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PAPER

Wearable microneedle graphene field-effect transistor sensors

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Abstract

Continuous monitoring of biomarkers in interstitial fluid (ISF) holds great potential for early disease detection and personalized medicine. This work presents a novel biosensing platform that combines microneedle technology with graphene field-effect transistors (GFETs) to enable sensitive, continuous analyte detection directly in ISF. Using spray-coating and laser lithography techniques, we create GFET channels on three-dimensional microneedle tips. The resulting devices show characteristic GFET behavior in solution. As a proof-of-concept, we demonstrate pH sensing capabilities with a sensitivity of 2.50% I_D /pH and the ability to detect changes as small as 0.04 pH units. Crucially, we show that these microneedle GFETs can function when inserted into *ex-vivo* human skin samples, with GFET gating occurring through the skin tissue. Finally, based on MTT assays and cellular imaging, no cytotoxic effect was observed which is an important prerequisite for human use.

1. Introduction

Wearable biosensors enable continuous monitoring of analytes, providing early detection of diseases such as cancer [1], monitoring of chronic conditions such as diabetes [2], and even allowing the development of personalized treatment strategies [3]. However, a key challenge when developing wearable biosensors is the continuous access to the biomarker.

The biomarker may be present in one or more biological fluids such as blood or sweat [4]. However, continuous sampling of blood is challenging and causes discomfort to the user [5]. On the other hand, sampling sweat is painless but only a limited number of analytes is present in sweat at concentrations similar to serum levels [6]. For instance, glucose sweat concentrations are $\sim 1/100^{\text{th}}$ of blood glucose levels [7], and subject to perspiration rate variations [8].

On the other hand, interstitial fluid (ISF), which surrounds cells within tissue, contains a wide range of

clinically relevant analytes, often well correlated with blood levels [9]. The biomarkers contained within the ISF include glucose [10], small ions such as Na^+ or K^+ [11], antibiotics [12], or signalling molecules such as cytokines [13]. The ISF is present at a fraction of a millimetre below the skin surface, in viable epidermis and dermis, and can be extracted using a range of non-invasive and minimally invasive methods [9]. The methods include the use of microneedles that only penetrate the top layers of the skin (epidermis into the dermis) and reverse iontophoresis which relies on a voltage driving the flow of ions between two electrodes to the skin surface [14].

The microneedles are typically only ~ 0.5 – 1 mm tall, which enables them to provide continuous access to the analytes in ISF but cause substantially less pain than hypodermic needles [15]. Microneedles can be organised into arrays, forming wearable patches with dozens of needles [3]. The large number of needles enables redundancy (if one or more needles fail to

penetrate the skin) but also possible multiplexing where different needles or needle groups target different analytes [16].

Graphene field effect transistors (GFETs) are a class of transducers that can deliver extremely high sensitivity for a wide range of target (bio)molecules [17]. For instance, Kumar *et al* detected SARS-CoV-2 spike protein with a GFET, obtaining a limit of detection of 88 zM [18]. GFETs can also sense pH [19, 20], small ions such as Na^+ [20], neurotransmitters [21] or proteins [22].

GFETs are typically constructed by contacting a graphene film with two electrodes (drain and source), creating a graphene channel [23]. The graphene film can be deposited using a range of techniques, including ink-jet printing [24, 25], spray-coating [20] or transfer of films grown by chemical vapour deposition [26]. The electrical properties of the graphene channel are then controlled by an electric field applied with a gate electrode. The gate is electrically separated from the graphene channel by a thin dielectric (such as SiO_2 or hexagonal boron nitride or by a solution, creating dielectric- and solution-gated GFETs, respectively [17, 27].

Charged species in the vicinity of the graphene channels can influence the electrical properties of the graphene channel, giving rise to GFET chemo- and bio-sensors [28]. GFET channels can be functionalised to make them sensitive and selective to the target analyte. This includes ion-selective membranes for sensing ions [29], or aptamers and antibodies for sensing a wide range of (bio)molecules [30, 31].

