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Glucose sensing based on hydrogel grating incorporating phenylboronic acid groups

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Abstract: We proposed a hydrogel grating sensor functionalized with phenylboronic acid (PBA) group for glucose concentration detection. A PBA functionalized polyacrylamide hydrogel film was first prepared via ultraviolet polymerization. Then, the diffraction grating was written on the hydrogel film via the femto-second (fs) laser point-by-point direct inscription. Binding between the PBA groups in the hydrogel and glucose molecules would lead to the swelling of hydrogel and the thus grating structure, thus modifying the diffraction properties of the grating. We experimentally characterized the swelling and transmission of the grating with different glucose concentrations. Sensitivity of the sensor was defined as variations in relative diffraction efficiency in response to glucose concentration changes, and was experimentally found to 0.61%/mM. The proposed sensor showed fast response towards the presence of glucose, and its reusability and biocompatibility were also confirmed. The use of fs-laser inscription technique does not require a pre-fabricated template, and would allow to directly modify the fabrication parameters such as scanning speed, pulse energy and frequency. Therefore, one is able to conveniently optimize the grating structure and improve the inscription efficiency. The proposed hydrogel grating could be potentially fabricated into wearable sensors, namely, contact lenses, for continuous monitoring of tear glucose level with rapid response.

1. Introduction

Hydrogels, due to their unique optical transparency and biocompatibility, have been extensively utilized in a variety of biomedical fields including biosensors [1–3], drug-delivery systems [4–6], tissue engineering scaffolds [7,8], microfluidics [9,10], and flexible electronics [11–13], to name a few. Among these applications, glucose sensing based on hydrogel materials have drawn tremendous attention in the academic community, as glucose detection is of considerable importance especially in clinical diagnosis and therapy of diabetes [14–17]. Besides, glucose-responsive hydrogel films could be fabricated in terms of contact lenses or integrated into commercial contact lenses to continuously monitor the glucose content in tears, thus favorable for wearable sensing applications [18–21]. This may constitute an alternative solution to the traditional "finger-prick" blood test, and provide a painless, noninvasive or mini-invasive detection route for long-term physiological glucose management.

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According to their sensing mechanisms, hydrogel-based glucose sensors can be generally categorized into two types. The first one is based on electrochemical sensing modality, in which the quantification of glucose concentration is based on the enzymatic reaction using glucose oxidase (GOD). Particularly, GOD catalyzes oxidation of glucose to gluconic acid and reduction of water to hydrogen peroxide. Then, hydrogen peroxide is further oxidized to produce oxygen, protons and electrons [22]. Concentration of glucose in tear could be thus quantitated by measuring the charge carriers generated in the abovementioned enzymatic reactions. Kim *et al.* developed a transparent and stretchable contact lens, which integrated a GOD-based glucose sensor with graphene-AgNWs antenna circuits for wireless communication [23]. Later, the same group also reported another contact lens that integrated GOD-based glucose sensor, AgNF-based wireless power-transfer circuits, and display pixels to visualize real-time sensing signals [24]. Recently, Guo *et al*. developed a multifunctional smart contact lens with an ultrathin MoS_2 transistors-based serpentine mesh [25]. These hydrogel glucose sensors based on the GOD enzymatic reaction had relatively high resolution and quick response towards glucose; however, their fabrication processes generally involved multiple etching and sophisticated photolithography in order to fabricate the specially patterned micro-electronic circuits for charge-carrier measurements and wireless communications. Besides, the chemical instability of GOD would also affect the long-term performance of the devices, and activity of the enzyme may be disturbed by environmental factors.

