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ARTICLE

A pH-regulated Drug Delivery Dermal Patch for Targeting Infected Regions in Chronic Wounds

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This work presents a low-cost, passive, flexible, polymeric pump for topical drug delivery which uses wound pH as a trigger for localized drug release. Its operation relies on a pH-responsive hydrogel actuator which swells when exposed to the alkaline pH of an infected wound. The pump enables slow release (< 0.1 µL/min) of aqueous anti-bacterial solution for up to 4 hours and sustains against up to 8 kPa of backpressure. Featuring a scalable layer-by-layer fabrication technique to expand the pump into a 2 × 2 array, the device can dispense 50 µl onto a 160 mm^2 dermal coverage within 4 hours. Robustness tests show that when integrated within a medical adhesive, the device can be worn around the forearm and can withstand various daily activities (non-intensive) for up to 12 hours. In-vitro experiments demonstrate a 58 times decrease of live P. aeruginosa after 24 hours of the pump assisted antibiotics treatment.

Introduction

Chronic wounds affect an estimated 6 million people in the US and cost the health care system $20-25 billion annually [1]. Unlike acute wounds, chronic wounds do not heal in an orderly or timely manner and usually remain unresolved for several months; sometimes the wound condition even worsens leading to significant morbidity. Chronic non-healing wounds are typically trapped in the inflammation phase of the normal healing process. This is the result of a combination of overlapping biological and physical factors such as tissue ischemia, hypoxia, impaired cellular metabolism, and excessive mechanical force/stress [2]. Conventional wound dressing products, including standard gauze pads/rolls or hydrogel/hydrocolloid products, are designed to fulfil one or multiple complicated requirements such as physical protection, exudates absorption, and optimal moisture levels restoration/maintenance [3–5]. All these materials aim at promoting tissue regeneration within a balanced moist environment. However, a more efficacious method is to target the chronic wounds based on different physicochemical and physiological parameters, acting as "biomarkers" of chronic wound, including temperature, moisture, nutrition factors, and inflammatory mediators [6].

Among the possible biomarkers, the pH level in wound bed is a key indicator for assessing the healing progress of chronic wounds as it can indirectly or directly influence all biochemical reactions taking place in the healing process. Healthy skin or acute healing wounds have a slightly acidic pH in a small range of 4 to 6, while chronic wounds have a more alkaline pH at a wide range of 7.3 to 10 (partly due to the proliferating bacterial colonies) [7,8]. Current dermal drug delivery systems can benefit from using pH responsive polymeric materials entrapping the desired drug into their polymeric network to achieve a pH-regulated wound dressing. One approach is to engineer the pH-sensitive monomers individually or copolymerize them into semi-interpenetrating polymer network (IPN) films to allow the diffusion of therapeutic agents through a swollen polymer matrix to infected wound beds [9,10]. Although inexpensive and convenient, pH-sensitive polymer/hydrogel carriers suffer from various limitations such as low mechanical strength, difficulty with drug loading, and local toxic reactions [11]. In addition, chronic wounds often exhibit a spatial irregularity of infection development due to their non-uniform healing rate, resulting in drastic pH variations throughout the affected area [12], and thus requiring precise administration over the release profile of drugs to avoid high dose induced adverse side effect [13,14].

A more spatially controllable alternative is to incorporate physical/chemical pH sensors into drug delivery modules "automated bandage". One such device involved a hydrogel-based dressing continuously monitoring wound infection through an array of immobilized colorimetric pH sensors and delivering antibiotics into the wound bed [15]. Although providing a 2D pH-controlled capability, this device still relied on a drug-diffusing scaffold to release the preloaded antibiotics. A more advanced design is a multilayer bandage consisting of an array of potentiometric pH sensors and thermo-responsive hydrogel drug carriers on a flexible heater. The wound infection is monitored by the pH sensor and once a critical pH value is detected, the heater actuates the hydrogel carriers to initiate the antibiotics release [16]. This, however, requires a complicated fabrication process and an on-board electronic module, limiting its application and clinical deployment.

In this work, we describe a simple alternative solution, i.e., a smart dermal patch utilizing a pH-sensitive hydrogel as an actuator

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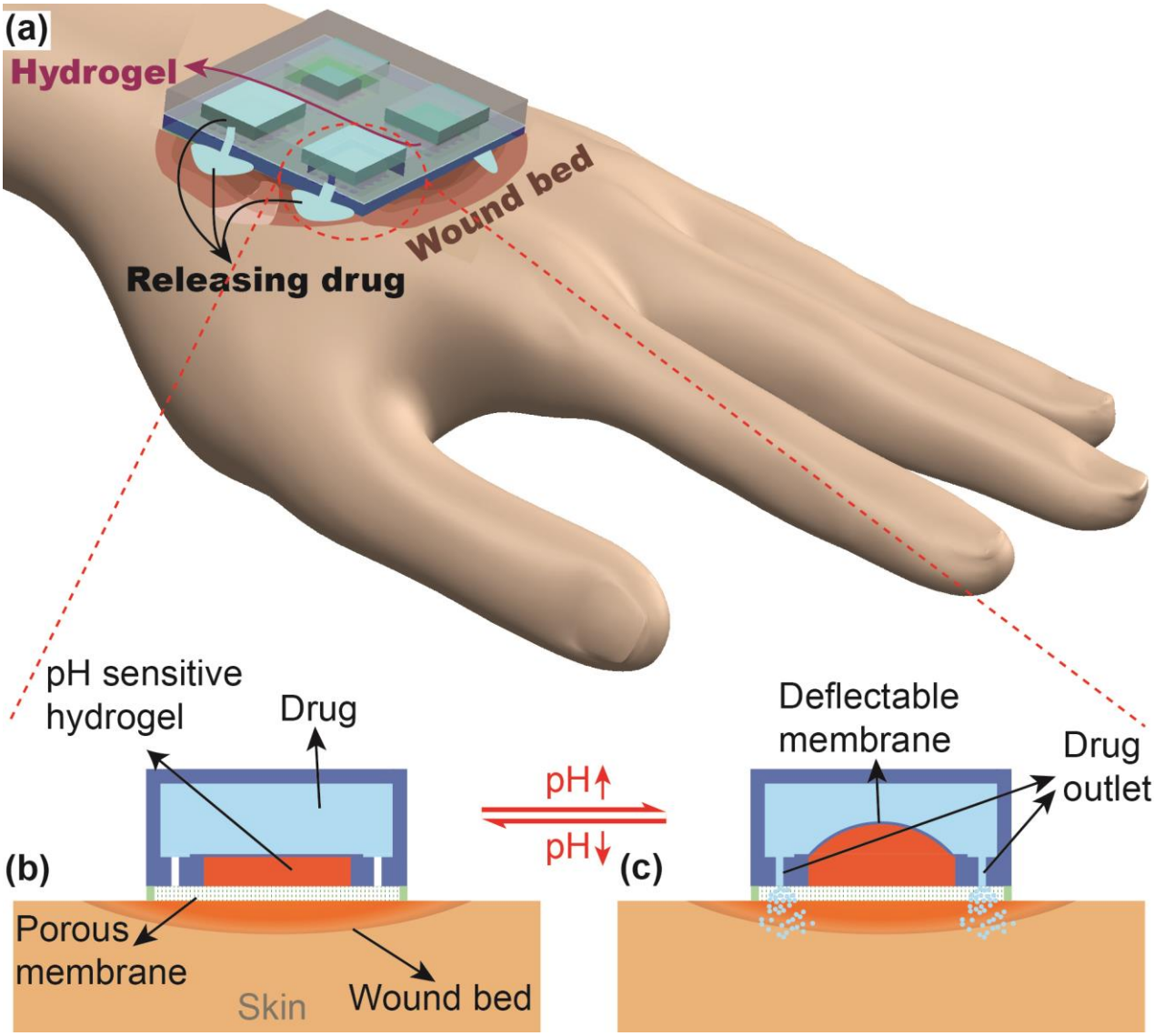

