Bioinspired structural color patch with anisotropic surface adhesion

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Patch plays an important role in clinical medicine for its broad applications in tissue repair and regeneration. Here, inspired by the diverse adhesion, anti-adhesion, and responsive structural color phenomena in biological interfaces, we present a hybrid hydrogel film with an adhesive polydopamine (PDA) layer and an anti-adhesive poly(ethylene glycol) diacrylate (PEGDA) layer in an inverse opal scaffold. It was demonstrated that the resultant hydrogel film could serve as a functional tissue patch with an excellent adhesion property on one surface for repairing injured tissues and an anti-adhesion property on the other surface for preventing adverse adhesion. Besides, because of the responsive structural color, the patch was imparted with self-reporting mechanical capability, which could provide a real-time color-sensing feedback to monitor the heartbeat activity. Moreover, the catechol groups on PDA imparted the patch with high tissue adhesiveness and self-healing capability in vivo. These features give the bioinspired patch high potential in biomedical applications.

INTRODUCTION

Biomedical patches have been attracting remarkable attention for their extraordinary application values in the areas of wound repair and tissue regeneration (1–7). A patch could be highly promising in restoring tissue functions when it matches the mechanical, biological, and chemical properties of native tissues (8–11). Although recent years have seen a rapid development of the patches by combining cells with scaffold materials (12, 13), it remains a major challenge that the patches cannot anchor themselves to the injured tissue and tend to escape from the wound quickly (14–19). To address this issue, self-adhesive patches have emerged by using various adhesives that enable tough adhesion to biological tissues through reactive chemistry or tailoring surface topography (20–25). However, the excellent biocompatibility and strong cell adhesion ability of these patches would induce its attachment to the regenerated or neighboring tissues, which leads to severe inflammation consequences and hinders the healing process (26–28). In addition, a strategy for real-time monitoring of the self-adhesive capability of these patches, which is crucial for evaluating the effectiveness of the repair system, is lacking. Thus, previously unknown patches with anisotropic surface adhesion and self-reporting feature are still highly anticipated.

Here, inspired by the diverse adhesion, anti-adhesion, and responsive structural color phenomena in biological interfaces (29–36), we present a composite patch with the desired features, as shown in Fig. 1. Taking lessons from the mechanisms of mussel adhesion and eyeball lubrication (35–39), a polydopamine (PDA) hydrogel and a poly(ethylene glycol) diacrylate (PEGDA) hydrogel were used as the adhesive and anti-adhesive surfaces of the patch, respectively. Although researchers have developed various materials inspired by those biological surfaces (40–42), the construction of a composite material with anisotropic surface adhesion properties combining these two distinct mechanisms remains a challenge. Thus, we here combined the adhesive PDA hydrogel and anti-adhesive PEGDA hydrogel by using a three-dimensional inverse opal scaffold, which not only could provide interconnected pore for the hydrogel cross-linking but also imparted the composite material with brilliant structural color. It was demonstrated that the resultant hydrogel film could serve as a functional tissue patch with an excellent adhesion property on its one surface for repairing injured tissues and an anti-adhesion property on the other surface for preventing adverse adhesion and reducing friction to neighboring tissues. Besides, because of the unique responsibility of the hydrogel structural color, the patch was imparted with a self-reporting mechanical capability, which could provide real-time color-sensing feedback to monitor the adhesive and repair process of the patch during the heartbeat. These features give the bioinspired patch high potential for versatile wound healing and other biomedical applications.

RESULTS

In a typical experiment, the PEGDA hydrogel inverse opal scaffolds were first fabricated by replicating silica colloidal crystal templates, as shown in Fig. 2A. To obtain these colloidal crystal templates, mono-dispersed silica nanoparticles were self-assembled on the surface of a glass slide and formed a closely packed array through solvent evaporation, as demonstrated by the scanning electron microscopy (SEM) images shown in Fig. 2B. The glass slide, along with the colloidal crystal, was treated with thermal sintering at 600°C to enhance the mechanical strength of the templates. A pregel solution of PEGDA was injected into the templates to infiltrate the nanopores because of capillary action. By polymerizing the pregel solution through ultraviolet (UV) light, a composite hydrogel was formed with an embedded colloidal crystal layer, as shown in Fig. 2C. Last, a free-standing PEGDA film containing an upper layer of an inverse opal scaffold and a bottom layer of a solid hydrogel was obtained by etching the colloidal crystal templates by using hydrofluoric acid. The inverse opal layer exhibited periodic porous structure, as shown in Fig. 2D.

