Article title: Graphene nanocomposites for transdermal biosensing

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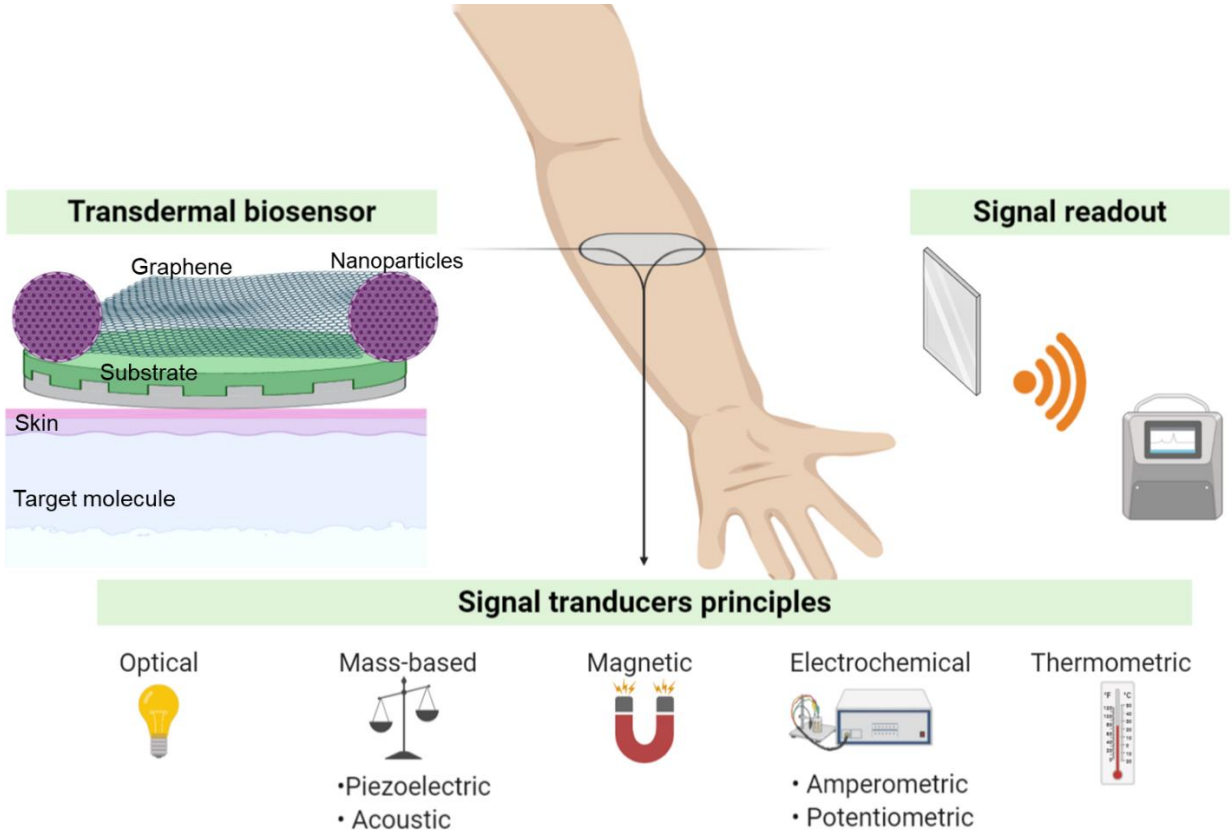
Abstract:

Transdermal biosensors for the real-time and continuous detection and monitoring of target molecules represent an intriguing pathway for enhancing health outcomes in a cost-effective and non-invasive fashion. Many transdermal biosensor devices contain microneedles and other miniaturized components. There remains an unmet clinical need for microneedle transdermal biosensors to obtain a more accurate, rapid, and reliable insight into the real-time monitoring of disease. The ability to monitor biomarkers at an intradermal molecular level in a non-invasive manner remains the next technological gap to solve real-world clinical problems. The emergence of the two-dimensional material graphene with unique material properties and the ability to quantify analytes and physiological status can enable the detection of critical biomarkers indicative of human disease. The development of a user-friendly, affordable, and non-invasive transdermal biosensing device for continuous and personalized monitoring of target molecules could be beneficial for many patients. This focus article considers the use of graphene-based transdermal biosensors for health monitoring, evaluation of these sensors for glucose and hydrogen peroxide detection via *in vitro*, *in vivo*, and *ex vivo* studies, recent technological innovations, and potential challenges.