Fig. 1 (a) Conceptual illustration of a pH-regulated wound dressing patch for autonomous drug delivery to the infected regions of chronic wounds; (b) no drug is released at non-infected region where pH is low; (c) pH-sensitive hydrogel swells against a deflectable membrane where pH is high, allowing drug to be pumped out from the drug chamber into the wound.

dispensing antibiotics from an array of mm-scale pumps, Fig. 1 (a). Each pump consists of two polymeric chambers, in which the top chamber serves as the drug reservoir and the bottom one houses the gel actuator. A thin flexible membrane separates the two chambers and forces the drug out of the reservoir once deflected by the swollen gel at high pH values (indicating infection). Since the pumps and their output channels are made of hydrophobic polymer (PDMS), the aqueous drug is not released unless being actuated by the deflectable membrane.

The working mechanism principle of the device is straightforward, Fig. 1 (b-c). When the pump is in contact with the infected and inflamed regions of the wound bed (high pH), the hydrogel will start to swell and push against the thin membrane, thus releasing the drug to the wound. At non-inflamed regions (low pH), the hydrogel de-swells or stays in the shrunken state, therefore stopping the release. Hence, the entire wound could benefit from selective delivery of drugs targeted to only the infected regions using this closed-loop drug delivery scheme. Moreover, since the polymeric wound dressing patch is fabricated through a scalable layer-by-layer process, the device can be expanded to an n x n array or a customized pattern to fit the geometry of the chronic wound.

Experimental Section

Device fabrication

The pH-regulated drug delivery device shown in Fig. 2 (a) is fabricated from three PDMS (Sylgard 184, Dow Corning, Auburn, MI) layers. The top layer is the liquid drug reservoir (11 × 11 × 1.8 mm^3), the bottom layer is the pH-sensitive hydrogel chamber (7 × 7 × 1.6 mm^3), and the intermediate layer is a thin layer of PDMS (0.15 mm thick), serving as the deflectable membrane. All layers are bonded together via plasma and two drug outlets (0.6 mm diameter) are created along the gel chamber wall at a distance of 10 mm to connect

