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ARTICLE

Two-dimensional photonic crystal hydrogel biosensor for colorimetric detection of penicillin G and penicillinase inhibitor

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A simple penicillinase functionalized two-dimensional photonic crystal hydrogel (2DPPCH) biosensor was developed for colorimetric detection of penicillin G and penicillinase inhibitor. The penicillinase can specifically recognize penicillin G and catalyze it to produce penicilloic acid, which decreases the pH of the hydrogel microenvironment and shrinks the pH-sensitive hydrogel. The particle spacing decrease of 2D photonic crystal array induced by the hydrogel shrinkage further causes a blue-shift in diffraction wavelength. While the hydrolysis reaction is repressed upon treatment with clavulanate potassium (a kind of penicillinase inhibitor), no significant change in diffraction wavelength is found. The detection of targets can be achieved by measuring the Debye diffraction ring diameter or observing the structural color change in the visible region. The lowest detectable concentrations for penicillin G and clavulanate potassium are 1 μM and 0.1 μM, respectively. Moreover, the 2DPPCH is proved to be of high selectivity and excellent regeneration property, and shows satisfactory performance for penicillin G analysis in real water samples.

Introduction

Beta(β)-lactam antibiotics have a pivotal role in the global pharmaceutical industry owing to their huge production and wide application.[1,2] Penicillin is a kind of β-lactam antibiotic that can effectively prevent and treat infectious diseases of humans and animals.[3,4] For this reason, it is often illegally added into the feed to promote animal growth.[5] However, excessive penicillin in animals will cause the accumulation of antibiotic residues in animal products [6] and can be discharged into the environment with feces and urine.[7] In addition, the pharmaceutical industry[8] and extensive clinical usage[9] are also two major sources of penicillin residues. These residues may lead to the development of antibiotic-resistant bacteria[10] and allergic reactions,[11] posing a substantial threat to public health.

To circumvent these potential threats, some electrochemical[12-14] and optical (such as fluorescence,[2,15,16] SERS,[17] Rifs[18]) sensors have been developed to detect penicillin residues during recent decades. These analytical methods generally use enzymes, labelled antibodies, aptamers or imprinted nanoparticles as bioreceptors to generate various quantifiable photoelectric signals. Although excellent results have been achieved, many of them often need sophisticated apparatus and professional personnel at appointed laboratories, and on-site monitoring of penicillin residues remain a challenge. Hence, it is necessary to develop a simple, economical and visually detectable penicillin G sensors.

Recently, photonic crystal hydrogel (PCH) sensors have attracted enormous attention owing to their high sensitivity and visual detection. Photonic crystal (PC) is a periodic array with unique structural color that consists of two or more different dielectric constants materials.[19] Asher and co-workers reported a photonic crystal hydrogel (PCH) sensor for the first time by combing the PC with responsive hydrogel.[20] Via the target triggered shrinkage or swelling of hydrogel, the PC shows particular optical signal changes that is visible to the naked eye. To date, numerous sensors based on PCH have been developed. Among them, three-dimensional photonic crystal hydrogel (3DPCH) sensors are extensively applied to the determination of biomolecule,[21-25] metal ion,[26] pressure,[27,28] pH,[29] and temperature.[30] However, the inherent shortcomings of the 3DPC template such as long preparation time and fragility[31] still limit their wide application. Afterward, Asher's group[32] developed a method for rapidly preparing two-dimensional photonic crystal (2DPC) at air/water interface, which could self-assemble into ordered 2DPC array (> 200 cm^2) within two minutes. Furthermore, the 2DPC not only possesses structural color, but also can produce a bright Debye diffraction ring under monochromatic light irradiation. By measuring the diameter of the Debye diffraction ring, the 2D array particle spacing change can be calculated and used for a quantitative signal. The entire detection process of 2DPC only needs a laser pointer and a ruler, which simplifies the detection process and avoids the use of complex equipment. Some excellent works have been reported based on this principle.[33-38]

In this study, we attempt to report a penicillinase functionalized 2D photonic crystal hydrogel (2DPPCH) film for colorimetric detection of penicillin G and penicillinase inhibitor. As seen in Fig. 1, the penicillinase is immobilized on the carboxyl-rich 2DPCH to prepare a 2DPPCH biosensing film. In the presence of penicillin G, penicilloic acid is produced following the hydrolysis reaction which results in a decrease of pH. This decreases the electrostatic repulsion

