Bio-inspired self-healing structural color hydrogels

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Biologically inspired self-healing structural color hydrogels were developed by adding a glucose oxidase (GOX)- and catalase (CAT)-filled glutaraldehyde cross-linked BSA hydrogel into methacrylated gelatin (GelMA) inverse opal scaffolds. The composite hydrogel materials with the polymerized GelMA scaffold could maintain the stability of an inverse opal structure and its resultant structural colors, whereas the protein hydrogel filler could impart self-healing capability through the reversible covalent attachment of glutaraldehyde to lysine residues of BSA and enzyme additives.

A series of unprecedented structural color materials could be created by assembling and healing the elements of the composite hydrogel. In addition, as both the GelMA and the protein hydrogels were derived from organisms, the composite materials presented high biocompatibility and plasticity. These features of self-healing structural color hydrogels make them excellent functional materials for different applications.

Results and Discussion

In a typical experiment, the GelMA hydrogel inverse opal scaffolds were fabricated by replicating silica colloidal crystal templates. These colloidal crystal templates were prepared by the self-assembly of silica nanoparticles in silica capillaries or on glass slides, which closely packed and finally formed an ordered structure during dehydration (Fig. 2A). This ordered packing of the nanoparticles endowed the colloidal crystals with interconnected nanopores throughout the templates, which enabled infiltration of the GelMA pregel solution. After the pregel solution penetrated the nanopores and filled all of the voids of the templates by capillary action, the solution was polymerized to form a hydrogel by UV light. Finally, the inverse opal scaffolds were obtained by etching the silica nanoparticles, leaving an inverse opal GelMA hydrogel scaffold (Fig. 2B). This kind of scaffold displays various brilliant structure colors (Fig. S1), which is an important feature of the materials.

To impart to the structural color hydrogel the capability of self-healing, the glutaraldehyde cross-linked BSA hydrogel with enzyme additives of GOX and CAT was filled into the inverse opal scaffold. In this process, the pregel was first prepared with a

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Fig. 2A. This ordered packing of the nanoparticles endowed the colloidal crystals with interconnected nanopores throughout the templates, which enabled infiltration of the GelMA pregel solution. After the pregel solution penetrated the nanopores and filled all of the voids of the templates by capillary action, the solution was polymerized to form a hydrogel by UV light. Finally, the inverse opal scaffolds were obtained by etching the silica nanoparticles, leaving an inverse opal GelMA hydrogel scaffold (Fig. 2B). This kind of scaffold displays various brilliant structure colors (Fig. S1), which is an important feature of the materials.

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In this paper, we present the desired self-healing structural color hydrogels by constructing them with a composite nanostructure, as indicated in Fig. 1. This nanostructure was composed of a methacrylated gelatin (GelMA) hydrogel inverse opal scaffold and a filler of glutaraldehyde cross-linked BSA hydrogel with enzyme additives of glucose oxidase (GOX) and catalase (CAT). The polymerized GelMA hydrogel scaffold in the composite materials could guarantee the stability of both the inverse opal structure and its resultant structural colors, whereas the protein hydrogel filler could impart the materials with self-healing capability through the reversible covalent attachment of the glutaraldehyde to lysine residues of BSA and the enzyme additives. As both the GelMA and protein hydrogels are derived from organisms, the composite materials had high biocompatibility and plasticity. It was demonstrated that a series of new structural color materials with one-dimensional (1D) linear microfiber, 2D pattern, and 3D photonic path structures could be developed by assembling and healing the composite structural color hydrogel elements. These features make our self-healing structural color hydrogels highly promising for different applications, such as counterfeit prevention, integrated optics, and biomedical engineering.