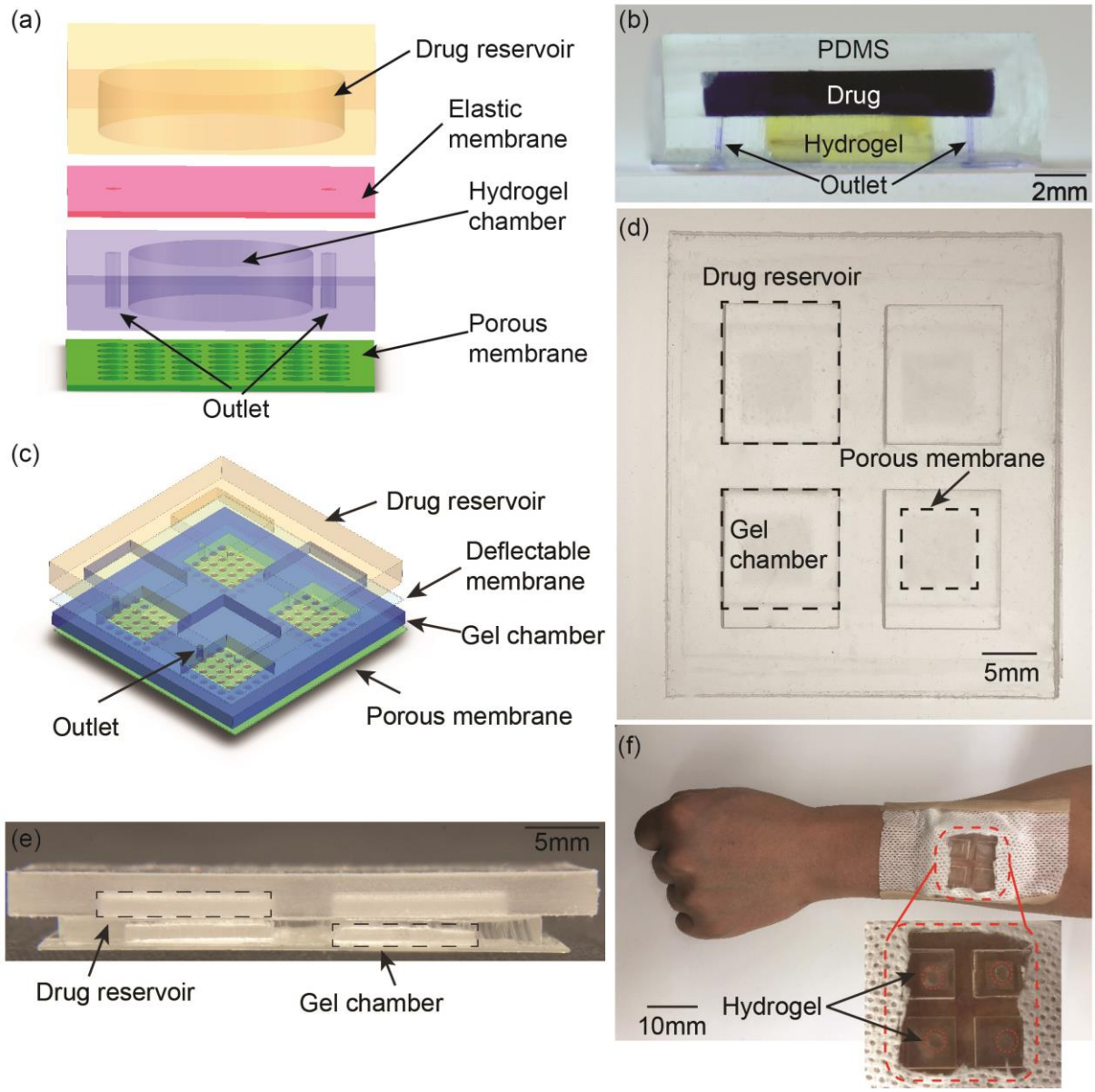

Fig. 2 (a) 3D exploded view of the autonomous pH sensing and drug delivery dressing patch, from top to bottom: drug reservoir, deflectable elastic membrane, pH sensitive hydrogel chamber with micro-outlet holes (for releasing the drug), and laser-cut porous membrane; (b) Photograph of a single pump cell; (c) The conceptual 3D illustration of the 2 × 2 array containing 4 pairs of drug reservoirs/gel chambers, designed to deliver 4 times of volumes of a single cell; (d) Top view of the fabricated 2 × 2 drug delivery array; (e) Side view of the fabricated 2 × 2 drug delivery array; (f) Photograph of the patch (2 x 2 array) worn on the forearm.

the drug reservoir to the wound bed. Due to the hydrophobicity of the PDMS, the micro-outlets also help to prevent the leakage of aqueous solution at zero external force. A 0.15 mm thick GelBond® film (Lonza Group AG, Switzerland) is laser-machined to form a 13.5 mm diameter porous plate with 60-70 µm holes. This plate is attached to the gel chamber, exposing the hydrogel to the aqueous contents of the wound bed while simultaneously providing mechanical protection.

The pH-sensitive hydrogel is poly mAA-co-AAm gel, prepared by mixing two pre-gel solutions [17,18]. Solution A is made by mixing 100 µL of methacrylic acid (mAA, Sigma Aldrich), 335 mg of acrylamide (AAm, Sigma Aldrich), 100 µL of tetra-methyl-ethylene-diamine (TEMED, Sigma Aldrich), and 3.27 mg of methylene-bis-acrylamide (crosslinker, Sigma Aldrich) in 1.2 ml of de-ionized (DI) water. Solution B is prepared by dissolving ammonium persulfate (APS, Sigma Aldrich) in DI water (80 mg/ml). The hydrogel is formed by mixing the sonicated solution A and B in a volume ratio of 5.9:1 and curing in room temperature for one hour.

A fabricated single cell device is shown in Fig. 2 (b); the drug chamber is loaded with a blue-dyed aqueous solution for testing purpose while the hydrogel chamber is filled with a yellow-dyed pH-sensitive hydrogel. A 2 × 2 array of pumps can also be fabricated through the same process; the drug delivery capability of the 2 × 2 array is expected to be 4 times as much as that of a single cell, Fig. 2 (c). The top and side view of the fabricated 2 × 2 array device are shown in Fig.2 (d) and (e), respectively. Fig. 2 (f) illustrates the patch (2 x 2 array) incorporated into a medical adhesive gauze and

attached to the forearm. Two silicone tapes are used at the top and bottom edges to provide additional mechanical robustness.

Swelling kinetics of the pH-sensitive hydrogel

The feasibility of the pH-regulated drug delivery scheme in this work is demonstrated by using a pH-sensitive hydrogel, poly (mAA-co-AAm) gel, whose swelling kinetics depends on the pH variations of the surrounding environment. When the hydrogel is exposed to high pH levels, the carboxyl group (-COOH) of MAA is ionized to $-COO^-$, increasing the internal electrostatic repulsion among the polymer chains and consequently swelling the polymer network. On the other hand, the electrostatic repulsion force decreases as the $-COO^-$ combines with the H^+ ions to the form of -COOH, reducing the electrostatic repulsion force and thus resulting in a shrunken configuration at low pH levels [19].

The dynamic swelling behavior of the pH-sensitive hydrogel was tested by casting a hydrogel film (6 x 6 x 1.6 mm^3) on the porous substrate and immersing them in five different pH solutions (pH 4, 5.5, 7, 8.5 and 10) for 240 minutes, where the pH 4 and 5.5 are in the typical pH of the normal human skin [7], the pH 7 and 8.5 are in the typical pH range of an infected wound, and the pH 10 represents an extremely infected wound-milieu[7,20]. The swelling ratio is defined as the surface area ratio between the swollen hydrogel at certain time to that of its initial state. A high resolution camera was used to record the gel surface area change and all the pH solutions were commercial pH buffer solutions purchased from VWR™.

Drug delivery characterizations

For characterizing the drug delivery capability of the pump, a wound tissue phantom was created by using a 0.5 %w/v agarose gel substrate made with phosphate buffered saline (pH 7.4). Subsequently, two platinum wire electrodes were inserted into agarose gel (2 cm apart) and connected to a voltage of 3 V; this resulted in an increased pH near the cathode, simulating the bacterial infections, Fig. 3 (a). A graduated capillary tube (I.D of 1.22 mm) was connected to the top outlet of the pump to allow for both the continuous measurement of flow rate and hydrostatic pressure. In order to overcome the physical skin barrier and consequently force the drug into the infected dermal wound, transdermal and subcutaneous drug delivery usually require the micro-pumps to provide an adequate active pressure to counteract the opposing dermal pressure, i.e., a nonzero backpressure of about 2 kPa [21] with any additional encountered subcutaneous pressure [22]. An experiment to test the capability of the fabricated pump to handle the backpressure was set to measure the flow rate using the same manner as above by connecting the pump to a pressure-regulated nitrogen source instead of an open-end capillary tube, Fig. 3 (b).

