A low-cost flexible pH sensor array for wound assessment

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Abstract

The pH level in a chronic wound bed is a key indicative parameter for assessment of the healing progress. Due to their fragility and inability to measure multiple wound regions simultaneously, commercial glass microelectrodes are not well-suited for spatial mapping of the wound pH. To address this issue, we present an inexpensive flexible array of pH sensors fabricated on a polymer-coated commercial paper (palette paper). Each sensor consists of two screen-printed electrodes, an Ag/AgCl reference electrode and a carbon electrode coated with a conductive proton-selective polymeric (polyaniline, PANI) membrane. Laser-machining is used to create a self-aligned passivation layer with access holes that is bonded over the sensing and reference electrodes by lamination technology. Characterization of the pH sensors reveal a linear ($r^2 = 0.9734$) relationship between the output voltage and pH in the 4-10 pH range with an average sensitivity of -50 mV/pH. The sensors feature a rise and fall time of 12 and 36 s for a pH swing of 8-6-8. The sensor biocompatibility is confirmed with HaCaT immortal human keratinocyte cells.

Keywords: Chronic wound, pH sensing array, paper substrates, rapid prototyping, laser micromachining

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1 Introduction

The pH level in the wound bed is a key indicative parameter for assessing the healing progress of chronic wounds [1,2]. Unlike healthy skin or healing acute wounds that have a slightly acidic pH (5.5-6.5), infected chronic wounds often exhibit pH values higher than 7.4 due to the alkaline byproducts of proliferating bacterial colonies. In many cases, the irregular vascular structure of the chronic wounds causes a heterogeneous distribution of infection in the wound bed, resulting in drastic pH variations throughout the affected area [3]. Commercial pH microelectrodes can be used to measure wound pH values, however, they are fragile and provide single point measurements, thus limiting their efficacy for 2D mapping and real time therapeutic feedback [4]. A more practical alternative would be a flexible array of pH sensors that can conformally wrap around the wound and generate a map of pH levels, thus revealing the location and severity of bacterial infections.

There has been no shortage of efforts in the miniaturization of pH sensors and their fabrication on flexible substrates. The majority of these sensors have been potentiometric [5–7] or ion sensitive field effect transistors (ISFETs) [8–10] fabricated on various polymeric substrates such as polyimide, parylene, or polyethylene terephthalate (PET). Although many of these sensors satisfy the operational metrics (i.e. sensitivity, resolution, and dynamic range) for wound pH mapping/monitoring; they all require cleanroom-based fabrication processes which are time-consuming and costly [11]. An interesting work by Schreml et al. uses luminescence imaging (time-domain luminescence of FITC and ruthenium(II)tris-(4,7-diphenyl-1,10-phenanthroline) for 2D pH mapping with a 5 μm spatial resolution [3,12]. This technique, however, requires complex chemical manipulations for the preparation and immobilization of the indicator dyes

into the microparticles. Another approach reported by Nocke et al. uses a pH sensor based on the chemomechanical transduction force generated by a pH sensitive hydrogel wrapped around a metallic wire (pH variations result in swelling/shrinking of the hydrogel which in turn changes the wire impedance) [13]. Although potentially flexible and wearable if woven into a fabric, the process of weaving the sensors in an array format without damaging the hydrogel is not trivial.

Recently, there has been an increasing interest in the low-cost paper based analytical systems, which are not only flexible but can also be disposed and recycled [14]. Two groups have recently reported on paper-based resistive [15] and potentiometric [16] pH sensors. The resistive one uses a simple fabrication process for printing carbon nanotubes on paper and uses their nonlinear response to $[H]^+$ for measuring pH. The potentiometric one, in contrast, utilizes an ion selective membrane covering carbon nanotube electrodes to measure the pH. The use of carbon nanotubes in these sensors significantly increases their cost and counteracts the rationale behind using paper as a low cost substrate material. In addition, issues such as integration of the reference electrodes on the same substrate, packaging, and passivation were not addressed and/or investigated; thus, reducing the impact of this work on real-life practical applications such as wound monitoring.

In this paper, we present an inexpensive and flexible array of pH and reference electrodes fabricated on a paper substrate. The sensor array is fabricated using out of cleanroom fabrication techniques including rapid prototyping tools (laser micromachining and laminating machines) and readily available substrates (polymer-coated palette paper), thus allowing for low cost mass production. This method features a simple technique using shadow/stencil mask patterning and creating a self-aligned passivation layer with access ports in the same setup. In Section 2, we will discuss the sensor array design and fabrication followed by the experimental and characterization setup in Section 3. This will be continued by results/discussion and conclusions in Sections 4 and 5.