Significance

Structural color hydrogels have been widely studied and used in different applications, such as in switches, optical devices, etc. However, because the deterioration and accumulation of damage of these materials are inevitable during applications, the creation of bio-inspired structural color materials with increased survivability is still desired for both fundamental research and practical applications. In this study, inspired by creatures in nature with spontaneous healing from injury and recovering of functionality, we demonstrated a self-healing structural color hydrogel by filling a healable protein hydrogel into an inverse opal scaffold. A series of new structural color materials with 1D linear microfiber, 2D pattern, and 3D photonic path structures could be constructed by assembling and healing the composite structural color hydrogel elements.
Healing the hybrid material, the refractive index of the materials. Although the infiltration of the will decompose into H2O and O2 by the CAT enzyme to avoid λ equation, linked BSA hydrogel with GOX and CAT additives. (of a GelMA hydrogel inverse opal scaffold and a filler of glutaraldehyde cross-the structural color hydrogel. obtained (Fig. 2 different diffraction peaks and structural colors could also be Gels were transferred to a closed environment with a certain humidity for the polymerization of the infiltrated pregel in the pores of the inverse opal, the structure color hydrogels should be dehydrated and then immersed in the pregel solution in a vacuum environment. After these steps, the structure color hydrogels were transferred to a closed environment with a certain humidity for the polymerization of the infiltrated pregel in the inverse opals (Fig. 2C). Finally, a hybrid inverse opal hydrogel with brilliant structure color was achieved.

The formation of the structural colors of the hydrogels was ascribed to their orderly arranged nanostructure, which imparts to the inverse opal hydrogel and its derived hybrid hydrogel a unique photonic band gap (PBG). This PBG leads light with certain wavelengths or frequencies to be located in and reflected instead of propagating through the materials. As a result, the colloidal crystal templates, a series of nanopores of the inverse opal, the structure color hydrogels should be dehydrated and then immersed in the pregel solution in a vacuum environment. After these steps, the structure color hydrogels were transferred to a closed environment with a certain humidity for the polymerization of the infiltrated pregel in the inverse opals (Fig. 2C). Finally, a hybrid inverse opal hydrogel with brilliant structure color was achieved.

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It is noteworthy that our strategy could even heal the microfiber segments with different structural colors. To demonstrate this, hybrid hydrogel microfiber segments with blue, green, and red structural colors were assembled together. The joints of these microfiber segments were simply treated with glucose. It was found that although having different structural colors, neighbor segments could still adhere together tightly and form an integrated microfiber. The combined microfiber inherited the multiplex structural colors of each segment and preserved the good elasticity of the original microfibers (Fig. 3B). This indicated that the enzyme-mediated hybrid structural color hydrogel exhibits excellent self-healing properties with high recovery and reversibility.

To investigate the self-healing property of GelMA and the proteins’ hybrid structural color hydrogel system, hybrid hydrogel microfibers with the same inverse opal nanostructures and composite protein materials were fabricated and cut into segments. Then, the segments of the structural color hydrogels were brought together slightly to ensure that their surfaces were in full contact. It was found that by simply connecting two of the segments, they could not adhere to each other and remained independent. However, with the addition of glucose, the two segments could adhere tightly to each other and form an integrated microfiber (Fig. 3A). Although the repaired traces could not be hidden and the reflection peak width at the fracture increased slightly (Fig. S4), the self-healing structural color microfiber maintained its vivid structural color through the whole body. In addition, the self-healing microfibers show elasticity as good as their original elasticity. Thus, the enzyme-mediated hybrid structural color hydrogel exhibits excellent self-healing properties with high recovery and reversibility.

To demonstrate the versatility of the self-healing structural color hydrogels in assembling other shapes besides cylindrical, three pieces of different hybrid hydrogel films were used for structural color pattern construction. By adding glucose to the intersection line, these films were stitched together to form an

![Fig. 1. Schematic diagram of the self-healing structural color hydrogel. (A) Fabrication of the self-healing structural color hydrogel. It was composed of a GelMA hydrogel inverse opal scaffold and a filler of glutaraldehyde cross-linked BSA hydrogel with GOX and CAT additives. (B) Self-healing process of the structural color hydrogel.](image)