Keywords: transdermal biosensing; graphene; microneedles; glucose; hydrogen peroxide

Graphical abstract



Caption

Schematic diagram of the non-invasive transdermal biosensor based on graphene and nanoparticles, a signal readout panel and signal transducer principles (resulting in data being collected and transmitted to the device).

Introduction:

The global wearable sensors market size was noted to be approximately $150 million in 2016 and is anticipated to reach $2.86 billion by 2025 **[1];** a large part of this market is focused on non-invasive glucose monitoring **[2]**. Glucose biosensors for the management of diabetes account for approximately 85% of the world's biosensor market **[3]**. Wearable biosensors have garnered considerable attention due to their high sensitivity, selectivity, straightforward replacement, portability, minimally invasive nature, low cost, continuous, real-time analysis, and non-invasive chemical measurements of biomarkers in biological fluids **[4]**. Recent advances in electronics have enabled devices to shrink from room-size to pocket-size, with rapid response time, high speed, and reduced power dissipation. These miniaturized and automated biosensors have become less expensive and allow for more rapid on-site and *in situ* testing and analysis than traditional medical devices that require bulky, fragile, expensive, and labor-intensive instrumentation **[4]**. Advanced sensing platforms are used in state-of-the-art biomedical devices, which incorporate a wide range of sensing matrices efficiently in a small form such as electromechanical, biological, electrocardiogram, electromyogram, and electroencephalogram sensing, thereby providing efficient storage and processing functionality **[5]**. Such advanced biosensors use optical and electrochemical sensing processes for the monitoring of electrolytes (e.g., pH, sodium, potassium, and calcium), metabolites (e.g., glucose and lactate), pathogens (e.g., bacteria and viruses), and hormones in biofluids such as tears, sweat, intestinal fluids, and saliva. These biosensors have been miniaturized to improve the wearability of the devices.

The most common design of a biosensor comprises of two functional units: i) a bioreceptor (for example, an enzyme, aptamer, antibody, or nucleic acid), which directly interacts with the analyte and is responsible for specific and selective detection of the target, and (ii) a transducer (for example, an electrochemical or optical device), which translates the biorecognition into a signal and transmits the signal to the device. The key mechanisms of biorecognition include adsorption, microencapsulation, bonding, and cross-linking. Based on the type of reaction, various types of transducers can be employed such as electrical, optical, conformational, thermal, or acoustic devices **[6]**. The transducer functions by converting one form of energy to another. Some sensors use a labeling approach, in which fluorescent probes are used to detect the interaction of the target analyte with the probe; others use label-free approach, in which the interaction between the target analyte and the probe is directly measured. Signal transduction is achieved using a variety of computational approaches, which translate the signal into clinically relevant information. Wearable sensing technologies have revolutionized healthcare monitoring, enabling early detection of disease at a treatable stage, prevention strategies, treatment strategies, and continuous monitoring of a disease progression in response to a specific treatment modality. Wearable biosensors have the advantages of compactness, robustness, low cost, fast reaction times, and low maintenance costs. The major advantage of wearable sensing technologies is point-of-care analysis of diseases in rural areas and developing countries where healthcare facilities and well-trained personnel to operate the diagnostic tools are lacking.

