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COMMUNICATION

Visible light powered self-disinfecting coatings for influenza viruses†

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Influenza A viruses, the pathogens responsible for the recent swine flu outbreak and many historical pandemics, remain a threat to the public health. We report herein the fabrication of self-disinfecting surfaces from photoactive building nanocrystals, which can inactivate influenza viruses rapidly, spontaneously and continuously under visible light illumination.

Introduction

Influenza A viruses, the pathogens that are responsible for the recent H1N1 outbreak and many historical pandemics, remain a threat to the public health.[1–3] On average, the influenza virus caused three deadly pandemics per century since the seventeenth century.[1] Current measures against influenza mainly rely on antiviral drugs and vaccines.[2] Using antiviral drugs might help to reduce the symptoms and contain virus spreading, but their stockpiling is not economical due to unpredictable outbreak of pandemics. While vaccination is considered as the most effective measure to prevent virus infection, continuous evolution of influenza viruses makes the design of effective vaccines extremely difficult prior to an outbreak. Seeking effective solutions that can prevent rapid spreading of the virus is of particular importance.

Influenza viruses are generally spread through contact with contaminated surfaces and virus-containing aerosol.[3] The influenza viruses could survive a remarkably long time on contaminated surfaces, for example over 24 h on hands, stainless steel and plastics,[4] and spreading through aerosol was supported by other studies, particularly in the case of short distance infection and prolonged airborne period.[5–7] But no matter by which pathway the influenza virus will be transmitted, it is practically impossible to sterilize such public areas with large flowing population every minute using the traditional disinfection techniques. We recognize that visible-light-driving photocatalysts, such as chemically doped TiO_2, may be used for self-disinfecting coatings; however, their current use is still focused on anti-bacteria purpose.[8–17]

Herein, we report the fabrication of self-disinfecting surfaces on which influenza viruses and other hazardous pathogens can be, spontaneously and continuously, disinfected by visible light. This is achieved by forming semiconductor coatings that could absorb visible light and produce active oxidative species. As illustrated in Fig. 1, when fomites and virus containing aerosols contact the surface as schemed, as-produced reactive species could oxidize their amino acid residues on the envelope proteins of the virus, which inactive these proteins and disinfect the virus.

Such self-disinfecting surfaces are fabricated from nano-crystals (NCs) of $CuInZn_4S_6$ (CIZS) with band gaps within the visible light range. The NCs are synthesized from diethyldithiocarbamate precursors of zinc, copper and indium with a designed molar ratio of 4 : 1 : 1. The synthesis is achieved by the hot-injection method using oleic acid as the capping agent. It is also worth mentioning that the fabrication of such coatings is achieved by directly coating photoactive nanocrystals on the substrates followed by a sintering process. Such a simple wet chemical approach ensures effective fabrication of such a disinfecting surface at low cost.

Methods

Synthesis of $CuInZn_4S_6$ nanocrystals and self-disinfecting surfaces

In a typical synthesis,[18] 0.8 mmol of zinc diethyldithiocarbamate, 0.2 mmol of copper diethyldithiocarbamate, 0.2 mmol of indium diethyldithiocarbamate and 2 mL of oleic acid were mixed in 10 mL 1-octadecene in a 50 mL three neck flask under nitrogen flow and vigorous stirring. The flask was heated slowly to 180 °C and 1 mL of oleylamine was added after the solid was completely dissolved. After 2 min, the flask was cooled rapidly by adding ethanol until it reached room temperature. The product was washed by toluene and methanol twice and finally stored in 5 mL toluene. As-prepared CIZS nanoparticles were characterized by XRD (Fig. S1(a)†), which confirmed the zincblende structure, EDS for element ratio (data not shown) and absorbance spectrum (Fig. S1(b)†), which showed the intense absorbance in the visible-light range with a band gap estimated around 2.21 eV. A transmission electron microscopic (TEM) image (Fig. S1(c)†) of the NCs suggested a uniformed NC size centered at 20 nm, which is consistent with that estimated from the