In addition to the tests of the device using the electrolyzed agarose gel substrate, we also assessed the pump's capability to deliver drug over a set of different constant pH buffer solutions (control experiments). This was conducted by fixing the device (single cell) on top of different pH buffers (pH 4, 5.5, 7, 8.5 and 10) and monitoring the dispensed volume through the two hydrophobic outlets (0.6 mm diameter) for up to 4 hours. Additionally, we also conducted another control experiment to investigate how the pump responses to the possible pH fluctuations in chronic wounds. For this, the pump was subjected to a high-low pH cycle, where two different pH buffers (pH 7 and 5.5) were alternated, each representing the typical pH level

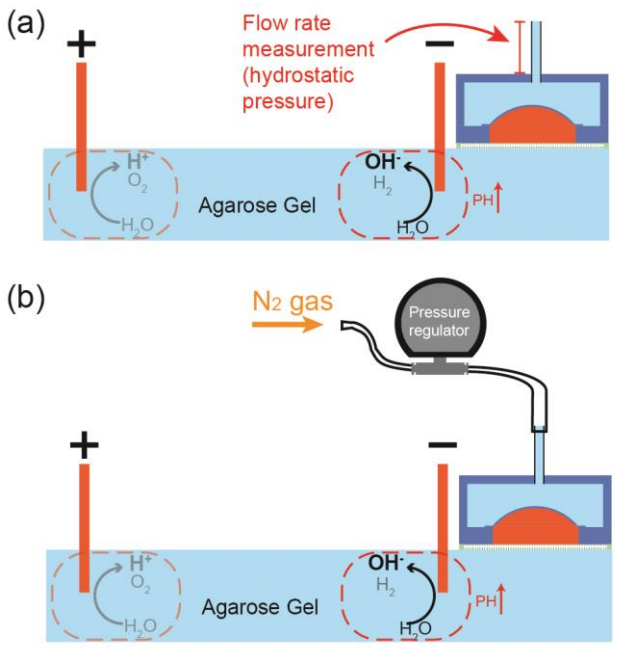

Fig. 3 Experiment setup for flow rate measurement. The single cell sits close to the cathode of a hydrolyzed agarose gel substrate (simulating an alkaline wound bed) with flow rate measured: (a) through an open-end graduated capillary tube or (b) against variable backpressure controlled by a pressure-regulated nitrogen source.

in the wound region (pH 7) and in the normal skin pH range (pH 5.5). Within each cycle, the pump was allowed to dispense the aqueous load over two hours at high pH (pH 7) first and then for one hour at low pH (pH 5.5). A total of three cycles were conducted and the dispensed volume was continuously monitored at intervals of 20 minutes up to 9 hours. All the pH solutions were commercial buffers purchased from VWR™.

Robustness characterizations

In order to deliver the drug to the wound, the fabricated device needs to be part of an adhesive-backed dressing robust enough to be wearable and stay on the wound with no or minimum movement for several hours. This was investigated by attaching the patch (2 x 2 array) on the human forearm with the adhesion provided by a medical gauze and two silicone tapes while monitoring the mechanical/structural stability of the patch for a total of 12 hours. The 12 hours experiment was divided to three treatment periods (each 4 hours), mimicking a typical daily treatment. The subject was not allowed to perform any intensive physical activities/excises during this period (mimicking normal daily activities or office work).

We also investigated the effects of skin temperature on the functionality of the device, since temperature can increase in affected regions (typically < 5°C [23,24]). Although the swelling kinetics of the pH-sensitive polyelectrolyte hydrogel used in this work is mainly due to the different ionic osmotic pressure between the gel network and the surrounding solution [25,26]; the ambient temperature also can affect the hydrogel swelling/shrinking through increasing/decreasing the thermodynamic interaction between the gel and solvents [27]. We investigated the drug delivery capability at various three temperatures (21°C, the room temperature for other experiments in this work; 27°C,

close to the normal skin temperature [28]; and 34ºC, within the range of the infected wound temperature). These were done at two different pH levels (4 and 7).

***In-vitro* assessment of antibiotics delivery on bacterial infections**

The *in-vitro* tests were undertaken to evaluate the efficacy of releasing antibiotics through the pump in inhibiting bacterial growth. The experiments used *Pseudomonas aeruginosa* (25668^{TM}, American Type Culture Collection), which is a common gram-negative bacteria found in chronic wounds and can increase the pH levels on infected regions [8,29]. The antibiotic loaded in the drug chamber was 10 mg/ml Tobramycin (VWR). The colony-forming unit (CFU) count was used to analyze the antibacterial activity of released Tobramycin.

The *in-vitro* efficacy experiment was conducted by inoculating two 3.5 ml of 0.5% Lysogeny Broth (LB) solution with 50 µl *P. aeruginosa* stock solution each using a 24 well plate. One sample (LB) was placed in contact with the pump (single cell), employed for testing the treatment efficacy; the other was left untreated for uninhibited bacteria growth (no pump engaged), used as the negative control. Both experiments were done for 24 hours at room temperature. The antibacterial efficiency of Tobramycin was then investigated by recording the CFU count at the time of inoculation (initial CFU) and after the 24 hours incubation period (final CFU) in order to compare the CFU count change for both solutions. The analysis was conducted in six distinct columns for each scenario.

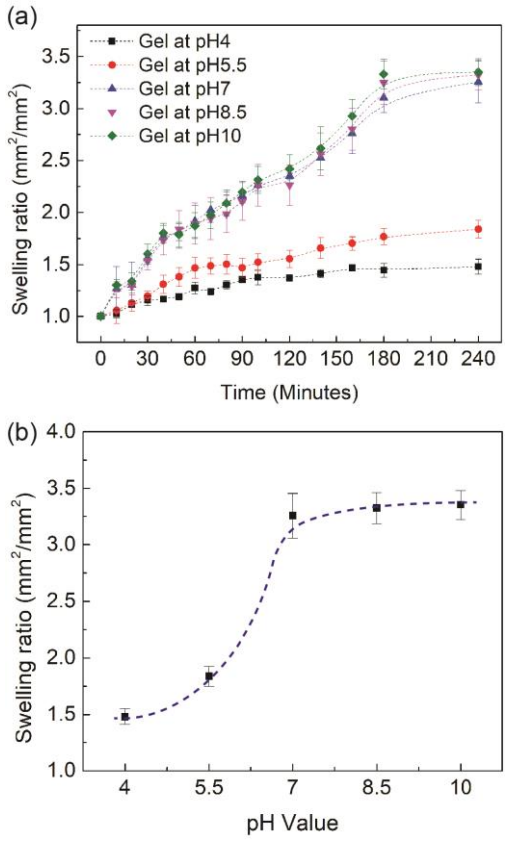

Fig. 4 Characterization of the pH sensitive hydrogel swelling attached to a porous substrate in pH 4, 5.5, 7, 8.5 and 10 buffer solutions for 240 minutes. (a) The hydrogel swells at different pH buffers over 240 minutes; (b) At equilibrium swelling within 240 minutes, the hydrogels present a swelling ratio of x1.48 at pH 4 and x1.82 at pH 5.5, which increases to x3.2, x3.32 and x3.4 at pH 7, pH 8.5 and 10, respectively; overall, the hydrogels illustrate a positive sigmoidal swelling kinetics corresponding to an ascending pH value. Each data represents a measurement in triplicate.