The second type of hydrogel-based glucose sensors adopts the optical sensing modality. In particular, the majority of these sensors would incorporate, as a recognition agent for glucose, the phenylboronic acid (PBA) or its derivatives, which are able to selectively bind with carbohydrate, thus resulting in swelling of the hydrogel and changing its transmission or reflection properties [26]. For example, using self-assembly technique together with free-radical solution polymerization technique, Alexeev *et al*. developed a crystalline colloidal array (CCA) embedded within a polymer network of polyacrylamide-poly (ethylene glycol) hydrogel film incorporating PBA groups. The hydrogel with a photonic crystal structure would swell with increasing concentration of glucose, thus shifting the wavelength of its diffracted light. Sensitivity of the sensor was in the range of 10-12 nm/mM [27]. Based on the similar fabrication route, Ruan *et al*. proposed to embed polystyrene (PS) CCA within the 4-aldehydephenylboric modified poly (vinyl alcohol) (PVA) hydrogel. Experimental results showed that this hydrogel sensor would shift the diffraction wavelength by ~100 nm as the glucose concentration varied from 0 to 50 mM [28]. Note that the self-assembly of polymer is typically time-consuming and may introduce localized imperfections in crystalline structure. Besides, these two sensors mentioned above also have a highly nonlinear response to glucose concentrations. In 2018, Lin *et al.* fabricated a PBA-based hydroxyethyl methacrylate (HEMA) contact lens and monitored glucose level by detecting changes in thickness of hydrogel film with a smartphone camera [20]. Although the sensing principle of this sensor device was straightforward, resolution of the sensor was relatively low. In the same year, Elsherif *et al.* took the advantage of the nanoimprint-lithography technique to fabricate polyacrylamide hydrogel grating film incorporating PBA. The changes in glucose concentrations in test analytes would vary the grating periodicity, and the sensitivity of the sensor was found to be ~12 nm (change in periodicity)/mM [21]. Utilizing this route, a nano-/micro-structured template had to be pre-fabricated typically using E-beam lithography or focused ion-beam patterning technique. Therefore, it is somehow lack of flexibility for researchers to directly modify the structural parameters, which are relevant to the diffraction properties (such as diffraction efficiency and diffraction angle) of the grating. Note that incorporating PBA and its derivatives into hydrogel does not always result in swelling of the hydrogel when interacting with glucose. For instance, Tierney *et al*. deposited a polyacrylamide hydrogel film incorporating with PBA and N-(3-dimethyl-aminopropyl)-acrylamide (DMAPAA) on an optical fiber tip to form a F-P interferometer [29,30]. Depending on the concentration of DMAPAA used in the recipe,

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the hydrogel may either swell or shrink with increasing glucose concentrations. They explained that with the presence of DMAPAA, carbohydrate molecules are prone to crosslink between two PBA groups, forming a bis-boronate-sugar complex. This would increase the crosslinking density of the hydrogel, thus resulting in shrinkage of the hydrogel. On the other hand, without the presence of DMAPAA, carbohydrate molecules are supposed to crosslink with a single PBA group upon diol-binding, thus resulting in hydrogel swelling [30]. Zhang *et al*. had also discussed the swelling and deswelling behavior of boronic acid-containing hydrogel interacting with glucose [31]. Finally, PBA groups could also be incorporated into micro-gel materials for glucose sensing. Chowdhury *et al*. theoretically demonstrated a poly(N-isopropylacrylamide) (PNIPAM) microgel-based photonic structured sensor incorporating PBA for glucose sensing [32]. Gold nanoplates were periodically embedded into the microgel film to realize refractive index modulation. Theoretical sensitivity of the sensor was as high as ~85.6 nm/mM at pH 7.4, when detecting the Bragg wavelength shift in response to glucose-induced sensor expansion. The lowest limit of detection was estimated to be 0.34 mM at pH 7.4. This microgel-based sensor had impressive performances; however, fabrication of the sensor was somewhat sophisticated, as phase-mask lithographic techniques as well as multiple rounds of sol-gel processes were involved.

Hydrogel sensors based on fluorescence detection could also be utilized for glucose monitoring. For example, Deng *et al.* proposed a 2-hydroxyethyl methacrylate (HEMA) hydrogel doping rhodamine fluorescent dyes. With the increase of glucose concentration, the fluorescent color would evolve from pink to blue, and one could use cameras on smartphones to analyze the RGB signals for quantification of the glucose level from 23 µM to 1 mM [33]. Badugu *et al.* demonstrated to incorporate a glucose-responsive fluorophore (Quin-C18) in silicone hydrogels, which are the primary hydrogel materials for commercial contact lens. As the glucose concentration increased from 0 to 100 mM, a ~50% decrease in the output fluorescence intensity was observed [19]. Compared to the hydrogel sensors with nano-/micro- structures, sensors based on fluorescence-detection may be less complicated in their fabrication; however, excitation of fluorophore normally uses ultraviolet (UV) light source that could be phototoxic to human skins or eyes. Moreover, the long-term stability of the fluorophore may also be problematic.

In this paper, we proposed a hydrogel grating sensor functionalized with PBA group for glucose concentration detection. To fabricate the sensor, a polyacrylamide hydrogel film incorporating PBA was prepared via UV polymerization. Then, a diffraction grating was fabricated using the femto-second (fs) laser point-by-point direct inscription technique. The PBA groups in the hydrogel were able to bind glucose molecules, which would lead to the swelling of hydrogel and thus the grating structure. The swelling process of hydrogel and the diffraction properties of the grating were experimentally characterized in response to different glucose concentrations. Sensitivity of the sensor was defined as variations in relative diffraction efficiency per mM, and was experimentally found to 0.61%/mM. The proposed sensor showed fast response towards the presence of glucose, and its reusability and biocompatibility were also confirmed. The use of fs-laser inscription technique does not require a pre-fabricated template, and would allow to directly modify the fabrication parameters such as scanning speed, pulse energy and frequency. Therefore, one could conveniently optimize the grating structure and improve the inscription efficiency. The proposed hydrogel grating may be potentially fabricated into contact lens for wearable applications such as monitoring of tear glucose content with rapid response.

