



Minimally invasive and continuous glucose monitoring sensor based on non-enzymatic porous platinum black-coated gold microneedles

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ARTICLE INFO

Article history:

Received 7 November 2020

Accepted 27 December 2020

Available online 30 December 2020

Keywords:

Microneedle electrode array

Interstitial fluid

Electrodeposition

Electrochemical impedance spectroscopy

Microelectromechanical systems

Chronoamperometry

ABSTRACT

Individuals with diabetes can considerably benefit from continuous glucose monitoring (CGM). Addressing this challenge, a proof-of-concept for a pain-free CGM was presented based on a highly porous platinum black (Pt-black)-modified non-enzymatic microneedle electrode arrays (MNEAs). Porous Pt-black was electrodeposited on microneedle substrates using potentiometry by employing a charge density of -2.5 mA cm^{-2} . The surface of the porous Pt-black-modified microneedles (MNs) was optimally packed using Nafion (Nf) biocompatible ionomer. The dimensions of the fabricated microneedles were $650 \mu\text{m} \times 110 \mu\text{m} \times 150 \mu\text{m}$ in height, width, and thickness, respectively. The surface morphology of the modified MNs was studied using scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDX). The microneedle sensor showed a wide glucose dynamic range (1 – 20 mM) in phosphate buffer saline (PBS) and artificial interstitial fluid (ISF) *in-vitro* with good sensitivities (PBS: $5.786 \mu\text{A mM}^{-1} \text{ cm}^{-2}$, ISF: $4.380 \mu\text{A mM}^{-1} \text{ cm}^{-2}$), detection limits (PBS: $10 \mu\text{M}$, ISF: $22.5 \mu\text{M}$), stability (2% lost at the end of 7 h), selectivity, reproducibility (2.32% RSD), and a response time of 2 s with good glucose recoveries in ISF (98%–102.5%). The device exhibited good stability (7 days) as a sensor *in-vivo* and lost its functional activity after 7 days due to electrode biofouling.

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1. Introduction

Individuals with diabetes mellitus require frequent blood checks for optimal glucose management. Accurate and real-time glucose monitoring is vital in patients with diabetes in order to define food habits, physical activities, and medication, which may affect the disease [1,2]. The most commonly used method for checking glucose concentrations comprises blood sampling from the fingertip using a tiny lancet that involves a slight prick on the skin surface, causing a pain that may be unacceptable to the elderly and children. Numerous commercially viable electrochemical detection-based glucometers are available to assist diabetic patients in routine blood glucose checks. However, testing always requires frequent pricking of fingers.

In order to avoid this practice, several CGM devices are commercially available to offer real-time glucose measurements for

each five-minute time interval [3]. The device is operational once implanted under the subcutaneous tissue and works for nearly 10 days [4]. Such devices are basically fabricated using enzymes such as glucose oxidase (GOx) and glucose dehydrogenases (GDH). The devices continuously monitor the glucose concentrations in the subcutaneous tissue through a needle that penetrates into the dermal compartment to access the interstitial fluid (ISF). The advantages of CGM sensors include wearability and transmission of data to a digital display device using a wireless transmitter. In spite of the advantages, these devices are known for complete invasiveness and associated discomfort with biofouling effects [5]. Moreover, the enzymatic microneedle-based amperometric sensors lack stability and are affected by pH, temperature, oxygen concentration, and humidity [6].

Microneedle (MN) based non-enzymatic amperometric sensors, fabricated using the microelectromechanical systems (MEMS) technology, provide a compromise [7]. Recently, nanoporous material-based non-enzymatic sensors gained interest as alternative methods for glucose sensing through direct oxidation on modified electrodes [8,9]. Similar to the CGM devices, the non-enzymatic MN

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sensors are capable of continuously monitoring glucose levels in the ISF. Enzyme-free MN sensors, unlike the enzymatic MNs, exhibit several advantages such as simplicity of fabrication, stability, free from oxygen limitation, or high sensitivity due to larger electroactive surface areas (EASA) [10]. These advantages are due to the MEMS technology-based micron-size needle fabrication that allows the penetration of the epidermis (50–500 μm) but not that of the dermis (500–2000 μm), unlike the penetration observed with the surgical hypodermal needles (6 mm). Considering the above-mentioned reason, the MNs are minimally invasive and less painful than CGM devices, thereby minimizing discomfort and improving the treatment quality in patients with diabetes [11].

MNs with micron-size dimensions were initially used as transdermal drug delivery devices [12] and analytical sensors for painless detection of clinically relevant analytes, for insulin injection [13], CGM [14], along with their usage as biosensors for the detection of various analytes [15]. Recently, Goud et al., demonstrated a new electrochemical wearable microneedle sensor array for minimally invasive and continuous L-Dopa monitoring towards parkinson disease management [16]. The L-Dopa sensing relied on square-wave voltammetry and chronoamperometry measurements at unmodified and enzyme-modified electrodes, respectively. Windmiller et al., developed a minimally invasive carbon paste-loaded microneedle electrode array for lactate biosensing. Enzyme-loaded carbon paste microneedle electrode arrays are used for the selective detection of lactate at potential +0.15 V vs. Ag/AgCl [17]. Wang group demonstrated a new bio-component microneedle array platform, for amperometric glutamate biosensing. The microcavities of the microneedle entrapped with the enzymes glutamate oxidase through electropolymerization of poly(o-phenylenediamine) thin film to prevent electro-oxidation of coexisting electroactive interferents [18]. Li et al., demonstrated one-touch-activated blood multidagnostic system for detection of cholesterol. The device comprises a hollow minimally invasive biocompatible microneedle and multiplex paper-based sensor, containing the functions of blood collection, separation, and detection in one device that function by one-touch activation using finger-power [19]. Detection of glucose based on glucose oxidase MNEAs involves the reduction of hydrogen peroxide, which occurs at high (mediator-less biosensors) [20] or lower overpotentials (redox mediator-based MN biosensors) [21]. Both types of sensors showed a high interference and low stability of the mediator on the electrode surface [22]. In order to overcome these difficulties, non-enzymatic highly porous nanocomposite-based MNs allowed a compromise in terms of higher overpotentials, sensitivity, electrode stability, and interference-free glucose detection [23]. The highly porous platinum nanocomposite with micrometer-sized pores drastically enhanced the EASA and the current densities, when coated onto the MNEAs [24].

For selective and interference-free detection, non-enzymatic highly porous nanocomposite modified MNEAs should be packaged into bio-compatible and protective polymer films. Nafion is an attractive packaging polymer owing to its ease of handling and availability. Its fluorocarbon hydrophobic backbone and hydrophilic sulfonic acid groups form a transparent membrane with strong anion exclusion in precluding many redox-active interfering compounds to reach the electrode array surface [25].

In the present study, we investigated a highly porous non-enzymatic Pt-black Au-MNEA based sensor to determine glucose levels via a minimally invasive procedure in ISF and animal models. A silver MN reference electrode array was fabricated by depositing chlorine (Cl_2) using potentiometry and studied for CGM. The highly porous Pt-black MN electrode was developed by electrochemical deposition through application of a negative potential of $-2.5 \text{ mA} \cdot \text{cm}^{-2}$ against the Ag/AgCl reference electrode in a solution containing chloroplatinic acid and HCl. The Pt-black-modified MNs and

AgCl reference MNs were characterized and used for CGM in both *in vitro* and *in vivo* models. The MNEAs were carefully packed in a bio-compatible Nafion membrane in order to prevent electrode biofouling in the animal models. The fully-packed MNs were also analyzed at several glucose concentrations both in PBS and ISF in order to validate the sensor applicability. The optical and microscopic images of the bare and modified MN electrode arrays, as well as the schematic representation of the *in vivo* CGM in rat model, is presented in Fig. 1a-e.

2. Experimental

2.1. Chemicals and instrumentation

Refer supplementary information

2.2. Fabrication of the Au-MNEAs

The fabrication of the Au-MNEA was performed as per methods described previously [26]. Briefly, the 150- μm thick stainless steel substrates (316 L grade) were patterned using a jet of wet chemical etchant, ferric chloride (FeCl_3) under a 2-kgf/cm^2 pressure for a 60 s time interval. On the patterned stainless steel substrate, a thin Au layer was electroplated and the MNs were punched out of the plane (90°) using a jig (Fig. S1a-c). The MN tip positions and contact pads were masked using a block of PDMS and Parafilm, respectively, in order to prevent parylene passivation. A 5 μm thick parylene coating was used to passivate the Au layer. After releasing the parafilm and the PDMS block, the needle tip areas were electrodeposited with the Pt-black catalytic layer (Fig. S1d).

2.3. Preparation of the Au/Pt-black/NF MNs

The Au MNs were modified with porous Pt-black using electrodeposition in a three-electrode setup (Fig. S2a). The deposition was carried out in an electrolytic bath comprising 2.5% hydrogen hexachloroplatinate (IV) hexahydrate, lead diacetate trihydrate (0.05%), and HCl (0.01 M). The function of the lead diacetate trihydrate in the electrolytic bath is to decrease the over-potential, and increases the rate of PtCl_6^{2-} to Pt(0) reduction [27]. Pb ions adsorbed on the electrode surface can also act as nucleation sites for Pt islands. Pb ions decrease the conversion of Pt(IV) to Pt(II) during electrodeposition and lowers the energy barrier for the reduction of Pt(IV) to Pt(0) [28]. The Pt-black electrodeposition was carried out by employing a fixed current density of $-2.5 \text{ mA} \cdot \text{cm}^{-2}$ for 200 s vs. the Ag/AgCl external RE in a three-electrode setup. The prepared Au/Pt-black MNs were packed using a Nafion: ethanol mixture (1:6% v/v) by dip-coating procedure. The needles were dip-coated for 60 s in the Nafion ionomer solution and dried at 50°C on a hot magnetic stir plate for 40 s followed by overnight drying at RT (24°C).

2.4. Preparation of the AgCl MNs

The silver (Ag) microneedles were modified with chloride (Cl) using electrodeposition. The deposition was carried out in an electrolytic bath comprising aqueous 0.1 M HCl. The Cl was deposited on the Ag MNs using potentiometry by applying a fixed current density of $10 \text{ mA} \cdot \text{cm}^{-2}$ for 60 s using Pt-coil as a cathode.