![Fig. 2. (A–C) SEM images of (A) colloidal crystal template, (B) inverse opal scaffold, and (C) hybrid self-healing hydrogel surface. (Scale bars: 500 nm.) (D) Optical images and absolute reflection spectra of six kinds of hybrid self-healing hydrogel microfibers.](image)
indexed pattern of an integrated film with blue, green, and red structural colors (Fig. 4A). To investigate the practical value of the self-healing hybrid hydrogel materials, a tensile test was performed to quantitatively evaluate the mechanical stability of the repaired sample. It was found that the self-healing film could keep its integrated structures not only in the assembled units but also in the repaired section (Fig. 4B). Thus, the self-healing structural color film is sufficiently flexible to resist an external tensile force (Fig. S5). In each assembled unit, it could be observed that all of them showed an equal ratio of stretching with the whole film, which caused the blue shift of their structural colors. These colors’ blue shift should ascribe to the gradual decreasing of the interplanar distance $d_{111}$ of the (111) diffracting planes during the stretching of the inverse opal materials. In addition to the simple indexed pattern of structural colors, a much more complex 2D pattern, such as Chinese Taiji (Fig. 4C), could also be constructed by using the same self-healing assembly strategy.

Besides the 2D patterns, the self-healing assembly strategy could also be used for the development of 3D structural color materials that have potential values in the areas of art creation, counterfeit prevention, and 3D integrated optics, etc. To demonstrate these concepts, a GelMA and proteins hybrid yellow structural color hydrogel film was cut into pieces of different sizes. These pieces were stacked together from large to small (Fig. 5A). Because of the complete porous inverse opal structure of these pieces, the filler self-healing protein hydrogels in the inverse opal scaffolds from the surface of the pieces could touch each other. Thus, these pieces could form an integrated 3D pyramid structure by a self-healing assembly strategy (Fig. 5B).

With the same method, we could also construct 3D structural color objects in a hydrogel block. In this process, hybrid blue structural color hydrogel pieces with different 2D green triangle patterns were first prepared by using the above process. Then, the pieces were stacked together and treated with glucose (Fig. 5C). Finally, a transparent blue hydrogel block containing a 3D triangle-stacked structural color object was generated (Fig. 5D).

By designing the objects with more complex shapes and structural colors, advanced counterfeit prevention tags could be achieved. By using slender structural color microfibers instead of the triangle pattern, a 3D integrated photonic path could be...
developed in the hydrogel block (Fig. 5 E and F). Because of the existence of the PBGs in the hydrogel block and its encapsulated photonic paths, the hybrid hydrogel could show excited 3D green or invisible optical paths under green or blue light irradiation, respectively (Fig. 5 G and H). This implies that the materials could be used as new carriers for a 3D optical waveguide or optical communication.

As both the GelMA hydrogel inverse opal scaffold and the filler of the protein hydrogel were derived from organisms, the self-healing structural color hybrid hydrogels should have the same high biocompatibility. To demonstrate this, the hybrid hydrogels before and after self-healing were all used for a hepatocellular carcinoma (HepG2) cell culture, respectively. The inverse opal GelMA hydrogel scaffold was also cultured with cells for the control group. It was found that the HepG2 cells could adhere and grow on the surface of the hybrid hydrogels irrespective of the repair process. These cells formed tight cell–cell connections both on the hybrid hydrogel film and on the healing section of the film after 24 h culture, as shown in Fig. 6 A–D and Fig. S6. The cell viabilities on the structural color hybrid hydrogels before and after self-healing, as well as on the commercial multiwell and on the inverse opal GelMA hydrogel scaffold, were also investigated quantitatively by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays, as presented in Fig. 6E. It could be observed that the viability of the HepG2 cells shows no obvious differences on these hydrogel substrates. Thus, the self-healing hybrid hydrogels were suitable for cell culture and reproduction. It is noteworthy that in many cases tissue engineering required hydrogels have no self-healing function, which greatly limits their application. However, with our strategy, both non- and self-healing hydrogels could be combined as a hybrid together through cross-linking nanoscaffolds, and the resultant hydrogels could be endowed with the self-healing function to remedy the restrictions of the hydrogel biomaterials in biomedical applications.

Conclusion

In summary, we have developed self-healing structural color hydrogels with a GelMA inverse opal scaffold and BSA protein filler with GOX and CAT enzyme additives. The GelMA scaffold in the hybrid hydrogels guaranteed the stability of the inverse opal structure and the resultant structural colors, whereas the protein filler could impart the hydrogels’ self-healing capability through the reversible covalent attachment of the glutaraldehyde to lysine residues of proteins. We have demonstrated that a series of unprecedented structural color materials with 1D linear structures, 2D patterns, and 3D counterfeit-prevention objects and photonic path structures could be created by assembling and healing the hybrid hydrogel elements. In addition, the biocompatibility and biological applicability of the GelMA and proteins hybrid structural color hydrogels were also demonstrated. These features of the self-healing structural color hydrogels indicated their versatile values in different areas.