2. Experiments and results

2.1. Reagents

Acrylamide (AA), N, N'-methylenebis(acrylamide) (BIS), 3-acrylamide phenylboronic acid (3-APBA), 2,2-diethoxyacetophenone (DEAP), dimethylsulfoxide (DMSO), D-(+)glucose, Phosphate buffer saline (PBS) solution, Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS) and Cell Counting Kit-8 (CCK-8) were purchased from Sigma Aldrich. Murine

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fibroblast cell line (L929 cell) was provided by Cell Bank of Chinese Academy of Sciences (Beijing, China). All reagents were directly used without further purification.

2.2. Fabrication of polyacrylamide hydrogel film

The fabrication process of polyacrylamide hydrogel film was briefly presented as follows [21]. We first prepared a monomer solution by adding AA (0.558 g), Bis (0.023 g), 3-APBA (0.382 g), DEAP (20 µL) in 1 ml DMSO, and stirred the solution using a magnetic stirrer, till the solutes were completely dissolved. Then, 80 µL prepolymer solution was directly dropped on a glass side, which was prewashed in acetone, ethanol, and deionized water, respectively, in an ultrasonic bath for 10 minutes for each agent. To ensure the uniformity of the hydrogel film, we placed a coverslip on top of the solution. The thickness of the film was controlled by inserting different numbers of spacers (scotch tapes) between the glass slide and the coverslip.

Photopolymerization process was carried out by illuminating the prepolymer solution using a UV lamp (SJMAEA, wavelength: 365 nm) with an illumination intensity of 12 mW/cm^2. After 5 minutes of illumination, a transparent hydrogel film was obtained. Finally, the hydrogel film was peeled off from the glass side and stored in deionized water in a dark environment. We also characterized the components of the hydrogel film with FTIR spectroscopy (Fig. S1 in Supplement 1).

2.3. Point-by-point fs-laser grating inscription on the hydrogel film surface

The grating inscription was performed using a fs-laser nano-/micro-machining system from Newport Corporation. The fs-laser features a pulse duration of 306 fs, a repetition rate up to 200 kHz and a maximal pulse energy of 28.2 µJ. As shown in Fig. 1, the linearly-polarized beam passes through a rotatable half-wave plate, a Glan polarizer, a beam expander and a quarter-wave plate in turn, which could not only control the pulse energy but also change the polarization of the fs-laser beam from linear to circular in order to reduce the anisotropic refractive-index changes on the hydrogel film.

Finally, an objective lens (40×, NA = 0.75, EC Plan-Neofluar, Zeiss) was used to focus the beam on the surface of polyacrylamide hydrogel film mounted on the 5-axis micro-positioning motorized platform integrated in the Newport µFab system. The grating fabrication was thus performed by exposing hydrogel film to fs-laser pulses while moving the motorized platform in designed spatial patterns. We set the scanning speed of the platform to be 200 µm/s, and the pulse frequency and energy to be 1.1 kHz and 18.4 nJ, respectively. A CCD camera was used to monitor the real-time fabrication process. The formation of diffraction grating could be attributed to the periodic refractive index modulation of polyacrylamide hydrogel due to two-photon polymerization of residual monomers and/or two-photon absorption induced photolysis. For the latter, the chain scission of C-C bond occurred, and finally the polyacrylamide was degraded to AA due to reaction of free radicals [34]. Both light-material interaction mechanisms could induce local material density variations with refractive index changes. However, further experimental quantitative investigation of refractive index change mechanism is out of the scope of this paper and will be studied in the future. When launching white light to the grating, its appearance would exhibit different colors viewing from different angles, thus indicating its diffractive properties (Fig. 1). In Fig. 2(a), we also showed an optical microscopic image (40× objective lens) of the obtained grating with a periodicity of ~5 µm. By launching a He-Ne laser onto the hydrogel grating, we could observe clear transmitted diffraction patterns projected onto a screen (Fig. 2(b)).