Several types of wearable bioelectronics based on transdermal biosensors have been developed using nanomaterials **[7]**. Microneedles have extensively been incorporated within wearable biosensors **[8-10]**. Microneedles have been fabricated from various materials (e.g., silicon, stainless steel, palladium, titanium, nickel, polymer, plastic, glass), in different shapes (e.g., cylindrical, conical, pyramidal), with varying lengths (hundreds of micrometres to ~ 1.5 millimetres), and types (solid and hollow) **[11]**. Microneedles have been used for transdermal delivery of several agents, including insulin, vitamin B, calcein, proteins, DNA, and vaccines; delivery of these agents has been demonstrated using *in vitro*, *in vivo*, and *ex vivo* studies. Some of the earliest microneedles for transdermal drug delivery were fabricated by Henry et al. **[12]** A wide range of microneedle-based cosmetic products are also available in the market; these products are generally used for skin care treatments. Smaller microneedles are used at home to improve skin texture; larger microneedles are employed in the clinic to treat skin scars and hyperpigmentation. Hollow microneedles (1.5 mm in length) have been used to deliver the influenza vaccines Intanza or IDflu **[13]**. In addition to transdermal delivery of drugs, microneedles have also been used for transdermal biosensing of glucose and biomarkers in interstitial fluid (ISF) **[14]**. For example, microneedles have been used for *in situ* detection of glucose in ISF **[15]**. Microneedles have advantages over conventional self-monitoring blood glucose monitoring devices such as acquisition of low amounts of blood, less pain, and less discomfort. Efforts focused on the application of microneedles for analyte detection at the transdermal level have been reviewed elsewhere **[10, 16, 17]**. Despite significant advancements made in the use of microneedles for transdermal biosensing, there are several limitations to this technology **[8, 10, 16]** such as; i) controlled skin penetration of microneedle electrodes to a known skin depth; ii) the use of microneedles is associated with pain, needle phobia, and tissue damage; iii) limitations to on-chip analyte collection and analytical performance; iv) the performance of on-chip bioreceptor, sensing, and transduction systems; and v) device issues such as biocompatibility, stability, and surface biofouling.

Real-time transdermal detection of analyte using microneedles is still in its infancy. However, nanomaterials-based biosensors have emerged for the detection of biomolecules owing to the unique physiochemical, mechanical, optical, electrical, and thermal properties of nanomaterials in comparison to their bulk counterparts **[18]**. Nanomaterials are found in a wide variety of shapes such as zero-dimensional (0D) structires (e.g., nanoparticles and quantum dots), one-dimensional (1D) structures (e.g., nanowires and nanotubes), two-dimensional (2D) structures (e.g., graphene and other single-layer materials)and three-dimensional (3D) structures (e.g., foams and aerogels). Owing to their size-dependent chemical, physical and optical features, the design and development of sensors with nanoscale materials has potential to propel the field of biosensing. The integration of microneedles with nanomaterials to create transdermal electrodes is a current area of research activity. A promising approach involves the use of graphene in transdermal biosensors **[19, 20]**. Graphene is a two-dimensional sp^2 hybridized material **[21]**. It was first exfoliated by Novoselov et al. using the scotch tape method; Geim and Novoselov accepted the Nobel Prize in Physics in 2010 for the isolation of graphene **[22]**. Graphene exhibits a high specific surface area, interesting mechanical properties, superior electrical conductance, as well as good electron and carrier mobility **[23]**. These features make

graphene an ideal material for use as an electrical surface and for sensing target molecules. Graphene derivatives include pristine graphene, graphene oxide, reduced graphene oxide, porous graphene, graphene quantum dots, and three-dimensional graphene. A schematic showing the structures of the graphene derivatives is shown in **Figure 1.** Graphene has been used in many biomedical applications, including drug delivery, disease diagnosis, photodynamic therapy, photothermal therapy, and wound healing **[23]**. There are four main synthesis routes to prepare graphene, including mechanical exfoliation, chemical exfoliation, chemical reduction, and chemical vapour deposition **[23, 24]**. **Tables 1 and 2** summarize the key features of graphene-based materials for biosensing systems as well as the advantages/disadvantages of using graphene materials as biosensors, respectively. The size, shape, morphology and functional groups present on the edges or basal planes of graphene affect the performance of a biosensor. The size, shape and porosity of graphene influence the distribution of layers of graphene and the interstacking distance between layers. For example, homogenous integration of graphene with other structures allows a large high specific surface area available to transport electrons and to adsorb target analytes. Several studies have been reported on the optimization of performance of a biosensor via tuning the size and morphology of graphene **[25]**.