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† Electronic supplementary information (ESI) available: XRD, UV-Vis absorbance, TEM, AFM of as-prepared nanocrystals and as-fabricated self-disinfecting surfaces, disinfection of influenza A virus by TiO_2 (P25) with UV irradiation as reference control, photoinactivation of influenza A virus envelope proteins and photoinactivation of trypsin. See DOI: 10.1039/c2nr30388d

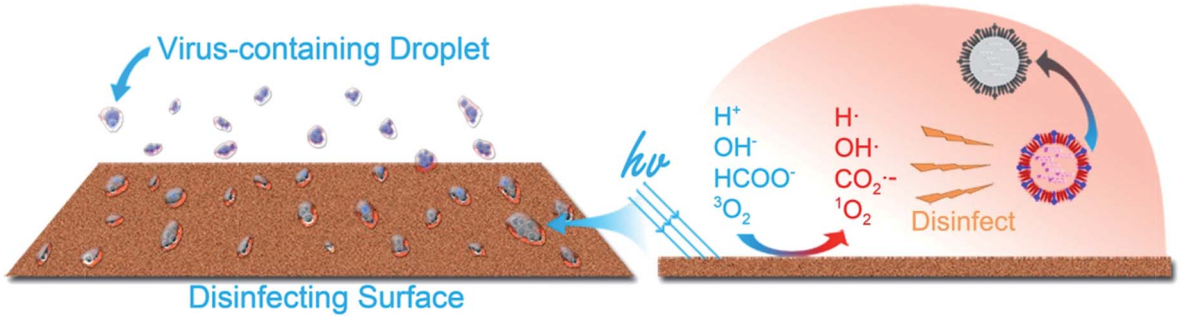

Fig. 1 Illustration of disinfecting virus on a self-disinfecting surface powered by visible light.

XRD pattern using the Debye–Scherrer equation. Moreover, Fig. S1(d)† showed a representative atomic force (AFM) image of a coating surface with smooth morphology.

A designated amount of CIZS toluene solution was drop-coated onto glass cover slips (Ted Pella, Inc.) with a surface concentration ~5 μg mm^{-2}. The coatings were dried under the room temperature without light illumination and followed by the surface ligands exchange with cysteamine. In a typical process, glass cover slips with dried $CuInZn_4S_6$ nanocrystal coating were placed into 24 well plates; then 500 μL cysteamine (5 mg mL^{-1}) water solution were added to each well. After 30 min, solution was sucked out and every sample was rinsed by 1 mL DI-water twice to remove the excess cysteamine. Finally samples were dried under the room temperature in a covered box to prevent the light. For ligands removed samples, as-coated cover slips were placed in a glass container and nitrogen carried water vapor was supplied. The samples were sintered at 400 °C for 4 h while heating rate was 5 °C min^{-1} and cooled slowly to room temperature.

TiO_2-coated surfaces were prepared for control at the same surface concentration using commercial TiO_2 (P25, Degussa) suspended in H_2O (10 mg mL^{-1}) as the coating solution.

Disinfection of influenza A virus

Disinfection effects were examined by immersing the coated slips with 300 μL solution of A/PR/8/34 H1N1 influenza A virus (PR8) on ice, the titer of which was 3.57 × 10^4 $TCID_{50}$ per mL (50% Tissue Culture Infectious Dose per mL) on MDCK (Madin Darby canine kidney) cell line as determined by the Reed–Muench method; meanwhile bare slips, TiO_2 coated slips and dark controls were set up in the same configuration. Visible light illumination was applied by a solar light simulator (Solar Cell & Module Test Equipment, PV Measurements, INC.) equipped with an ultraviolet (UV) filter (HOYA HMC 58 mm UV(0)). After 1 h illumination, virus activities were measured using the same TCID50 assay on the MDCK cell line. To simulate the virus spreading through aerosol, droplets of virus solution (15 μL) were deposited on the 5 μg mm^{-2} CIZS coated slips, which were then placed on ice. Virus activities were assayed after only 15 min using the same Reed–Muench method.

