Supporting Information

Double Trouble for Viruses: Hydrogel Nanocomposite Catches Influenza Virus While Shrinking and Changing Color

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Materials and Methods

All chemicals were purchased from Sigma (Germany) and used without further purification, unless otherwise noted. α-Amino-ω-azido PEG, PEG-MW. 3000 Dalton was purchased from RAPP Polymer (Germany). Dendritic polyglycerol cyclooctyne (dPG-C) with a number average molecular weight (Mn) of 10,000 Dalton and polyethylene glycol derivatives were synthesized as previously reported. Influenza virus A/X31, a recombinant human influenza strains and VSV viruses propagated in BHK-21 cells were used in this study.

Synthesis Experiments

*Thiolated-PEG-diazide (SH-PEG-DIA)*

The synthesis of SH-PEG-DIA was previously reported. Briefly, to get the thiolated-PEG-diazide, COOH-PEG-DIA (0.300 g, 0.047 mmol) was dissolved in dimethylformamide (minimal amount, 8 ml) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.014 g, 0.07 mmol) and N-hydroxysuccinimide (0.001 g, 0.0087 mmol) were added, followed by the addition of cysteamine (0.009 g, 0.12 mmol). The reaction mixture was degassed for 1 hour under argon atmosphere at stirred at room temperature overnight. The product was purified by dialysis in H$_2$O followed by the removal of the solvent by freeze-drying. The product was obtained and subsequently analyzed by $^1$H NMR, $^{13}$C NMR spectroscopy, and gel permeation chromatography (GPC).

$^1$H-NMR (700 MHz, δ (ppm), CDCl$_3$): 3.78-3.19 (m, PEG), 3.30-3.20 (m, 4H), 2.90-2.87 (t, 2H). $^{13}$C-NMR (700 MHz, δ (ppm), CDCl$_3$): 70.66 (PEG), 50.754 (PEG), 29.773 (PEG).

Elemental analysis: %C: 54.07 ± 0.04, %N: 0.64 ± 0.04, %H: 8.677 ± 0.001, %S: 1.36 ± 0.03.
Gold Nanoparticle Synthesis and Sialic Acid Conjugation

The synthesis of AuNP (20 nm) conjugated with sialic acid was performed as previously reported. Briefly, 10 ml of 1.25 mM sialic acid was prepared in distilled water and mixed with an aqueous solution of HAuCl$_4$ (0.005 M, 1 ml) was brought to reflux at 80 °C at constant stirring at 1200 rpm for 20 min. The color of the solution changed from clear/yellow to light red wine. The suspension was then allowed to cool to room temperature. After that, the suspension (1 ml) was centrifuged for 10 min at 13,000 rpm. The supernatant was removed and the sediment redispersed in distilled water (1 ml).

**Figure S1.** GPC graph of the obtained SH-PEG-DIA.
UV-Visible characterization of AuNP-SA

Figure S2. UV-vis spectra of AuNP-SA displaying a plasmon shift at $\lambda = 525$ nm.

Infrared (IR) spectrum of AuNP-SA

Figure S3. IR spectra of conjugated sialic acid AuNP.

A/X31 (H3N2) Virus Detection in Solution

Stock solutions of Influenza virus A/X31 (H3N2) ($1.2 \times 10^9$ pfu/ml, 0.31 mg/ml protein) was prepared in 10 mM PBS and diluted into the concentrations of $4 \times 10^8$, $2 \times 10^8$, $1 \times 10^8$, $5 \times 10^7$, $1 \times 10^7$, and 0 pfu/ml. The previous final solutions of AuNP-SA were added into the viruses
solutions at equal volume (100 µl each). After being incubated for 30 min at 37°C, the color is recorded by a digital camera and quantified by UV-Vis spectra. As a control for specificity, to the previous final solutions of SA-AuNP were added an unspecific protein solution (0.22 mg/ml proteins of VSV virus). The obtained solution did not undergo any color change. The color difference or indifference was quantified by UV-Vis measurements using a plate reader.

**Characterization of the SA-AuNP-X31 response time**

![Graph showing Optical density of the complex SA-AuNP-X31 formation overtime at 610 nm.](image)

**Figure S4.** Optical density of the complex SA-AuNP-X31 formation overtime at 610 nm.
Specificity in Solution

**Figure S5.** UV-vis spectrum of SA-AuNP solution with specific or unspecific viruses to sialic acid showing the absence of interaction of the SA-AuNP with VSV virus but only with A/X31 (H3N2) IAV. The control was plain SA-AuNP solution with PBS.