![Fig. 5](image-url)

**Fig. 5.** Construction of 3D structural color objects. (A and B) Schematic diagram and optical image of a yellow pyramid-structured structural color hydrogel. The hydrogel had an integrated structure and could be hung in the air in B. (C and D) Schematic diagram and optical image of a blue hydrogel block with a 3D triangle stacked green object inside. (E–H) Schematic diagram and optical images of the 3D integrated photonic paths in a hydrogel block. The images were taken under natural light (F), 550 nm monochromatic light (G), and 450 nm monochromatic light (H), respectively. (Scale bars: 5 mm.)

![Fig. 6](image-url)

**Fig. 6.** (A–D) CLSM images of the HepG2 cells cultured on hybrid hydrogels before (A and C) and after (B and D) self-healing. A and B are the bright-field microscopy images, and C and D are the calcein-AM fluorescent images. (Scale bar: 100 μm.) (E) Results of the HepG2 cell MTT assays cultured on different hydrogels for 24 h.
Methods

Materials. Eight kinds of SiO₂ nanoparticles with sizes 200 nm, 210 nm, 290 nm, 300 nm, and 320 nm were purchased from Nanjing Dongqian Biological Technology Co., Ltd. The GelMA hydrogel was self-prepared. Gelatin (from porcine skin), methacrylic anhydride, and MTT were purchased from Sigma Aldrich. Calcein-AM (molecular probe) was purchased from Life Technologies, and glutaraldehyde was derived from Aladdin. BSA, prepared. Gelatin (from porcine skin), methacrylic anhydride, and MTT were purchased from Nanjing 5904.

Preparation of Inverse Opal Scaffold. The inverse opal scaffolds were fabricated using a sacrificial template method. The colloidal crystal templates were obtained at invariant temperature and humidity by a vertical deposition method. In brief, the colloidal crystal templates were prepared with the self-assembly of silica nanoparticles in silica capillaries or on glass slides. The SiO₂ nanoparticles (50 wt%) with a variety of particle sizes (200 nm, 210 nm, 230 nm, 250 nm, 270 nm, 290 nm, 300 nm, and 320 nm) were dispersed in water, showing a good monodispersity. For the preparation of colloidal crystal fiber templates, the SiO₂ solution was injected into silica tubes (d = 1.36 mm) and formed an ordered fiber cluster structure during a dehydration procedure at 40 °C for 15 d. Then the fiber templates were calcined at 750 °C for 5 h to improve their mechanical strength. Finally, the silica tubes were removed and the free templates were obtained. The colloidal crystal film templates (with thickness of about 0.5 mm) were also prepared to obtain different patterns under the same condition. The silica nanoparticles self-assembled on glass slides with a silica solution (ethanol:water, 2:1 v/v) concentration of 20 wt% at 4 °C for 24 h, and then the glass were calcined at 450 °C for 5 h to improve their mechanical strength. The inverse opal structural color hydrogels scaffold was obtained based on these colloidal crystal templates. The GelMA pregel solution (0.2 g/mL) was infiltrated into the silica templates by capillary force, and the solution was polymerized to form a hydrogel (with refractive index about 1.387) by exposure to UV light. The fabricated inverse opal GelMA scaffold exhibiting a color of green was obtained by etching (with 4 wt% hydrofluoric acid) the silica nanoparticles, leaving an inverse opal GelMA scaffold. These inverse opal scaffolds with different patterns could also be obtained by exposure to UV light with mask templates.