2.4. Swelling property of grating sensor

We studied the swelling properties of the hydrogel film in response to glucose solutions with different concentrations. The complexation mechanism of PBA to carbohydrate in aqueous solution follows two steps (Fig. 3(a, b)). The first one is hydroxylation. PBA exists in an

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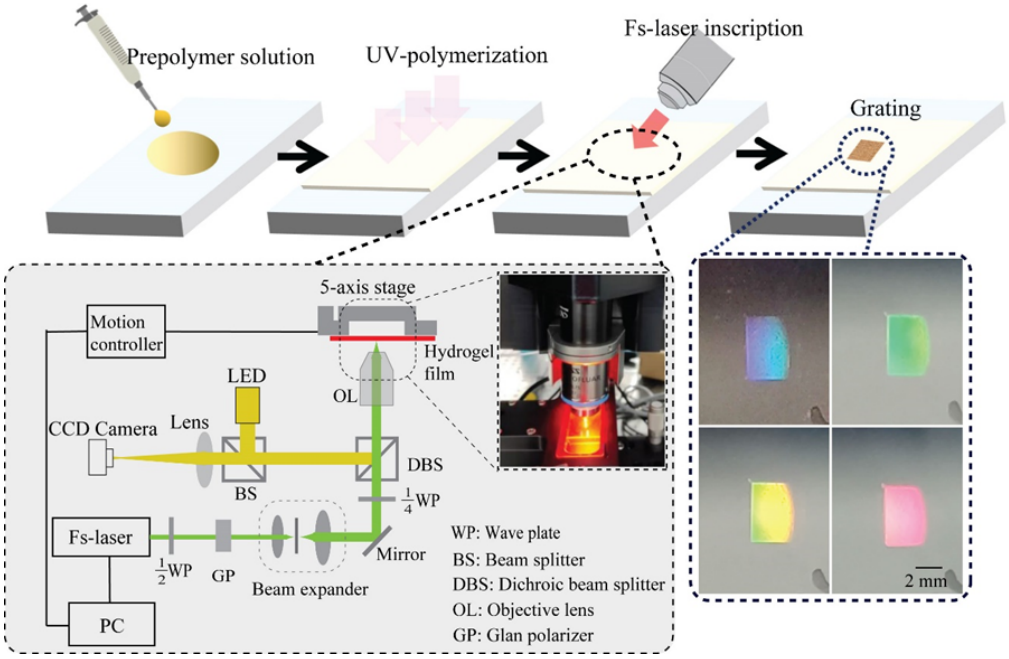

Fig. 1. Fabrication process of the hydrogel grating

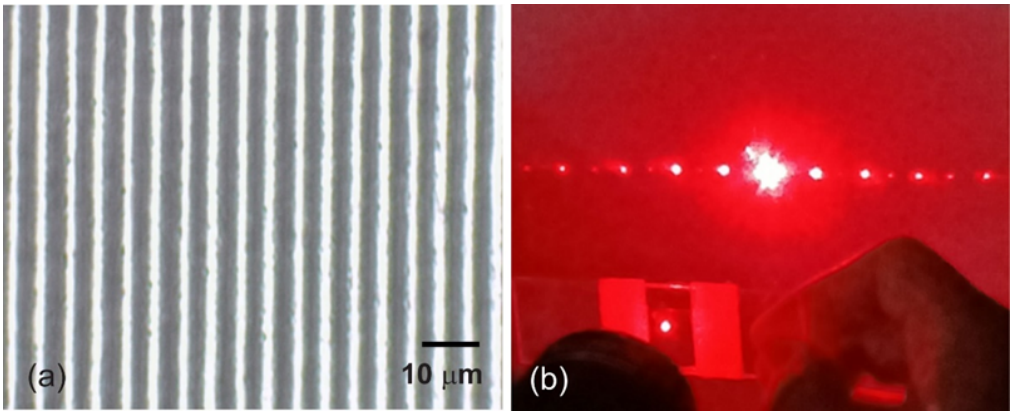

Fig. 2. (a) Microscopic image of the hydrogel grating, (b) Transmitted diffraction pattern on a screen obtained by projecting a He-Ne laser to the hydrogel grating

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uncharged trigonal planar form and hydroxylates to form a tetrahedral borate anion. The second step is esterification reaction. When PBA interacts with cis-diol molecules, a cyclic ester is formed and dissociates into the hydrogen ion and the stable boronate anion. The increased Donnan osmotic pressure thus leads to volumetric swelling of hydrogel [26], which occurs in all of the 3 dimensions. In our experiment, the hydrogel film was first equilibrated in PBS (pH: ~7.4, 24 °C) for 1 h, and then placed in glucose-PBS solutions with the glucose concentrations ranging from 0 to 50 mM with 10 mM increment. In each individual solution, the hydrogel grating was immersed for 1 h, and thickness of the film cross-section was measured using the optical microscope. As shown in Fig. 3(c), thickness of the film cross-section increased from ~128 μm to ~156 μm, as the glucose concentration increased from 0 to 50 mM. Besides, a good linear correlation between film thickness and the glucose concentration was confirmed (Fig. 3(d)).