Pristine graphene, graphene oxide, reduced graphene oxide, graphene integrated with metal nanoparticles, graphene integrated with metal oxides, graphene integrated with quantum dots, and polymeric nnaocomposites have been used to monitor glucose, hydrogen peroxide (H_2O_2), disease biomarkers, DNA, RNA, proteins, enzymes, genes, adenosine triphosphate (ATP), dopamine, ascorbic acid, uric acid, cancer biomarkers, bacterial/fungal/viral strains, heavy metal ions, antibodies, and many other target molecules **[25, 26]**. Additionally, graphene may be modified with biomolecules such as enzymes and antibodies for biomedical applications. Recently, human-like robot wearable tactile sensors containing 3D microstructured graphene integrated with polydimethylsiloxane (PDMS) have been reportedfor detection of capacitive pressure with high sensitivity, flexibility, low detection limit, and stability **[27-29]**. Structures that contained layer-by-layer sandwiching of graphene with polymers showed appropriate capactive pressure sensing functionality. Graphene-polymer composites have also been used for electrochemical the detection of cortisol, glucose, and cytokines in sweat **[30-32]**. To date, however, there has been no comprehensive review on transdermal biosensing using graphene-based nanocomposites. The majority of literature has focused on graphene-based electrochemical and fluorescent biosensors. Therefore, this focus article is intended to provide key insights into advances in graphene-based transdermal biosensors. Specifically, we discuss how graphene can be integrated with metallic nanoparticles and microneedles to develop efficient transdermal biosensors. We critically highlight groundbreaking and innovative studies that have potential to impact the field of transdermal biosensing.

Graphene-based transdermal biosensors

Accurate and reliable measurement of glucose levels is not only of crucial importance to diabetic patients but also to premature neonates and others **[40]**. The currently available gold standard approach requires the collection of a blood sample **[41]**. A finger-stick calibration is a major limitation associated with conventional glucose detection methods; moreover, this approach

is associated with indiscriminate extraction of blood and substantial dilution of blood before quantification. The incorporation of graphene within integrated devices for electrochemical detection of glucose has received significant attention **[42]**. Nanomaterials are incorporated within the highly conductive surface of graphene due to its high specific surface area, electrical conductivity, and ability to adsorb proteins and biomolecules. Lipani et al. **[19]** reported on a transdermal glucose monitoring pixel array system based on graphene. Glucose was extracted transdermally via electroosmotic forces through follicular pathways and then monitored via an array of miniature pixels, thus providing calibration-free detection of glucose levels. They demonstrated measurement of glucose levels using a process that considered the area and volume of pixel arrays. In this study, two graphene platforms were used for glucose detection: (a) a graphene-based film (prepared by chemical vapour deposition (CVD)) was modified with Pt nanoparticles, and (b) a graphene ink-based electrode; at this electrode, glucose oxidase reacted with glucose to generate H_2O_2. CVD is a widely used method to prepare graphene; the graphene film is commonly deposited on Ni or Cu substrate and then transferred to another substrates (e.g., PDMS, poly(methyl methacrylate, or poly(ethylene terephthalate). This substrate-supported method has widely been used to fabricate graphene-based devices and electrodes **[26]**. Graphene prepared using the CVD method has been used for the detection of biomolecules such as glucose, DNA and H_2O_2. The electrode was shown to detect glucose levels over six hours. Continuous monitoring of ISF glucose was demonstrated in mammalian skin *ex vivo* tissues and in an *in vivo* model. **Figure 2** represents the *ex vivo* extraction–detection experiments with graphene arrays of various sizes. Graphene integrated with Pt nanoparticles showed higher detection efficiency than pristine graphene; this result was attributed to the higher specific surface area and higher electrical conductivity of the nanoparticle-modified graphene. This calibration-free non-invasive strategy to monitor glucose opens new mechanisms for glucose monitoring that do not rely on invasive blood sampling.

Overexpression of H_2O_2, a reactive oxygen species, is associated with many types of neurodegenerative diseases and forms of cancers **[43]**. H_2O_2 is also a by-product of enzymatic reactions **[44]**. Accurate and rapid detection of H_2O_2 is a focus of research activity for this reason. Graphene nanostructures have widely been used for the detection of H_2O_2 **[45]**. Integration of nanoparticles with graphene improves the selective and sensitive monitoring of H_2O_2. Integration of nanomaterials on microneedle surfaces as transdermal electrodes is another area of wearable electronic device research. Jin et al. **[20]** described an electrochemical biosensor based on microneedles that were integrated with the combination of reduced graphene oxide and Pt nanoparticles for transdermal detection of H_2O_2. The microneedle electrode containing reduced graphene oxide and Pt nanoparticles showed high sensitivity. In this example, microneedles were employed to evaluate the *in vivo* H_2O_2 detection. The integrated graphene-Pt nanoparticle electrode was protected by a polymer layer to prevent mechanical damage during skin insertion. The polymer surface was instantly dispersed in ISF, which exposed the graphene-Pt integrated surface for detection of the target molecule. Real-time monitoring of H_2O_2 was carried out on pigskin and mice. **Figure 3** shows the *in situ* and real-time monitoring of H_2O_2 in biological tissues and pig skin. The biosensor contained a three-electrode system; the reduced graphene oxide-Pt-microneedle, Pt-coated microneedle patch, and Ag/AgCl coated microneedle patch were used as