2.5. Electrochemical measurements

The electrochemical analyses were carried out in a three-electrode configuration (CompactStat potentiostat) consisting of a working electrode (WE) (bare and modified MNs), a counter electrode (CE) (bare Au MN), and a reference electrode (RE) (AgCl MN).

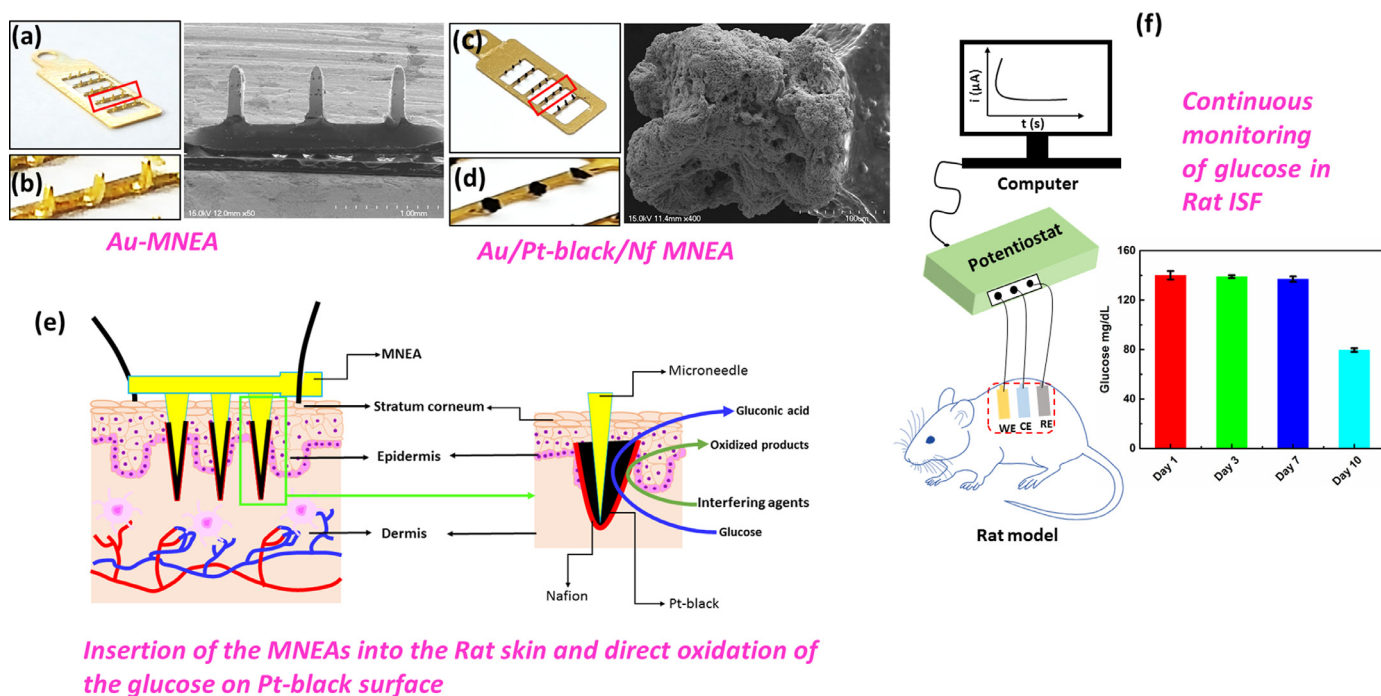


Fig. 1. Optical and SEM Micrographs of the fabricated MNEAs before and after the catalytic Pt-black layer deposition (a-d). Schematic representation of the MNEA insertion in the animal skin models (e) for the potential application of the developed MNEAs for CGM in rat models.

The cyclic voltammograms (CVs) were carried out in $10 \times$ PBS (pH = 7.4, 24 °C), at a sweep rate of 50 mV s^{-1} within a potential range of -0.8 V to $+0.8 \text{ V}$ vs. AgCl MN. The electrochemical impedance spectroscopy (EIS) measurements of MNs were carried out in 0.9% NaCl and DI water at room temperature (RT) (24 °C) using a three-electrode setup. The spectra were measured by selecting an alternating current perturbation voltage with a root mean square value of 0.05 V in a frequency range of 0.1 Hz – 1 MHz . The Pt-black electrodeposition was carried out using a mini-potentiostat from IVIUM technologies (Eindhoven, Netherlands) in a three-electrode configuration where, Au MN, Pt wire, and external Ag/AgCl were used as WE, CE, and RE respectively. The amperometric measurements (i vs. t) were carried out using a three-electrode configuration in $10 \times$ PBS (pH = 7.4, 24 °C) and artificial ISF by applying an external potential of $+0.12 \text{ V}$ against the AgCl reference MN electrode at RT (24 °C) (Fig. S2b). The electrode response was measured after background current stabilization and the analyte was spiked sequentially with increasing glucose concentrations at 50 s time intervals.

2.6. Preparation of ISF

Briefly, the artificial ISF was formulated by adding 2.5 mM CaCl_2 , 5.5 mM glucose, 10 mM HEPES (pH: 6.8), 3.5 mM KCl, 0.7 mM MgSO_4 , 123 mM NaCl, 1.5 mM NaH_2PO_4 , and 7.4 mM saccharose. The solution pH was adjusted to 7.0 using 1 N HCl.

2.7. In-vivo studies

For animal studies, the modified MNs were inserted into the subcutaneous tissue of male Sprague-Dawley rat models. All the experimental animals were handled at the Gachon University Animal Care Center. The animal studies were performed by following the procedures of the Animal Care Committee of the Gachon University (animal experiment approval number: GIACUC-R2019028, Gachon University, Animal Experiment Committee). The MNEAs were attached to a transparent adhesive tape (3 M Tegaderm™

film, 3 M Deutschland GmbH, Neuss, Germany) and applied on the dorsal side of the rats ($n = 10$) by pressing it with a cohesive elastic band (3 M Coban™) and monitored in order to establish a stable background current. The MN sensitivity was assessed through the oral glucose tolerance test (OGTT), carried out 24 h after needle fixation. Blood samples from the tail vein were collected and analyzed using a commercial blood glucose monitoring device (Accu-chek® active, Roche diagnostics GmbH, USA).

2.8. Histological analysis

The histogram analysis was performed by a specialized anatomic histopathologist. The skin tissue specimens were prepared consistently for light microscopy (fixation, dehydration, embedding, micro sectioning, and staining with Hematoxylin and eosin stain; H&E). The skin samples were carefully sectioned to $5\text{-}\mu\text{m}$ thick sections and stained. The skin sections were examined using a light microscope at 400X magnification and analyzed using the ImageJ software (ImageJ bundled with 64-bit Java 1.8.0_112, NIH, USA)

3. Results and discussion

3.1. Characterization of the MNEAs

3.1.1. EDX and XRD analysis

The EDX experiments were performed to confirm the atomic ratios and elemental composition of the modified electrodes (SEM/EDX, Fig. 2a-c), by carrying out measurements over the randomly selected areas. The EDX of the Au/Pt-black modified electrode surface shows almost Pt (due to platinum in metal salt) and minor amounts of Cl (due to adsorbed chloride from the metal salt on the MNEA surface) (Fig. 2a). The elemental composition of the Au/Pt-black/Nf MN shows major quantities of Pt stemming from metal salt, and minor quantities of Cl from metal salt and C, O, F, and Na from the Nafion ionomer (Fig. 2b). The EDX of the AgCl reference MN show major quantities of Ag stemming from metal, and

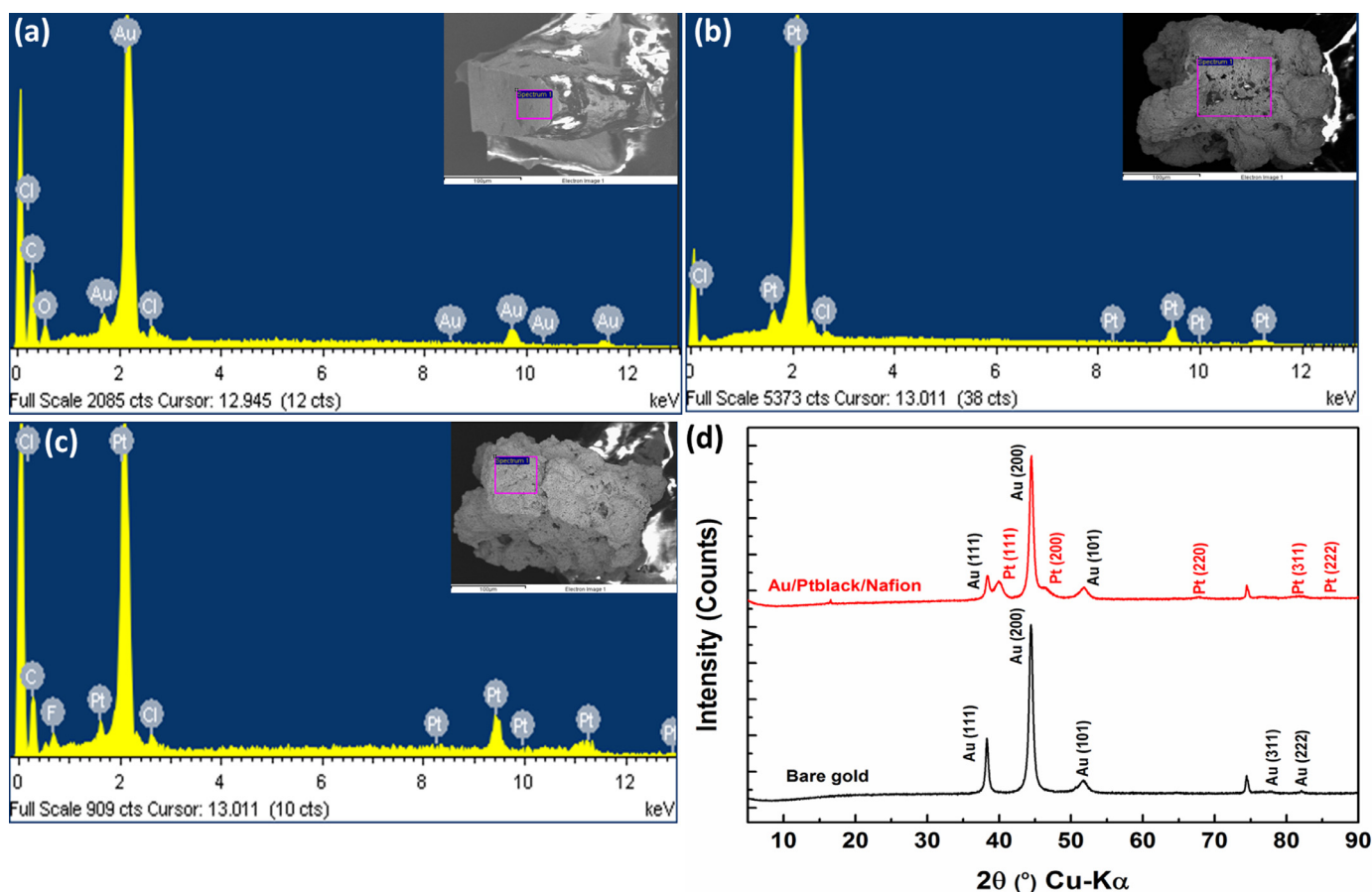


Fig. 2. SEM-EDX elemental analysis of the bare Au-MNEAs (a), Au/Pt-black microneedles (b), Au/Pt-black/Nf microneedles (c) endorsing the presence of Pt and Cl in Pt-black and Pt, Cl, F, C in Pt-black and Nf modified MNs, XRD spectra of the bare Au-MNEAs and Au/Pt-black/Nf microneedles (d).

minor quantities of chlorine (Cl), obtained during electrodeposition from HCl (Fig. S3).

