A pH-Mediated Electronic Wound Dressing for Controlled Drug Delivery

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A pH-mediated electronic wound dressing for controlled drug delivery

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Abstract

Topical administration of drugs in a timely manner according to the physiological need at the wound site can enhance the healing rate of chronic wounds. Existing wound dressings offer passive release of a single drug which may be ineffective for healing. Here we demonstrate an electronic wound dressing that enables active topical drug delivery in response to electrically induced pH change for potential treatment of chronic wounds. In this platform, the pH of the dressing is controlled using electrical field using an electronic circuitry. We show that this allows precise electrical control over the temporal profile of pH-mediated drug release. This engineered dressing is comprised of micro-fabricated electrodes serving as anode and cathode, pH sensitive hydrogel and control electronic circuitry. Anode is coated with pH sensitive PEGDA/Laponite hydrogel layer containing drug loaded chitosan nanoparticles (ChPs). Applying a DC voltage between the electrodes results in local change in pH near the electrodes due to combination of movement of ions from electro-osmosis and local redox reactions. In basic environments found near anode, the ChPs release their drug content due to the dehydration process, while in acidic environments the release profile is negligible. Turning off the DC voltage results in immediate pH recovery and cessation of drug release.

We confirm the biocompatibility of the dressing and show that the pH shift resulting from the applied electrical voltage does not affect the wound pH significantly. This platform can provide

precise control on the temporal profile and drug dosage, improving the therapeutic effectiveness while reducing unwanted side effects.

Introduction

Normal wound healing has three phases: inflammation, proliferation and remodeling with wound contraction. Wounds are chronic when they have failed to progress through the normal stages of healing and therefore enter a state of pathologic inflammation [1]. Different pathologies can cause chronic wounds such as diabetes, vascular insufficiency, neurological defects, and advanced age. These wounds continue to be of major concern for health care providers; increasing healthcare costs. An aging population, increasing incidence of diabetes and obesity is making the treatment of chronic wounds more challenging [2]. More than 7 million individuals are affected by chronic wounds in the US and require regular screenings by medical professionals. More than $3 billion per year is spent on the associated care [2]. Furthermore, chronic wounds are prone to severe complications, which not only affect the time to heal but also have a negative impact on the patients' quality of life [3].

Existing wound dressings such as Alginate, foam, hydrogel dressings are designed to cover the wound to maintain humidity and apply appropriate pressure on the wound [4] [5] [6] . Some of these dressings may provide a passive release of one drug such as a growth factor to facilitate the wound healing [7], which alone may not be sufficient to complete the healing process [8]. Infection is a common problem of chronic wound, which can delay the healing process. Thus, current treatments for infected wound necessitate combination therapy to enhance tissue regeneration and limit infection [9] [10].

Smart wound dressings can provide superior healing support by enabling the on-demand

release of multiple drugs [11]. The controlled release of drugs can provide more efficient therapy by reducing side effects and enhancing patient compliance [12]. Smart wound dressings are typically made of stimuli-responsive particles and a controller [9]. There are two different types of stimuli-responsive particles used for drug delivery depending on the source of stimulation factor. If the system responds to local changes in environment, it is called self-regulated or a closed loop system and this normally occurs with enzyme or competitive substances. On the other hand, externally regulated mechanisms governed by outside stimulation forces such as ultrasound, temperature, electric and magnetic fields provide more user control [13].

Use of an externally regulated stimuli-responsive system enables the immediate treatment and precise release profile control by adjusting the rate and dosage, externally. Recently, we have used temperature as a stimulus for a smart dressing through use of the thermo-responsive particles [9] [14] . However, the wound's temperature varies from patient to patient and the temperature of the environment prevents precise control on release mechanism. Recently, pH has been introduced as an alternative stimuli-factor for drug delivery applications; a slight pH difference between healthy and cancerous tissue was exploited to design anticancer drug delivery systems [15]. For example, biocompatible polymers such as chitosan made from the chitin shells of crustaceans, can undergo pH dependent swelling due to the protonation of amino group (pKa ~6.3). This has been used to release an encapsulated drug such as tumor necrosis factor alpha (TNFα) in the local acidic environment of tumor tissues [16]. pH responsive materials have also been widely used for oral drug delivery to protect the drugs from harsh environments in stomach and to improve the drugs absorbance in the intestine [16]. However, these studies were limited to passive delivery of the drugs by swelling and dehydration of pH

responsive materials. In addition, there is a lack of precise control over the release profiles.

Incorporation of pH responsive materials for active delivery in management of chronic wounds would be a superior alternative, as it is not significantly affected by external environmental factors such as temperature [17] . Only severe infection of the chronic wound might change the pH significantly. And such a change can only have a beneficial effect on would through pH based release of drug into the wound without any adverse effect.

In this research, we engineered a pH responsive wound dressing capable of on-demand release of drugs by electronically controlling local pH. Use of an electronically regulated system enables on-demand delivery by temporal control of the release profile.