the working, counter, and reference electrodes, respectively. The surface morphologies of counter and reference electrodes are shown in **Figure 3 b**. The Pt-reduced graphene oxide microneedle patch was applied on the skin surface (**Figure 3 c**). **Figure 3 d** shows the detection of H_2O_2 by the Pt-reduced graphene oxide that was integrated with microneedles.

The transdermal biosensing applications of graphene have been discussed above. In addition to transdermal biosensing, graphene has widely been used in flexible electronics and wearable sensing devices such as mechanical sensors (e.g., breath, pulse, motion, and acoustic sensors), electrophysiological sensors (e.g., ECG, EMG, EEG, and EOG devices), fluid sensors (e.g., glucose and organic molecule sensors), and gas sensors (e.g., humidity, NO_2, NH_3, and acetone sensors). These sensing technologies are beyond the scope of this focus article. However, we refer the reader to more specialized review papers that have been published in the area of graphene-based sensing systems **[46-48]**.

Conclusion and future outlook

Transdermal biosensors have made significant progress in recent years due to their capabilities for selective and sensitive detection of analytes, low cost, ease of use, ease of implementation, portability, and small size. However, the successful clinical translation of these devices is not straightforward. There are significant challenges related to the viable commercialization of these sensors such as challenges associated with large-scale fabrication, challenges associated with cost-effective fabrication, surface biofouling from biological fluids, on-chip calibration, instabilities of the biorecognition and bioreceptor assays under extreme environments, challenges associated with functionality during prolonged use of the device, challenges associated with reproducibility of the device, and regulatory approval. In addition to these limitations, an important consideration is the privacy of the medical data that is recorded by a biosensor. In addition, successful clinical translation efforts must take into account the regulatory framework. Considerable advances have been made in the field of microneedle-based transdermal biosensing systems as a first step towards intradermal molecular monitoring. However, no microneedle transdermal biosensor is commercially available at this time. Graphene can improve the accuracy and reproducibility of signals in transdermal biosensors owing to their electrical conductance, high specific surface area, and mechanical strength. Further extensive research is needed for commercial translation of graphene-based transdermal biosensing devices.

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Table 1: Summary of key features of graphene-based materials for biosensing

Features and application	**Graphene**	**Graphene oxide**	**Reduced graphene oxide**	**3 D graphene network (foam)**	**Ref.**
synthesis	mechanical exfoliation, chemical vapour deposition	Hummers method, modified Hummers method, Tour's method	reduction of graphene oxide	chemical vapour deposition by using Ni foam template	34
surface area (m^2g^{-1})	2630	466-500	833-1000	850-1275	35
electron mobility ($cm^2\ V^{-1}\ s^{-1}$)	15000-50000	Insulator	0.05-200	4050	36
Application in biosensing	Glucose (because of the fast electron transport process of graphene)	Glucose, cholesterol, DNA (because of the catalytic activity of the enzymes and unique electrochemical properties of graphene oxide. In addition, graphene oxide can make π–π bonds between the conjugated systems and DNA))	Gluocose, cholesterol, hydrogen peroxide (H_2O_2), DNA (because of high specific surface area, and superior electric conductivity. In addition, reduced graphene oxide can make π–π bonds between the conjugated systems and DNA)	Gluocose, hydrogen peroxide (H_2O_2) (because of the high specific surface area, mechanical flexibility, and electric conductivity)	25

Table 2: Advantages and limitations of using graphene materials for biosensing.