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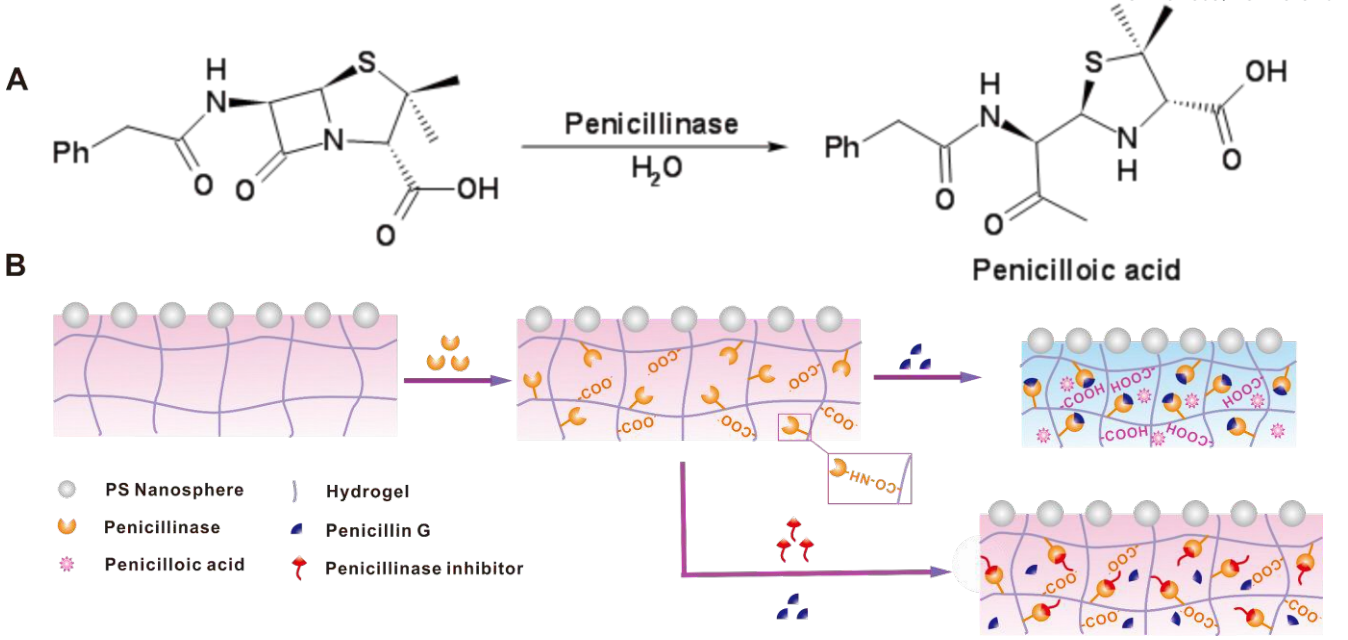

Fig. 1 (A) The hydrolysis reaction between penicillinase and penicillin G. (B) Schematic illustration of the 2DPPCH biosensor for the detection of penicillin G and penicillinase inhibitor.

between carboxylates and further reduces the osmotic pressure of the 2DPPCH film. According to Donnan equilibrium, the reduction of osmotic pressure will trigger the shrinkage of the 2DPPCH film and the narrowing of the particle spacing, leading to a blue-shift in diffraction wavelength. While clavulanate potassium is added, the hydrolysis reaction would be repressed, and thus little changes of particle spacing and diffraction wavelength can be found. Furthermore, Since the diffraction wavelength of 2DPPCH is located in the visible region during the detection process, the brilliant structural color change can be observed by naked eye. Compared with other traditional methods, the 2DPPCH biosensor is more portable and intuitive, affording a useful platform for on-site penicillin G contamination monitoring and penicillinase inhibitors screening.

Experimental Section

Materials and reagents

Acrylamide (AAm), *N*, *N*′-methylenebisacrylamide (BisAA), acrylic acid (AAc), *N*-hydroxysuccinimide (NHS), 2-(*N*-morpholinyl)ethanesulfonic acid (MES), 1-(4-(2-hydroxyethoxy)-phenyl)-2-methyl-1-propanone (Irgacure2959), *N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), penicillinase and potassium clavulanate were purchased from Sigma-Aldrich (Missouri, USA). Penicillin G sodium, clindamycin hydrochloride, lincomycin hydrochloride, chloramphenicol and fluconazole were purchased from Aladdin (Shanghai, China). All other reagents were analytical grade. Common glass slides were washed with piranha solution (H_2SO_4/H_2O_2 = 7:3 v/v).[39] High temperature sterilized ultrapure water was used for the preparation of all solutions.

Preparation of the 2DPC array

First, monodisperse polystyrene (PS) nanoparticles were prepared by soap-free emulsion polymerization,[40] purified by repeated centrifugation and dispersion. Then, the PS dispersion solution (25 wt%) was mixed with n-propanol at a volume ratio of 2:1, and slowly injected into the water surface at a constant speed by tip-directing technique.[32] Since the surface tension of n-propanol is much smaller than water, the PS microspheres can spread rapidly and spontaneously self-assemble into 2DPC arrays as the n-propanol evaporated. Finally, the 2DPC array was transferred onto a clean glass slide from the surface and dried in air (Fig. S1, Supporting Information).

Preparation of the responsive 2DPPCH film

Typically, the pregel solution was consisted of 10 wt% AAm, 0.5 wt% BisAA, 1 % (v/v) AAc and 1%(v/v) Irgacure 2959. After mixing thoroughly, the solution was injected into a mold which was constructed by placing a 125 μm thick parafilm spacer between a clean glass slide and a glass slide with 2DPC array. The pregel solution was copolymerized under 365 nm UV light for 15 min to form a two-dimensional photonic crystal hydrogel (2DPCH) film. The 2DPCH film was washed out from the mold and rinsed several times with ultrapure water. Then, the 2DPCH film was activated with 0.1 MEDC and 0.1 M NHS for 30 min. Lastly, the activated film was soaked into the penicillinase solution and reacted overnight at 4 ℃ to

obtain a two-dimensional penicillinase functionalized photonic crystal hydrogel (2DPPCH) film.