Results and Discussion

The proposed pH responsive wound dressing is composed of an electronic driver, microelectrodes fabricated on a flexible substrate, and a PEGDA/Laponite hydrogel containing chitosan particles (ChPs) as drug carriers (**Figure 1**). Zinc and copper wires serve as anode and cathode respectively. Anode was covered with the hydrogel containing the drug carriers, while the cathode electrode was left uncovered. Application of an electrical voltage changes the local pH at the hydrogel surrounding the anode electrode. There is no significant pH alteration away from the electrode. There is no change in wound pH. Change in pH is a result of multiple factors. The applied voltage creates a flow of positive and negative ions in the presence of electric fields. Positive ions move away from the anode to create a local basic environment. Moreover, the electrolytic microenvironment also results in redox reactions at anode and cathode. The combined effect is a more basic environment at the anode. The concentration of ions moving toward the cathode electrode is negligible in a bulk solution (wound exudate) and does not

change the bulk pH. The pH change in hydrogel at the working electrodes causes the ChPs to release a desired dosage of drugs *in situ* due to the dehydration process. This local pH change is reversible by removing the applied field, which can be controlled temporally to provide electronic control over pH within the hydrogel dressing for controlled drug release.

To develop pH responsive drug delivery platform, ChPs were fabricated as pH sensitive materials. The nanoparticles with a drug encapsulation efficiency of 18.5 % were fabricated using ionically cross-linked approach detailed in experimental section. In summary, the electrostatic interaction between positively charged chitosan (0.2% in citric acid) and negatively charged tripolyphosphate (TPP) (1 mg/ml in water) lead to the formation of ChPs under ultrasonication. The concentration of chitosan solution and ultrasonication speed and duration, controls the size of the particles.

As previously shown, Chitosan is a polycation polymer which responds to pH variation above the 6.5 and experiences deformation in non-healing chronic wounds [18]. ChPs deprotonates in high pH and its charge accumulation changes. ChPs demonstrated a zeta potential of +40, +25, +1.5 and -10 mV respectively in the solution with pH equal to 2, 5.5, 7.4 and 14, which confirms the pH responsivity (**Figure 2A**). To study the behavior of the chitosan particles loaded in the hydrogel four points in different range of pH were selected. In basic condition (pH=14) chitosan particles have the greatest size change, and subsequent drug release. pH 2 was selected as a representative of the acidic condition where particles have very low activity. While the pH of 5.5 was selected as a pH of normal skin and particles did not show any significant activity. Finally, pH of 7.4 was chosen as a natural point and the starting point of a significant change in particles size.

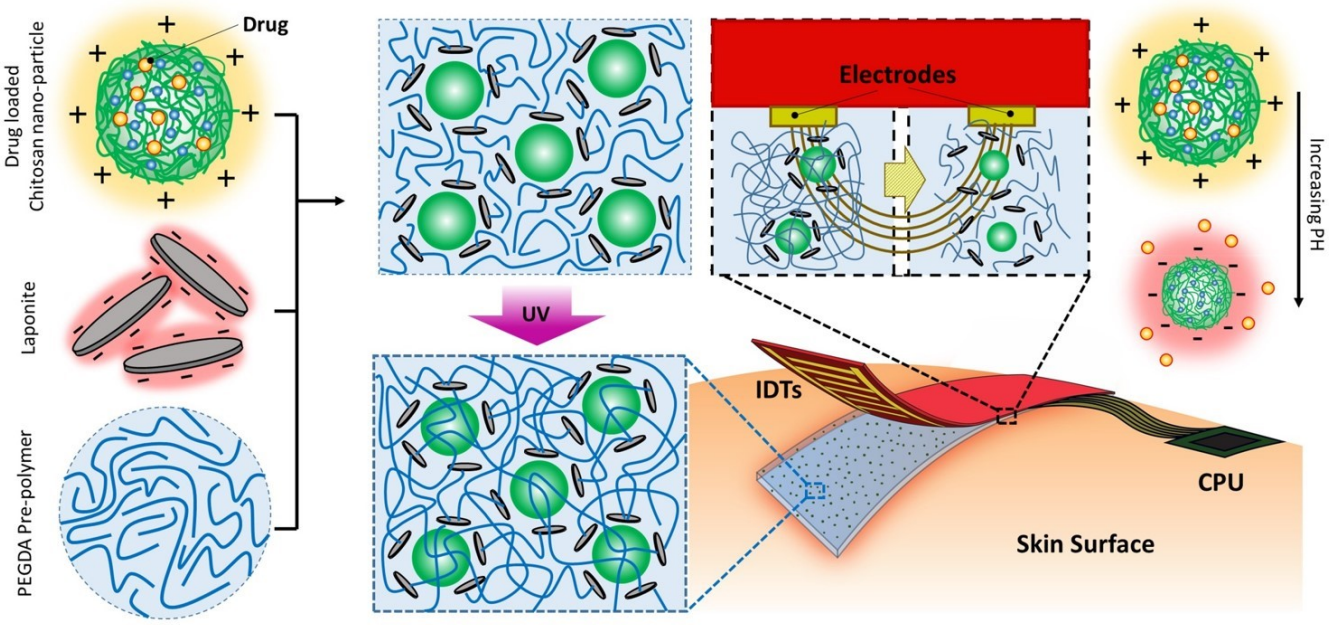

Figure 1. Schematics of an integrated electronic wound dressing for the transdermal drug delivery in which a pH responsive hydrogel layer carrying drug loaded chitosan nanoparticles was placed on a flexible electrode and connected to a microcontroller that will power them up. The inset shows the mechanism of the drug release with the application of electrical voltage. The electrostatic interaction between negative face of Laponite and positively charged chitosan nanoparticle in an acidic environment is reduced in basic environment and leads to the drug release.