2 Sensor design and fabrication

Fig. 1(a) shows the schematic of a 3×3 array of pH sensors on a paper substrate. Fig. 1(b) shows the cross section of the sensor array embedded into a wound dressing. Each sensor consists of two electrodes, an Ag/AgCl reference electrode and a carbon electrode coated with a conductive proton-selective polymer (polyaniline, PANI). The operation of the pH sensors is based on the well-studied protonation and de-protonation of nitrogen atoms in the polymer chains of PANI [17], Fig. 2. In acidic solutions the polymer is doped with H^+ ions to create the emeraldine salt (ES) form of PANI, which is known for its high electrical conductivity. The resulting surface charge increases the electrical potential of the sensing electrode relative to the reference electrode. When the polymer is exposed to the alkaline solutions, the captured H^+ ions are neutralized, resulting in a decreased polymer surface charge/potential. The deprotonated or neutralized form of PANI is referred to as its emeraldine base (EB) form and is not electrically conductive. The pH-dependent electrochemical equilibrium between the ES and EB moieties of PANI results in an inverse relationship between the electrochemical potential of the sensing electrode and the pH of its environment.

The fabrication process of the sensor array is illustrated in Fig. 3. First a single layer of adhesive tape (3M[®] MagicTape[™]) is attached to the polyethylene-coated side of the palette paper substrate (palette paper is used for oil/acrylic painting and has two different sides, one is polyethylene-coated while the other is similar to a regular paper surface), Fig. 3(a and b), and laser-machined (Universal Laser Systems, Inc., Scottsdale, AZ) to create a stencil mask, Fig. 3(c). The laser machining process ablates the cellulose acetate backing of the tape, leaving behind the stencil mask. To eliminate the need for subsequent alignment, the access ports/openings in the insulating part of the array (named sensor ports in Fig. 1(a)) is fabricated at the same time by laser machining, Fig. 3(c). Next, the carbon (Mgchemicals[®] Graphite

Conductive Coating) and silver (118-09, CreativeMaterials, Ayer, MA) inks are screen-printed onto the tape-covered palette paper, Fig. 3(d). The inks are allowed to cure at 100 °C for 30 min. After curing, the tape mask is peeled off leaving 9 carbon and 3 silver electrodes on the palette paper, Fig. 3(e). Subsequently, a layer of silver chloride is deposited on top of the silver electrodes by submerging the substrate into a 1.0 M NaCl solution and passing a 1 mA current for 2 min, Fig 3(f). This is followed by a DI-water rinse and dry-out at 70 °C for at least 3 hours.

The reference electrode electrolyte was prepared by mixing KCl with an UV curable epoxy (Henkel Loctite[®] 3105) in a 1:1 ratio to form a homogenous paste. The paste was then cast on the Ag/AgCl electrodes and cured under UV light for 10 minutes, forming a transparent solid electrolyte (SE), Fig. 3(g). The polyaniline solution was prepared by dissolving 25 mg of polyaniline emeraldine base in 25 mL dimethyl sulfoxide (DMSO). The base form was used since it is soluble in organic solvents such as DMSO and ensures a uniform coating on the carbon electrode. The solution was stirred for 5 hours before use. A 4 μ L volume of PANI solution (ion selective membrane) was drop-cast on the carbon electrodes and allowed to dry for 24 hours at room temperature, Fig. 3(h). Next, the PANI layer was doped with HCl in a vacuum chamber for 5 hours. The HCl vapor introduced H⁺ ions into the polyaniline emeraldine base (PANI-EB) film and produced the polyaniline emeraldine salt (PANI-ES). During the doping process the PANI base film changed color from dark blue to dark green; this form has a higher conductivity as compared to emeraldine base. The electrodes were then rinsed with DI-water and blow-dried using nitrogen. Finally, the substrate was folded in half to create a structure with 9 pH sensors and their interconnections sandwiched between two layers of palette paper bonded together using a hot roll laminator (Apache AL13P Professional) set at 130 °C, Fig. 3(i). This step produced a strong irreversible bond between the polyethylene-coated faces of the paper.