Disinfection of hepatitis C virus

CIZS-coated slides were immersed in 400 μL HCV solution with a titer of 4900 ffu per mL (foci forming unit per mL) under illumination for 1 h on ice. Then HCV virus was titrated by an immunofluorescence assay. Briefly, Huh-7.5.1 cells were seeded in a 96-well plate at the density of 3 × 10^3 cells per well. The HCV supernatant collected after the CIZS treatment was 10-fold serially diluted in complete Huh-7.5.1 cell growth medium and inoculated onto Huh-7.5.1 cells in triplicate for 72 h at 37 °C. Infected cells then were fixed with methanol and detected by immunohistochemical staining for the HCV protein NS5A. By counting the number of the NS5A antigen positive foci at the highest dilution, the virus titer was assessed and presented as ffu per mL.

Disinfection of bacteria

Briefly, 35 μL of *E. coli* with a titer of 2 × 10^5 cfu per mL (colony formation units per mL) was added onto each CIZS coated surface and illuminated for 1 h. Then *E. coli* was plated out on agar to measure the remaining titer after incubation for 18 h at 37 °C.

Results

Disinfection results of influenza A virus

The disinfecting abilities of these coatings were examined using the A/PR/8/34 H1N1 influenza A virus (PR8) as an example. Fig. 2(a) showed the activity of the virus after exposure to the CIZS coated surfaces and visible light illuminated for 0.5, 1, 1.5 and 2 h, exhibiting remarkable infectivity loss of 73%, 86%, and 92% and 94%, respectively. To confirm the disinfection effect, Fig. 2(b) compared the virus infectivity after exposure to glass, 5 μg mm^{-2} CIZS coated and TiO_2 (commercialized P25) at the same concentration, a widely used photo-oxidizing catalyst, coated surfaces for 1 h. With visible light illumination, the TiO_2-coated surface reduced the infectivity of the virus by 8%, which was significantly less effective than the CIZS-coated surface (83%), while the glass surface showed no effect. Without visible light illumination, no significant change in infectivity was observed. This result clearly demonstrates the effectiveness of using visible-light-adsorptive semiconductors as self-disinfecting surfaces. The significantly lower disinfecting capability observed for the TiO_2 surface was due to the higher band gap of TiO_2 (2.21 eV[18] for $CuInZn_4S_6$ and 3.1 eV for TiO_2) that excludes it from harvesting visible light.

To simulate actual circumstances where small volumes of virus-containing aerosols or liquid were deposited on a surface through coughing, sneezing or direct contact, droplets of PR8 virus solution (~15 μL) were applied on the CIZS surface and the results were summarized in Fig. 2(c). 74% of virus infectivity was neutralized shortly after only 15 min, suggesting a highly effective and rapid route to disinfect influenza virus using visible light. However, we were