A hybrid hydrogel of Poly (ethylene glycol)-diacrylate (PEGDA) and Laponite was used as the dressing material in which ChPs carrying the desired drug were embedded. PEGDA is an inert and biocompatible hydrogel, which is not cell adhesive and is a promising material for wound dressings. In addition, Laponite as a synthetic silicate nanoparticle consists of nanometric disks (25 nm in diameter and 0.92 nm in thickness) with non-toxic degradation products (Na^+, $Si(OH)_4$, Mg^{2+}, Li^+). Laponite nano-discs have negatively charged faces and positively charged edges, were added to PEGDA. The self-association of the positively charged ChPs and negatively

charged Laponite faces can prolong the release of drugs inside the ChP nanoparticles [19]. The competition between the positively charged ChPs and other cations in solution (Ca^{2+}, Na^{+}, and K^{+}) determines the amount of encapsulated ChPs into hybrid hydrogel. The stronger electrostatic interaction between chitosan nanoparticles and Laponite, the higher the encapsulation efficacy would be. The strength of this interaction calculated by the ChPs charge, which depends on pKa of chitosan and the pH of the solution. The dissociation of ChPs from Laponite happens when other cations take the place of ChPs by decreasing its charge at pH above 6.5 (pKa). Following the ChPs dissociation in basic pH, dehydration of ChPs forces drug to be released (**Figure 1**). The size of ChPs decreased from 458 ± 8 nm in pH 2, to 141.77 ± 20 nm in pH 14 which represents this dehydration process. The ChPs have the size of 255 ± 12 nm and 341± 10 nm in the pH of 7.4 and 5.5 respectively (**Figure 2B**).

Copper electrodes with thickness of 300 nm were fabricated on a parylene layer as a flexible substrate using standard photolithography, and then electroplating was used for electrodeposition of zinc an anode electrode. Zinc and copper electrodes served as anode (positive terminal) and cathode (negative terminal). The application of a DC electrical field lead to local pH change attributed to multiple factors, primary among which is the movement of charged ions toward electrodes of opposite polarity (electro-osmosis). Other factors include possible redox reactions of electro-active species found in wound exudate (eg. Sodium, potassium, chloride etc) and electrolysis of water [20]. The net observable effect is a result in a pH gradient between electrodes at the application of DC voltage between them. The motion of positive ions such as H^{+} toward the cathode leads to temporary acidic pH around it. At the same time, negative ions move toward the anode resulting in basic pH around anode. The local pH change around electrodes is temporary and the removal of the applied electrical stimulation

results in recovery of original pH due to the reverse diffusion of hydrogen, hydroxyl and other ions [20]. The kinetics of ions is voltage dependent, which affect the rate of pH change around electrodes, as with 4 V, the rate of pH changing was faster than with 2.5 V. The maximum pH of 14 was achieved in 2 seconds by applying 5 V (**Figure 2C**). The rate of pH change also has a direct relationship with the distance from electrode. A pH of 12 was achieved in 2 seconds around anode with the application of 4 V (**Figure 2C**), however it took 5 minutes to reach that pH in distance of the 2.5 cm from anode (**Figure 2F**). In addition, the pH buffering capacity of the electrolytic microenvironment (phosphate buffer solutions (PBS) in the experiments) keeps pH constant in the region far from electrodes and prevents abrupt pH changes in the solution. Electrical stimulation around anode (4V and 2.5V) for one-minute duration confirmed the transient pH change near the anode. The basic pH around anode returns to its initial value after discontinuing electrical stimulation (**Figure 2D**). We demonstrate the reversibility and reproducibility of pH change around anode by the application of electrical voltage in an on/off pattern (**Figure 2D, E**).

We conducted *in vitro* drug release test at different pH which could be a result of electrical stimulation as explained earlier. FITC was used as a drug model to study the release profile. The drug release profile followed distinctive patterns at different pH. At pH above 6.5, some of the positive-charged chitosan particles were balanced, thus the ionic interaction between chitosan and TPP was diminished and caused them to dissemble. In addition, the electrostatic attraction of chitosan particle and Laponite faded away due to dropping the particles zeta potential. However, in acidic pH, the protonated chitosan formed a strong ionic bond with TPP and retained drug inside the particle. **Figure 2G** shows that 3.54% and 7.46% of drug was released after 6 hours in pH of 2 and 5.5, respectively. However, 62% and 100% of drug was released in

pH 7.4 and 14 within same time, respectively. The release rate was also controlled by switching the electrical voltage on/off which makes the hydrogel basic/acidic (**Figure 2H**). Figure 2H shows the temporal control of the drug release profile with application of different pH. Higher pH results in greater release rate.The dissociation of ChPs from Laponite happened at pH above 6.5, and the ChPs were substituted with another cation like Na^{+} due to the reduction in ChPs positive charge. The replacement of ChPs by simple cation like Na^{+} or Ca^{2+} in acidic pH is difficult to achieve due to the stronger interaction that positively charged ChPs could make with negative face of Laponite [21]. The release profile was accelerated with increase of pH from 7.4 to 14. More OH- groups enhanced the degradation rate of the PEGDA and chitosan particles.