3 Experimental and characterization setups

3.1 Qualitative inspection

A scanning electron microscope (field-emission SEM, Hitachi S-4800) with an acceleration voltage of 10 kV was used to understand the microstructure of the PANI coating and SE on the printed carbon and Ag/AgCl electrodes. High resolution images were obtained to study the microstructure of the electrodes before and after the coating.

3.2 Preparation of the pH solutions

These experiments made use of various pH solutions in the range pH 4–10. Solutions with pH values 4, 7, and 10 consisted of commercial stock buffer solutions (Pinnacle pH Buffers, Nova Analytics) used directly. Additional solutions (pH 5, 6, 8, and 9) were prepared by mixing different ratios of the commercial solutions. Prior to all experiments, the pH of the solutions was verified with a commercial pH meter (Model IQ125, IQ Scientific Instruments, USA).

3.3 Reference electrode characterization

To investigate the effect of thickness of the electrolyte on the performance of the reference electrode, various thickness ranging from 200 μm to 3 mm were tested with respect to a commercial Ag/AgCl reference electrode

In order to evaluate the stability of the reference electrode at various temperatures, multi-temperature experiments were carried out at controlled conditions from 23 °C to 37 °C using water bath; these temperatures are physiologically relevant, as they span the range of typical skin and core temperature of humans [25]. The experiment was performed with 200 μm solid electrolyte coating. During each measurement at set temperatures the potential readings were recorded against a conventional Ag/AgCl reference electrode for 10 min.

Conductivity measurements of test solution were performed to assess the KCl leakage for the proposed solid reference electrode and results were compared with commercial reference electrodes. In this test the electrodes were immersed in 10 ml deionised water (DI) and under

continues stirring the conductivity of the solution was measured with an LCR meter (GW Instek LCR-819) for 24 h. Since the temperature of the solution will influence the leakage of the KCl from the polymeric matrix, leakage test was conducted at temperatures ranging from 25 °C and 37 °C.

3.4 Potentiometric measurements

The performance of the pH sensor array was evaluated via potentiometric measurements conducted in pH- and temperature-controlled solutions. These measurements were collected using data acquisition software (Agilent's IntuiLink) via a digital multimeter (Agilent 34401A) set to high impedance mode to minimize current draw from the sensor. For each test, the sensor array was immersed in a buffer solution for 2 minutes, and the potential between the reference and working electrodes was then recorded. This test was repeated for various solutions of pH 4, 5, 6, 7, 8, 9, and 10 (a wound-relevant range). The sensitivity of the sensor was extracted from these data. The sensors were rinsed with DI-water and blow-dried with nitrogen between each test to prevent cross-contamination.

3.5 Sensor stability

The response time of the sensor was measured by two methods. The first method consists of immersing the sensor in a low pH buffer solution and subsequently adding an alkaline buffer solution, all done while recording the output voltage of the sensor in real-time. In the second method, the sensor was placed in an alkaline solution, and an acidic buffer solution was subsequently added. During both experiments, the solution was continuously stirred. To evaluate sensor stability, the devices were immersed in different pH buffer solutions and the potential between the working and the reference electrodes was recorded as a function of time at a sampling frequency of 1 Hz over a period of 24 h (since these disposable sensors are not expected to be used much longer than that).

The effect of temperature variation on the pH measurements was evaluated by repeating the pH characterization described above using buffers at various temperatures within the physiologically-relevant range of 25–35 °C (i.e., close to human skin temperature).

3.6 Robustness

The quality of the interconnect passivation was investigated by measuring the leakage current between traces in a modified design. The experimental setup for these tests is illustrated in Fig. 4. Each test sample consisted of two conductive traces patterned on palette paper and sealed with a thermally laminated layer of various insulating materials (75 μm -thick Kapton[®] tape, Scotch[®] MagicTape[™], and palette paper). The samples were soaked in phosphate-buffered saline (PBS) solution and a 1 V bias was applied between two adjacent traces. The leakage current between the two electrodes was monitored over a period of 24 hours using a multimeter with the data acquisition setup as described above. For control samples, two conductive traces without insulating layer were also tested under the same conditions. In all cases, the solution was stirred constantly during the experiments using a magnetic stirrer.