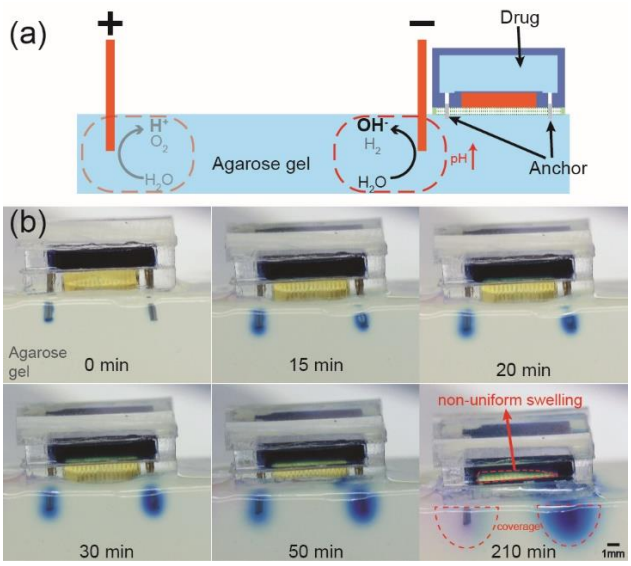

Fig. 5 (a) Experiment setup used to demonstrate the pH-regulated wound dressing patch (single cell) capability to release the drug through two hydrophobic micro-channels into a simulated wound bed; (b) Time-series photographs of blue-dyed water (working liquid) dispensed by swelling of the pH sensitive hydrogel on agarose gel. After 210 minutes, each outlet port covers a ~5 mm diameter circular region in agarose gel, indicating a 40 mm^2 wound area coverage.

The CFU counting was done by taking 20 µl LB from the two samples and mixing them separately with 180 µl Sterile LB. The two mixtures were then diluted five times with a ratio of 1:10 in series using a 96 well plate. The diluted solutions were then plated on agar plates and incubated for another 24 hours at 37ºC. The CFU counts were done through a calibrated microscope.

Results and Discussion

pH-sensitive hydrogel swelling kinetics

As mentioned previously, the poly (mAA-co-AAm) gel exhibits swelling/shrinking behavior when the surrounding pH level increase/decreases. Fig. 4 (a) shows the results of the hydrogel swelling kinetics measured over a period of 4 hours; within 240 minutes, the hydrogels are able to achieve a swelling ratio of x1.48 ± 0.07 at pH 4 and x1.82 ± 0.09 at pH 5.5, which can increase to x3.2 ± 0.2, x3.32 ± 0.14 and x3.4 ± 0.13 at pH 7, pH 8.5 and 10. There is no statistically significant difference in hydrogel swelling between 3 hours and 4 hours (p value = 0.3, 0.06, 0.18, 0.32, and 0.46 for gel swelling at pH 4, 5.5, 7, 8.5 and 10, respectively), indicating that the hydrogel reaches equilibrium within 4 hours. Fig. 4 (b) illustrates the hydrogel swelling ratio as a function of pH, showing a positive sigmoidal behavior. At the normal human skin pH range of pH 4 to 5.5, the gel exhibits maximum/equilibrium swelling ratios of x1.48 and x1.82, respectively; whereas in chronic wound regions corresponding to pH 7, 8.5 and 10, the swelling ratios increase to x3.2, x3.32 and x3.4. The results indicate that the maximum volume-transition would occur at a certain pH level or a small range of pH

values (6 < pH < 7), which is higher than the pH of healing/non-infected wounds.

Drug delivery performance

We first demonstrated the pumping action using Evans blue-dyed DI water (surrogate for aqueous solution of antibiotics) through two 0.6 mm diameter micro-channels adjacent to the negative electrode, Fig. 5 (a). Fig. 5 (b) presents a single cell delivering blue-dyed water into the agarose gel over 210 min. At the outlet port near the cathode, the Evans blue was somewhat decolorized from the raised pH level [30]; nonetheless, the blue color was still clearly visible at the other outlet >10 mm far away from the cathode. This phenomenon identified a gradient pH distribution induced by the hydrolysis of agarose gel; regions closer to the cathode experienced higher pH values. Moreover, the generated pH gradient caused a non-uniform hydrogel swelling and consequently a small difference in expelled volume between the two outlet ports. Each outlet exhibited a ~5 mm diameter circular coverage in agarose gel in 210 minutes, Fig. 5 (b); it indicated that the pump can deliver the drug to a 40 mm^2 infected region (each outlet providing ~20 mm^2 surficial coverage) at a 10 mm lateral resolution (spatial distance between two outlets).

The quantitative characterization of drug delivery capacity of the pump is presented in Fig. 6. Fig. 6 (a) demonstrates the use of PBS in agarose gel in combination with an electric potential for creating a pH gradient that can serve as a testbed for pH-responsive devices. The pH values were measured by a commercial micro pH probe, showing the pH changes observed within 0.5 mm of the cathode and anode in response to electrolyzing the agarose gel (made with pH 7.4 PBS) over 165 minutes. The former illustrates a pH increase from 7.4 to 10.6 and the latter presents a pH decrease from 7.4 to 1.6, which indicates a generation of a pH gradient over the 2 cm separation between the cathode and anode. Fig. 6 (b) presents the output volume from the pump through a 1.22 mm diameter capillary tube; in 180 minutes, the dispensed volume reached 12.2 ± 3.6 µL and the flowrate was stable between 0.05 µL/min and 0.1 µL/min, with average of 0.082 µL/min and maximum of 0.13 µL/min. At the same time, the hydrostatic pressure was continuously monitored showing a graduated increase up to 100 Pa, Fig. 6 (c). The results reveal that the pump can operate at a backpressure of 0.1 kPa to deliver the drug at 0.082 µL/min over 3 hours. The drug delivery capability of the pump under an escalated backpressure is presented in Fig. 6 (d); the flowrate is inversely proportional to the applied backpressure, with the average flowrate decreasing from 0.082 µL/min to 0.022 µL/min as the backpressure increases from 0.1 kPa to 8 kPa. The device performance in terms of flow rate (constant 0.082 µL/min over 3 hours) and maximum back pressure (8 kPa), illustrate its utility as a drug dispenser or pump for