X-ray diffraction (XRD) analysis was carried out to analyze the crystalline phase of the Platinum in the developed Au/Pt-black/Nf MNs (Fig. 2d). The unmodified MNs shows Au (111), Au (200), Au (220), and Au (311) characteristic peaks corresponding to crystalline Au at $2\theta = 38.2^\circ, 44.4^\circ, 64.7^\circ$, and 77.6° , respectively, which confirms the face-centered cubic (fcc) structure of Au (ICDD reference pattern no: 00-004-0784); the highest peak intensity was observed at the Au (111) plane [29]. In case of Au/Pt-black/Nf modified electrode the characteristic diffraction peaks at about $2\theta = 39.74, 46.30, 67.64, 81.80$ and 85.92 were detected, which corresponds to (111), (200), (220), (311) and (222) planes of the fcc lattice of metallic platinum (ICDD reference pattern no: 00-004-082), suggesting the presence of crystalline Pt on the Au-MNEA [30].

3.1.2. SEM analyses

The SEM analyses were performed to validate the morphology of the MNEA at major stages of electrode modification. The bare Au MN shows an excellent needle-to-needle uniformity as specified by the design (Fig. 3a, i & ii) with a smooth surface morphology. The needles show a length and width of $650 \mu\text{m} \times 110 \mu\text{m}$ and the size of stainless-steel substrate was found to be $150 \mu\text{m}$. The smooth surface turned into extended complex dendritic structures after the Pt-black deposition on the MN tips (Fig. 3b). The complex and well-defined dendritic structure was transformed into a porous structure after packaging with the Nafion ionomer, as shown in Fig. 3c. The SEM image of the bare Ag MN electrode shows a smooth surface morphology which, changed into a porous

and highly rough surface after the Cl electrodeposition (Fig. 3d). The major factors of consideration during the preparation of the well-defined Pt-black porous structure are the time interval and the optimal negative charge density. A charge density of -2.5 mA cm^{-2} was chosen as an optimal value for adequate hydrogen bubbling on the gold electrode surface for generating a well-defined porous structure without damaging the MN surface. A time interval of 200 s was chosen as optimal time to completely coat the well-porous structure on the MN tip surface. Another critical and carefully optimized parameter was the distance between the WE and CE. A well-defined porous structure of the Pt-black surface was formed when the gap between the WE and CE was greater than 1 cm. An AgCl reference MNEA was constructed using the galvanostatic method. During the electrochemical reaction, a gray color porous structure was deposited on the Ag MN surface to release the AgCl MN (Fig. S4, a & b).

3.1.3. XPS analysis

To analyze the electrodeposition and coating of nafion on the modified electrode, elemental dispersive X-ray and elemental mapping analysis was performed on the Au/Pt-black/Nf electrodes. The survey spectrum for Au/Pt-black/Nf show peaks corresponding to Pt (due to the presence of Pt-black) and C, F, S and O (due to the presence of Nafion) (Fig. 4). The high-resolution region scans of C1s, O1s, S2p, F1s, and Pt4f for Au/Pt-black/Nafion MN along with the respective peak fitting components are shown in Fig. 4b-f. Binding energies (BEs) of carbon (C1s) are observed at 285.0 eV, 287.0 eV, 289.5 eV, 291.9 eV and 294.11 eV (Fig. 4b). The main peak detected at 291.9 eV correlates to the carbon content present within the polytetrafluoroethylene backbone ($-\text{CF}_2-\text{CF}_2-$).

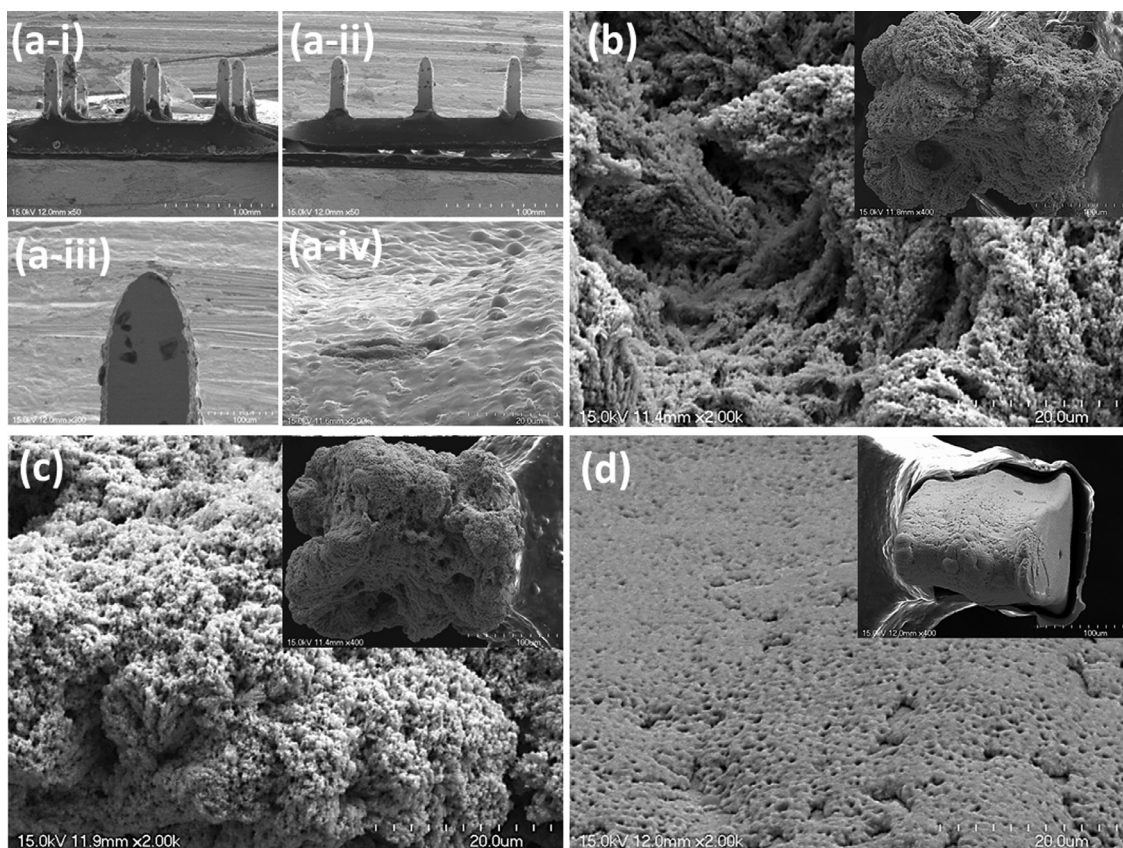


Fig. 3. SEM micrographs of bare Au MNs (a), Au/Pt-black (b), Au/Pt-black/Nf (c), and AgCl MNs (d) showing a drift change in the surface morphology after major steps of electrode modification.