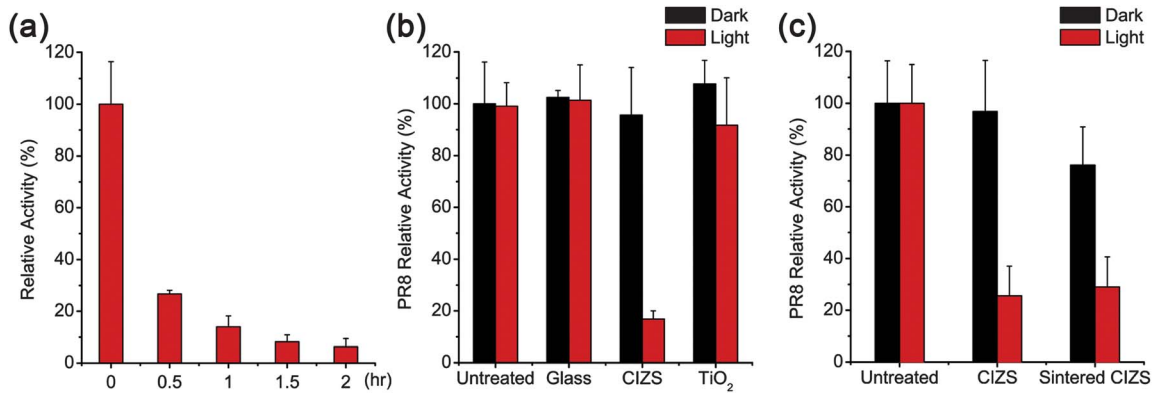

Fig. 2 Disinfection of influenza A virus. (a) Disinfection efficiency of CIZS-coated surfaces over time. (b) Disinfection efficiency of CIZS- and P25-coated surfaces with and without illumination for 1 h. (c) Disinfection efficiency of the CIZS-coated surfaces prepared with and without the sintering process.

aware that the un-sintered CIZS-coated surfaces might not be stable enough for practical uses. Mechanically stronger CIZS coatings were therefore prepared by sintering the nanocrystal coatings at 400 °C under nitrogen and water vapor; removal of the capping ligands from the nanocrystals and sintering process converted the nanocrystals into highly robust CIZS coatings. As expected, such robust coatings could disinfect 71% of the virus within an exposure time of 15 min. Considering abundant semiconductor materials that are photoactive within the visible light wavelength, this study opened up a new avenue to the design and fabrication of self-disinfecting coatings.

Disinfection results of hepatitis C virus and bacteria

The general applicability of such a disinfecting surface was further examined using hepatitis C virus (HCV). Like influenza virus, HCV is an enveloped virus but currently has no vaccines available. Each NC-coated surface was immersed in 400 μL HCV solution with a titer of 4900 ffu per mL (foci forming unit per mL) and illuminated for 1 hour. As shown in Fig. 3(a), HCV infectivity was reduced by 85%, while no significant changes were observed for without light exposure and bare glass treated group. Immunofluorescence analysis of HCV samples using the antibody against the viral core protein was carried out and the representative images are shown in Fig. 3(c–e). Compared to untreated HCV (Fig. 3(c)), the fluorescence-positive cells (infected by HCV) were greatly reduced (Fig. 3(d)) when HCV was treated with an as-fabricated self-disinfected surface for 1 h of illumination. Under dark conditions, surface treatment has no effect (Fig. 3(e)). As-fabricated CIZS surfaces also demonstrated their disinfecting effects on bacteria, another diverse microbe group that is highly related to human health. A common bacterium, *E. coli*, was used in this study. As shown in Fig. 3(b), only 21% of *E. coli* retained their activity while no significant change was observed in control groups.

Discussion

The disinfection mechanisms may involve inactivation of hemagglutinin (HA) and neuraminidase (NA), the two critical envelope proteins governing influenza virus infection. The HA receptor-binding domain mediates the entry of flu virus into the host cell, while NA effectively cleaves the glycosidic linkages of neuraminic acid and allows virions to be released from the host cells. HA is named by its ability to aggregate red blood cells and this hemagglutination ability is also used to quantify influenza viruses. To examine the disinfecting effects of CIZS, quantification of PR8 viruses was carried out by a hemagglutination assay (details described in the ESI†) and HA units (HAU) were determined.

As shown in Fig. 4(a), HAU of PR8 dropped by 50% after 2 h exposure to visible light on the CIZS-coated surface compared to the same treatment under darkness, while no difference was observed between light and dark conditions on native or bare glass treated PR8. The result indicated that the hemagglutination ability of PR8 was impaired by the CIZS coated surfaces, most likely due to adverse effects on HA. We also examined whether NA is affected by the CIZS surface. The enzymatic activity of NA was measured using 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid sodium salt hydrate as the substrate, which upon cleavage by NA would generate products emitting fluorescence at 450 nm when excited at 365 nm. After exposure to the visible light on different surfaces for 1 h, NA activity was measured and the spectra were plotted in Fig. 4(b). (For experiment details please refer to the ESI†.) The result showed that CIZS treated NA lost 90% activity, compared to native NA and bare glass treated NA with illumination and CIZS treated under darkness.