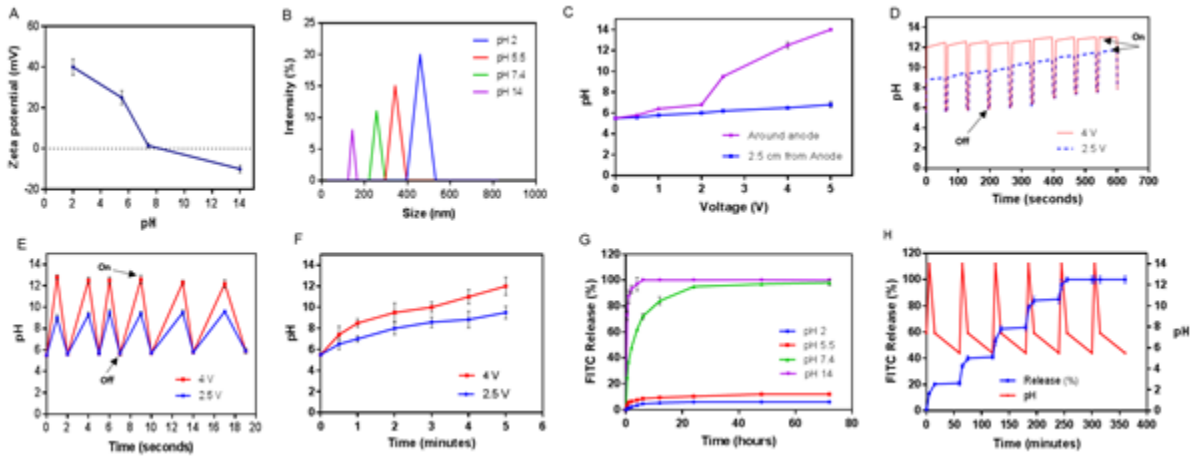

Figure 2. Evaluation of pH responsive drug-eluting patch in different pH. A) ChPs charge is pH responsive according to the Zeta potential result in different pH B) The reduction in ChPs size by changing the pH from 2 to 14 confirmed the de-swelling process of ChPs, which was due to the decrease in positive charge of ChPs. C) The rate of pH changing depends on the applied voltage and the distance from electrode, the pH of 14 was achieved in 2 seconds with 5 V however, in distance of 2.5 cm from anode, the pH changing was so slow. D) The application of electrical field for one minutes lead to the increase in pH around anode, and after discontinuing the electrical stimulation, the pH returned to its initial value and this process was reversible. E) The dramatic pH changes around electrode was transient and controllable by the application of electrical field. F) The rate of pH changing in distance of 2.5 cm from electrode with constant application of electrical field for 5 minutes. G) Release profile of FITC (as a drug model) from ChPs embedded in PEGDA/Laponite hydrogel in different pH showed the abrupt release of drug in pH 14

and slow release of drugs in acidic pH. H) Graph showing the control of drug release rate with pH changing. Large release slope in pH 14 decreased when pH became 7.4 and then 5.5.

Hydrogel Characterization

Physicochemical properties of PEGDA/Laponite/ChPs in different pH, degradation of hydrogel and diffusion of drug play a critical role on drug release profile and require precise characterization. The addition of chitosan particle to PEGDA/Laponite increased the pore size of the hydrogel as shown in SEM image (**Figure 3A)**. Comparing the swelling ratio of PEGDA/Laponite with and without ChPs proved that the addition of ChPs increased the swelling ratio from 290 % to 390% after one-hour incubation in pH 5.5 due to the presence of hydrophilic chemical groups such as NH_4^+ in structure of ChPs (**Figure 3C**). In addition, more porosity facilitated the water penetration to the hydrogel (**Figure 3D**). The amine groups of chitosan are neutralized in basic pH, however; the negative charged of Laponite remains causing the electrostatic repulsion of Laponite layer [22]. Therefore, more space would be provided to absorb water (**Figure 3D**), which lead to burst swelling around 800% in pH 14 after one hour. In pH 14, OH^- attacks