Graphene materials	Advantages	Limitations	Ref.
pristine graphene	higher specific surface area; electron mobility	absence of oxygen containing functional groups	37
graphene oxide	high dispersibility in water; presence of functional groups onto the planes and edges of graphene	limited amount of adsorption places	37
reduced graphene oxide	good electron transportation features	low amount of functional groups	37
three-dimensional graphene foam	large specific surface area	low amount of functional groups	38, 39
functionalized graphene composites (graphene paper/PtAu–MnO_2 nanocomposites)	higher specific surface area than pristine graphene and other graphene materials	the stability of the adsorbate changes with the adsorption strategy such as; physical and chemical adsorption	25, 38

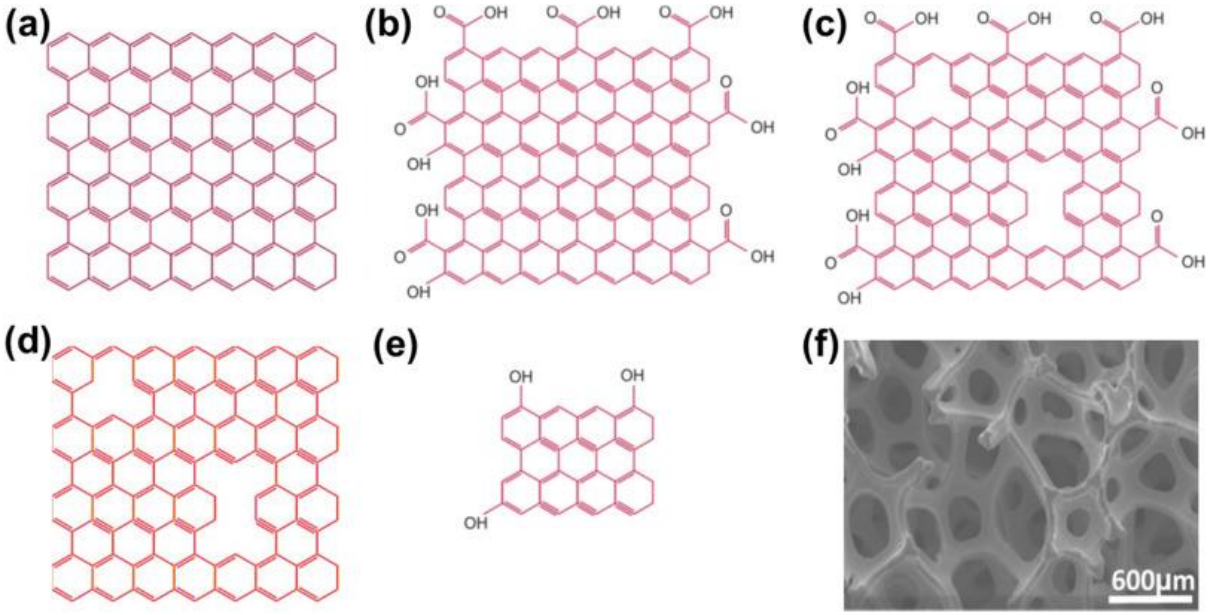

Figure 1: A schematic illustration of structures of various forms of graphene. (a) Graphene - a sp^2 hybridized model of carbon atoms in a repeated fashion, (b) graphene oxide – chemical synthesis of graphene facilitates the formation of functional groups onto the surface and basal plane of graphene, (c) reduced graphene oxide – chemical reduction of graphene oxide shows defects and vacancies introduced into graphene as a result of reduction, (d) porous graphene – pores of varying size into the sheets of graphene (e) graphene quantum dots – zero dimensional graphene which has bandgap and reveals excellent photoluminescent features and (f) three dimensional graphene foam – three dimensional interconnected architecture of graphene and found in the form of foam, aerogels and sponge. Reproduced with permission of Elsevier, Copyright 2018 **[33]**.

