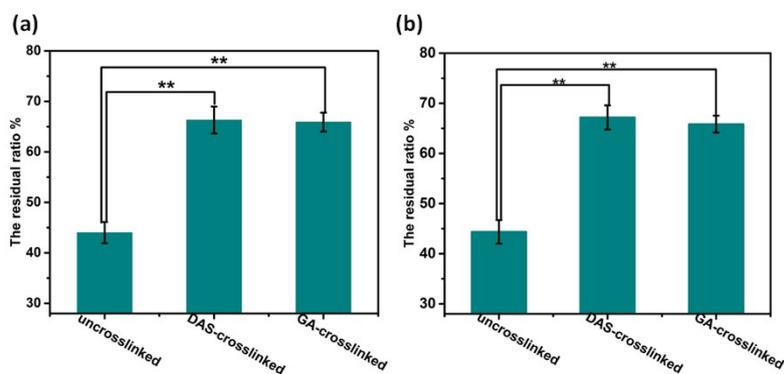
#### 4. Film stability of uncrosslinked and GA-crosslinked multilayers.

The GA-crosslinked multilayered film was prepared through immersing the substrate with (PAH/CAT)<sub>5</sub>-PAH multilayer film into a GA solution (0.5 wt% in 0.1 M PBS buffer with pH=7.4) for 5 min, followed by rinsing with copious deionized water to remove residual GA. To check the film stability of both uncrosslinked and GA-crosslinked multilayered films, we treated these two multilayers with a solution of NaOH (aq, 0.5 M, pH=13.7) for 50 min and checked the corresponding UV-visible spectra before and after alkaline immersion. For the uncrosslinked (PAH/CAT)<sub>5</sub>-PAH multilayered film (**Figure S4a**), the only  $44.5 \pm 2.3\%$  of the original multilayer left on the substrate by comparing the absorbance at 220 nm before and after alkaline treatment: the residual percentage =  $(Abs_{\text{after}} - Abs_{\text{before}}) / Abs_{\text{before}} * 100\%$ . For the GA-crosslinked (PAH/CAT)<sub>5</sub>-PAH multilayered film, **Figure S4b** shows a residual ratio of  $65.8 \pm 1.7\%$ , indicating the improved film stability toward harsh conditions after crosslinking.



**Figure S4.** UV-vis spectra of (a) the uncrosslinked and (b) the GA-crosslinked (PAH/CAT)<sub>5</sub>-PAH multilayered films.

Moreover, we have analyzed the significant level between the above three percentage values as summarized in **Figure S5**. The results showed that for both evaluations at 200 nm and at 220 nm, the uncrosslinked film displayed similar significant difference from the crosslinked ones, indicating the crosslinking has enhanced the film stability in a significant way.



**Figure S5.** The residual film ratio after alkaline treatment by calculating the absorbance at (a) 200 nm and (b) 220 nm. \*\* means high significance level.