Preparation of Bio-Inspired Self-Healing Structural Color Hydrogels. The bio-inspired self-healing structural color hydrogels were prepared based on the enzyme additives of the GOX and CAT. The glutaraldehyde (0.5 wt%) cross-linked BSA (12.5 wt%) with hydrogel with GOX (0.2 wt%) and CAT (0.8 wt%) was filled into the inverse opal scaffold. In this process, the pH of the pregel solution was adjusted to 7.0. The inverse opal scaffold was dehydrated for 2 h at 35 °C and quickly filled with the pregel solution (with refractive index about 1.352) in a vacuum environment for 20 min. After these steps, the structure color hydrogels were transferred into a closed environment with a certain humidity at 4 °C for another 3 h for polymerization of the infiltrated pregel in the inverse opals. Finally, the hybrid structural color hydrogels with good visibility and brilliant structural colors were prepared. In addition, by using different sizes of silica nanoparticles, a series of hybrid hydrogels with different diffraction peaks and structural colors could also be obtained. The optical microscopy images of the inverse opal colloidal crystal templates, inverse opal scaffold, and hybrid hydrogels were obtained under the same conditions by a digital camera (Canon5D Mark II). The reflectance spectra of these samples were recorded at a fixed glancing angle, using an optical microscope equipped with a fiber-optic spectrometer (Ocean Optics; USB2000-FLG).

The Construction Process of Structured Structural Color Hydrogels. The self-healing property of cross-linked protein hydrogel systems was investigated by cutting the hybrid structural color hydrogels into two segments. Then, two segments of the hybrid structural color hydrogels were brought together slightly to ensure the two surfaces were fully contacted and stimulated with external glucose (0.1 mg) for 3 h under a closed condition at 4 °C. Finally, the enzyme-mediated hybrid structural color hydrogels, exhibiting excellent self-healing properties, were prepared. In another experiment, three kinds of hybrid hydrogel fibers with different structure colors were assembled together under the same conditions. The 2D pattern and 3D photonic path structures could also be developed by assembling and healing the composite structural color hydrogel elements. Obtained by using mask templates of UV light, these inverse opal scaffolds in different shapes were first dehydrated for 2 h at 35 °C and quickly filled with the prepared solution in a vacuum environment for 20 min. Then, the 2D pattern and 3D photonic path structures could also be assembled together with an external glucose for 3 h under a closed condition at 4 °C. The optical microscopy images of the samples were obtained under the same conditions by a digital camera (Canon5D Mark II).

Cell Culture. Cells were regularly cultured and passaged with DMEM supplemented with 10% FBS and 1% penicillin-streptomycin in a humidified incubator at 37 °C with 5% CO₂. The structural color hydrogels were first disinfected by exposure to UV light for 2 h and rinsed with sterile PBS solution three times before cell culture. Then the HepG2 cells, cultured on the surface of the structure color hydrogels, were treated in traditional ways. The cells were seeded on the surface of hydrogel films (1 cm²) in a six-well tissue culture plate (2 × 10⁴ cells per well) for 24 h. The viability of HepG2 cells cultured in different structural color hydrogels was analyzed. Briefly, cells were first cultured on the surface of the structure color hydrogel films for 24 h, then MTT/PBS solution (5 μg/mL) was added, and the cells were incubated for another 4 h. Then the cell viability was quantified by the MTT assays according to the manufacturer's instructions. To test different cell viability under the same conditions, the cells cultured on the tissue culture plate were set as control experiments. The mean value and SD of five parallelized experiments for each sample were recorded. The morphology of cells was also observed. After being cultured on the surface of the structure color hydrogel films for 24 h, the cells were stained with calcein AM (2 μg/mL, 2 mL per well) for 20 min at 37 °C, followed by being rinsed twice with PBS and fixed with glutaraldehyde (2.5%, 2 mL per well) for 6 h at 4 °C. Finally, the cells were observed using an inverted fluorescence microscope.

Characterization. Reflection spectra were obtained at a fixed glancing angle, using an optical microscope equipped with a fiber-optic spectrometer (Ocean Optics; USB2000-FLG). SEM images of samples were taken by a scanning electron microscope (Hitachi S-3000N). Microscopy images of the samples were obtained with an optical microscope (Olympus BX51) equipped with a CCD camera (Media Cybernetics Evolution MP5.0) and a digital camera (Canon5D Mark II). The stiffness of the hydrogel materials was characterized by Single Column Table Top Systems (5943; Intron).

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