To impart one side of the PEGDA hydrogel film with an adhesive surface property, we introduced a mussel-inspired PDA hydrogel

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into the inverse opal scaffold. In this process, a pregel solution was first prepared containing biocompatible gelatin as a hydrophilic polypeptide skeleton to provide primary amine groups and PDA as a cross-linker to cross-link the gelatin backbone. The mechanism diagram of the reaction is shown in fig. S1. Then, the PEGDA hydrogel film was dried naturally and soaked in the pregel solution with vacuum compression. The inverse opal layer of the film with interconnected voids was completely filled with the PDA pregel solution, while the solid layer remained uncontaminated. After 30 min, the cross-linked PDA network formed in situ in the inverse opal scaffold, as shown in Fig. 2E and fig. S2. Thus, a hybrid hydrogel film with a PEGDA layer and a PDA layer was achieved.

Because of the periodic arrangement of the ordered nanostructure of the inverse opal layer, the PEGDA hydrogel film had unique structural color, which was ascribed to the generation of a photonic bandgap (PBG). Light of a certain wavelength located in the PBG is prohibited from propagating and therefore is selectively reflected. Thus, the inverse opal PEGDA scaffold exhibited bright structural colors.
and characteristic reflection peak. The corresponding reflection peak position for a normal incident beam could be estimated by Bragg’s law (43)

$$\lambda = 1.633 d n_{\text{average}}$$

where $\lambda$ is the reflection peak wavelength, $d$ is the distance between the diffracting planes, and $n_{\text{average}}$ is the average refractive index of the substrate. When the compositions remained unchanged, $n_{\text{average}}$ is constant, implying that the reflection peak position $\lambda$ depends on the size of the pores, which could be derived from the colloidal crystal template nanoparticles. A series of PEGDA hydrogel inverse opals with different diffraction peaks and structural colors could also be acquired, as shown in fig. S3. Besides, the hybrid hydrogel film also had structural color after PDA infiltration, and the corresponding reflection peak was red-shifted because the infiltration of PDA increased $n_{\text{average}}$ and thus $\lambda$, as shown in Fig. 2F and fig. S4.

As the hybrid film consisted of one PDA layer and another PEGDA layer, it had an anisotropic surface adhesion property on each side. To confirm this, we used the hybrid hydrogel film to culture NIH-3T3 cells. As a control, a glass slide was used to culture the same cell type. Fluorescent images were taken after 2 days, which suggested that the NIH-3T3 cells could hardly adhere or grow on the surface of the PEGDA layer, as shown in Fig. 3 (A and C). By contrast, the cells could adhere and grow well on the surface of the PDA layer, as shown in Fig. 3 (B and D), with a higher cell density observed than in the control group (Fig. 3E). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was conducted to quantitatively evaluate the cell viabilities on the hybrid hydrogel film, as shown in Fig. 3F. It was demonstrated that the cell viability remained the lowest value on the side of the PEGDA layer. Despite the fact that PEGDA had well-known biocompatibility, low cell adhesion resulted in few cells staying on the surface of the PEGDA layer after rinsing with phosphate-buffered saline (PBS). Besides, compared with the control group, PDA hydrogels showed better cell viabilities, which suggested stronger adhesion and better growth of the cells. To further investigate the adhesion of cells on PEGDA hydrogels, we fabricated a series of the PEGDA hydrogels with different polymer solution concentrations for cell culture. No obvious differences were observed on these PEGDA hydrogels (fig. S5). These results indicated that the PEGDA side of the hybrid hydrogel has good anti-adhesion property, whereas the PDA side exhibits good biocompatibility and cell adhesion ability. The hybrid hydrogel film was used as a tissue patch. To obtain excellent adhesion, we fabricated a series of the PDA hydrogels with different polymer solution concentrations for adhesion test, as shown in figs. S6 and S7. The adhesion performance improved with the increase of dopamine concentration, while an excessive amount of dopamine resulted in a highly viscous pregel solution that is difficult for infiltration. Therefore, an optimized dopamine concentration of 3 weight % (wt %) was set for the fabrication of the hybrid inverse opal hydrogel patch throughout the following study. After introducing PDA into the inverse opal scaffold, the interconnected voids were completely filled, while a nonporous PDA hydrogel formed an outermost layer, which would be attached to the tissue. Therefore, the adhesive properties of the patch were characterized on the basis of the PDA hydrogel. Besides, we tested the lap shear strengths of the PDA layer and the PEGDA layer to further claim the adhesion performance. As shown in fig. S8, the PDA layer exhibited strong adhesion to porcine skin with an adhesion strength of 0.9 N/cm². By contrast, the PEGDA layer showed no adhesion. By virtue of the adhesion property of the PDA layer, the resultant patch was adhered tightly on the surface of the porcine myocardium tissue, as shown in Fig. 4. The PDA side of the patch could be firmly bonded to the surface of the porcine myocardium tissue, and the PEGDA side faced outward and exhibited vivid structural colors. Besides, the patch remained intact and adhered tightly to the tissue, even suffering from the process of bending, distorting, water soaking, and stretching. Moreover, the patch showed motion-responsive color change. These properties make the hybrid hydrogel film a robust tissue patch to adapt to the dynamic environments.