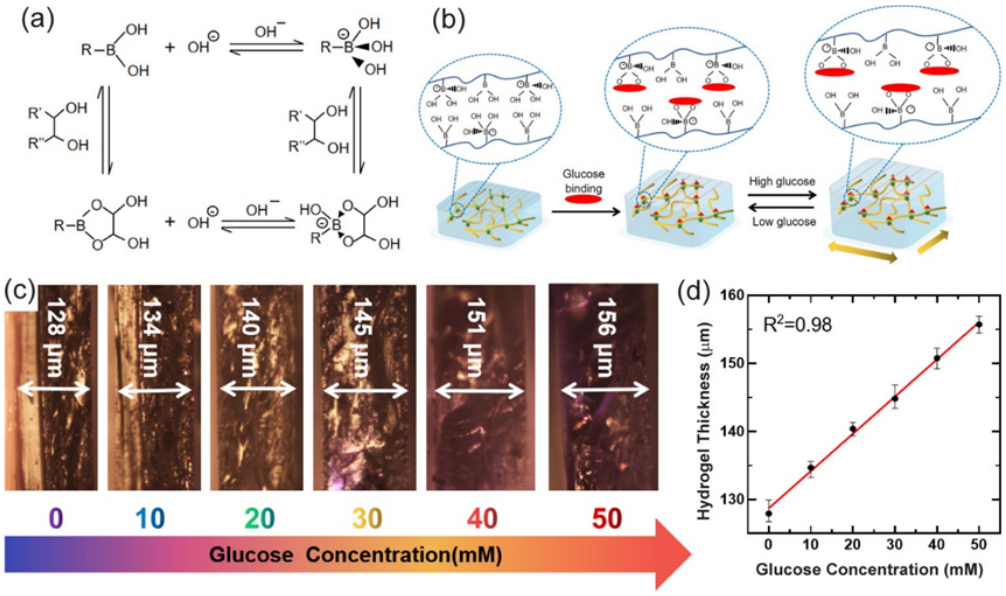

Fig. 3. (a) Complexation mechanism of PBA to carbohydrate; (b) Schematic of the swelling process of the hydrogel due to glucose binding; (c) Thickness of the hydrogel film interacting with glucose-PBS solutions with different concentrations, (d) Linear fitting of the hydrogel film thickness as a function of glucose concentration.

In order to characterize how the grating periodicity changes in response with glucose concentration, we monitored the transmitted diffraction pattern of the hydrogel grating, while changing the concentration of glucose solutions. Experimentally, a He-Ne laser was directed onto a free-standing hydrogel grating film placed on the bottom of a reservoir filled with glucose solution. The diffraction pattern was then captured by a CMOS camera (FLIR, BFLY-U3-23S6C-C). In order to conveniently measure the distance between diffracted beams with different orders, we placed a lens with a focal length of 40 mm tightly beneath the grating to focus the diffracted beams on the camera. Concentration of the glucose solution in the reservoir was then varied from 0 to 50 mM with 10 mM interval, and diffracted patterns were recorded for each concentration after an immersion of 60 mins.

As shown in Fig. 4(a), the interspace between the 0 and ± 1 order diffracted beams could be directly measured on the CMOS camera. Thus, the diffraction angle could be calculated using the formula $\theta_d = tan^{-1}\ (l/h)$, where h is the distance between the grating and the CMOS chip. Subsequently, the grating periodicity (d), can be calculated from the formula $dsin\theta_d = m\lambda$, where m is the diffraction order, and λ is the wavelength of the light source. Swelling of hydrogel

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grating would directly result in the increase of grating periodicity constant and the decrease of the diffraction angle. As shown in Fig. 4(b), the interspace between the 0 and +1 order diffracted beam decreased from 6.10 mm to 5.55 mm, and diffraction angle decreased from 7.3° to 6.7°, when the concentration of the glucose solution increased from 0 to 50 mM (Fig. 4(d)). Meanwhile, the periodicity constant of the sensor increased from ~5.00 μm to ~5.44 μm (Fig. 4(c)). A linear correlation between periodicity constant and glucose concentration was also confirmed.