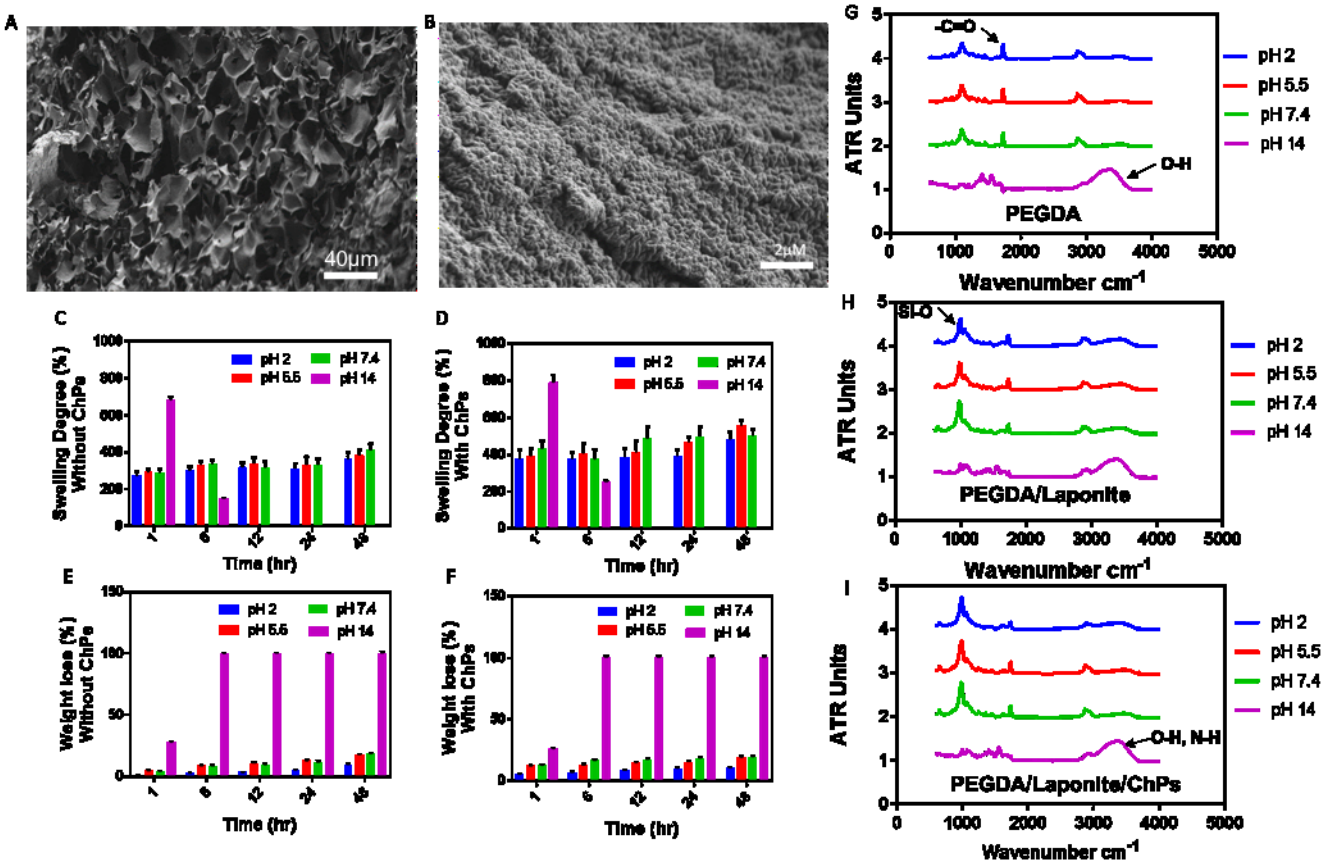

Figure 3. Hydrogel characterization of pH responsive drug-eluting patch in different pH. A-B) The SEM result showed the increase in porosity of the hydrogel by the addition of chitosan nanoparticle. (A: PEGDA/Laponite/ChPs (Scale bar: 40µm), B: PEGDA/Laponite (Scale bar: 2µm)). C) The abrupt increase in swelling degree in basic pH after one hour, D) and the role of chitosan nanoparticle in increasing the swelling degree of hydrogel. E) The rapid degradation of hydrogel in pH 14 due to the hydrolysis of PEGDA, after 6 hours the whole hydrogel was disappeared. F) The addition of ChPs increased the degradation rate of hydrogel G-I) The appearance of new peaks and disappearing of the old peaks in FTIR result at pH of 14, in compare to other studied pH, confirmed the degradation of hydrogel. Each peak belongs to specific chemical active group in hydrogel (G, PEGDA, H, PEGDA/Laponite, I, PEGDA/Laponite/ChPs)

the carbonyl group in acrylate ester of PEGDA and accelerates the degradation which cause the decrease in swelling degree after 6 hours [23]. Taken all of these aspects together, the faster drug release in basic pH compared to acidic pH can be explained through the rapid degradation

and burst swelling ratio of hydrogel. After 6 hours' incubation in pH 14 the hydrogel was degraded completely (Figure 3E, F), this can be confirmed by the FTIR result as well. The similarity between PEGDA/Laponite and PEGDA/Laponite /ChPs FT-IR confirmed the complete intercalation of chitosan nanoparticle in Laponite, the chitosan amine groups fully covered by Laponite hydroxyl group. However, after one-hour incubation in pH 14, a wide band around 3300 to 3450 cm^{-1} (the stretching vibration of N–H and hydroxyl group) appeared related to the chitosan de-intercalation from Laponite. The band at 1560 and 1080 cm^{-1} belonged to the chitosan amide II (N–H bending vibrations coupled to C–N stretching vibrations) groups, and glycosidic linkage (ether bond), respectively [24]. The presence of Laponite inside the hydrogel was verified by FTIR analysis (Figure 3 G-I). The infrared spectrum of the PEGDA containing Laponite was reported with a peak in 1000 cm^{-1} which correspond to Si-O stretching in Laponite. Other peaks at 1000 cm^{-1} corresponded to Si–O stretching in Laponite and the peak around 2882 cm^{-1} corresponded to alkyl-CH stretch in PEGDA and the peak at 1732 cm^{-1} is assigned to the –C=O stretching from ester bonds in PEGDA which in pH 14, it was disappeared (**Figure 3G, H, I)** [25].