Instruments and Measurements

The particle size distribution and diameter of PS nanoparticles were characterized by a nanoparticle size instrument (NS-90, OMEC). The polymerized hydrogel was prepared under a 365 nm UV lamp (03-II, SCIENTZ). A bright Debye diffraction ring was obtained by a 405 nm Laser Pointer and its diameter was measured with a common ruler. The digital camera (D7100, Nikon) was used to record all the optical images. The micromorphology of the 2DPC array and the 2DPCH film was performed on a field emission scanning electron microscope (FESEM, Carl-zeiss Sigma HD).

Detection of penicillin G and clavulanate potassium

For penicillin G detection, the 2DPPCH film was first equilibrated with 50 mM NaCl solution such that it became a non-close-packed array. Next, the uniform size 2DPPCH films were separately immersed in penicillin G solutions at concentrations between 0 to 10 mM for 30 min. For potassium clavulanate detection, the equilibrated 2DPPCH films were respectively treated with various concentrations of potassium clavulanate solutions for 30 minutes at room temperature. Subsequently, 6 mM penicillin G solution was added and incubated for 30 minutes. In the whole experiments, the Debye diffraction ring diameter of each sample was recorded by a home-made Debye diffraction measurement device.

Results and discussions

Fabrication and characterization of 2DPPCH film

The fabrication of the 2DPPCH film was started with the fixation of 2DPC array to an acrylic acid-acrylamide copolymer to obtain a 2DPCH film. Then the carboxyl-rich 2DPCH film was activated with EDC/NHS and finally the amino-containing penicillinase was attached to the film by forming amide covalent bonds. Among these processes, the key step is to prepare a 2DPCH film with low-defect and high-quality, which is a prerequisite for observing a clear Debye diffraction ring and accuracy detection. Thus, the quality of the synthesized PS nanoparticles was first characterized by dynamic light scattering. The results show that PS nanoparticles have a narrow particle size distribution (Polydispersion Index (PdI) = 0.012) with an average diameter value of 602.4 nm (Fig. S2, Supporting Information). Scanning electron microscopy (SEM) was further used to investigate the microstructures of 2DPC and 2DPCH. As exhibited in Fig. 2A and 2B, the 2DPC template not only has a periodic order array but also possesses a uniform monolayer structure. Additionally, Fig. 2C and 2D reveal that the 2DPC array is successfully embedded onto the hydrogel matrix and maintains perfect structural characteristics. The SEM images verify the well quality of the 2DPCH film, and hence ensure the reliability of measurement results in whole experiments.

Diffraction of the 2DPPCH

2DPCH has many interesting optical characteristics due to the spatially arranged periodic structure. In particular, it has a very strong

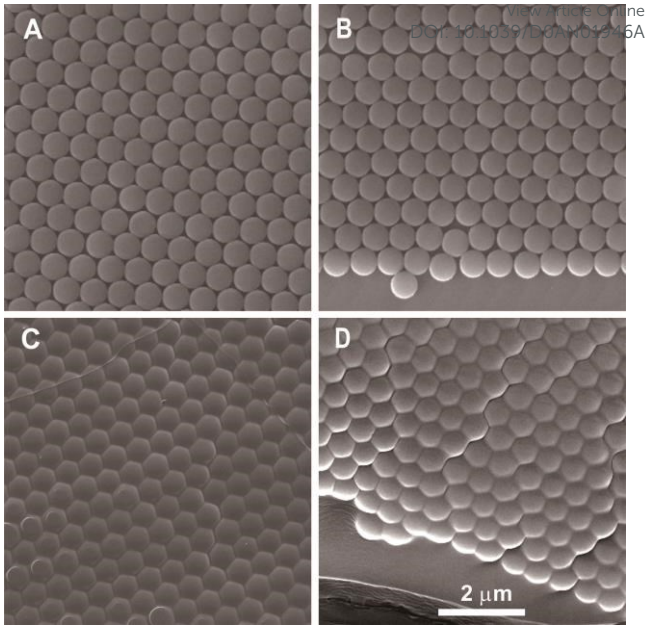

Fig. 2 SEM images of (A) the surface of the 2DPC array. (B) the edge of the 2DPC array. (C) the surface of the 2DPCH film. (D) the edge of the 2DPCH film.

forward diffraction phenomenon for incident light,[41] so a bright Debye diffraction ring can be obtained when utilizing monochromatic light to vertically illuminate the 2DPCH. The particle spacing of 2DPCH can be regulated by the hydrogel volumetric change in response to different concentration analytes, and calculated by measuring the Debye ring diameter.[42] Here, a home-made Debye diffraction measurement device shown in Fig. 3 was applied to measure the particle spacing changes induced by enzymatic reactions. The Debye diffraction follows the Bragg diffraction

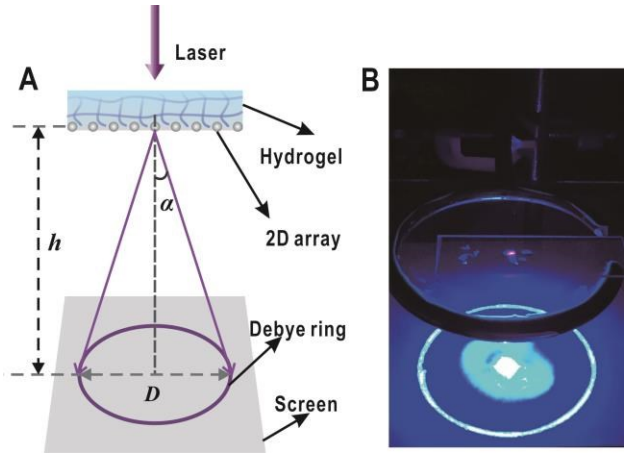

Fig. 3 (A) The formation of the 2DPC Debye diffraction ring. (B) Image of the home-made Debye diffraction ring measurement device.