Leakage of liquid electrolyte from standard reference electrodes can be a source of contamination in the test solution. To avoid this, we used a KCl solid electrolyte with an adhesive binder. We evaluated the leakage of KCl from the solid electrolyte (SE) using conductivity measurements of test solution. For this test the electrodes were immersed in 10 ml deionized water (DI) and was continuously stirred while the conductivity of the solution was measured with an LCR meter at 1 kHz (GW Instek LCR-819) for 24 h. The leakage test was conducted at temperatures ranging from 25 °C to 37 °C. The results were compared to equivalent experiments using commercial reference electrodes.

3.7 In vitro characterization

The biocompatibility of the materials used in the sensor construction. A cell line of immortal human keratinocytes (HaCaT) was used to evaluate the biocompatibility of the materials used in the sensor construction (carbon, silver, and PANI). The cells were cultured in Dulbecco's Modified Eagle Media (DMEM), supplemented with 10% fetal bovine serum (Gibco) and 1% Penicillin-Streptomycin (Gibco) in a humidified 5% CO₂ atmosphere at 37 °C. Cell culture media was changed every two or three days and cells were passaged when they reached 70% confluency. For the cell viability tests, 1 ml cell suspension (2×10^4 cells/ml) was seeded in a 24-well plate coated individually with carbon, silver, and PANI. Wells with no coating were used as control. Cell viability was assessed using calcein AM/ethidium homodimer-1 live/dead[®] assay (Life technologies) according to the manufacturer's instructions following 1, 3 and 7 days of culture. The cells were then imaged using a Nikon Eclipse Ti-S fluorescence microscope. The total number of live and dead cells was quantified using NIH ImageJ software and the cell viability was determined as the ratio of live cells to the total cell number.

Cell proliferation was investigated using PicoGreen[®] DNA quantification assay. At each time point (1, 3 and 7 days), the samples were lysed for 2 h with 500 µl of 50 µg/ml proteinase k at 37 °C and pelleted by centrifugation at $18,000 \times g$ at 4 °C for 10 min. Samples were then incubated with PicoGreen[®] working solution, diluted 1:200, for 5 min at room temperature, protected from light. Fluorescence of the whole sample mixture was measured in 96-well plates (Thermo Fisher Scientific, Waltham, MA), at excitation and emission wavelengths of 485 and 520 nm, respectively, using a microplate reader (Fluostar, Ortenberg, Germany).

4 Results and discussion

4.1 Qualitative characterization and scalability

Fig. 5 show photographs of the sensor array in its unfolded and folded configurations. The final laminated device is shown in Fig. 5(c). The overall size of the array is $3\text{ cm} \times 3\text{ cm}$ with the pH-sensitive working areas/openings being 2 mm in diameter. The number, spacing density, and size of the sensors can be modified during the design to accommodate specific wound sizes and geometries.

As illustrated in Fig. 5(d) our fabrication technology can be easily modified to create a broad range of dimensions for custom applications. For example, Fig. 5(e) shows a magnified view of a sensor array with active area of 1 mm diameter and interconnection width as small as $300\text{ }\mu\text{m}$, whereas Fig. 5(f) presents traces of a scaled-up patch with active area of 10 mm diameter and interconnection width of 3.5 mm. The up- and down-scaling is limited by the spot size of the laser beam, the effective working space of the laser system ($82\text{ cm} \times 45\text{ cm}$), the screen printing process, and the conductive inks used in the process. Our results show that although the laser process is able to make masks as small as 0.1 mm, the final smallest patterns are limited to about 0.3 mm. The $3\text{ cm} \times 3\text{ cm}$ dimension is comparable in size to many commercial chronic wound dressings, making it suitable for integration with such dressings. Furthermore, a 3×3 array provides a sufficient number of sensing regions for precise wound assessment while maintaining minimal circuit complexity for rapid interfacing with commercial microcontrollers (via an A/D interface). Thus, our characterization experiments focused on a 3×3 array of sensors with electrode diameter of 2 mm.

Fig. 6 shows the SEM images of the working and reference electrode. SEM images of the working electrode before and after coating with PANI shows uniform and conformal coating of the PANI-Emeraldine base on the carbon electrode with an average thickness of $4\text{ }\mu\text{m}$, Fig. 6 (a,

b). SEM images of the reference electrode shows a thickness of 200 μm for the SE screen-printed Ag/AgCl electrode, Fig. 6 (c). Higher magnification of the surface shows the micro roughness on the composite which is caused by the of KCl particles distributed into the in the polymer binder, Fig. 6 (d).