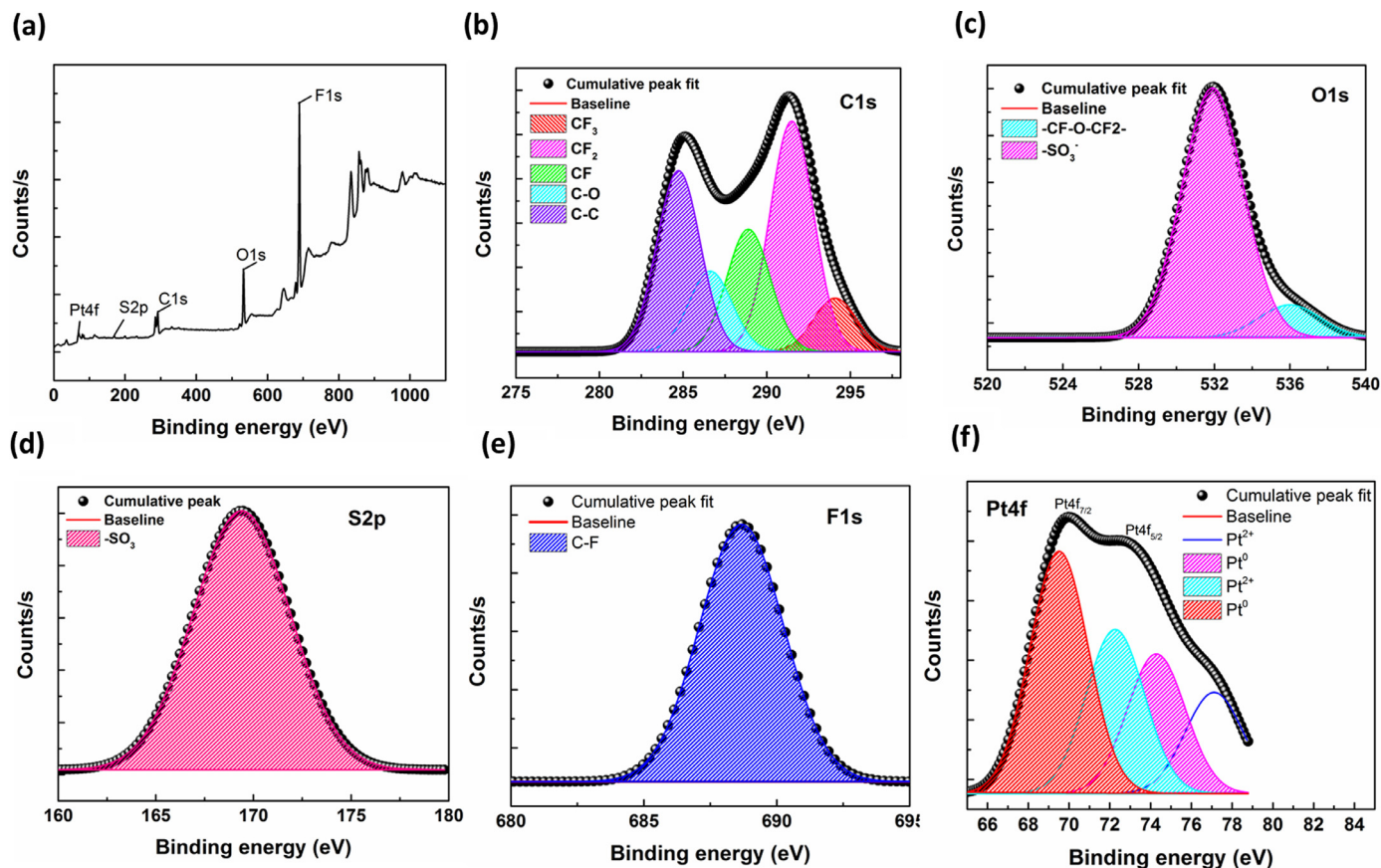


Fig. 4. XPS spectra of the Au/Pt-black/Nf MNEA (a) showing a high resolution elemental spectra for C1s (b), O1s (c), S2p (d), F1s (e) and Pt 4f (f).

The shoulder peak at 294.11 eV is ascribed to the –CF₃ functional group. The peaks at BEs 289.5 eV and 287.0 eV endorsed to carbons in the –CF and –OCF₂– configurations, respectively. The peak at BE 285.0 eV is due to the adventitious carbon [31]. Two peaks are detected for deconvoluted O1s spectra: a 535.98 eV higher binding energy peak and a 532.6 eV lower binding energy peak (Fig. 4c). All the peaks were normalized by shifting of the C–C peak to 285 eV. The higher BE peak 535.98 eV and lower BE peak 532.6 eV is ascribed to the oxygen present in the ether functional group and sulfonate groups, respectively [32]. Both the F1s and S2p deconvolution region scans show a distinct peak at 688.6 eV and 169.9 eV, respectively (Fig. 4d & e) [32]. In Pt 4f deconvolution region spectra, a pair of peaks with lower BEs at 70.28 eV and 74.27 eV are ascribed to the 4f_{7/2} and 4f_{5/2} peaks of Pt⁰, respectively [33]. An additional pair of peaks with higher BEs at 72.27 eV and 76.12 eV are assigned to the 4f_{7/2} and 4f_{5/2} peaks of Pt²⁺ respectively (Fig. 4f) [33].

3.2. Electrochemical characterization of the MNEAs

3.2.1. EIS

The electrochemical impedance spectra of the MNs were measured in 0.9% NaCl by applying a range of frequency from 0.1 Hz to 1 MHz in order to probe the electrochemical characteristics of the needle after the Pt-black and nafion modification. The measured impedance spectra of the modified electrodes at different steps of the electrode modification were fitted to an equivalent circuit model. The electrode impedance was governed by electrode interfacial impedance modeled as the double layer capacitance (CPE) and a solution resistance in series (R_s). The CPE is represented to T • (jω)^P, where T and P are adjustable parameters, j is the imaginary unit, and ω is the angular frequency (= 2πf, f is the frequency). [34]. Table. S1 summarizes the extrapolated fitting results of the measured EIS spectra for the circuit elements shown in Fig. 5a. The electrodeposition of Pt-black nanomaterial led to a decrease in 1/T, suggesting an enhanced surface roughness of overall electrode area, while P for bare MNs and modified MNs was ~1, suggesting that the interfacial impedance of electrode was mostly ascribed to the capacitive reactance. From the fitting analysis of the measured spectra in 0.9% NaCl, it was found that the impedance property of the fabricated MNs was well explained by the designed equivalent circuit model shown in Fig. 5a. Bare Au MN electrode with parylene insulation having an electroactive surface area of 1.004 cm² (Fig. S5a) shows a very high impedance in comparison with the needles modified with Pt-black, thus showing a drastic decrease in impedance. This effect was due to a rise in the electrode-active surface area of the MNs (17.5 cm²) after the Pt-black modification (Fig. S5b). The as-fabricated MNEA without parylene insulation shows a high impedance in comparison with the MNs coated with parylene (except for the MN tips) (Fig. 5a). The parylene coating aids in elimination of the direct contact between the MN and skin surface, thus completely eliminating the skin impedance during animal studies.

3.2.2. Cyclic voltammetry (CV)

The electrochemical characterization of the unmodified and modified MNEA was assessed by CV experiments performed in a three-electrode configuration. The CV of the bare Au MNEA shows no significant redox peaks (Fig. 5b, curve i) and was electrochemically inactive in the selected potential window, whereas the Au/Pt-black electrode (Fig. 5b, curve ii), shows redox peaks corresponding to the metallic Pt on the electrode in a potential ranging from –0.7 to –0.2 V vs. AgCl MN [35]. The CV of the Au/Pt-black/Nf electrode (Fig. 5b, curves iii), shows redox peaks similar to those of the Au/Pt-black electrode with an additional peak in the potentials

ranging from 0.0 V to +0.3 V. The CV of the Au/Pt-black/Nf electrode supplemented with 4 mM of glucose shows 3 pairs of peaks in the potentials ranging from –0.7 V to –0.2 V; +0.0 V to +0.3 V and +0.4 V to +0.7 V [36]. Upon glucose addition a clear increase in current magnitude at the corresponding peak potentials was observed, suggesting the response of the modified electrode towards glucose addition (Fig. 5b, curves iv).

Upon running a voltammogram in a potential window (–0.8 V to +0.8 V) the glucose molecules were directly oxidized on the tips of the Pt-black modified MNs into gluconic acid by the glucose adsorption followed by hydrogen removal at the C₁ position (–0.7 V to –0.2 V). Subsequently, the water dissociates to produce hydroxide (OH) anions tailored by the oxidation of glucose (Glu_{ads}) by the hydroxide anions (OH_{ads}) (+0.0 V to +0.3 V). Glucose may also get oxidized by the platinum oxide layer (PtO) formed by the evolution of oxygen in electrocatalysis, thus endorsing a MN response to the glucose addition (+0.4 V to +0.7 V) (Fig. 5c) [37]. The voltammograms of the Au/Pt-black/Nf electrodes at various concentrations of glucose addition in 10 × PBS, pH 7.4, is shown in Fig. 5d. The current magnitudes at the aforementioned peak potentials of the modified MNs increased along with the glucose concentration in the buffer solution from 1 mM to 20 mM. The voltage of +0.12 V vs. AgCl MN was selected as an optimum for the direct glucose oxidation in order to overcome the interference from the common interfering agents in the ISF.

3.2.3. Electrocatalysis of glucose at the modified electrode

The mechanism of electrocatalysis of glucose at Au/Pt-black/Nf microneedle was studied by CV in 10 × PBS (pH 7.4). A typical CV of glucose oxidation on Pt-black surface is shown in Fig. 5c. Upon the addition of 4 mM glucose, the CV shows the three distinct peaks. The peak-1 represents the dehydrogenation of the glucose molecule at the hemiacetal carbon 1 atom (C₁) and adsorption of the glucose molecule onto the platinum surface (Fig. 6a). The adsorption and dehydrogenation occurred at a potential region of –0.7 V to –0.2 V vs. AgCl MN reference electrode with the release of the first hydrogen atom considering as a rate-limiting step and the region is known as “hydrogen region”. The peak-2 represents the electro oxidation of the chemisorbed glucose molecules which occurs in a potential range from 0 to +0.3 V vs. AgCl MN reference electrode, with the aid of adsorbed hydroxide anions produced by the dissociation of the water molecules (Fig. 6b, i and ii). As the two electro-active species, such as hydroxyl anions and glucose molecules were adsorbed on the Pt-black surface the region is called as “double-layer region”. The peak-3 may represent the direct catalytic oxidation of the bulk glucose solution, which occurred in a potential range from +0.4 V to +0.67 V vs. AgCl MN reference electrode (Fig. 6c). In this potential range the Pt-black surface might have been covered by a monolayer of oxygen, resulting in the formation of PtO layer and the region is called as “oxygen region” [38,39].

3.2.4. Scan rate and pH study

In order to investigate the reaction kinetics, CVs of Au/Pt-black/Nf MNEAs were performed with 5 mM of glucose at different scan rates ranging from 10 → 100 mV.s^{–1} in 10 × PBS (pH 7.4.). Fig. 7a shows a shift in the oxidation peak potential (E_{pa}) towards positive side as scan rate increases, which is significant for the diffusion controlled process at the electrode surface [40]. To understand the diffusion controlled process on the electrode surface, the Randles–Sevcik equation was used at the electrode: I_{pa} = 2.69 × 10⁵ n^{3/2}AD^{1/2}Cv^{1/2}, where I_{pa} is the anodic peak current, n is the number of electrons transferred, A is the active surface area, D is the diffusion coefficient, C is the concentration of analyte (mol/cm³), and v is the scan rate (mV/s). Fig. 7a shows a gradual increase in the anodic (I_{pa}) and cathodic peak currents