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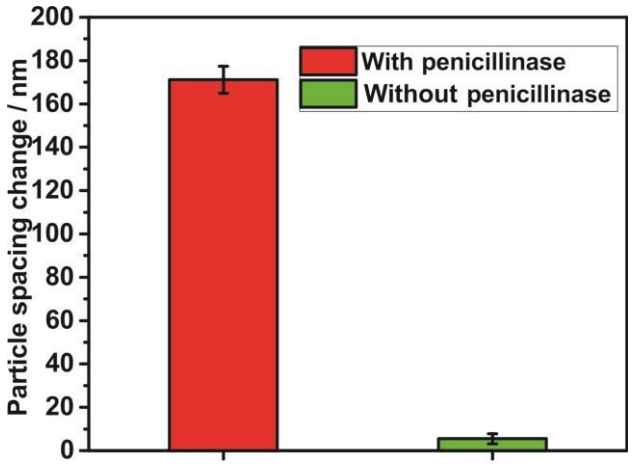

Fig. 4 Particle spacing changes of the PCH films that functionalized with or without penicillinase upon adding 6 mM penicillin G. The error bars indicate the standard deviation of three assays.

equation $\sin\alpha = 2\lambda_{laser}/\sqrt{3}d$, where λ_{laser} (405 nm) is the monochromatic light diffraction wavelength, α is Debye diffraction angle that can be calculated by the formula $\alpha = \tan^{-1} D/2h$, in which D is the diameter of Debye diffraction ring, h (7 cm) is the vertical distance between the plane of 2DPCH film and the Debye diffraction ring. Eventually, the particle spacing d can be measured by the following simplified equation.

$$d = \frac{4\lambda_{laser}\sqrt{(D/2)^2 + h^2}}{\sqrt{3}D}$$

Feasibility of 2DPPCH film

To verify the feasibility of the 2DPPCH biosensor, the activated 2DPCH and 2DPPCH films were soaked into 6 mM penicillin G solution, respectively. As seen in Fig. 4, the particle spacing change of 2DPPCH is 171.1 ± 6.2 nm, but the value of 2DPCH without penicillinase is 5.5 ± 2.3 nm. The remarkable difference demonstrates that the 2DPPCH can be used as a novel sensing platform for the detection of penicillin G.

Effect of penicillinase concentration on 2DPPCH response

The amount of penicillinase immobilized on the 2DPPCH directly affects its catalytic hydrolysis efficiency to penicillin G. In order to obtain the optimal assay performance, the activated 2DPCH films functionalized with different concentrations of penicillinase were incubated with 6 mM penicillin G solutions, respectively. The results show that the change of particle spacing increases gradually with the growing penicillinase concentration from 0 to 800 U mL^{-1}, and the changes are ignorable with higher concentration (Fig.5A). Therefore, penicillinase with a concentration of 800 U mL^{-1} was used to prepare a sensitive 2DPPCH biosensing film.

Optimization of ionic strength and hydrolysis time

Considering that the volumetric state of the charged hydrogel can be affected by the ionic strength in the aqueous solution,[43] the ionic strength dependence of particle spacing was studied upon exposure of 2DPPCH films to varying salt concentrations. It can be found that the particle spacing decreases obviously as the NaCl concentration increases from 0 to 50 mM, and a slight decrease is observed when the concentration is higher than 50 mM (Fig. 5B). This phenomenon may be due to the interaction between Na^+ cations and the carboxylate ions that causes a lowered repulsive force among carboxylates, leading to the decrease in osmotic pressure and the volume of the hydrogel. To eliminate the effect of ionic strength, 50 mM NaCl was added to all subsequent test solutions.

Besides, the influence of hydrolysis time on 2DPPCH response was investigated. The particle spacing change increases from 66.1 ± 4.2 nm to 172.5 ± 2.3 nm as the reaction time increases from 3 min to 30 min, and reaches a plateau after 30 min (Fig. 5C). In this experiment, an incubation time of 30 minutes was chosen for obtaining a quick and accurate result.