We believe that the inactivation of HA and NA is attributed to oxidative species (*e.g.*, singlet oxygen species and reactive free radicals) produced upon illuminating the CIZS, such as $^1O_2$, H˙, $O_2^{\cdot-}$, OH˙, and $CO_2^{\cdot-}$.[19–21] These species were known to be capable of reacting with amino acid residues, such as His, Trp, Tyr, Met, Cys and Arg, and altering protein properties.[22–25] Furthermore, the oxidation can also cleave a polypeptide chain and induces formation of crosslinked protein aggregates.[26,27] Specific to H1N1 influenza virus, the H1 binding site contains Asp, His, Leu, Trp, Tyr, Thr, Gly and Gln, while the N1 active site contains Glu, Asp, Val, Arg, Gln, and Tyr.[28,29] Such residues can react with the oxidative species, leading to the disinfection effect observed. Moreover, it is also possible that these reactive species penetrate viral particles and cause damages to genetic materials, resulting in the loss of infectivity.[30,31]

For the other instance, hepatitis C virus, which shares a similar virion organization with influenza virus, has an envelope decorated with viral glycoproteins, E1 and E2, which are important for viral infectivity. Thus, it was thought that HCV might be neutralized by a CIZS-coated surface through singlet oxygen species and free

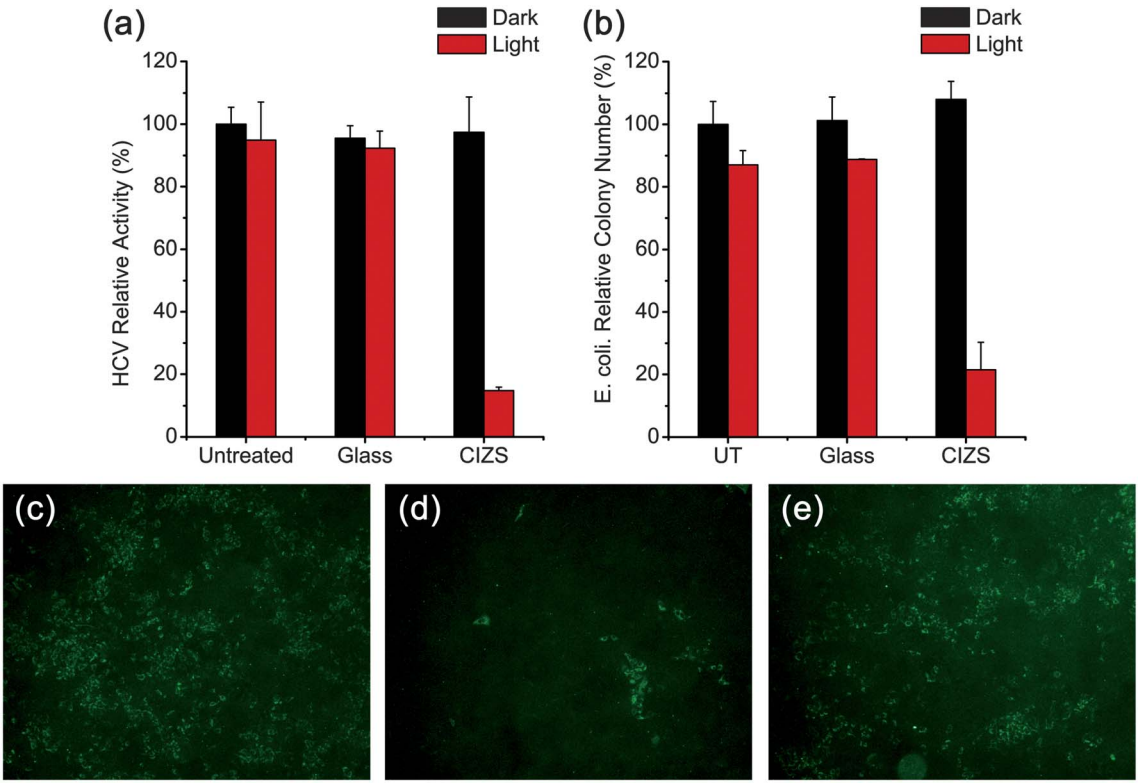

Fig. 3 Disinfection of hepatitis C virus and bacteria. (a) Disinfection of HCV and (b) *E. coli* by a CIZS-coated surface after 1 h illumination and fluorescence images of the HCV-infected cells on the CIZS-coated surface: (c) control, (d) with and (e) without the illumination.

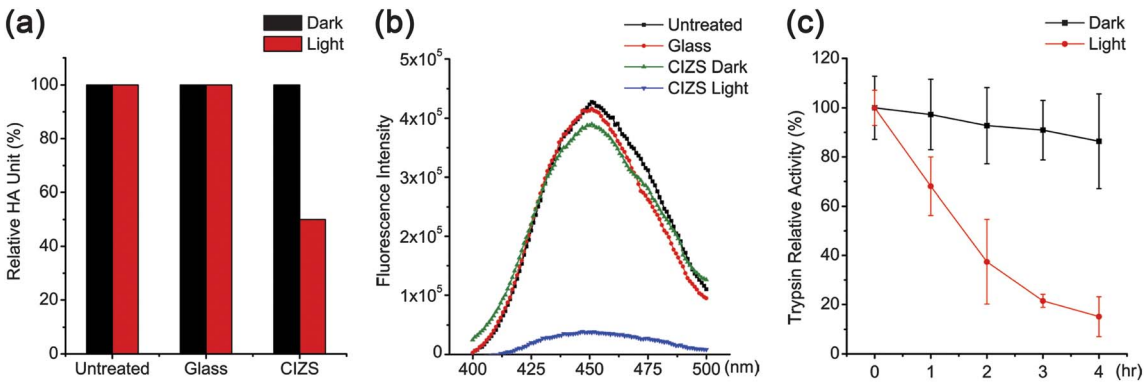