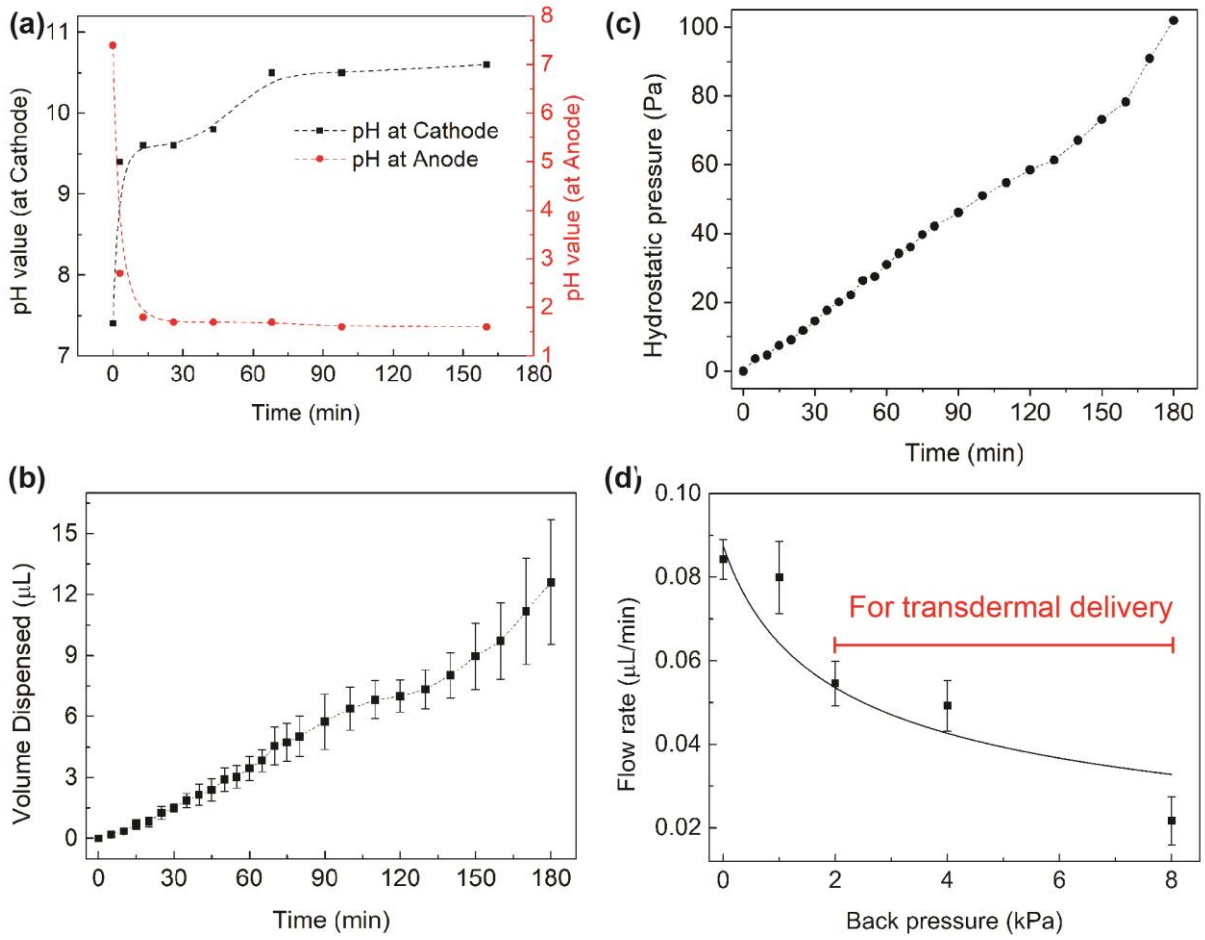

Fig. 6 (a) pH change within 0.5 mm of both the cathode and anode over time; (b) Volume dispensed by pH-regulated pump (one cell) at minimal backpressure (< 100 Pa) shows a linear response over 180 minutes, each data represents a measurement in quadruplicates; (c) Linear increased backpressure (hydrostatic); (d) Flow rate vs. backpressure measurements.

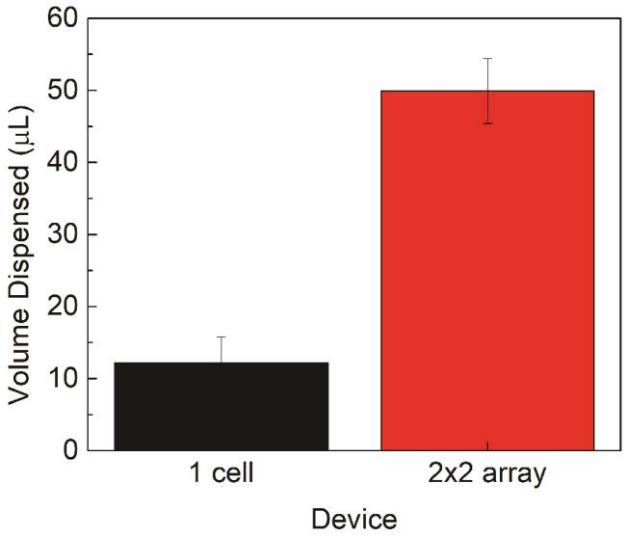

Fig. 7 Experimental comparison of the drug delivery capacity between the 2 × 2 array and a single cell for 180 minutes shows a 4.4 times increase in the released drug using the array (50 µL) instead of the single cell (12.2 µL). Plots represent the average of N=3 devices; error bars represent 1 standard deviation.

transdermal drug delivery applications requiring a slow flow rate over an extended period [31,32]. It is worth noticing that although we demonstrated the pump delivering drug on a high moisture level of testbed (the electrolyzed agarose gel substrate), it is also feasible for the pump to operate functionally on a low moisture level environment by integrating the pump with a commercial medical bandage capable of maintaining certain moisture level or further scaling down the gel size from current mm-scale to micro or sub-mm scale.

Fig. 7 shows the comparison of the drug delivery capacity between a 2 × 2 array and a single cell; within 240 minutes, the 2 x 2 array can deliver a volume of 50 ± 4.5 µL, about 4.4 times of 12.2 ± 3.6 µL of the single cell with an increased surficial coverage from 40 to 160 mm^2 assuming each cell in the 2 x 2 array performing identically. The result demonstrates the linear relationship between the number of the incorporated single cell and the drug delivery capacity. It also further implies that extending to an n × n array is expected to provide an n^2 x 40 mm^2 coverage with 10 mm resolution of drug delivery to a heterogeneous chronic wound.

In addition to testing the drug delivery capability of the pump on a mimicked heterogeneous wound bed (uneven distributed pH values), two control experiments were conducted to investigate the pump delivery capability over constant or alternating pH buffer solutions. Fig. 8 (a) shows the delivery performance of the pump (single cell) on a set of pH values, from 4 to 10, over 4 hours. At both pH 4 and 5.5, the pump delivered 0 µL volume, which confirmed that the device can stay at an "off" state at the normal skin pH range. When transferring the device to pH 7, the device was able to reach an averaged dispensed volume of 10 µL, which then increased to 11 µL and 11.4 µL by increasing the pH to 8.5 and 10, respectively. This result verified that when working in the chronic wound bed, the pump can deliver the drug at a volume/rate proportional to the surrounding pH level. Overall, the results demonstrate a positive sigmoidal relationship between the dispensed volumes and the pH. Importantly, it also implies a phase-transition of pH > 6 in the infected chronic wound region, which can enable and trigger the device to achieve the desired drug delivery capacity.