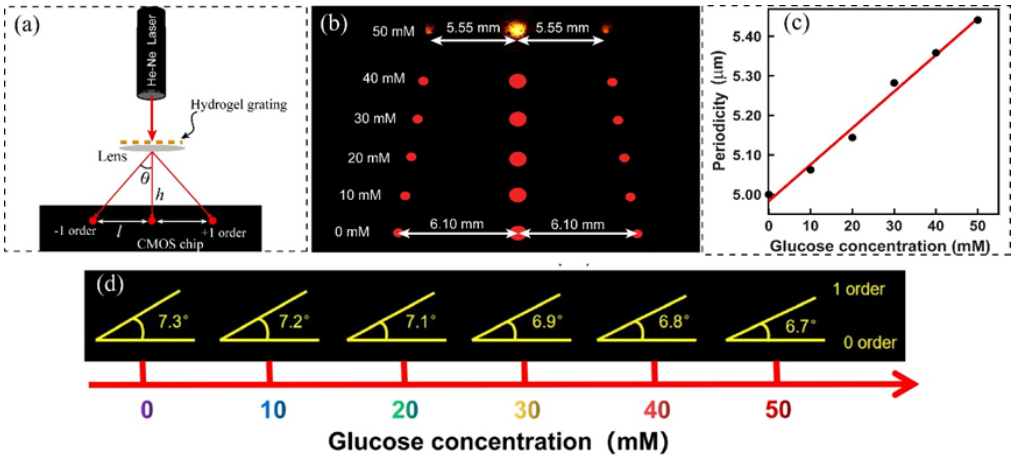

Fig. 4. (a) Schematic of the experimental setup for observing the diffraction pattern; (b) Evolution of diffraction pattern generated by the hydrogel grating in response to glucose solutions of different concentrations; (c) Calculated grating periodicities of the grating with different glucose concentrations; (d) Evolution of the diffraction angle for the 1st order fringe.

Moreover, we also confirmed the cyclic usability of the hydrogel grating. Based on the diffraction measurements above, we first immersed the hydrogel grating in pure PBS buffer and 10 mM glucose-PBS solution for an hour, respectively, and calculated the corresponding grating period. Then, the hydrogel grating was reset by placing it into acetate buffer (pH = 4.6) for 15s and pure PBS buffer for one hour. As reported by Ref. [21,26,35], the relative acidic environment would facilitate the dissociation of glucose, since the glucose-PBA complex is prone to hydrolysis in such environments. We repeat the above-mentioned process for 5 times. As shown in Fig. 5, the periodicity of grating increased to ~5.08 μm in the glucose concentration of 10 mM and then decreased to the original ~4.98 μm in PBS solution. The results indicated that the sensor had good recovery ability without hysteresis in each cycle. The hydrogel grating was also stored in PBS buffer in a dark environment for more than 1 month, and the cyclic measurements were still repeatable.

2.5. Glucose sensing performance of the hydrogel grating

The glucose sensing tests was performed by measuring the diffraction efficiency of the hydrogel grating. As shown in Fig. 6, we immobilized the hydrogel grating on a coverslip and measure the power of the +1 order diffracted beam in response to different glucose concentrations. Thus, the diffraction efficiency (DE) could be calculated as the power ratio of the +1 order diffracted beam to that of the incident beam. As shown in Fig. 6(a), a collimated incident beam emitted from a LED (Thorlabs, M625F2, wavelength: 625 nm) was directed to pass an aperture and then launch normally to the hydrogel grating. The hydrogel grating was immersed in a reservoir filled with glucose-PBS solutions. The glucose concentration in the solution was initially 0 mM and increased by 10 mM every ~50 mins by adding proper amount of glucose into the solution while

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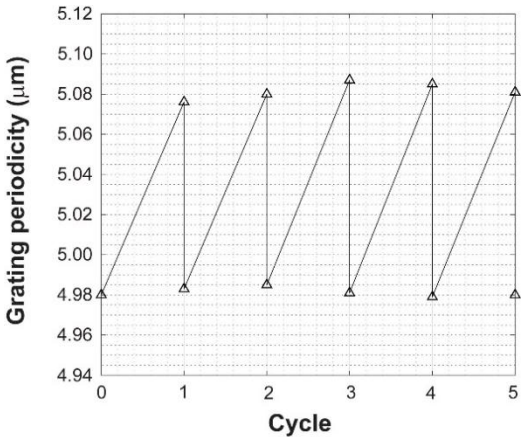

Fig. 5. Cyclic measurements of the grating periodicity

very slightly stirring the solution to accelerate the dissolution. The mean diffraction efficiency (DE) was calculated by averaging the power data, which were collected after the diffracted power became stabilized. For the 10 mM measurement, we considered the diffracted power stabilized 20 minutes after changing the concentration, and for measurements of other concentrations, we considered the power stabilized 10 minutes after changing the concentration. The equilibrium time could be further shortened by diminishing the thickness of the hydrogel films or by using microgel films for grating fabrication. When using hydrogel grating film with thickness on the order of microns, we expect the equilibrium time to be ~1-2 min [21]. Standard deviations were also calculated using the same data for the DE calculation. The relative diffraction efficiency (DE/DE_0) that was defined as the ratio of the DE for an individual glucose concentration with respect to the initial DE_0 for 0 mM glucose solution was measured.