It was anticipated that the existence of the PDA hydrogel filler endowed the structural color patch with a self-healing ability due to the coexistence of the supramolecular bonds and covalent bonds in the hydrogel network. To test this, we fabricated two groups of heart-shaped structural color patches with different reflection peaks and cut them into pieces, as shown in Fig. 5A. Then, two complementary pieces of the patch with different structural colors were brought in contact at room temperature (Fig. 5B). As shown in Fig. 5C, the two pieces of patches could tightly adhere to each other and form an integrated heart-shaped patch after 3 hours. Besides, each part could keep its original structural color after being pieced up. The self-healing reversibility of the structural color patch was proved in fig. S9, which showed a persistent level of fracture strain at several healing cycles at the same damage site. This self-healing ability endows the structural...
color patch with high recoverability and reversibility. Notably, the inverse opal–based structural color patch provided a photonic sensing platform through dynamic color change. To demonstrate this capability, we performed a mechanical test. The patch displayed a gradual change in the reflection color from orange red to green after being stretched by using a vernier caliper, as shown in Fig. 5D and movie S1. Along with the elongation of the patch, the reflection spectrum was blue-shifted from 604 to 550 nm, as shown in Fig. 5E. The dynamic color shift was ascribed to the gradual decreasing of the interplanar distance \( d \) of the (111) diffracting planes during the stretching of the patch. Besides, the patch remained intact during stretching. To further explore the mechanical strength of the patch, we performed a tensile test, as shown in Fig. 5F. Compared with the bare PEGDA inverse opal scaffold film, the mechanical strength of the structural color patch was slightly decreased because of the presence of the PDA hydrogel filler in the interconnected voids of the inverse opal scaffold, which restricted the extent of deformation of the pores during stretch. Nevertheless, the structural color patch was sufficiently flexible, with a fast and reversible mechanochromic response, making it highly potential for sensing applications.

Given the above-described excellent features, including anisotropic adhesive property, self-healing ability, and structural color–based sensing ability, the bioinspired patch was used for monitoring of cardiac activity in vitro, as shown in Fig. 6A. To simulate a heart beating, we put a balloon inside of a duck heart, which was subjected to intermittent inflation and deflation by using an air pump. A patch was adhered firmly to the heart tissue. Throughout the experiment, the strength of the patch was sufficient to support the dynamic mechanical loading of the heart beating activities of a duck cardiac. Along with the simulated beating activity, the structural color of the patch was changed reversibly from a fixed observation position, as shown in Fig. 6B. This was mainly ascribed to the changes in the Bragg glancing angle, which was induced by the expansion and contraction of the heart during the beating. As a result, the color of the patch experienced a synchronous red-to-green-to-red transition during a complete expansion-contraction heartbeat cycle. The reflection peaks in the whole process were shifted from 610 to 565 nm, which were read out at a fixed vertical angle using an optical microscope equipped with a fiber-optic spectrometer (Fig. 6, C and D). Moreover, the frequency of the structural color transition corresponded to that of the heart beating activity, as shown in movie S2. These results further demonstrated that the bioinspired structural color patch could have potential in clinical operations.

We further evaluated the capacity of the patch on a wet surface by conducting an in vivo experiment on a beating mouse heart. The PDA side of the patch exhibited stability and strong adhesion even with slurries and continuous movements, as shown in movie S3. This strong adhesion was ascribed to the supramolecular and
covalent bonds in the PDA cross-linked hydrogel. This property is exceptional because developing a cardiac patch is conventionally challenging due to the presence of the epicardium, which acts as a lubricant to reduce friction. Besides, the patch showed a vivid iridescent color. Along with the rhythmic expansion and contraction of the heart, the structural color of the patch was changed reversibly. This performance makes the patch highly suitable for tissue engineering applications. Despite the many exciting functionalities, challenges remain and endeavors must be made for practical surgical applications. As most of the patients are fixed with the drainage tubes after surgical treatment, it is thus anticipated that by introducing a specialty optical fiber, postsurgical monitoring of inner organs based on structural color changes of the patch could be achieved through the drainage tubes.