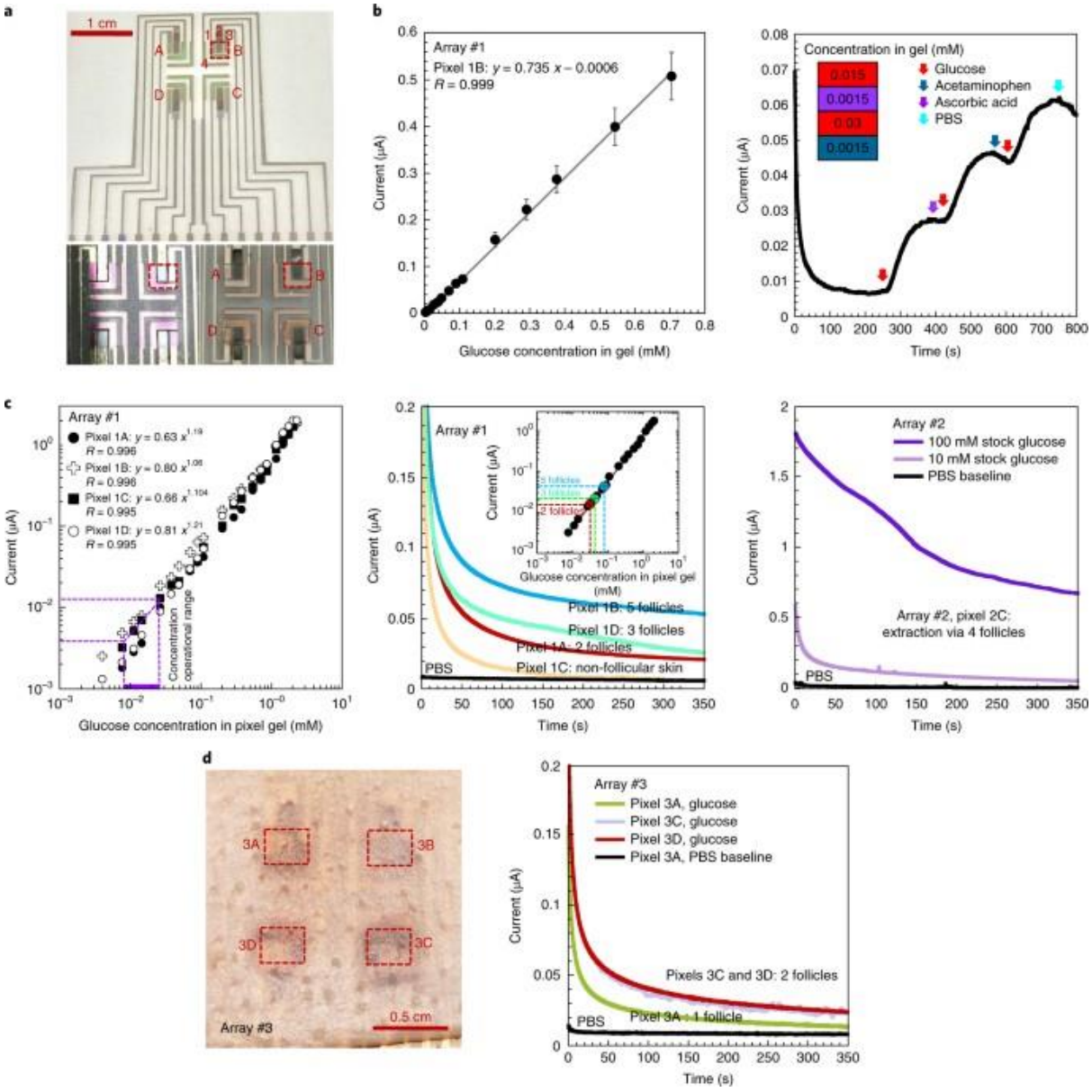

Figure 2: (**a)**, Electrodes one to three used in extraction–detection (4, unused). (**b)**, Left: linear response of a pixel sensor to 0.006–0.7 mM glucose. Right: response to glucose, PBS, acetaminophen and ascorbic acid. (**c)**, 10 mM subdermal glucose was extracted across porcine skin *ex vivo* for 5 min. Left: sensitivity calibration curves for the four pixel devices, demonstrating very similar current–concentration dependencies. Middle: detected current versus time after glucose extraction. Right: detected current versus time after extractions using the same pixel device for 10 and 100 mM subdermal glucose concentrations. Extracted, in-gel glucose concentrations agree with calculations based on the follicular extraction flux and the number of follicles probed. (**d)**, Left: visual correlation between number of follicles probed by each array pixel. Right: current detected after extraction of 10 mM subdermal glucose. Reproduced with permission from Nature Publishing Group **[19]**.