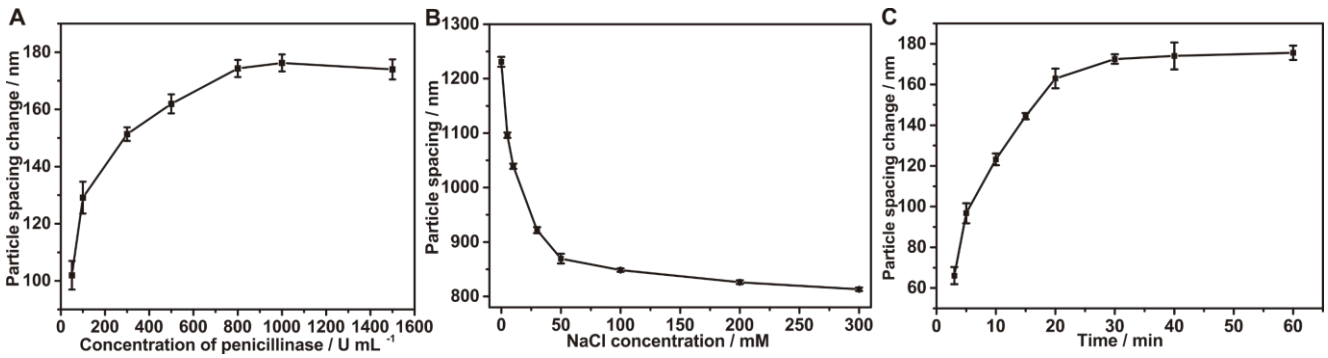

Fig. 5 (A) The particle spacing change of 2DPPCH upon treatment with different penicillinase concentration in the presence of 6 mM penicillin G. (B) The influence of different ionic strengths on the particle spacing of 2DPPCH. (C) The effect of hydrolysis time on the particle spacing change of 2DPPCH in the existence of 6 mM penicillin G. All solutions contain 50 mM NaCl. The error bars indicate the standard deviation of three assays.

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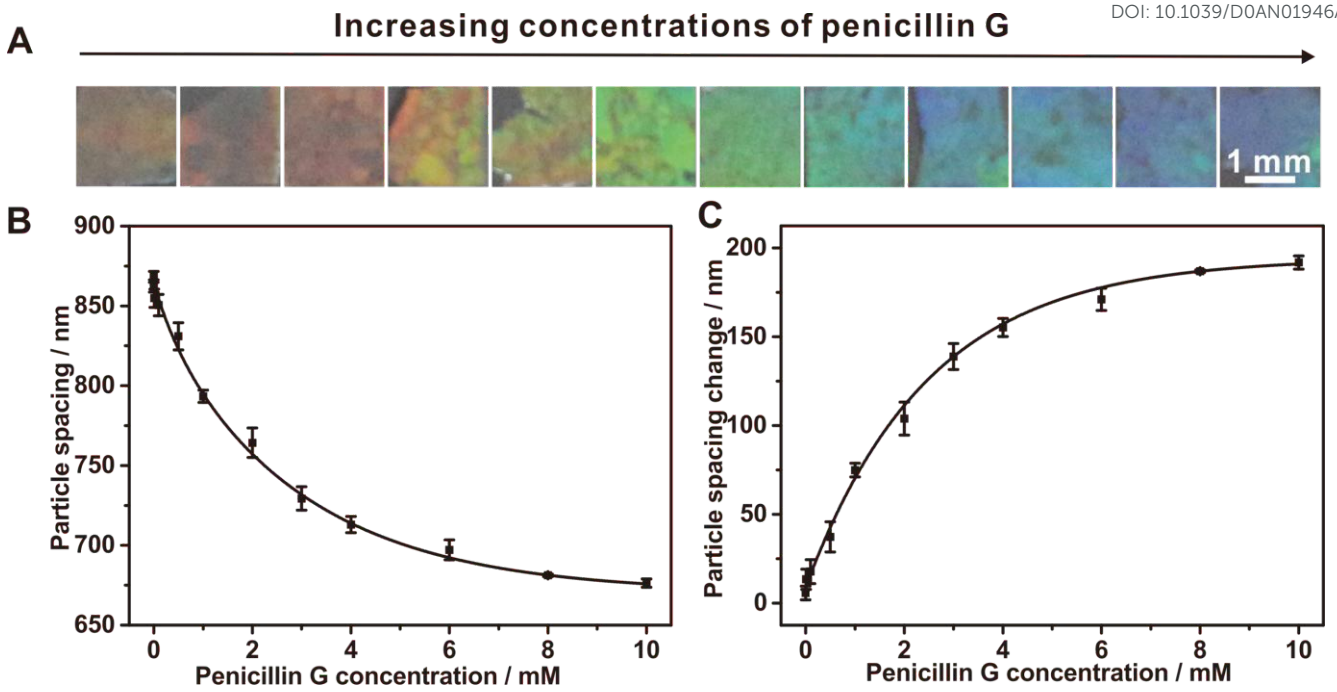

Fig. 6 (A) Optical images of the 2DPPCH films at different concentrations of penicillin G (The concentration of penicillin G from left to right is 0 μM, 1 μM, 10 μM, 100 μM, 500 μM, 1 mM, 2 mM, 3 mM, 4 mM, 6 mM, 8 mM, 10 mM, respectively). (B) The particle spacing versus different penicillin G concentrations. (C) The relationship between the particle spacing change and the concentration of penicillin G. All of the above reaction solutions contain 50 mM NaCl. The error bars indicate the standard deviation of three assays.

Detection capability of 2DPPCH film

Under the optimized conditions, the ability of the 2DPPCH biosensor for quantitative detection of penicillin G was assessed. Fig. 6A and 6B show that the particle spacing is 868.2 nm and the structural color of the 2DPPCH film is red in the absence of penicillin G. After adding penicillin G, the particle spacing decreases gradually with an incremental penicillin G concentration, which causes a shift of structural color to blue. In addition, it is observed that the particle spacing change increases clearly along with the penicillin G concentration increasing from 1 μM to 6 mM, and tends to a balance at concentrations higher than 6 mM (Fig. 6C). In contrast, the minimum detectable concentration of the 2DPPCH biosensor (1 μM) for penicillin G is comparable to the previously reported sensors listed in Table S1 (Supporting Information). Moreover, the visual analysis of penicillin G can be realized by observing the structural color change of the 2DPPCH and without any additional signal reagent or sophisticated instruments, a laser pointer can complete the entire detection, which makes the biosensor more intuitive and convenient.