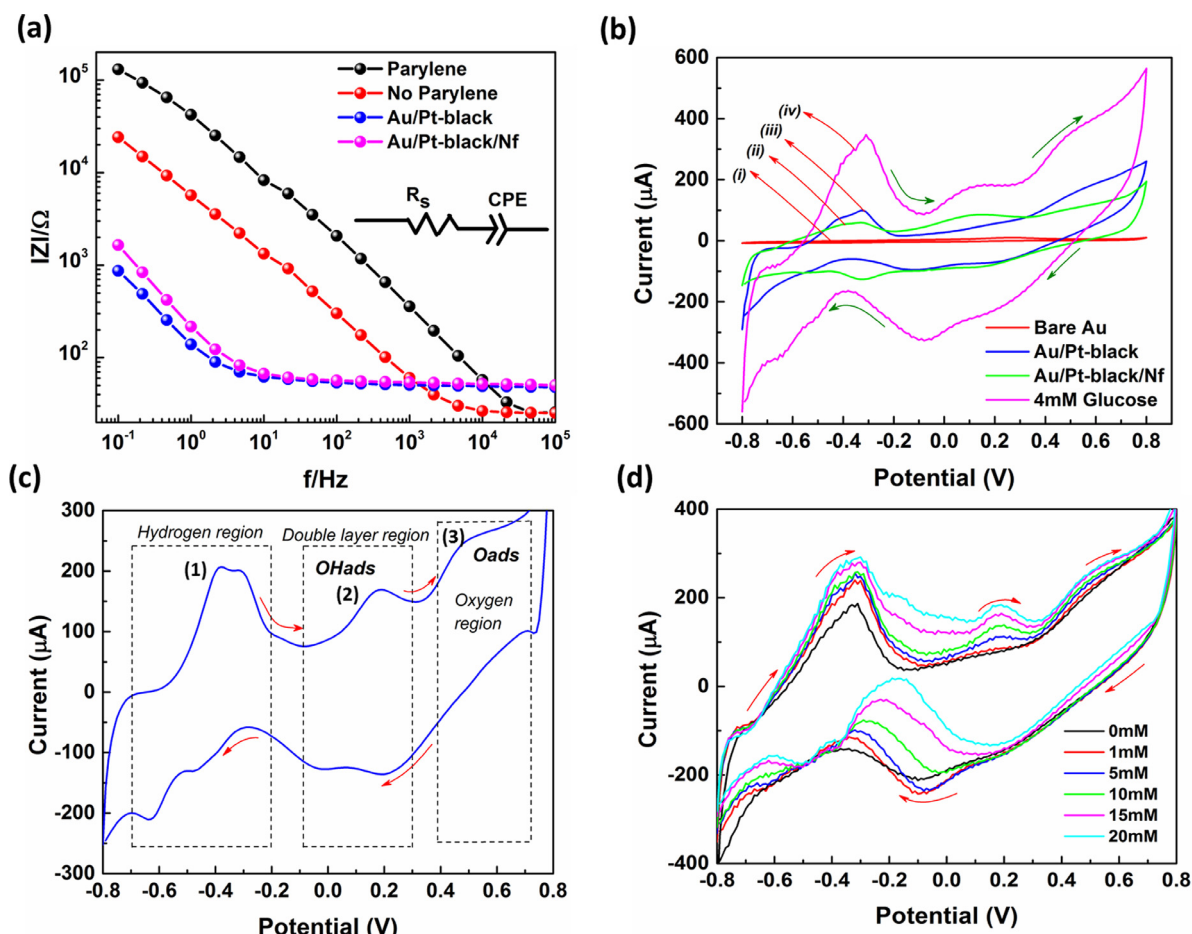


Fig. 5. Impedance spectra of the bare Au MNs with and without parylene coating (i and ii), Au/Pt black MN (iii) and Au/Pt black/Nf MN (iv) carried out in 0.9% NaCl at a frequency range of 0.1 Hz to 1 MHz (a), CV of the bare Au (i), Au/Pt-black (ii), and Au/Pt-black/Nf MNs (iii) with 4 mM glucose addition (iv) at a scan rate of 50 $\text{mV} \cdot \text{s}^{-1}$ in 10 × PBS (pH = 7.4) (b), CV of the Au/Pt-black/Nf MN in 10 × PBS (pH = 7.4) with 5 mM glucose addition at a scan rate of 50 $\text{mV} \cdot \text{s}^{-1}$ in 10 × PBS (pH = 7.4) (c), CV of the Au/Pt-black/Nf MN in 10 × PBS (pH = 7.4) with increasing concentrations of glucose between 0 and 20 mM at a sweep rate of 50 $\text{mV} \cdot \text{s}^{-1}$ (d).

(I_{pc}) with increase in scan rate. An excellent linearity of the anodic peak current (I_{pa}) was detected in the scan rate ranging from 10 → 100 $\text{mV} \cdot \text{s}^{-1}$. The regression equation for the anodic peak current with respect to the increasing scan rate (v) was found to be $I_{pa} (\mu\text{A}) = 2.1674v + 51.188$ (Fig. 7b). The linear relationship for the square root of the scan rate ($v^{1/2}$) vs. I_{pa} intensities are shown in Fig. 7c. The linearity of ($v^{1/2}$) vs. I_{pa} clearly displays that glucose oxidation on modified MNEA follows diffusion controlled kinetics, which is ideal for glucose oxidation [41]. The regression equation for ($v^{1/2}$) vs. I_{pa} was found to be $I_{pa} (\mu\text{A}) = 28.1534 v^{1/2} - 30.2836$. The effect of pH on the Au/Pt-black/Nf MNEA was investigated using CV with 5 mM of glucose in PBS (10mM) with a pH range between 5.4 → 10.4 at 50 $\text{mV} \cdot \text{s}^{-1}$ sweep rate. When the pH is raised, the E_{pa} shifts towards negative side, and the maximum I_{pa} was observed at a pH of 7.4 as shown in Fig. 7d. The correlation between pH and the I_{pa} in Fig. 7d clearly shows that the I_{pa} increased gradually from 5.4 → 7.4 and then decreased from 8.4 → 10.4. Hence, the maximum I_{pa} was observed at pH 7.4 demonstrating the greater electrocatalytic activity of Pt-black/Nf at pH 7.4. Fig. 7d also shows a linear correlation between the pH vs. E_{pa} of glucose oxidation. The regression coefficient of pH vs. E_{pa} was found to be 0.9892 and the regression equation calculated is $E_{pa} = 0.05895(\text{pH}) - 0.24275$. The slope value obtained from the linear regression of pH vs. E_{pa} is $-58.95 \text{ mV} \cdot \text{pH}^{-1}$, which is $\sim -59 \text{ mV}$, and this clearly suggests that the oxidation of glucose on Au/Pt-black/Nf modified MNEA involves 2 e^- and 2 H^+ (1:1) as per

the Nernst equation [42]. Therefore, a pH of 7.4 was selected as an appropriate pH for the entire electrochemical study of glucose oxidation at the Au/Pt-black/Nf MNEAs.

3.2.5. Response studies of modified MNEAs in PBS and ISF

The analytical characterization of the modified MNs towards glucose addition was assessed by chronoamperometric i vs. t curves at a fixed applied electrode potential of +0.12 V ($E_{app} = +0.12 \text{ V}$ vs. AgCl MN). The potential was carefully selected to overcome the overvoltage and any interference caused by the interferences present in the ISF. Fig. 8a & 8c shows the amperometric response curves of the Au/Pt-black/Nf bioelectrode in 10 × PBS and ISF, respectively, for the successive step addition of various glucose concentrations in PBS (pH = 7.4) and ISF at E_{app} of +0.12 V vs. AgCl MN. The sensor response characteristics in both electrolytes, ISF and PBS are summarized in Table 1. The modified electrodes show a linear increase in peak current at +0.12 V with the successive addition of glucose concentrations in both electrolytes. The MN electrodes show a wider glucose dynamic range of 1 – 20 mM both in PBS and ISF (Fig. 8b & 8d). The electrodes show a detection limit (DL) of 10 and 22.5 μM in PBS and ISF, respectively. The DL was calculated by using the formula $3 \times \text{SD}/\text{slope}$; where SD is the standard deviation of the blank electrode response and slope of the calibration curves is the sensitivity of the electrode in presence of glucose [43]. It is demonstrated in Table 1 that the sensitivity of the modified electrode in PBS ($5.786 \pm 0.17 \mu\text{A} \cdot \text{mM}^{-1} \cdot \text{cm}^{-2}$) is more than that of in ISF

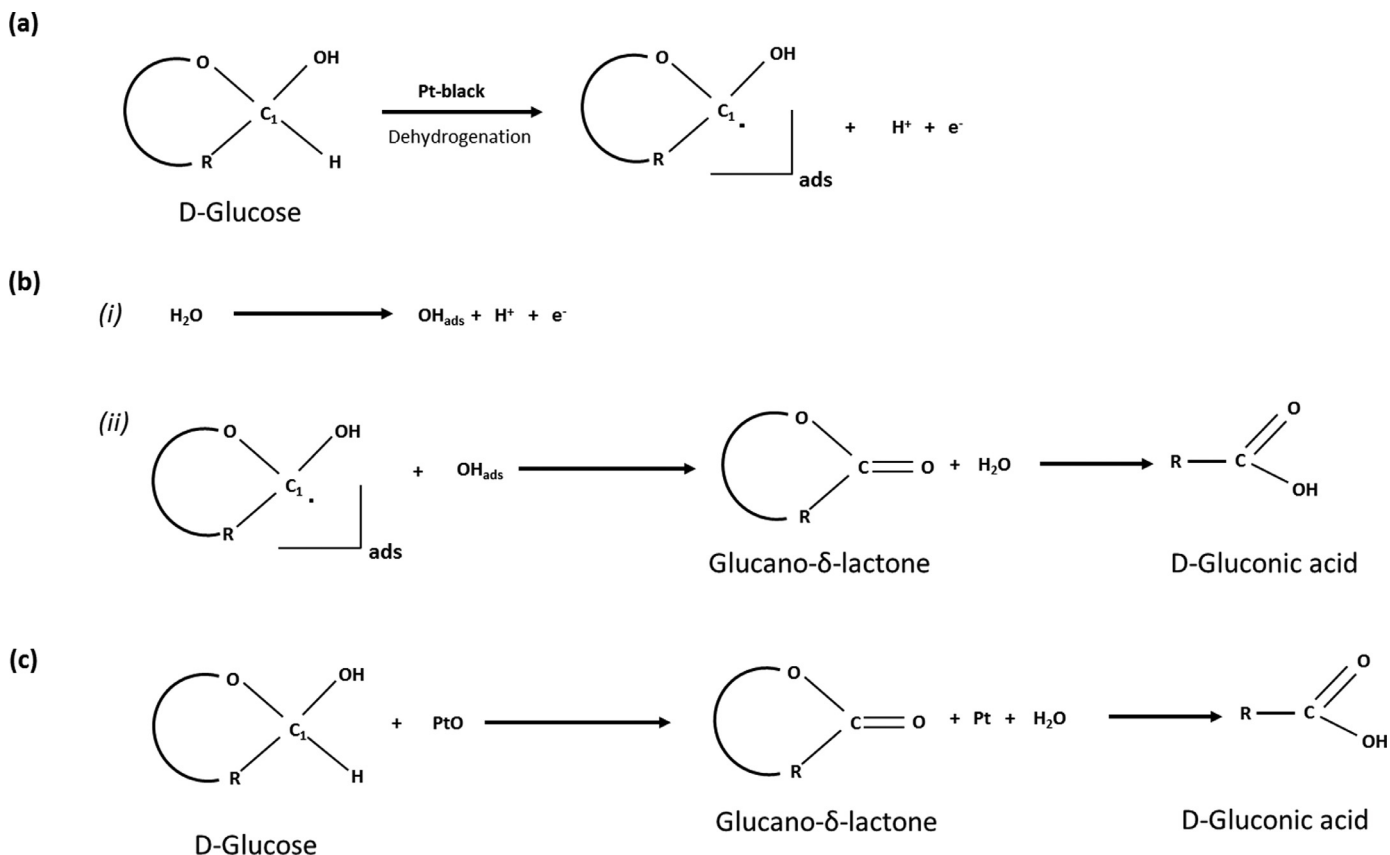


Fig. 6. Schematic representation for the possible mechanism of glucose oxidation on the Pt-black surface at near neutral solution (pH 7.4).