The other control experiment was to further assess the "on-off" characteristics of the pump in response to fluctuating pH values in chronic wound region. The device was transferred among the alternative pH values between 7 and 5.5 with the dispensed volume continuously monitored up to 9 hours, covering three high-to-low pH cycles. The result is shown in the Fig. 8 (b). During the first cycle, the pump delivers the aqueous load to a volume of 5.2 µL at pH 7 over 2 hours; at pH 5.5, the pervious dispensed volume is maintained for over

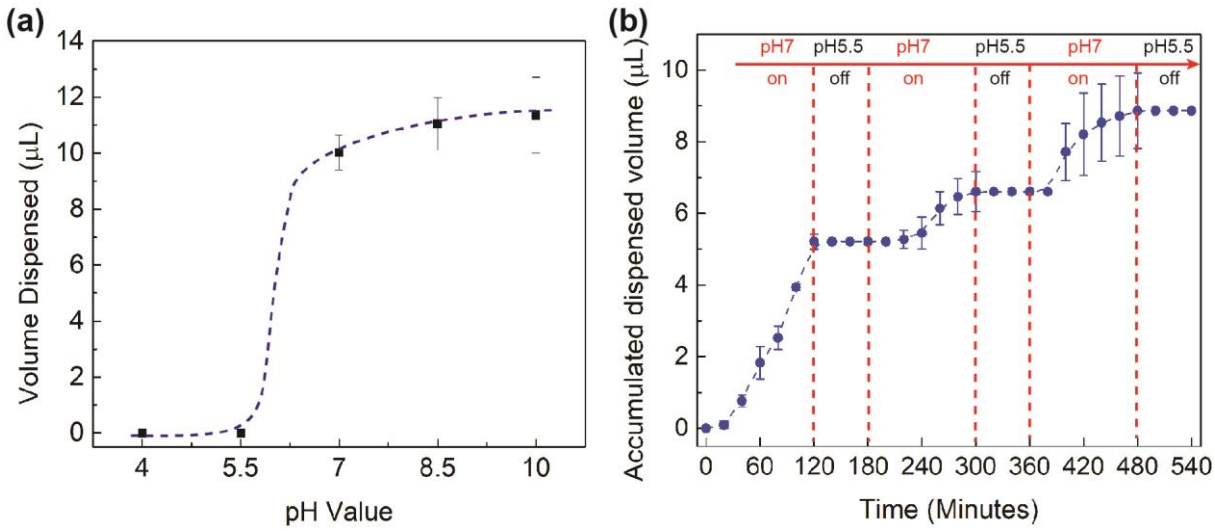

Fig. 8 The control experiments investigating the pump delivering drug (a) at different constant pH levels, the pump can deliver the aqueous load at a range of 10 to 11.4 µL over 4 hours from pH 7 to 10 whereas maintains a zero delivery (thus zero passive delivery rate) from pH 4 to 5.5; (b) at alternate pH values of 7 and 5.5 over 9 hours (three cycles of high-to-low pH), the device presented an "on-off" response achieving a total dispensed volume of 8.9 µL. Each data represents a measurement in triplicates.

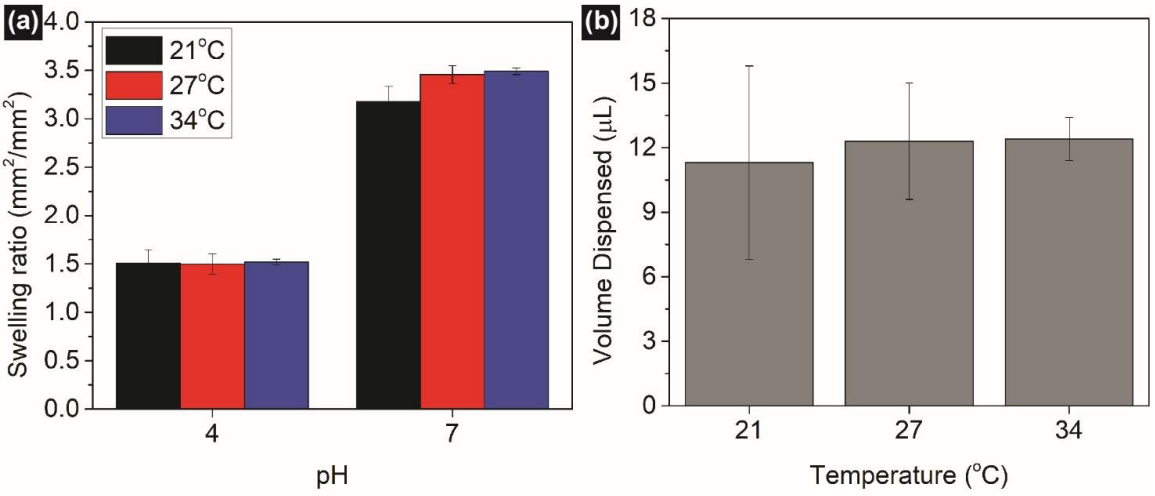

Fig. 9 (a) pH-sensitive hydrogel swelling kinetics at different temperatures (21°C, 27°C and 34°C) and pH levels (4 and 7). At pH 4, the hydrogel swelling shows negligible temperature dependence (~x1.5 swelling ratio); whereas at pH 7, the hydrogel swelling exhibits a slight temperature dependence (x3.2 at 21°C to x3.45 at 27°C and x3.5 at 34°C). (b) Drug delivery capacity of the single cell at different temperatures, 12.4 µL and 11.3 µL dispensed at 34°C and 21°C, respectively. Each data represents an experiment in triplicates.

1 hour. Moreover, the next two cycles illustrate a similar pattern; the pump begins drug release only when in high pH and stops dispensing when transferred to low pH. Finally, over 9 hours, the pump reaches a total dispensed volume of 8.9 µL. Both control experiments indicate that when working in the non-infected or healed wound regions (both at pH < 6), the device stays at a zero passive drug delivery rate; whereas, when serving the infected region (pH > 6), the device starts pumping the drug.

Robustness

As discussed previously, the device is designed to be functionally wearable for at least 4 hours (representing one working period of the device). This was tested by integrating the patch (2 x 2 array) into a medical adhesive film and fixing the system on the human forearm. The mechanical stability tests were conducted for up to three treatment cycles (each 4 hours) under normal daily activities. During a total of 12 hours, the device was firmly attached to the skin and there were no drop-off or pop-out, thus confirming the system's capability of working under normal use conditions.