Clavulanate potassium, a representative penicillinase inhibitor, was applied to explore the analysis capability of the 2DPPCH for penicillinase inhibitor by disturbing the hydrolysis reaction. The particle spacing changes of the 2DPCCH pretreated with various clavulanate potassium concentrations were obtained after reacted with 6 mM penicillin G. As illustrated in Fig. 7A, the particle spacing change decreases following the growing concentrations of clavulanate potassium and the minimum detectable concentration for clavulanate potassium reaches 0.1 μM. All of the above results suggest that the 2DPPCH biosensor can be established to screen penicillinase inhibitors.

Selectivity and regeneration property of 2DPPCH film

The selectivity is a vital standard in judging the performance of the biosensor. In this study, the selectivity of the 2DPPCH film was investigated by measuring the responses of 2DPPCH to four other common non-β-lactam antibiotics, including clindamycin hydrochloride, lincomycin hydrochloride, chloramphenicol, and fluconazole. As presented in Fig.7B, among these antibiotics, the 2DPPCH biosensing film shows the maximum response signal to clindamycin hydrochloride, but only a 9.4 ± 1.6 nm change in particle spacing is observed. However, a 171.1 ± 6.2 nm particle spacing change is found upon treatment with penicillin G. The high selectivity of the 2DPPCH film might be attributed to the high specificity of penicillinase for catalyzing the penicillin G hydrolysis.

The regeneration property of the 2DPPCH was also examined. As shown in Fig. 7C, after five cycles of penicillin G treatments followed by simple washing with 50 mM NaCl solution, no significant difference is found in response to penicillin G. The satisfactory regeneration property of the 2DPPCH may benefit from the good enzyme activity and the stability of hydrogel network.

Penicillin G analysis in real samples

To examine the capability of the developed 2DPPCH biosensor for penicillin G analysis in complex water samples, three simulated

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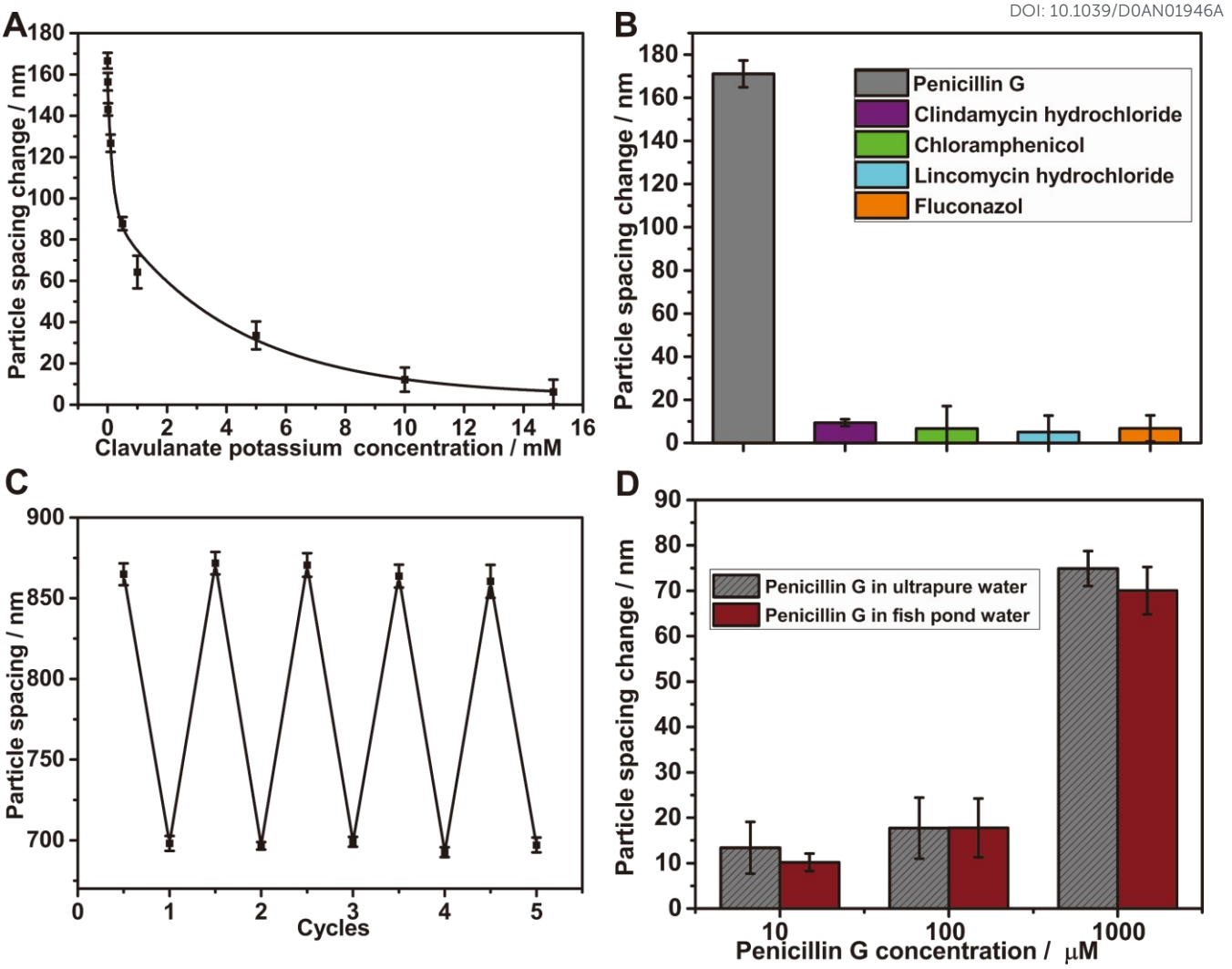