Table 1

Comparison of the analytical figures-of-the merit for our fabricated Au/Pt-black/Nf MNEA sensor in PBS and ISF with other reported non-enzymatic and enzymatic sensors.

Electrode Configuration	Sensitivity ($\mu\text{A mM}^{-1} \text{cm}^{-2}$)	Dynamic range (mM)	Detection limit (μM)	Electrolyte	Reference
PANI@CuNi/GC (Non-enzymatic)	1030	0.1–5.6	0.2	NaOH	[40]
Ni/ITO (Non-enzymatic)	576.7	0.2–10	50	NaOH	[41]
CuO NFs-ITO (Non-enzymatic)	873	0.2–1.3	0.04	NaOH	[44]
CuNWs/GTE (Non-enzymatic)	1100	0.05–6.0	1.6	NaOH	[45]
GCE/PAA-Ni (Non-enzymatic)	N.R	5–12	0.64	NaOH	[46]
PEDOT: PSS/CuO/MWCNTs-PEGs (Non-enzymatic)	663.2	0.0–10	0.23	NaOH	[47]
CuO/PANI-NF/FTO (Non-enzymatic)	N.R	0.25–4.6	0.24	NaOH	[48]
3D Co ₃ O ₄ /Ni (Non-enzymatic)	13,885.0	0.04–3.6	1.0	NaOH	[49]
Pt/MWCNT/MN (Non-enzymatic)	17.73 $\pm$ 3	3.0 – 20	N.R	PBS	[50]
PMMA/MN (Non-enzymatic)	N.R	0 – 20	N.R	ISF	[6]
AuMN/Au-MWCNT/pMB/GDH (Enzymatic)	405.2 $\pm$ 24.1	0.05–5	70	ISF	[4]
AuMN/h-PG/FcSH/GDH (Enzymatic)	50.86	0.1 – 10	50	ISF	[51]
Au/Pt-black/Nafion (Non-enzymatic)	5.786 $\pm$ 0.17	1 – 20	10	PBS	This work
Au/Pt-black/Nafion (Non-enzymatic)	4.380 $\pm$ 0.21	1 – 20	22.5	ISF	This work

N.R: Not reported.

(4.380 $\pm$ 0.21 $\mu\text{A mM}^{-1} \text{cm}^{-2}$) due to better diffusion of the glucose into the catalytic Pt-black layer. The higher sensitivity in turn led to an improved detection limit (DL) in the case of the modified electrode in PBS (10 μM) compared to the ISF (22.5 μM).

A comparative analysis on the figures of merit for various prominent non-enzymatic sensors and minimally invasive non-enzymatic and enzymatic glucose MNs reported in the past decade shows that the present Pt-black-coated minimally invasive needles offer a wide linear range of detection and an improved detection limit with acceptable sensitivity and good stability of the non-enzymatic MNs for CGM. The sensor showed lower sensitivity in comparison to the studies reported in [40, 41, 44, 49, 50 and 51], but shows higher dynamic range compared to the reports [40, 41, 44, 45, 46, 47, 48, 49, 50, 51, 6 and 4]. The main advantages of

the present study are the prevention of the parylene reactive-ion etching step which is economical for the mass production of MNs and fabrication of the AgCl MN reference electrode for sensing, the optimization of the electroplating conditions, histogram analysis of the rat skin inserted with the MNs, and response studies of the developed MNs *in-vitro* and *in-vivo* ISF. In order to validate the application potential of the non-enzymatic minimally invasive MN sensor, the modified Au/Pt-black/Nf electrode array was applied for the estimation of four different glucose concentrations (3.0, 5.0, 8.0, and 12.0 mM) spiked in ISF. The results show a good recovery range (98.0%–102.5%; $n = 5$) and found no significant difference ($P = 0.572$) between the measured and the spiked glucose concentrations. The detailed information and the recovery rates with % RSD's is given in the Table. S2. We recorded four different glucose

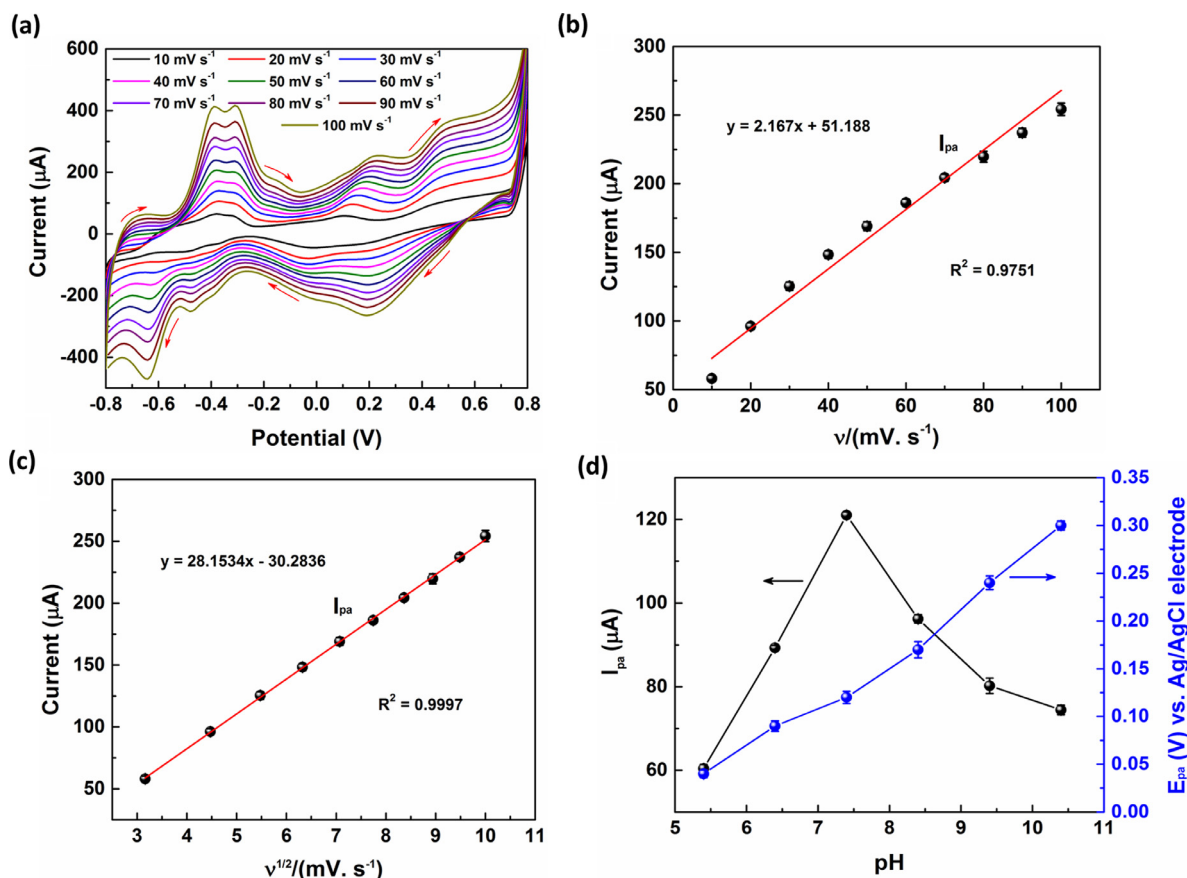


Fig. 7. CVs of Au/Pt-black/Nf MNEA at various scan rates ranging from 10→100 mV s⁻¹ in 10 × PBS, pH 7.4 containing 5 mM of glucose (a), the linear relationship between the scan rate vs. I_{pa} (b) and square root of scan rate vs. I_{pa} (c) with regression equations. The relationship of pH with E_{pa} and I_{pa} carried out at different pH ranging from pH 5.4 → 10.4 in the presence of 5 mM of glucose at 50 mV s⁻¹ (d). Each data point represents the average of three independent experimental values ($n = 3$), with the range indicated in the standard error bars (d).

concentration spikes at 3.0, 5.0, 8.0, and 12.0 mM respectively, and applied to the modified microneedle electrode arrays. We carried out five different measurements for each glucose concentration. The average of the five measurements ($n = 5$) was recorded and the percentage of the recovery was tabulated. It could be seen from Table. S2 that the glucose concentrations in the spiked artificial ISF samples were 2.94, 4.95, 8.20, and 12.18 mM with the corresponding recoveries of 98.0% ($n = 5$), 99.0% ($n = 5$), 102.5% ($n = 5$), and 101.5% ($n = 5$), respectively. The results showed a good recovery range for the five measurements and we found no significant difference ($P = 0.572$) between the spiked and the measured glucose concentrations using the fabricated MNEA. Hence, the Au/Pt-black/Nf modified microneedle array could be effectively used for an accurate glucose determination in ISF samples.