Bringing GFET sensing to the microneedle tips would enable the translation of the wide range of existing GFET sensors to microneedles, unlocking ultra-sensitive, versatile and continuous sensing of a wide range of analytes directly in the ISF. However, a key challenge is the fabrication of the GFETs on the three-dimensional (3D) microneedles, which is out of reach of traditional fabrication techniques. In our previous work [32], we have addressed this challenge by building a custom spray-lithography setup and fabricated GFET channels on microneedles. In the present work, we explore the sensing properties of the GFET microneedle platform, investigate GFET behaviour in human skin and the overall safety of the device. We demonstrate pH sensing using the GFET on the microneedle tips and modulation inside human skin, uncapping the potential of GFET microneedle biosensors towards wearable, real-time and multi-analyte sensing in point-of-care and home diagnostics.

2. Results and discussion

GFET channels were deposited on microneedle tips to maximise access to the ISF. A diagram of the device is shown in figure 1(a). The microneedles are square-based pyramids, with a graphene channel

running through the middle and source (S) and drain (D) electrodes on their sides. The tips of the microneedles penetrate the epidermis and reach the dermis, bringing the graphene channels in contact with the ISF. The GFETs are driven through a separate set of gate (G) microneedles. A gate-source voltage (V_{GS}) and drain-source voltage (V_{DS}) are applied, with the drain current (I_{D}) and gate current (I_{G}) monitored.

2.1. Device fabrication

Microneedles' 3D shape makes manufacturing the GFETs on the microneedle tips challenging and incompatible with standard lithographic or GFET manufacturing techniques. The steep walls make uniform coating with films such as graphene or photoresist challenging [33], while the non-planar surfaces make focusing during photolithography particularly difficult [32]. In our previous work [32], we have developed a new fabrication approach based on spray-coating and laser lithography. The process relied on spray-coating to coat the microneedles with metal ink (to render them conductive), photoresist (for lithography) and graphene (to form the GFET channels). A special optical setup with a low numerical aperture was used to keep the laser focused across the microneedle height range while also avoiding possible collisions with the optics [32].

The devices are based on injection-moulded polycarbonate microneedles, developed and characterised by Sharma *et al* [34]. The substrate is 20×20 mm in size, with 64 microneedles (1 mm tall, 0.6×0.6 mm square-base pyramids) across four groups. The microneedles were coated with a metal film to make them electrically conductive, for instance, by sputter-coating gold or spray-coating a silver nanoparticle ink [32]. The metal film was subsequently patterned through a multi-step laser lithography process with a laser linewidth ranging from 50 μm to 72 μm , as described in our previous work [32]. This resulted in a gap on the microneedle tips.

To form the graphene channels on the tips, we used a liquid-phase exfoliated (LPE) graphene ink. The ink was prepared by ultrasonication of graphite powder in 2-propanol, with the addition of polyvinylpyrrolidone (PVP), which served as a biocompatible stabiliser [20, 35]. After sonication, unexfoliated material was removed through centrifugation, yielding a stable graphene dispersion with a concentration of $\sim 0.82 \text{ mg}\cdot\text{mL}^{-1}$ [20]. A complete characterisation of the ink properties is presented in our previous work [20].

The microneedles were spray-coated with the LPE graphene ink, yielding graphene FET channels with an initial resistance of $R_{\text{ch}} \sim 165 \Omega$ (over a 63 μm channel length, as shown in figure 1(b). An intense-pulse light (IPL) annealing was used to remove the PVP stabiliser [32, 36] without damaging the polycarbonate microneedles. The