Fig.7 (A) The particle spacing change of 2DPPCH films at different concentrations of clavulanate potassium in the presence of 6 mM penicillin. (B) Selectivity property of the 2DPPCH biosensor for penicillin G detection, all antibiotics are at a concentration of 5 mM. (C) Regeneration property of the 2DPPCH film for detecting 6 mM penicillin G. (D) The capability of the 2DPPCH film for the detection of penicillin G in real water samples. All of the above solutions contain 50 mM NaCl. The error bars indicate the standard deviation of three assays.

real samples with different concentrations were prepared by adding a standard penicillin G solution of known concentration to the fish pond water. The detection ability of this biosensor in simulated real samples is almost consistent with that in ultrapure water (Fig. 7D). The result indicates that the 2DPPCH biosensor has great potential for the on-site monitoring of penicillin residues.

Conclusions

In summary, we have constructed a label-free 2DPPCH biosensor for penicillin G and penicillinase inhibitor detection based on the enzymatic hydrolysis reaction between penicillin G and penicillinase. The analysis of targets can be accomplished via measuring the Debye diffraction ring diameter or observing the structural color change by naked eye, which greatly simplifies the analytical process and avoids sophisticated instruments. Furthermore, this assay demonstrates very high specificity and excellent regeneration property, and shows good performance for penicillin G detection in real samples. Therefore, the developed approach may provide a simple, visual and robust platform for the monitoring of penicillin and screening of penicillinase inhibitors.

Conflicts of interest

There are no conflicts of interest to declare.