Fig. 4 Inactivation of proteins: hemagglutinin (a) and neuraminidase (b) on a CIZS-coated surface and a bare glass surface with and without illumination for 2 h and trypsin (c) by CIZS nanocrystals with and without illumination.

radicals reacting with residues of E1 and E2, such as His, Trp, Tyr, Met, Cys, Arg, Lys, Gln, Leu, Val, *etc.*[32]

Based on the proposed mechanism, such an inactivation process should be applicable to any protein. We then tested the hypothesis on trypsin. Indeed, trypsin's activity dropped significantly with visible light exposure to 68%, 37%, 21% and 15% after 1, 2, 3 and 4 h treatment, respectively (Fig. 4(c)). For experiment details please refer to the ESI†. The active site of trypsin contains Ser, Asp and His,[33] among which His could be reacted with singlet oxygen species thus to inactivate trypsin. This result supports our hypothesis that the protein inactivation *via* photo-oxidation is non-selective and can be generally applied to proteins in addition to HA, NA and trypsin. These results suggest an essential conclusion: it is possible to construct general self-disinfecting surfaces powered by visible light for virus and pathogens from photoactive coatings.

Conclusions

Overall, we have demonstrated the fabrication of highly effective self-disinfecting surfaces from preformed NCs. Such surfaces show promising ability in non-discriminative disinfection of influenza A virus, HCV and *E. coli*. Several important features, including the use of visible but not hazardous UV light, non-selective inactivation of proteins, and capability of continuous disinfection, render such surfaces a practical and effective solution to reduce potential harmful infections of pathogens in public areas.

Acknowledgements

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