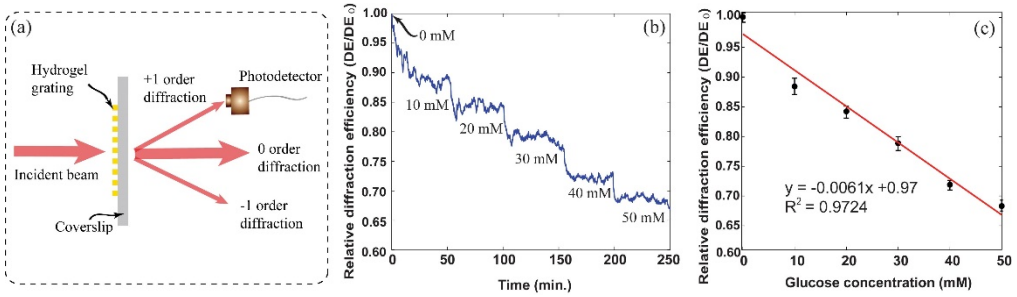

Fig. 6. (a) Schematic of the experimental setup for measuring the diffraction efficiency. (b) Relative diffraction efficiency (DE/DE_0) of the hydrogel grating as the glucose concentration varied. (c) Linear fitting of the relative diffraction efficiency in response to glucose concentration.

From the experimental results shown in Fig. 6(b), the relative DE of the grating was lowered with increasing glucose concentration. Since the hydrogel grating was immobilized on a rigid glass substrate, the changes in diffraction intensity and thus relative DE were mainly attributed to the increasing grating depth. We could also observe that once the glucose concentration was varied, the hydrogel grating showed a fast response, and the diffraction intensity varied immediately. In the 0 to 10 mM measurement, it took ~15 minutes for the grating to reach an equilibrium. However, for the subsequent concentration variations, the equilibrium time was less

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than 5 minutes. Besides, the relative DE has approximately a linear correlation with glucose concentration. The linear fitting in Fig. 6(c) suggested that sensitivity of the hydrogel grating sensor could be defined as 0.61% relative DE variation per mM concentration with a correlation coefficient of R^2= 0.9724. Note that according to the grating diffraction theory, the DE of a grating with rectangular index profile could be calculated as Eq. (1–(3)) [36]:

$$\eta_0 = 1 - 2D + 2D^2 + 2D(1 - D)\cos(\Delta\varphi) \tag{1}$$

$$\eta_m = \frac{4}{\pi^2 m^2} sin^2(\pi m D) sin^2(\Delta\varphi/2) \tag{2}$$

$$\Delta\varphi = \frac{2\pi}{\lambda}(h \cdot \Delta n) \tag{3}$$

where η_0 and η_m refer to the 0- and m- order ($m = \pm 1, \pm 2, \ldots$) diffraction efficiency, D is duty cycle parameter, $\Delta\varphi$ is the amplitude of phase modulation induced by the grating profile, λ is wavelength, Δn is the amplitude of refractive index modulation, h is the grating depth. Following Eq. (2), DE is a sine-squared function of $\Delta\varphi$ and thus h. Therefore, as the grating depth increased, the DE could be either enhanced or diminished, depending on whether $\Delta\varphi$ is located at the ascending edge or descending edge of the $\eta - \Delta\varphi$ curve. For gratings with Gaussian or sinusoidal index profiles, the $\eta - \Delta\varphi$ curves are somewhat different; however, for the 1-order $\eta - \Delta\varphi$ curves, there are always ascending edges and descending edges.

Finally, we would like to discuss the potential applicability of the proposed hydrogel grating sensor as a wearable contact lens. The proposed hydrogel grating could be directly fabricated in the form of contact lens or fabricated onto the surface of a commercial contact lens. In this case, the sensor should operate in its reflection modality, meaning that users should measure spatial displacements of the reflected +1/-1 order light in response to glucose concentrations, or measure the changes in diffraction efficiency (DE) of the corresponding reflected light as a function of glucose concentrations, similar to the case reported in Ref. [21]. Besides, glucose concentrations in human tear generally are less than 1 mM, as reported in several existing literatures (~0.2 mM in Ref. [37], 0.1-0.6 mM in Ref. [38]). In our experiments for glucose sensing, we demonstrated that the sensitivity of the hydrogel grating sensor was ~0.61% relative DE variation per mM concentration (~0.12% corresponding to 0.2 mM). However, fluctuations of the relative DE in the measured results could be seen, which was unfavorable for glucose detection especially for samples with relatively low concentrations. We note that these fluctuations arose due to several factors. (1) The light source (LED) has some inherent power fluctuations. (2) Environmental noises such as mechanical vibrations, and ambient light noise could also lead to some fluctuations. (3) During the grating fabrication by fs-laser, some localized imperfections may be introduced into hydrogel matrix due to light-matter interaction, and these imperfections would cause scattering of light. In the dynamic swelling process of the hydrogel, diffracted light intensity would be perturbed by the scattering. To alleviate these problems, one might use a more stabilized light source, design denoising proper algorithms to process the output signal, or employ additional denoising devices. Moreover, procedures to minimize environment noises could be considered. For example, the sensor could be integrated into a customized head-gadget, which is able to provide optical isolation and minimize mechanical vibrations. Finally, the fabrication recipe of the grating could be further optimized to create a more uniform grating structure in order to minimize the structural imperfections and thus the scattering of the grating sensor. In the current version of the paper, the main aim is to demonstrate that a glucose-responsive hydrogel grating could be conveniently fabricated via fs-laser direct inscription. As the future work of this sensor project, we will carry out the above-mentioned procedures in order to verify the applicability of the proposed hydrogel grating sensor for detection of tear samples.