Biocompatibility

Wound dressing should not be cytotoxic or should not negatively affect cellular growth. Thus, the engineered dressings were interfaced with the culture of human 3T3 cells and potential toxicity of the engineered dressing was assessed by applying different electrical voltage to electrode, which results in different values of pH in vicinity of electrodes. We did not observe significant change in cell growth by application of different electrical voltages for the range shown. The pH changes only happened in vicinity of the electrode not in the bulk solution and it does not affect the cell growth significantly.

The biocompatibility of electrical field and its effect on cell proliferation was investigated in several studies [26] [27] [28] [29]. Exposing fibroblast to continuous electrostatic field of 1000 V/cm caused the increase in DNA and collagen synthesis after 14 days, and high voltage pulsed current induce the activity of fibroblast [24] [26]. The suggested mechanism for the fibroblast increased cell activity was attributed to the opening of voltage sensitive calcium channel in cell membrane and upregulating of insulin receptor on the cell surface, which triggered the DNA and collagen synthesis [30] [31] .

The localized pH changes around electrode during electroporation showed decrease of cell viability. It was shown that the pH 12.5 and above caused cell lysis due to the damage of cell membrane by hydroxyl ions. However, no abnormal cell morphology was seen after the alkaline stimulation with pH value 12 for 10 seconds[16]. It was shown that there is a critical pH value of cell viability. Above pH 13, almost no cells survives. In addition longer pH shock lead to the less cell viability. The alkaline environment cause more severe damage to cells than acidic environment.

Here, scratch assay was employed to evaluate the effect of the pH change around the electrode on cells viability and migration. Three group were selected: group 1 as a control sample without application of the electrical voltage, group 2 and three were the samples exposed to 2.5 V and 4 V for 5 minutes. The viability of cells was assessed by live/dead assay for day one, three and seven (**Figure 4A**). Wound-edge Cells extended in to wound site and aligned perpendicular to wound direction in all groups. The pH changing around electrode did not show to have any toxic effect on cell growth at in vitro wound closure test.

The growth of cells was also watched in an independent experiment under microscope for 48 hours to measure the rate of wound closure. Although, there were significant differences between group 2 and 3 and control group (*P<0.05) at each time point, after 48 hours, the wound width percentage reached to less than 5 % in all groups of studies (**Figure 4C**). Here, the most important point was to assure the normal growth of cells after exposure to electric field since the temperature and pH changing induced by electric current may affect cell growth [24]. However, in our dressing the pH change happens in vicinity of electrode not in bulk solution (**Figure 2C**). Therefore, we did not see any toxicity and significant change in cell growth with voltage application.