4.2 Reference electrode characterization

Since the reference electrode has a solid electrolyte, the sensor requires a few minutes (~10 minutes) in order to reach a stable output voltage prior to taking the first measurement point (after submerging the sensor in the testing solution, the KCl in the film begins to dissolve, forming a KCl-saturated microchannel between the Ag/AgCl reference electrode and the solution). The polymer composite, in this case, replaces the electrolyte and the porous membrane in conventional reference electrodes. Various solid supporting matrices such as PVC, porous silica, Agar gel, and active carbon have been adopted with homogeneous mixes of salts and utilized as a replacement for the inner electrolyte and liquid junction in conventional Ag/AgCl reference electrodes [22]. For example, Granholm et al. [23] employed an injection molding method to fabricate an all solid state reference electrode. In this approach a melted mixture of polyvinyl acetate (PVAc) and KCl powder at 170 $^{\circ}\text{C}$ was deposited around an Ag/AgCl wire. Tymecki et al. [24] reported a miniaturized reference electrode by means of all screen printing technology in which the SE liquid junction was prepared by mixing UV curable adhesive with KCl powder. Our approach is similar, and our characterization shows satisfactory performance.

The thickness of the SE affects the initial conditioning (activation of the composite) and life time of the reference electrode. The life time of the reference electrode is limited by the leakage of KCl through diffusion at interface between the polymer matrix and the analytic solution. Therefore thicker polymeric composites (mass) will increases the life time of the reference electrode by providing a more stable saturated KCl environment around the reference electrode

for a longer time. In addition the dissolution of the KCl particles and forming an ionic contact between the Ag/AgCl electrode and the analytic solution is necessary for activating solid state reference electrode. Thus thicker electrolytes will require a longer conditioning (activation) before starting the actual pH measurement. Fig. 7 (a) shows the results of the effect of our SE thickness on conditioning time. For the 200 μm thick electrolyte, a stable potential was obtained after 10 min of immersion into the solution. However, for the electrode with thicker electrolyte such as 3 mm a stable potential was obtained after 80 min. For wound monitoring applications, it is an important to have a fast response in the early stage of placing the sensor on the wound, but a 10 min delay is acceptable (within the duration of typical physician office visit). In addition the polymeric matrix has stiffer mechanical property compared to the paper therefore making a thicker layer can decrease the flexibility of the device.

The results of the conductivity experiments show that the solid state reference electrode gives a similar performance to the conventional Ag/AgCl reference with a stability of ± 1 mV (Figure S1 in the Supporting Information).

Leakage of electrolyte from the reference electrode filling solution can cause contamination in the test solution. As shown in Figure 8b the measured conductivity of the solutions at 37 $^{\circ}\text{C}$ with the commercial reference electrode shows a sharp increase with an average of 0.32 S/m.h, whereas the solid reference electrode shows a significant lower change in conductivity over time, which indicates a small leakage of the ions into the measurement environment, Fig. 7(b). Fig. 7(c) shows the average increase in conductivity for SE at different temperatures from 1450 $\mu\text{S/m.h}$ at 37 $^{\circ}\text{C}$ down to 541 $\mu\text{S/m.h}$ at 25 $^{\circ}\text{C}$; this amount of leakage shows a good agreement with previously reported solid reference electrodes [23].

4.3 pH sensing (potentiometric) results

With the reference electrode providing a stable potential in all measuring solutions, the sensitivity and response time of the sensor should only be dependent on the pH sensitive working electrode (PANI). Fig. 8(a) shows the potentiometric response of five sensors as a function of pH. The data reveal a correlation coefficient of $r^2 = 0.9734$ and a linear relationship from 4-10 pH range with an average Nernstian sensitivity of -50 mV/pH. Fig. 8(b) shows the sensor response for increasing and decreasing pH solutions steps in the 4–10 pH range. Potential measurements in the range of 2–12 pH show a greater change in the output potential, with an average linear ($r^2 = 0.952$) sensitivity of -58.2 mV/pH (see Figure S2 in the Supporting Information). Solutions with high acidity contain a higher concentration of H^+ , which results in a greater number of (nitrogen) sites being protonated on PANI. The opposite phenomenon occurs with concentrated basic solutions, wherein the adsorbed H^+ is neutralized by OH^- within the analyte. The sensitivity result in the range of 2–12 pH agrees with previous reported electrodeposited PANI electrodes [6,17,18]. These reports have also shown that the sensitivity and dynamic range of PANI-based sensors strongly depends on the polymerization condition of the polymer [18]. Although the typical electro-polymerization processes of PANI in HCl has shown pH sensitivity close to the Nernstian slope at RT (~ 59 mV/pH), they have multiple drawbacks, including the requirement of using concentrated acid (1M HCl), the use of a toxic monomer (aniline) [19,20], and the complexity of approach for low-cost and disposable manufacturing. In contrast, our method (solution-cast onto the electrodes by dissolving it in DMSO) the pH sensitive membrane (PANI-EB) is advantageous over others in terms of the bio-compatibility of the materials, low cost, simplicity, and scalability [21].