**DISCUSSION**

In summary, we developed a bioinspired structural color patch with anisotropic surface adhesion features by integrating a PDA hydrogel into a PEGDA inverse opal scaffold. The resultant hydrogel film could serve as a functional tissue patch with an excellent anisotropic adhesion property on the PDA side and the PEGDA side. Besides, the presence of the inverse opal structure gave rise to a responsive structural color, which imparted the patch with a self-reporting mechanical capability. Thus, the patch enabled a real-time color-sensing feedback to monitor the adhesive and repair process of the patch during the heartbeat. Moreover, the PDA-based supramolecular and covalent bonds contributed to a self-healing function and a strong adhesive performance of the patch even in a wet and dynamic environment. These features give the bioinspired patch high potential for tissue engineering and other biomedical applications.

**MATERIALS AND METHODS**

**Materials**

Six kinds of SiO$_2$ nanoparticles with diameters of 220, 230, 270, 285, 300, and 315 nm were self-prepared. PEGDA 700, gelatin (from fish skin), dopamine hydrochloride, 2-hydroxy-2-methyl-1-phenyl-1-propanone (HMPP), MTT, dimethyl sulfoxide (DMSO), and PBS (0.01 M, pH 7.4) were purchased from Sigma-Aldrich. Sodium hydroxide (NaOH) and sodium periodate (NaIO$_4$) were acquired from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). NIH-3T3 cells were obtained from the Institute of Biochemistry and Cell Biology, the Chinese Academy of Sciences, Shanghai, China. Calcein-AM was obtained from Molecular Probes Co. Dulbecco’s modified Eagle’s medium (DMEM) and 0.25% trypsin-EDTA were purchased from Gibco, USA. Cellulose dialysis membranes (molecular weight cutoff, 8000 to 14,000) were derived from Shanghai Yuanye Bio-Technology Corporation (Shanghai, China). The 200–250-g Sprague-Dawley rats were provided by Jinling Hospital. Animals were treated in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, USA. All the animal care and experimental protocols were reviewed and approved by the Animal Investigation Ethics Committee of Jinling Hospital. The water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore) with resistivity higher than 18 megohm-cm.

**Preparation of PEGDA inverse opal scaffold**

The PEGDA inverse opal scaffolds were fabricated using a sacrificial template method. A series of SiO$_2$ nanoparticles with different particle diameters (220, 230, 270, 285, 300, and 315 nm) were dispersed in ethanol solution (20 wt %). The colloidal crystal templates were fabricated by self-assembly of silica nanoparticles on glass slides at constant temperature and humidity through a vertical deposition method for 3 hours. During the gradual evaporation of the solvent, the silica nanoparticles self-assembled into an ordered structure on the surface of the glass slide. The silica colloidal crystal templates were prepared after calcination at 600°C for 5 hours. High-temperature calcination causes slight softening of the silica nanoparticles, leading to partial adhesion between adjacent colloidal nanoparticles, thereby improving the mechanical strength of the templates. On the basis of these colloidal crystal templates, the PEGDA inverse opal scaffold hydrogels could be obtained. The as-prepared PEGDA pregel solution (0.2 g/ml) with HMPP (1%, v/v) was injected into the silica templates and gradually penetrated into the voids between the silica nanoparticles by capillary force. Then, the solution was solidified to form a hydrogel by UV light radiation. Last, the PEGDA inverse opal scaffold was fabricated by etching the silica nanoparticles by immersing the film in 4 wt % hydrofluoric acid. Various different reflection peaks of PEGDA inverse opal scaffolds were obtained by changing the sizes of the silica nanoparticles.
Preparation of bioinspired structural color patch with anisotropic surface adhesion

The bioinspired structural color patch with anisotropic surface adhesion was prepared on the basis of PDA hydrogels. A pregel solution was first prepared. Briefly, gelatin (7 wt %) was dissolved in PBS solution (0.01 M, pH 7.4) at 70°C. Dopamine hydrochloride (3 wt %) was added, followed by the solution of NaOH (0.4 M) was prepared to adjust the pH of the system to 7.0, and the mixture was shaken for 10 s. The PEGDA inverse opal scaffold was dried naturally for 2 hours and immediately immersed in the pregel solution under a vacuum environment for 30 min. The structural color patch was placed into a sealed and stable environment for another 12 hours at 37°C, allowing the pregel to polymerize. Last, the bioinspired structural color patch with anisotropic surface adhesion was prepared. Moreover, by using PEGDA inverse opal scaffolds with different sizes of the voids, a group of structural color patches with different reflection peaks could be acquired.