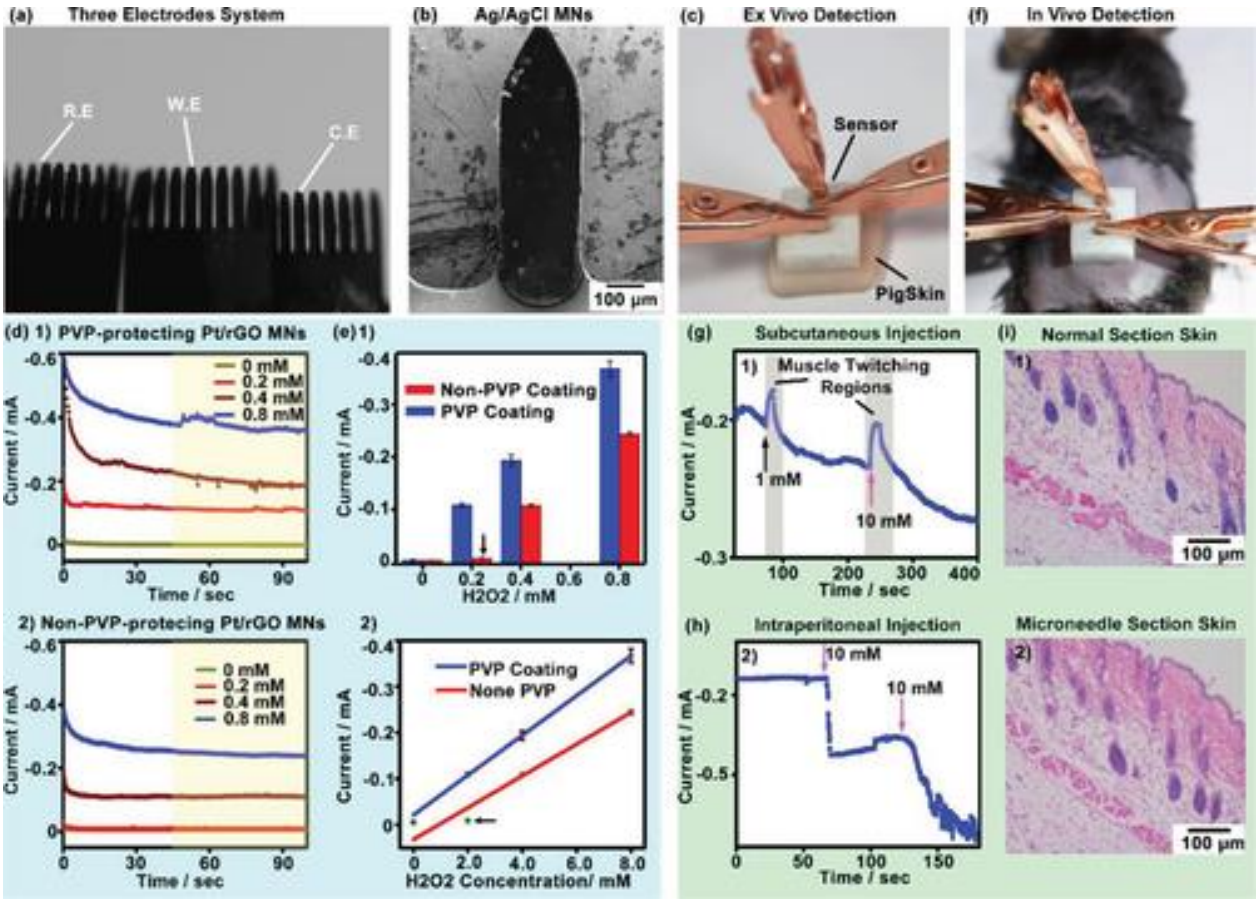

Figure 3: a) Optical microscopic image of three-electrode platform comprising of counter, working and reference electrodes. b) Scanning electron microscopy (SEM) image of Ag/AgCl coated microneedle patch. c) Image of microneedle patch used on the pigskin. d) Amperometric responses of microneedles. e) Statistical analysis of the steady-state currents in (d), f) Photographic image of microneedle patch used on mice to monitor H_2O_2. g) Amperometric responses of microneedle patches on mice. The mice were s.c. injected with 1×10^{-3} and 10×10^{-3} M H_2O_2 solutions at the time points t = 70 s and t = 230 s (indicated with arrows). h) Amperometric responses of MN sensors upon the sensing on mice. The mice were i.p. injected with 10×10^{-3} M H_2O_2 solutions at the time points t = 70 s and t = 130 s (indicated with arrows). i) Optical microscopic images of skin irritation assay. Microneedle-treated skin tissue was stained with hematoxylin and eosin (H&E). Reproduced with permission from Wiley **[20]**