Fig. 9 presents the results of the response time experiments. The results show a rise time of 12 s for the pH swing of pH 6–8, Fig. 9(a). The fall time is 3 times slower (36 s) for a change

from pH 8 to pH 6, Fig. 9(b). Previous work on the PANI-based pH-sensitive electrodes have reported a faster response time (a few seconds), this is due to their thinner PANI layer deposited using electro-deposition of PANI [26] (drop-casting, in contrast, results in thicker films with slower response time). However, the (slower) results obtained by our method are adequate for wound monitoring applications, since the pH does not change drastically over a short period of time.

The effect of different PANI thicknesses was tested by using multiple iterations of PANI casting on the working electrode and testing the sensor as described above. From the data in Figure S3 (Supporting Information) one can see a minor increase of 1 mV/pH in the sensitivity of the sensor with the second and third coating; however, the response time of the sensor significantly increased (2 min and 4 min for electrodes with second and third layer coating of PANI). Therefore, although the thicker coating provides a small improvement in the sensitivity, this will come at the cost of a significant increase in the response time. In addition, for each PANI coating the solution was casted and allowed to dry, therefore multiple PANI coatings can significantly increase the complication and time of the fabrication process.

4.4 Sensor stability

Sensor stability and output drift are of major concern in real-life applications. For wound monitoring/assessment, we do not expect the sensors to be used for more than a day (24 hours), where the wound dressing will be regularly changed. Fig. 10(a). The results of the stability test show that during the first five hours there is no significant change in the output voltage. After that, the sensors exhibit a small drift (0.5 mV/h or 0.01 pH/h Av.), mainly attributed to the leakage of the electrolyte from the polymer matrix covering the Ag/AgCl reference electrode. Although the healing process for chronic wounds takes more than a few days, frequent (daily)

dressing replacement is required [27]. Therefore such drift is not very critical for wound monitoring applications and the sensor can be easily replaced with daily dressing changes.

Since this system is intended for medical applications, it is important to evaluate the sensors in temperatures that are physiologically relevant. The internal human body temperature is 37 °C and, depending on ambient conditions, the normal skin temperature can be between 25 °C and 35 °C. Fig. 10 (b) show the results of the pH measurements in various temperatures. The sensor has a small increase in sensitivity at elevated temperatures (23–37 °C) for 4–10 pH (~ 0.1 mV/T), which is in agreement with Nernstian theory. At 35 °C the sensor on average shows a negligible change of ± 1 mV in readings with respect to measurements at room temperature. Such small errors are acceptable for wound healing applications, given the large output of our sensor.

4.5 Robustness

Fig. 11 shows the results from the passivation quality characterization. The substrates laminated with Kapton[®] and Scotch[®] tapes both exhibited larger leakage currents (6 μ A and 10 μ A) than the palette paper laminated against another palette paper. Upon inspection of the samples, the cause of the leakage for both Kapton[®] and Scotch[®] tapes was determined to be the delamination of the tapes from the electrodes. In contrast, the samples packaged by laminated palette paper revealed no signs of current leakage. The results imply that the latter method produces a satisfactory moisture barrier and can be reliably used for packaging sensors to be used in wet/moist environments such as wounds.

4.6 In vitro results

All cells on the tissue culture plastic (TCP, control) or on different sensor materials exhibited healthy and flagstone shape morphology after 1, 3 and 7 days of culture, Fig. 9. Cells did not adhere to the Ag, carbon, PANI, and the SE; however, they were found to grow adjacent to the materials as illustrated in Fig. 12 (a(i)-e(i)) and (a(ii)-e(ii)). Cell viability was maintained at

> 90 % for all samples at all the examined time points, Fig. 13 (a). Cells became confluent on day 7 for all the samples. Total DNA content measured on days 1, 3, and 7 demonstrated significantly higher values for each successive time point, indicating retained proliferation capacity of cells grown on TCP, Ag, carbon, PANI and SE ($p < 0.05$) (Fig. 13 (b)). DNA concentrations did not vary between the control TCP and the other sensor materials (Ag, carbon , PANI, and SE). The above results have shown that the cells on different sensor materials exhibited similar morphology, viability and proliferation to the control TCP, indicating appropriate cyto-compatibility of individual sensor materials. In addition, results indicated the leakage of KCL from the SE is insignificant and does not cause any damage to the adjacent cells.