Self-healing performance of bioinspired structural color patch with anisotropic surface adhesion

By using mask templates, two groups of heart-shaped structural color patches with different reflection peaks were fabricated and cut into two pieces. Then, two segments were combined together to ensure that the two pieces were fully contacted. Last, the bioinspired structural color patch with anisotropic surface adhesion and brilliant self-healing properties could sustain its integrated heart shape under gravity.

Cell culture

NIH-3T3 cells were cultured with DMEM composed of 10% fetal bovine serum and 1% penicillin-streptomycin in a humidified incubator with constant temperature (37°C) and 5% CO₂. The structural color patches were treated with PBS for 1 week and immersed with ethanol (75%) under ultrasound, followed by sterilization with UV light irradiation overnight and successive washing with PBS solution several times before cell culture. The PBS treatment is a prerequisite step for cell culture experiment by which the patch can be diazoyed for cells to grow in a suitable environment. All groups of structural color patches were cut into the same size, similar to the area of one well of a 24-well plate. Then, the NIH-3T3 cells were cultured on the surface of the PEGDA layer and the PDA layer of the structural color patch, and the NIH-3T3 cells cultured on the surface of the glass with the same size was set as control group. The activity of NIH-3T3 cells cultured in two layers of structural color patches was investigated. The solution of MTT (5 μg/ml) was prepared and filtered. After cells were cultured on the surface of each layer for every 12 hours, the films were rinsed with PBS. The MTT solution (70 μl) was added in 600 μl of DMEM and cultured for another 4 hours at 37°C. Subsequently, the solution in the 24-well plate was removed, and 600 μl of DMSO was added to dissolve the formazan crystal in cells for further optical density (OD) value measurement. Three parallel experiments were performed for each sample. The morphology of cells was captured. After being cultured on the surface of each layer of structural color patch for 48 hours, calcein-AM (2 μg/ml, 2 ml per well) was added into each well to stain the cells for 20 min at 37°C, followed by rinsing three times with PBS. Last, the cells were observed using an inverted fluorescence microscope. In addition, a group of PEGDA layer of structural color patch, which was fabricated by different concentrations of PEGDA inverse opal scaffold (10, 20, 30, and 40%), was cultured with NIH-3T3 cells, and the following steps were the same.

In vivo experiment on a beating mouse heart

A mouse was used to evaluate the capacity of the patch on wet surfaces by conducting in vivo experiment on a beating mouse heart. First, the mouse was anesthetized by intraperitoneal injection of 10% chloral hydrate. Then, the mouse’s chest cavity was opened with surgical scissors to expose the heart. Immediately, a bioinspired structural color patch was adhered on the surface of the beating heart. The patch did not fully swell in a wet physiological environment. Videos were recorded with a metallographic microscope (Olympus, BX51) and captured with a color CCD (charge-coupled device) camera (Olympus, DP30BW).

Characterization

The microstructures of the bioinspired structural color patch with anisotropic surface adhesion were obtained with a field-emission SEM (Ultra Plus, Zeiss). Reflection spectra were measured with an optical microscope (Olympus, BX51), equipped with a fiber-optic spectrometer (Ocean Optics, USB2000-FLG). Fluorescence images of the samples were captured using an optical microscope with a CCD camera (Media Cybernetics Evolution MP5.0). The OD value was taken with a microplate reader (SYNERGY HTX). The stress-strain curves of the samples were measured with Single Column Table Top Systems (5943, Instron). All photographs were taken by the authors (photo credit: Yu Wang, State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, Southeast University, Nanjing 210096, China).

Statistical analysis

The one-way analysis of variance (ANOVA) statistical method was adopted to evaluate the statistical significance. A value of 0.05 was chosen as the significance level, and the data were labeled with a single asterisk for $P < 0.05$, two asterisks for $P < 0.01$, and three asterisks for $P < 0.001$.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/6/4/eaax8258/DC1

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