3.3. Operational stability and precision

The operational stability of the fabricated MNEAs was studied by measuring the current response towards glucose addition as a function of time (Fig. 9a). For a period of 7 h, 4 measurements were performed in a concentration range of 1 – 12 mM of glucose using the freshly prepared MNEA in 10 × PBS at pH = 7.4. At the end of the 4th measurement, the electrode shows a 2% decrease in the initial response, probably due to a decrease in the glucose diffusion rate into the catalytic layer with an increase in viscosity of the buffer solution. Other important factors for the clinical use of the MN-based sensor is reproducibility and repeatability. The reproducibility and repeatability of the fabricated MN electrode was investigated by measuring the intra- and inter-assay

relative standard deviations (RSD). The inter-assay precision was carried out in a glucose concentration range of 1 – 12 mM with three different MNEAs ($n = 3$) (Fig. 9b). The intra-assay precision of the MN was carried out in a range of glucose concentration from 1 – 20 mM with three replicate measurements of a single MNEA ($n = 3$) (Fig. 9c). The assays show an RSD of 1.94% and 2.32% for the intra- and inter-assay precisions, respectively, suggesting a satisfactory repeatability and reproducibility for the developed MN sensor.

3.4. Interference study

In order to demonstrate the specificity of the glucose MN sensor for possible ISF interfering agents on its response, the voltammograms were measured in 10 × PBS containing glucose and each interferent individually. The selectivity coefficient (SC) of the developed MN array for each interfering agent (0.5 mM) was computed by applying the formula, $SC = I_{g+i}/I_g$, where I_g and I_{g+i} are MN response at +0.12 V vs. AgCl MN for glucose (0.5 mM) in the absence and presence of each interferent, respectively. The results suggest no significant change in the MN response in the presence of the common ISF interferents (Fig. 9d), thus confirming a good selectivity of the MN sensor. The biocompatible protective film Nafion assured, to exert an electrostatic repulsion for low accessibility of the interferent oxidation onto the electrode surface [25].

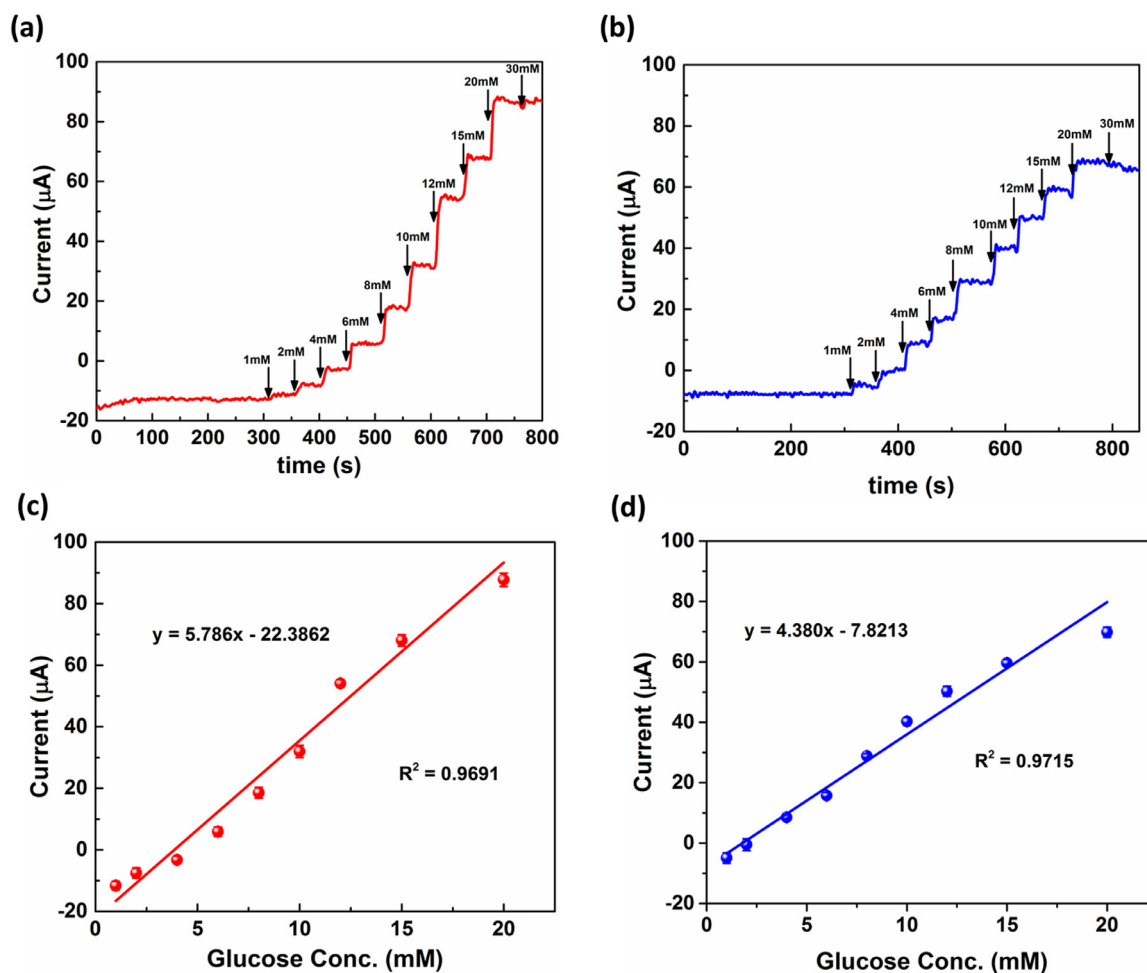


Fig. 8. Chronoamperometric response curves (i vs. t) for the Au/Pt-black/Nf MNEAs in $10 \times$ PBS and ISF, respectively (**a & b**) with successive addition of various glucose concentrations (1–30 mM) at an applied external potential of +0.12 V vs. AgCl MN. Linear calibration plots for the glucose response curves in PBS and ISF respectively, with linear regression equations (**c & d**). Each data point represents the average of three independent experimental values ($n = 3$), with the range indicated in the standard error bars (**d**).

3.5. Animal studies

For the *in vivo* studies, we precisely packed the modified MNs with bio-compatible Nafion membrane to prevent biofouling at the MN electrode surface. The needles were inserted manually using physical force and the length of the MN tip coated with the Pt-black sensing layer was 550 μm long. The WE (Au/Pt-black/Nf), RE (AgCl MN), and CE (bare Au MN) were attached to the dorsal skin of the rat model (3 M TegadermTM film, 3 M Deutschland GmbH, Neuss, Germany), using a cohesive elastic band (3 M CobanTM) (Fig. S6b) and monitored for a background current stabilization. Once the background current stabilized, the rats were fed orally with a 20% glucose solution (Fig. S6a). The ISF glucose values were electrochemically measured for every 5 min time intervals. The histogram analysis was carried out to verify the insertion depths of the MN arrays applied on the dorsal side of the subcutaneous tissue in the rat models. The study shows a MN array depth penetration of $598.70 \pm 2.1 \mu\text{m}$ ($n = 3$) suggesting that the needle insertion occurred across the stratum corneum (10–40 μm), the viable epidermis (epidermis thickness: 50–500 μm), and superficial dermis (dermis thickness: 500–2000 μm), as indicated in Fig. 10a. The ISF is usually present in the dermal compartment constituting 45% of the volume fraction in the mammalian skin with a larger amount in the dermis [23]. The results demonstrated that the height of the fabricated Au MNs was sufficient and sharp enough to penetrate

into the rat skin, for easy access of the ISF present in the dermal compartment, and to successfully sense the glucose level variations in the ISF during the OGTT test. Fig. 10b shows the regeneration of the epidermis following the coagulation changes in the underlying dermis with mild lymphocytic infiltration in the needle insertion site with very minimal damage to the epidermis.

The glucose solutions were administered orally thrice a week and the glucose levels were checked continuously in the subcutaneous tissue right after ingestion, as well as 1, 2, 4, and 6 h after ingestion, respectively. In order to correlate the rise in the ISF glucose levels to that in the capillary, the blood was simultaneously obtained using a commercial glucose meter (Accu-chek[®] active, Roche diagnostics GmbH, USA) and measured for increasing blood glucose levels. The measured blood and ISF glucose levels during the OGTT are shown in Fig. 11a–d. The currents measured from the fabricated modified MN and the capillary glucose levels estimated through the glucometer (Accu-chek[®] active, Roche diagnostics GmbH, USA) were compared and calibrated using a linear regression analysis by following the equation; $[CG] = a \times [OUT] + b$, where a is slope of calibration curve, CG is a calibrated glucose level, OUT is signal output from MN glucose sensor, and b is a constant of calibration curve [52]. The a and b parameters calculated are 0.413 and 110.92, respectively. The calibrated glucose levels from the fabricated device and the capillary glucose levels were recorded for 10 days. The device shows a good correlation between