At infected regions, the skin temperature typically increases several degrees of Celsius. This should not disturb the operation of the device, i.e., the operation should be solely dominated by the surrounding pH variations. Fig. 9 (a) shows the pH-sensitive hydrogel swelling kinetics at different temperatures and pH values (4 and 7 buffer, respectively). At pH 4, different temperatures caused no noticeable difference on the hydrogel swelling as the swelling ratio in each case was around x1.5 ± 0.1. A difference was observed only when the gel was at pH 7, in that case, the swelling ratio exhibited an increase from x3.2 ± 0.16 to x3.45 ± 0.1 and x3.5 ± 0.03 at 21°C, 27°C and 34°C. This is, however, small enough (x0.023/°C) not to significantly interfere with the pH-regulated drug delivery process, particularly in the limited temperature difference (< 5°C) between the uninfected and infected wound regions [23,24]. This was also confirmed by investigating the performance of the device on the agarose gel, i.e., when raising the temperature from 21°C to 34°C, the dispensed

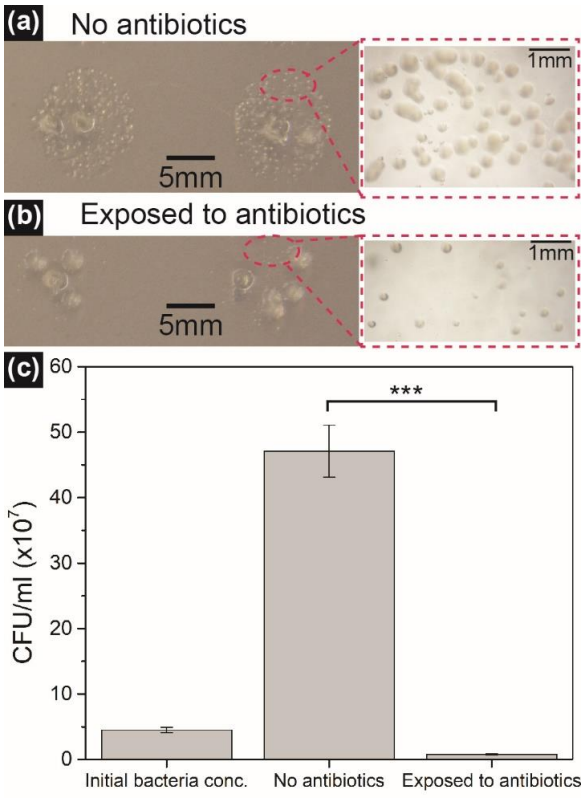

Fig. 10 Microscopic photographs of colony-forming bacteria (at four times dilution) after 24 hours (a) of uninterrupted growth in LB medium and (b) exposed to antibiotics released from the pump. The comparison shows a significant decrease in bacteria concentration. (c) The quantification of bacteria in terms of CFU/ml. Without antibiotics treatment, the initial bacteria concentration of $4.5x10^7$ CFU/ml is able to grow freely and reach $47x10^7$ CFU/ml (10.4 times increase); whereas with a 24 hours pH-triggered antibiotics treatment the concentration decreases to $0.8x10^7$ CFU/ml (5.6 times lower). Each data represents the average of six experiments. Three asterisk (*) indicates p value smaller than 0.001 ($p<0.001$).

volume only increased by ~10% (from 11.3 ± 4.3 µL to 12.4 ± 1 µL), Fig. 9 (b).

***In-vitro* experiments**

The *in-vitro* experiment was conducted by placing a single pump on top of 3.5 ml LB containing 50 µl *P. aeruginosa* and allowing the pump to release the antibiotics for 24 hours. The control experiment used the same amount of bacteria cultured in LB medium without the pump releasing the antibiotics, allowing a free growth of *P. aeruginosa*. The bacteria inhibition efficacy was evaluated by assessing the CFU assay of each sample after 24 hours incubation at 37ºC. Fig. 10 (a-b) show the comparison of bacteria colonies in the two conditions after four times of dilution, each using a ratio of 1:10. The microscopic photographs illustrated a significant reduction in the numbers of live bacteria before and after the pump-assisted antibiotics treatment. The amounts of CFUs differed by approximately one order of magnitude. This statistically significant difference (p value smaller than 0.001) was confirmed through the quantitative analysis of bacteria growth in terms of CFU/ml, Fig. 10 (c). The bacteria was cultured at the starting concentration of $4.5x10^7$ CFU/ml. After 24 hours incubation, it can either increase to $47x10^7$ CFU/ml or decrease to $0.8x10^7$ CFU/ml. The former, a 10.4 times increase, raised from the uninterrupted bacteria growth; whereas the latter, a 5.6 times of decreases, resulted from the pH-induced antibiotics treatment. Therefore, the total 58 times reduction of CFU illustrate the bacteria-elimination capability and feasibility of the device employed in the treatment of a bacterial infected chronic wound bed.

Conclusions

In this work, we presented a low-cost pH-regulated flexible passive pump for slow release of antimicrobial drugs within an infected wound environment. A single cell of the fabricated pump can achieve constant flow rates as small as < 0.1 µl/min over an extended period of time (4 hours) at backpressures of up to 8 kPa over 40 mm^2 spatial coverage. The device is scalable and multiple cells can be fabricated using the same process. Further characterizations revealed that the device start the pumping action at the infected wound regions over a wide range of pH (6 to 10) and can maintain a zero delivery rate at non-infected regions (pH < 6). Moreover, the device was shown to be capable of working functionally under normal daily activity for up to 12 hours within a wide range of temperatures (21ºC to 34ºC). The *in-vitro* bacteria inhibition capability was evaluated using CFU assay for two samples treated with and without antibiotics. A high efficacy in eliminating bacterial infection was illustrated by achieving a 58 times decrease of live *P. aeruginosa* after antibiotics release from the pump. It is also worth noting that this patch can incorporate other smart hydrogels, responsive to different stimuli (e.g., specific ions, glucose, etc.) in order to achieve a broader responsiveness to other wound biomarkers.

Conflicts of interest

There are no conflicts to declare.

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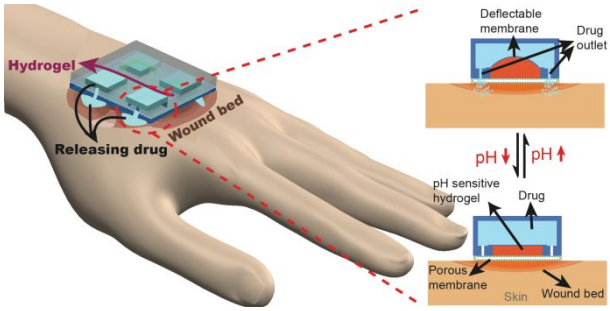

A low-cost, passive, and flexible dermal patch using wound pH to regulate topical drug delivery in chronic wounds