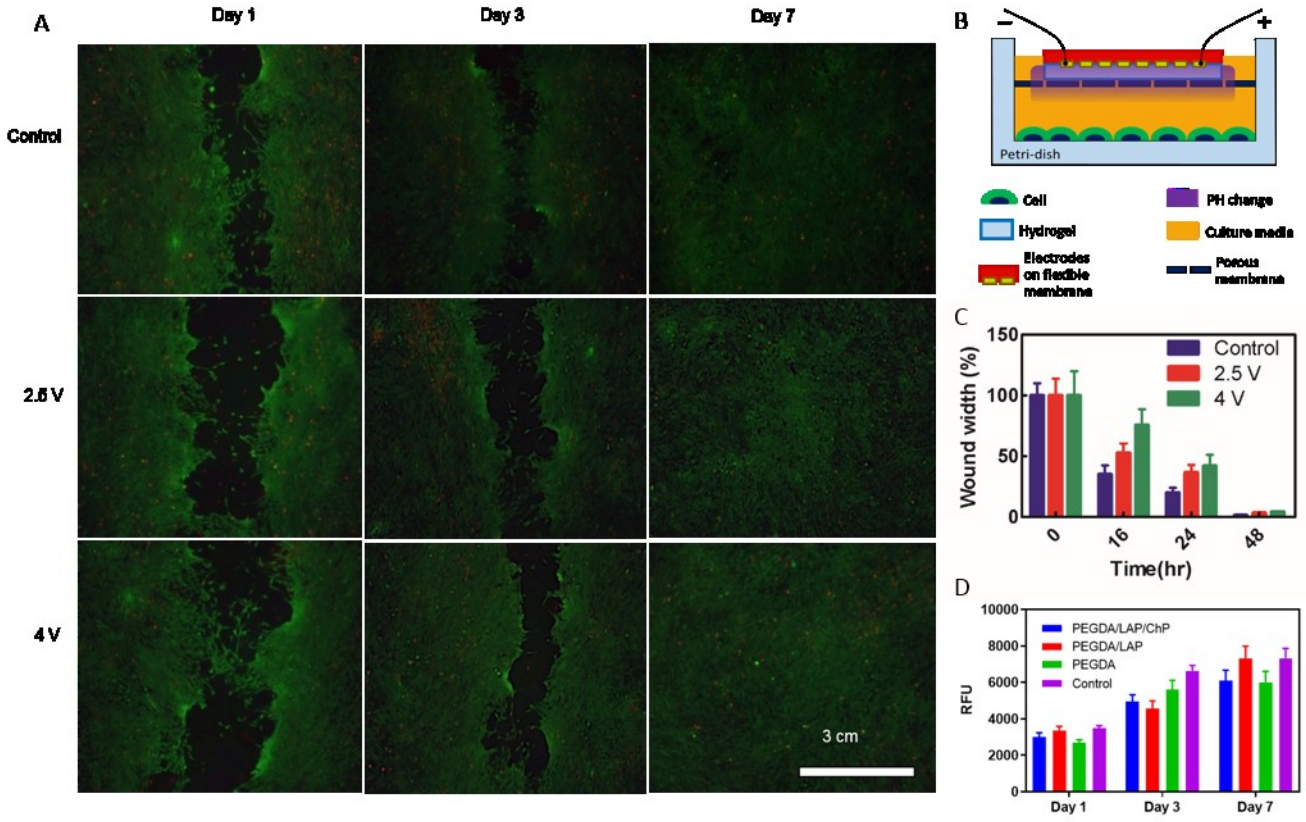

Figure 4. In vitro experiments to test the biocompatibility of the electronic wound dressing. A, B) In vitro scratch wound assay, application of 4 V, 2.5 V in contact with 3t3 for 5 minutes prior to the scratch test

and control group without exposing to electric field. The live/dead images showed the wound closure after 7 days with high cell viability as green color present live cells and red dots present dead cells. C) Result of wound closure measurement after 48 hours (p> 0.05) confirmed the normal growth of cells after exposure to electric field. D) PrestoBlue assay after 7 days of 3t3 plating on to hydrogels showed the biocompatibility of the proposed system.

We also put fibroblast in contact with our hydrogel wound dressing and conducted proliferation test to assess the cytocompatibility of the PEGDA/Laponite /ChPs hydrogel. No significant difference in proliferation activity of fibroblast was seen between PEGDA/Laponite, and PEGDA/Laponite /ChPs, PEGDA and control groups (*P> 0.05) (**Figure 4D**).

Conclusion

In this work, we developed an electronic wound dressing made of pH responsive material for treatment of chronic wounds. The proposed wound dressing consists of an electronic driver, microelectrodes fabricated on a flexible substrate, and PEGDA/Laponite hydrogel embedded with chitosan drug nano-carriers. We illustrated the on-demand delivery of drug due to the de-swelling (dehydration) of chitosan nanoparticle in response to an alkaline environment. Alkalinity is introduced around the electrode by electrical stimulation. This effect is transient and removal of electric field returns pH levels to normal. This external pH regulation mediated via electric field is ideal for external control of drug delivery. The wound closure and cell viability studies confirmed the biocompatibility of the electronic wound dressing. Controlled release of drug from chitosan nanoparticles was confirmed due to the alkaline environment around anode. Detailed experimental studies and characterization validate the function and efficacy of the wound dressing platform.

Experimental section

Material

Chitosan (molecular weight), Laponite, poly (ethylene glycol) diacrylate, DAPI were obtained from Sigma-Aldrich (St. Louis, MO, USA). 2-hydroxy-1- (4-(hydroxyethoxy) phenyl)-2-methyl-1-propanone (Irgacure 2959, CIBA Chemicals) was used as a photoinitiator (PI). Dulbecco's modified Eagle medium (DMEM), 0.05% trypsin-EDTA (1X), fetal bovine serum (FBS), Live/Dead kit and antibiotics (Penicillin/Streptomycin) were acquired from Invitrogen (Carlsbad, CA, USA). PrestoBlue® Cell Viability reagent was received from Life Technologies.

1. pH change from applied voltage:

We placed electrodes with spacing of 5 cm in 20 ml release media (PBS) with pH of 5.5. We measured pH changes around anode after applying electrical stimulation with pH meter. First, we applied different voltage from 0.5 to 5 V and measured the pH changes around anode during electrical stimulation. The maximum pH of 14 was achieved with 5 V after 2 seconds, therefore we compare the pH around anode after 2 seconds of electrical stimulation with voltage less than 5 V. In another experiment, we applied 2.5 and 4 V in the on/off pattern for 19 seconds to demonstrate the pH changing reversibility around anode. Then in a cyclic pattern with the interval of one minute, we applied 2.5 and 4 V for 9 cycles. We also calculated the rate of pH changing in the distance of 2.5 cm from anode and cathode with the application of 2.5 V and 4 V for 5 minutes. We repeated each test 9 times.

2. ChPs preparation:

We prepared 0.2 % chitosan aqueous solution in citric acid 5% containing drug (FITC), and then we ultrasonicated it for one minute, and added TPP solution in a ratio of 1:5 chitosan

solution/TPP and continued the ultrasonication process for 4 minutes. We centrifuged the solution to separate the chitosan Nanoparticle at 14000 rpm for 20 minutes. We removed supernatant and washed chitosan nanoparticle with four different pH buffer, pH 2, pH 5.5, pH 7.4 (PBS) and pH 14 and then dispersed in PBS. The size and zeta potential characterization was done with Zetasizer Nano-ZS90 (Malvern Instruments), the measurement was performed at a scattering angle of 90° at a temperature of 25°C.