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2.6. Biocompatibility test of grating sensor

The biocompatibility test was conducted according to ISO 10993-5 [39]. L929 cells (murine fibroblast cell line) and human primary conjunctival cell line (provided from Shantou University Medical College, Eye Research Institute) were used to evaluate the cytotoxicity of the grating sensor. Three comparison groups including the proposed hydrogel film, a commercial silicon contact lens, and the hydrogel film together with a commercial contact lens were completely immersed in the Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin at 37 °C for 24 h to acquire the leach liquor. The two kinds of cells were seeded in 96-well culture plates at a density of 1000 cells/well and incubated. After 24 h, cells were isolated and incubated with 10 µl CCK-8 solution and then tested using a UV-spectrophotometer (BioTek ELX800 kit, Power waveXS, USA) in an absorbance of 450 nm. Cell viability (%) refers to the ratio of the cell numbers in each group to that in the control group (DMEM without hydrogel film or commercial contact lens). As shown in Fig. 7, the result of the CCK-8 assay showed the cell viability of four groups in two cells was more than 90%. These results confirmed our grating sensor has comparable biocompatibility as the commercial contact lens.

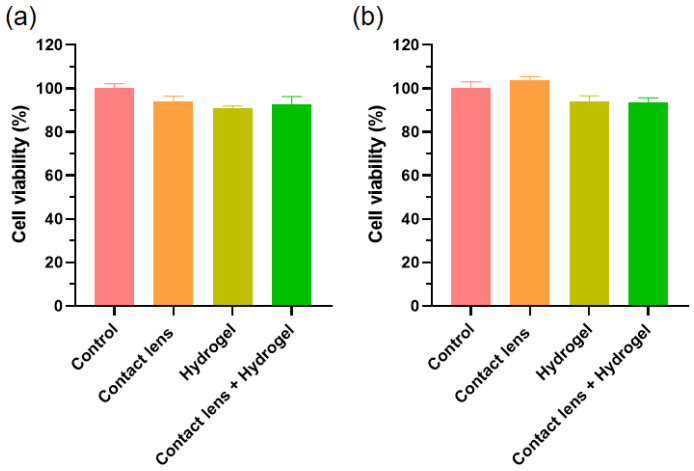

Fig. 7. Cytotoxicity test in vitro. (a) Cell viability of human primary conjunctival cell line. (b) Cell viability of L929 cells.

3. Conclusions

In summary, we proposed a hydrogel grating sensor functionalized with PBA group for glucose concentration detection. We firstly fabricated the polyacrylamide hydrogel incorporating PBA group via UV polymerization. Then, the grating structure was written on the hydrogel film using fs-laser direct point-by-point inscription. The hydrogel grating is glucose responsive, because the PBA groups could bind with glucose molecules, thus resulting in the swelling of the hydrogel. This would consequently modify the diffraction properties of the grating. Experimentally, we characterized the hydrogel film thicknesses and the diffraction properties of the grating in response to a series of glucose-PBS solutions with different glucose concentrations. The sensitivity of the sensor was defined as variations in relative diffraction efficiency per mM, and was experimentally found to 0.61%/mM. The reusability and biocompatibility of the hydrogel sensor was also confirmed. Compared to the nano-imprint lithographic technique, the use of fs-laser direct inscription technique would allow to conveniently optimize the fabrication parameters, and one does not need to pre-produce the template. The hydrogel grating proposed here has promising

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potential for applications in terms of wearable sensors for continuous monitoring of tear glucose with rapid response.

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Disclosures. The authors declare no conflicts of interest.

Data availability. Data underlying the results presented in this paper are not publicly available at this time but may be obtained from the authors upon reasonable request.

Supplemental document. See Supplement 1 for supporting content.

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