**Figure S6.** UV-vis spectrum of SA-AuNP solution with specific IAV A/X31 (H3N2) to sialic acid, with different concentrations of viruses. The control was plain SA-AuNP solution with PBS.
Preparation of Hydrogels for Virus Detection

Gold Nanoparticle Synthesis and Sialic Acid Conjugation for Hydrogels

The synthesis of AuNP (20 nm) conjugated with sialic acid was performed as previously reported with some modifications. Briefly, 10 ml of 1.25 mM sialic acid was prepared in distilled water and mixed with an aqueous solution of HAuCl₄ (0.005 M, 1 ml) was brought to reflux at 80 °C at constant stirring at 1200 rpm for 20 min. The color of the solution changed from clear/yellow to light red wine. The suspension was then allowed to cool to room temperature. After that, the suspension (1 ml) was centrifuged for 10 min at 13000 rpm. The supernatant was removed and the sediment redispersed in distilled water (200 µl) to obtain an intense red wine color.

Hydrogels and Bioimprinting Process

Prior to hydrogel formation, to the previous solution of Au-SA-virus conjugates (100 µl, 5 * 10⁷ pfu/ml) were added thiolated-PEG-diazide (10 mg) to get a concentration of 10 wt% PEG solution. The reaction mixture was left 30 min at rt to allow the thiol groups to bind to the AuNPs conjugates.

To this mixture of Au-SA-virus-PEG-diazide was added 125 wt% dPG-cyclooctyne and the bio-imprinted hydrogel was formed by mixing both components at a 2:10 ratio dPG:PEG. 10 µL of the mixture was added to small Eppendorf tubes to afford the final hydrogels with consistent shapes. The hydrogels were formed quickly after a few minutes. This group is called molecularly imprinted polymers “MIP.”

As a control group, hydrogels were formed the same way but without virus templates and were called non-imprinted polymers “NIP.”
Removal of Viruses

To mold the virus imprint into the hydrogel, the previously bound virus was removed by immersing the hydrogels in a big volume of stirring solution of 3.7 % hydrochloric acid (~ 100 ml) for two days. After reaching equilibrium, the hydrogels were washed in distilled water overnight. These samples were called “MDP.”.

**Figure S7.** Photographs of hydrogels with and without virus template. Pink are the hydrogels bio-imprinted during the virus template removal process. Purple are the hydrogels with virus template (before template removal). Hydrogels without template already expanded by ~ 33 % in size after a few hours in 3.7 % HCl compared to the hydrogels with template in distilled water.

**Figure S8.** Optical density of hydrogels bio-imprinted after virus template removal (“without template”) and hydrogel with virus template (“with template”) at 610 nm.
Rebinding of Virus

Rebinding of virus was performed by immersing the nanocomposite bio-imprinted hydrogels into 10 µl of 1.2 * 10^9 pfu/ml (0.31 mg/ml HA) overnight.

Figure S9. OD_{610} of hydrogels for different cycles of binding/removal of A/X31 (H3N2) virus in MDP and NIP for different hydrogels showing that imprinting was necessary to get the hydrogel response.

Specificity in Hydrogels

To assess the specificity, a nanocomposite bio-imprinted hydrogel was immersed into a solution containing unspecific virus (VSV virus, 0.22 mg/ml proteins).
Characterization of the SA-AuNP solution, non-imprinted and imprinted hydrogels

Figure S10. TEM images of SA-AuNP (d_{AuNP} ≈ 20 nm).
Figure S11. TEM images of hydrogels (a) with and (b) without imprinting showing various aggregation of AuNP around possibly viruses (a) and more dispersed particles in hydrogels without imprint (b).
SA-AuNP concentration

Gold structure was assumed to be face-centred cubic (fcc) and 100 % yield. The number of gold atoms in one gold nanoparticle is given by Equation (1)³:

\[
N = \frac{4\pi \rho r^3 N_A}{3 M}
\]

With \(M\), gold atom atomic weight, \(N_A\) Avogadro constant, \(\rho\), density of fcc gold, and \(r\), the radius of the particle.

The calculation gives \(\sim 250,000\) Au atoms in a 20 nm spherical gold nanoparticle. The initial concentration used to synthesize the SA-AuNP was 0.5 mM, the concentration of SA-AuNP is 2 nM and concentrated 10 X, thus a final concentration of SA-AuNP of 20 nM.

References