3. Electrode fabrication:

To fabricate the electrodes, the previously reported approach was used. In summary, first a 25 μm parylene was deposited on a silicon substrate. Then, the photolithography, Ebeam deposition and lift off process were employed to pattern the Ti/Au electrodes on the surface of the substrate. At the end, parylene was peeled off from silicon.

3. In vitro drug release test:

We re-suspended ChPs in PEGDA 10%, PI 0.5%, Laponite 1% aqueous solution and UV cross-linked for 3 minutes, and then the known mass of crosslinked sample with approximate dimension (1 cm ×1 cm) placed into 2ml of release media under constant stirring at 36.5°C. Four different release media with pH 2, pH 5.5, pH 7.4 (PBS) and pH 14 were used to study the effect of pH, on drug release. At each time interval, we replaced the whole 2 ml of released media with fresh media to guarantee the continuation of the release. We measured the concentration of released drug with ultraviolet-visible spectrometer (UNICAM Series 8700 model, Philips Co., Amsterdam, the Netherlands) using the calibration curve prepared by measuring the intensities of known concentrations in different pH in order to determine the concentration of the released drug models (FITC-dextran: λex 485 nm and λem 530 nm). We repeated this test three times,

each time with three samples for each group. Then we normalized the results and converted it to percentage cumulative.

4. SEM Imaging:

Freeze-dried PEGDA/Laponite and PEGDA/Laponite/ ChPs were coated with a thin layer of gold by a Bio-Rad E5200 auto sputter coater (England). We used Scanning electron microscope (CamScan MV2300 model, Oxford) with 5000X magnification to study the effect of addition chitosan particle on hydrogel pore size.

5. FTIR Study:

After one-hour soaking the samples in different pH, we freeze dried them and studied their chemical structures with Fourier transform infrared (FTIR) spectroscopy using a Spectrum 100 FTIR spectrometer (PerkinElmer, Massachusetts, USA) fitted with an ATR attachment with the range of 4000–600 cm^{-1} for scanning, and the resolution set of 1 cm^{-1}

6. Swelling and degradation test:

We immersed the known weight of Crosslinked samples in 4 different released media at 36.5°C for 1, 6, 12, 24 and 48 hours. At each time point, we took the samples out of the solution, removed the excess solution on their surface and weighed the samples using a high precision scale, and then we put samples in oven 45 °C to reach a constant weight in order to measure the degradation ratio.

Swelling degree: $(M\text{-}M_i/M_i) \times 100$

Degradation percentage: $(M_d\text{-}M_i/M_i) \times 100$

M is the swollen weight of sample, M_i is the initial dry mass of sample and M_d is the dried mass of immersed sample in release media. We repeated this test three times, each time with three samples for each group.

7. Biocompatibility:

We cultured Fibroblast in a DMEM containing 10% FBS and 1% penicillin/streptomycin at 37°C and 5% CO_2. We passaged Cells at 80% confluency. We added 100 000 cells into six well plates and incubated at 37 °C. PrestoBlue cell viability assay was used to measure cellular metabolic activity after treating cells with PEGDA, PEGDA/Laponite and PEGDA/Laponite/ ChPs. Each well was incubated for 1 h at 37 ◦C with 400 µL of a solution containing PrestoBlue reagent and cell culture media in a ratio of 1:9. By subtracting the samples fluorescence values obtained from reader (Synergy HT-Reader, BioTek, Winooski, VT) from the background, the fluorescence values were determined for each day. Higher fluorescence values were associated with greater total metabolic activity. The test was repeated three times for each sample.

Wound Scratch assay: Cells were seeded into six-well plates at 37 ◦C and 5% CO_2. Once a confluent monolayer of cells was achieved, with pipette tip, 1 mm wound width was created, then suspended cells were aspirated and fresh media was added to each well plate. Cell cultures were divided into three groups: group one and two were exposed to 2.5 V and 4 V for 5 minutes respectively and group three stayed as control group. We took Images at time 0, 16, 24, 48 hours after scratch with an inverted microscope, and the wound width percentage calculated at each time point as following: (W_t: the width of wound at time t, W_0 is the width of wound at time zero)

Wound width percentage: $W_t/W_0 \times 100$

8. Live/dead assay:

We performed live/dead assay to determine the viability of cells after exposure to 2.5 V and 4 V for 5 minutes. We used the Live/Dead kit (Invitrogen, USA) according to the manufacturer's instructions; ethidium homodimer-1 (2 µl/ml) and calcein AM (0.5 µL mL−1) were mixed in PBS. Cultures were incubated with the prepared solution at 37 ◦C for 15 min and were washed with PBS. Stained samples were imaged with an inverted fluorescence microscope (Nikon TE 2000-U, Nikon instruments Inc., USA).

9. Statistical Analysis:

Data were reported as mean ± standard error. One-way ANOVA and two-way ANOVA were employed for the analysis of the data using (GraphPad Prism 6.01) software. We had nine samples for each test. The p-value parameter illustrated the level of significance in comparison of data (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$).

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Summary:

This paper presents an electronic wound dressing made of pH-responsive drug-loaded hydrogel for treatment of chronic wounds. Local pH change is induced with the application of electric field between built-in electrodes. It demonstrates precise time controlled drug release, and confirms biocompatibility and in vitro wound closure using this pH-mediated electronic wound dressing.