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References

1 G. Ozcengiz and A. L. Demain, *Biotechnol. Adv.*, 2013, **31**, 287-311.
2 E. Song, M. Yu, Y. Wang, W. Hu, D. Cheng, M. T. Swihart and Y. Song, *Biosens. Bioelectron*., 2015, **72**, 320-325.
3 T. Netzker, M. Flak, M. K. Krespach, M. C. Stroe, J. Weber, V. Schroeckh and A. A. Brakhage, *Curr. Opin. Microbiol.*, 2018, **45**, 117-123.
4 L. Zhang, Y. Jin, H. Mao, L. Zheng, J. Zhao, Y. Peng, S. Du and Z. Zhang, *Biosens. Bioelectron*., 2014, **58**, 165-171.
5 V. N. Binh, N. Dang, N. T. K. Anh, L. X. Ky and P. K. Thai, *Chemosphere*, 2018, **197**, 438-450.
6 L. M. Durso and K. L. Cook, *Curr. Opin. Microbiol.*, 2014, **19**, 37-44.
7 A. S. Oberoi, Y. Jia, H. Zhang, S. K. Khanal and H. Lu, *Environ. Sci. Technol*., 2019, **53**, 7234-7264.
8 M. Milaković, G. Vestergaard, J. J. González-Plaza, I. Petrić, J. Kosić-Vukšić, I. Senta, S. Kublik, M. Schloter and N. Udiković-Kolić, *Sci. Total Environ.*, 2020, **706**, 136001.
9 L. Kovalova, H. Siegrist, H. Singer, A. Wittmer and C. S. McArdell, *Environ. Sci. Technol*, 2012, **46**, 1536-1545.
10 N. Kumar, W. Wang, J. C. Ortiz-Marquez, M. Catalano, M. Gray, N. Biglari, K. Hikari, X. Ling, J. Gao, T. van Opijnen and K. S. Burch, *Biosens. Bioelectron*., 2020. **156**, 112123.
11 S. M. Dorman, S. Seth and D. A. Khan, *J. Allergy Clin. Immunol. Pract*., 2018, **6**, 196-200.
12 Z. Li, C. Liu, V. Sarpong and Z. Gu, *Biosens. Bioelectron*., 2019, **126**, 632-639.
13 A. Mohammad-Razdari, M. Ghasemi-Varnamkhasti, Z. Izadi, A. A. Ensafi, S. Rostami and M. Siadat, *Microchim. Acta*, 2019, **186**, 372.
14 F. A. Scholl, P. V. Morais, R. C. Gabriel, M. J. Schöning, J. R. Siqueira and L. Caseli, *ACS Appl. Mater. Interfaces*, 2017, **9**, 31054-31066.
15 A. Pennacchio, A. Varriale, A. Scala, V. M. Marzullo, M. Staiano and S. D'Auria, *Food Chem*., 2016, **190**, 381-385.
16 P. H. Chan, H. B. Liu, Y. W. Chen, K. C. Chan, C. W. Tsang, Y. C. Leung and K. Y. Wong, *J. Am. Chem. Soc.*, 2004, **126**, 4074-4075.
17 Y. Chen, X. Li, M. Yang, L. Yang, X. Han, X. Jiang and B. Zhao, *Talanta*, 2017, **167**, 236-241.
18 P. Weber, B. R. Riegger, K. Niedergall, G. E. M. Tovar, M. Bach and G. Gauglitz, *Sens. Actuators, B*, 2018, **267**, 26-33.
19 H. Wang and K. Zhang, *Sensors*, 2013, **13**, 4192-4213.
20 J. H. Holtz and S. A. Asher, *Nature*, 1997, **389**, 829-832.
21 W. Chen, M. Fu, X. Zhu and Q. Liu, *Biosens. Bioelectron.*, 2019, **142**, 111499.
22 J. Qin, X. Li, L. Cao, S. Du, W. Wang and S. Q. Yao, *J. Am. Chem. Soc.*, 2020, **142**, 417-423.
23 Q. Li, S. Zhou, T. Zhang, B. Zheng and H. Tang, *Biosens. Bioelectron.*, 2020, **150**, 111866.
24 Y. Wang, Y. Li, Y. Zhang, K. Ren, H. Ju and Y. Liu, *Sci. China Chem*., 2020, **63**, 731-740.
25 X. Qiu, W. Chen, Y. Luo, Y. Wang, Y. Wang and H. Guo, *Anal. Chim. Acta*, 2020, **1093**, 142-149.
26 F. Xiao, Y. Sun, W. Du, W. Shi, Y. Wu, S. Liao, Z. Wu and R. Yu, *Adv. Funct. Mater.,* 2017, **27**, 1702147.
27 Y. Zhang, Y. Wang, H. Wang, Y. Yu, Q. Zhong and Y. Zhao, *Small*, 2019, **15**, 1902198.
28 Y. Yue and T. Kurokawa, *ACS Appl. Mater. Interfaces*, 2019, **11**, 10841-10847.
29 T. Wang, J. Liu and F. Nie, *J. Mater. Chem. B*, 2018, **6**, 1055-1065.
30 F. Liu, S. Zhang, X. Jin, W. Wang and B. Tang, *ACS Appl. Mater. Interfaces*, 2019, **11**, 39125-39131.
31 A. Yadav, A. Kaushik, Y. K. Mishra, V. Agrawal, A. Ahmadivand, K. Maliutina, Y. Liu, Z. Ouyang, W. Dong and G. J. Cheng, *Mater. Today Chem*., 2020, **15**, 100208.
32 J. Zhang, L. Wang, D. N. Lamont, S. S. Velankar and S. A. Asher, *Angew. Chem., Int. Ed.*, 2012, **51**, 6117-6120.
33 G. Li, F. Xiao, S. Liao, Q. Chen, J. Zhou, Z. Wu and R. Yu, *Sens. Actuators, B,* 2018, **277**, 591-597.
34 Z. Cai, A. Sasmal, X. Liu and S. A. Asher, *ACS Sens.*, 2017, **2**, 1474-1481.
35 Z. Cai, D. H. Kwak, D. Punihaole, Z. Hong, S. S. Velankar, X. Liu and S. A. Asher, *Angew. Chem., Int. Ed.*, 2015, **54**, 13036-13040.
36 Y. Wang, T. Xie, J. Yang, M. Lei, J. Fan, Z. Meng, M. Xue, L. Qiu, F. Qi and Z. Wang, *Anal. Chim. Acta*, 2019, **1070**, 97-103.
37 G. Murtaza, A. S. Rizvi, M. Irfan, D. Yan, R. U. Khan, B. Rafique, M. Xue, Z. H. Meng and F. Qu, *Anal. Chim. Acta*, 2020, **1117**, 1-8.
38 A. E. Coukouma, N. L. Smith and S. A. Asher, *Analyst*, 2015, **140**, 6517-6521.
39 F. Xue, Z. Meng, Y. Wang, S. Huang, Q. Wang, W. Lu and M. Xue, *Anal. Methods,* 2014, **6**, 831-837.
40 C. E. Reese and S. A. Asher, *J. Colloid Interface Sci.*, 2002, **248**, 41-46.
41 J. Zhang, L. Wang, J. Luo, A. Tikhonov, N. Kornienko and S. A. Asher, *J. Am. Chem. Soc.*, 2011, **133**, 9152-9155.
42 J. Zhang, X. Chao, X. Liu and S. A. Asher, *Chem. Commun.*, 2013, **49**, 6337-6339.
43 K. Lee and S. A. Asher, *J. Am. Chem. Soc.*, 2000, **122**, 9534-953.

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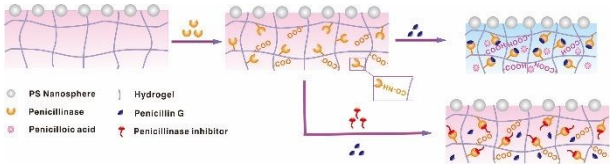

A simple 2DPPCH biosensor was developed for colorimetric detection of penicillin G and penicillinase inhibitor based on the enzymatic hydrolysis reaction between penicillin G and penicillinase.