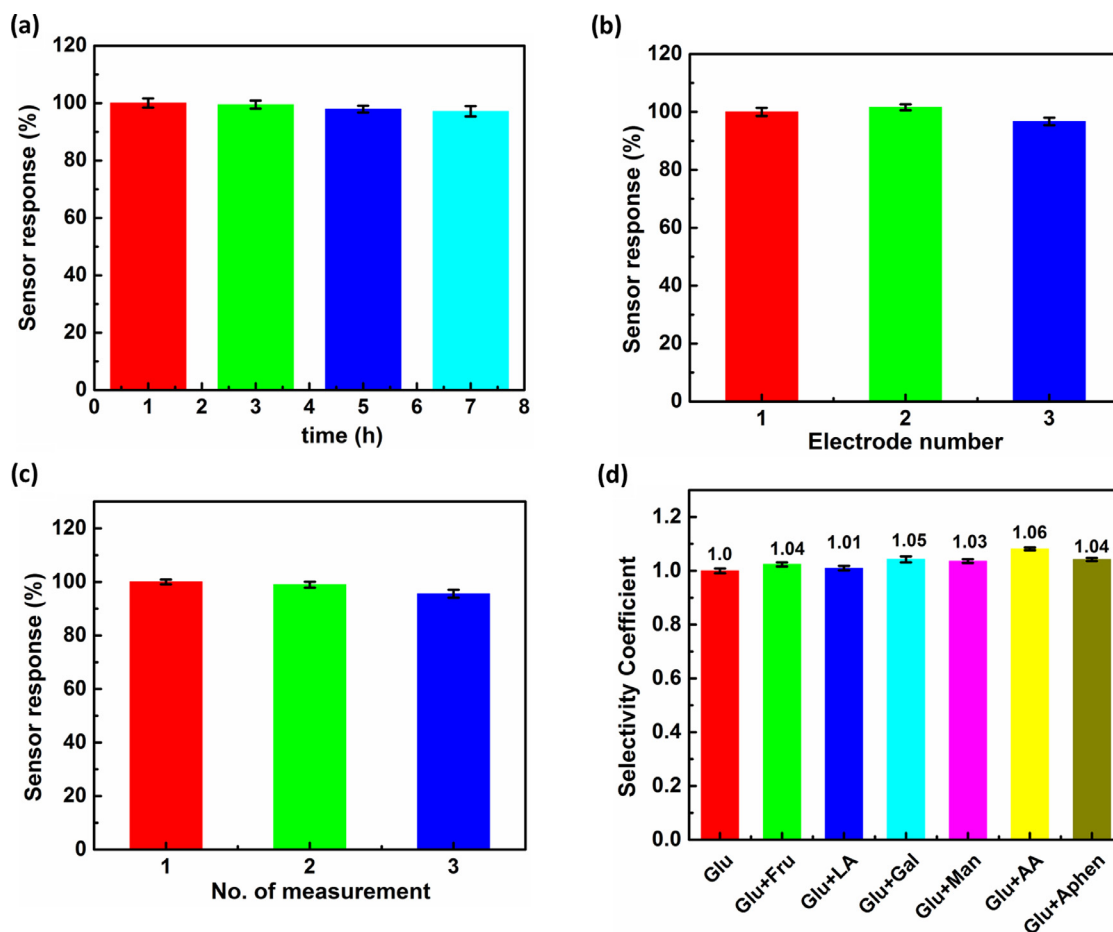


Fig. 9. Operational stability of the microneedles with the addition of glucose in a range of 1 – 12 mM after background current stabilization in PBS (pH = 7.4) at 24 °C (a), I vs. t plots for reproducibility (b) and repeatability (c) studies in a range of glucose concentrations (1 – 12 mM) at an applied external potential of +0.12 V vs. Ag/AgCl microneedle in 10 × PBS. Interference study of the sensor in the presence of common interferents (Fru: fructose, LA: lactic acid, Gal: galactose, Man: mannose, AA: ascorbic acid, and Aphen: acetaminophen) present in ISF, carried out in 10 × PBS at pH = 7.4 (a). Each data point represents the average of three independent experimental values ($n = 3$), with the range indicated in the standard error bars (d).

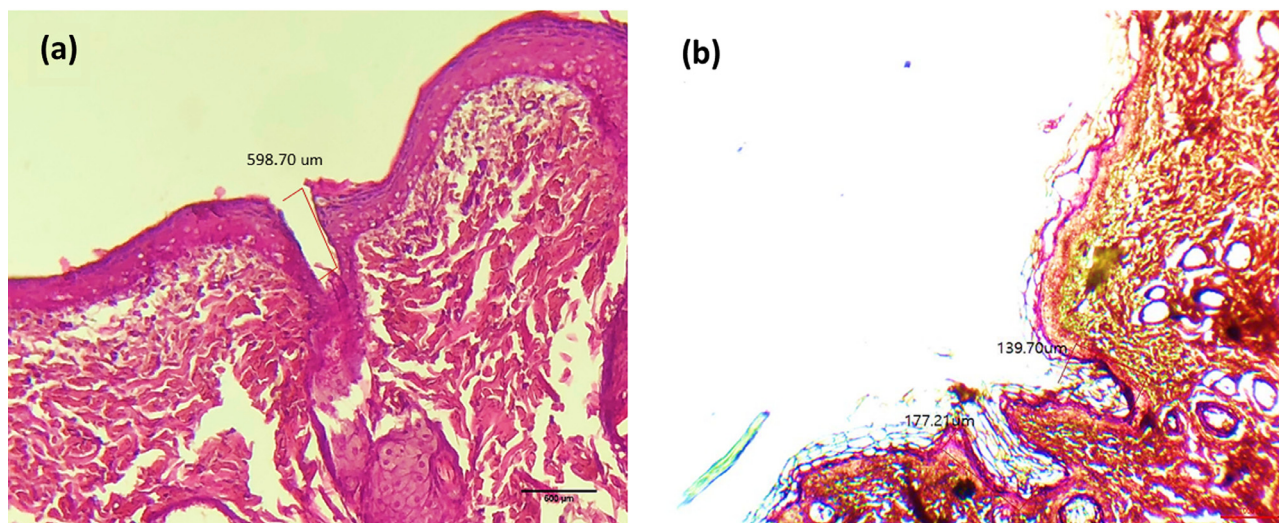


Fig. 10. Typical histological skin sections of rat model showing the Au MN penetration into superficial dermis (a). Tissue section taken on the onset of 8th day showing the regeneration of the epidermis (b).

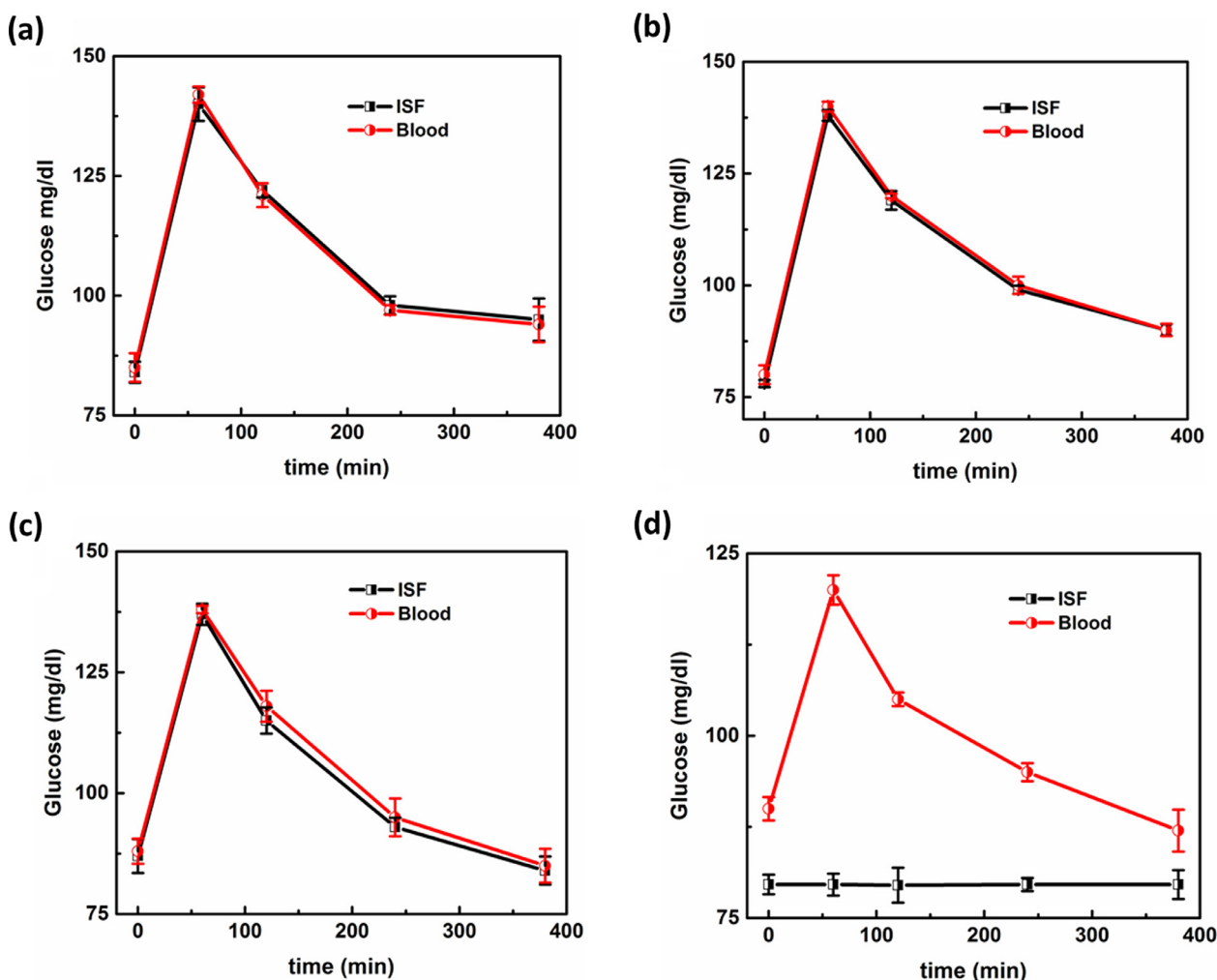


Fig. 11. CGM of the *in vivo* attached sensor for successive 10 days, following the attachment of the MNEA sensor to the rat skin: Sensor stability at Day 1 (a), Day 3 (b), Day 7 (c), and Day 10 (d). Each data point represents the average of three independent experimental values ($n = 3$), with the range indicated in the standard error bars (d).

the ISF and capillary glucose levels on days 1 and 7, whereas the needle completely lost its activity by the end of day 10. The loss in catalytic activity might be due to the biofouling effects on the MN surface over a prolonged period. The SEM images also reveals that the Pt-black coating on the needle surface is intact after 10 days of the continuous operation in rat sub-cutaneous skin models. The SEM images also show that no bending and breakage of the needle after 10 days of the MNEA operation in the rat model (Fig. S7).

Conclusions

The study validated the application of the MNEA as a painless and minimally invasive CGM device. The main impact of this study is the realization of a highly porous Pt-black microneedle electrode and the use of Nafion as a packaging polymer for interference free detection of glucose. The porous Pt-black enhanced the electroactive surface area of the Au MNs by 17 folds thus increasing the sensitivity of the sensor. The device showed a good dynamic range, detection limit and recoveries with acceptable RSD's in ISF. However, the *in vivo* sensor response after fixing the device onto a rat model skin shows a moderate lifetime of 7 days. We assume that the constraint in the *in vivo* device stability was due to the biofouling of the electrode surface with the host proteins. The obtained results are promising for the use of the fabricated MNEA-based non-enzymatic sensors as wearable devices in the field of medicine for the CGM in diabetic patients. In the future, studies should focus

on the effect of electrode size on the sensor response and coating conditions of the packaging polymers in order to enhance the performance of the MNs and decrease the biofouling of the electrode arrays during the *in vivo* studies. However, future studies should also be devoted to the toxicity associated with the usage of the developed devices, as well as the possible integration of electronics for wireless communication.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The study was supported by the National Research Foundation of Korea (No. NRF-2018R1C1B6009385, 2018M3A9F1023690, NRF-2019R1A2C1088680).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.electacta.2020.137691](https://doi.org/10.1016/j.electacta.2020.137691).

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