5 Conclusions

We developed an inexpensive, flexible array of pH sensors fabricated on a paper substrate for use in wound monitoring. The developed fabrication process takes advantage of low-cost materials, laser machining, and self-aligned passivation with lamination technology. The sensor performance in different buffer solutions of pH 4 to pH 10 showed a linear potential ($r^2=0.9734$) and a sensitivity of -50 mV/pH. The sensor array achieved targeted sensitivity, repeatability, stability, and biocompatibility making it appropriate for integration with low-cost wound dressings.

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7 RefereAnces

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Fig. 1. (a) A 3D exploded view of the 3×3 pH sensor array on paper with self-aligned encapsulation, (b) cross section showing the sensor embedded into a wound dressing.

Fig. 2. Chemical transformation of polyaniline emeraldine base (blue) to emeraldine salt (green) in presence of acidic and basic solutions.

Fig. 3. Fabrication process of the sensor: (a-c) laser-pattern the shadow/stencil mask; (d) screen print silver and carbon electrodes; (e) remove the tape mask; (f) chloridize the silver reference electrode; (g) cast the electrolyte paste on the Ag/AgCl and UV cure; (h) drop-cast PANI on the carbon electrode; (i) fold the isolation layer over the electrodes and bond by lamination.

Fig. 4. Schematic of the setup used for testing leakage current.

Fig. 5. Photographs of the fabricated pH sensor array, (a) fabricated electrodes on paper; (b) folding the encapsulation layer over the electrodes; (c) the final array of pH sensors after lamination, scale bar: 5 mm; (d) photograph showing the adjustability of the size sensor to the desired needs, scale bar: 8 mm; (e) close-up view of the smallest fabricated electrode, scale bar: 300 μm ; (f) close-up view of a large electrode with wide interconnections; scale bar: 3 mm.

Fig. 6. SEM image of the surface microstructure on the carbon electrode: (a) before and (b) after coating with PANI-EB. (c) SEM image of reference electrode with SE coating of 200 μm . (d) Higher resolution image of the KCl and UV curable glue composite.

Fig. 7.(a) Preconditioning time required for reference electrodes with different solid-state electrolyte; (b) KCl leakage measurement from commercial reference electrodes and solid-state reference electrode at 37 °C estimated by conductometric measurement within analyte; (c) KCl leakage measurement of solid-state electrolyte at different temperatures.

Fig. 8. (a) The measured sensor response to various pH levels between 4 and 10. The data show a linear response ($r^2 = 0.9734$) with an average sensitivity of -50 mV/pH. (b) sensor response to increasing and decreasing pH solutions from pH 4 to pH 10.

Fig. 9. Time response of the pH sensor, output swing for (a) pH step from 8 to 6 and (b) opposite swing from 6 to 8.

Fig. 10. (a) Long-term stability of the pH sensors at pH 6, pH 7 and pH 8 (pH values observed during chronic wound healing). (b) Effect of temperature variation upon the response of the pH in buffer solutions of pH4, pH7, and pH10.

Fig. 11. Electrical isolation quality of the laminated palette paper compared to Scotch® MagicTape™ and Kapton® tapes. The current was measured between two electrodes in PBS solution with an applied bias of 1 V.

Fig. 12. Representative phase contrast (a(i), b(i), c(i), d(i)) and their corresponding fluorescent images (a(ii), b(ii), c(ii), d(ii)) of cells grown on TCP, Ag, Carbon, PANI and SE after 3 days of culture. Cells were stained with calcein (green, live cells) and ethidium homodimer (red, dead cells) according to the live/dead assay. (e) Cell viability quantification. (f) Cell proliferation quantification. Scale bar = 100µm.

Fig. 13. (a) Cell viability quantification and (b) Cell proliferation quantification on TCP, Ag, Carbon, PANI and SE after 1, 3 and 7 days.