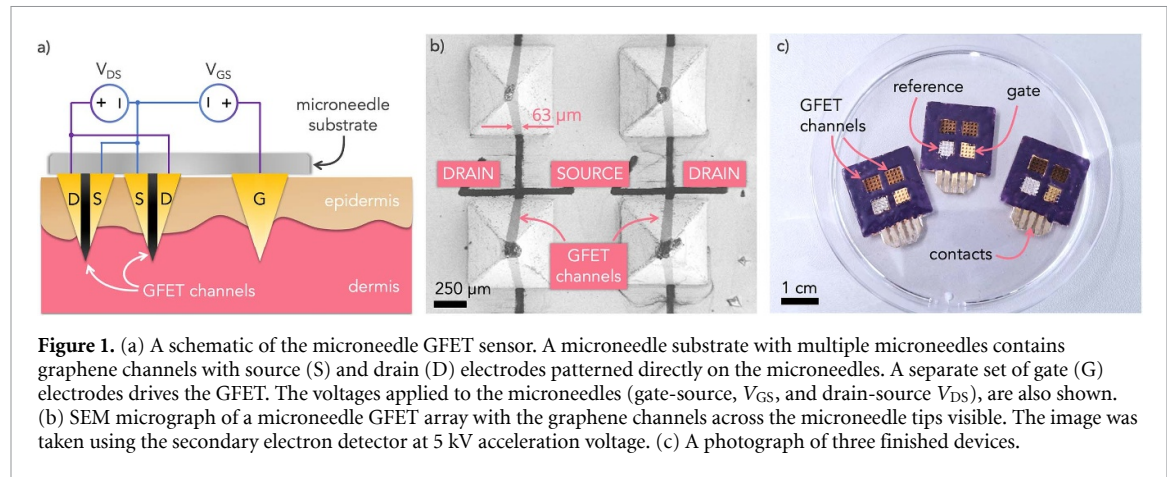


Figure 1. (a) A schematic of the microneedle GFET sensor. A microneedle substrate with multiple microneedles contains graphene channels with source (S) and drain (D) electrodes patterned directly on the microneedles. A separate set of gate (G) electrodes drives the GFET. The voltages applied to the microneedles (gate-source, V_{GS} , and drain-source V_{DS}), are also shown. (b) SEM micrograph of a microneedle GFET array with the graphene channels across the microneedle tips visible. The image was taken using the secondary electron detector at 5 kV acceleration voltage. (c) A photograph of three finished devices.

annealing approximately halved the resistance to $\overline{R_{ch}} \sim 90 \pm 21 \Omega$ [32].

Figure 1(b) shows a scanning electron microscopy (SEM) micrograph of the microneedles, with the graphene channels visible. The channel length (source-drain electrode spacing) was typically $67 \pm 9 \mu\text{m}$ [32].

A UV-curable varnish (visible as a purple coating in figure 1(c)) was applied to parts of the microneedles to prevent unwanted exposure to the skin or solutions during measurements. Additionally, a reference electrode was formed by manually depositing on the microneedles a commercial Ag/AgCl paste and drying it in an oven. The finished microneedle patches, shown in figure 1(c), have two GFET channels. Each channel consists of 16 patterned microneedles similar to the ones shown in figure 1(b), where one GFET channel passes across multiple microneedles. The two GFETs can be used to measure two different analytes, or one of them can act as a control, to e.g. correct for any changes due to the presence of redox molecules, long-term drift or temperature variations [37, 38]. Two further groups of 16 microneedles each serve as the gate and reference electrodes, respectively (figure 1(c)). At the edge of the substrates, five contacts are present with a 2.54 mm (0.1 inch) pitch, providing the electrical interface to the GFET electrodes.

2.2. GFET operation in solution

To demonstrate the modulation and GFET function, the finished devices were immersed in a buffer solution and biased with a custom-built source meter unit (SMU). The SMU (described in supporting information section S1) was designed to interface with the five contacts on the microneedle test strip and apply V_{GS} and V_{DS} of up to ± 1.2 V. It has fixed current ranges, of ± 10 mA for I_D and ± 1 mA for I_G which have been chosen to match the currents typically observed with the present microneedle GFET devices.

Figure 2(a) shows a typical transfer curve observed for our microneedle GFET under $V_{DS} = +0.2$ V and V_{GS} sweeping between -0.4 and $+0.4$

V. This response is consistent with previous literature observations [20, 32] for solution-gate GFET devices. Crucially, I_G remained at less than 1% of the I_D change, at ± 0.004 mA, as compared to I_D between 2.03 and 3.14 mA. This is an important indicator that the device acts as a GFET and that Faradaic currents from electrode or solution redox processes are negligible [20, 37].

The field-effect mobility (μ_{FE}) can be calculated by the following formula [39]:

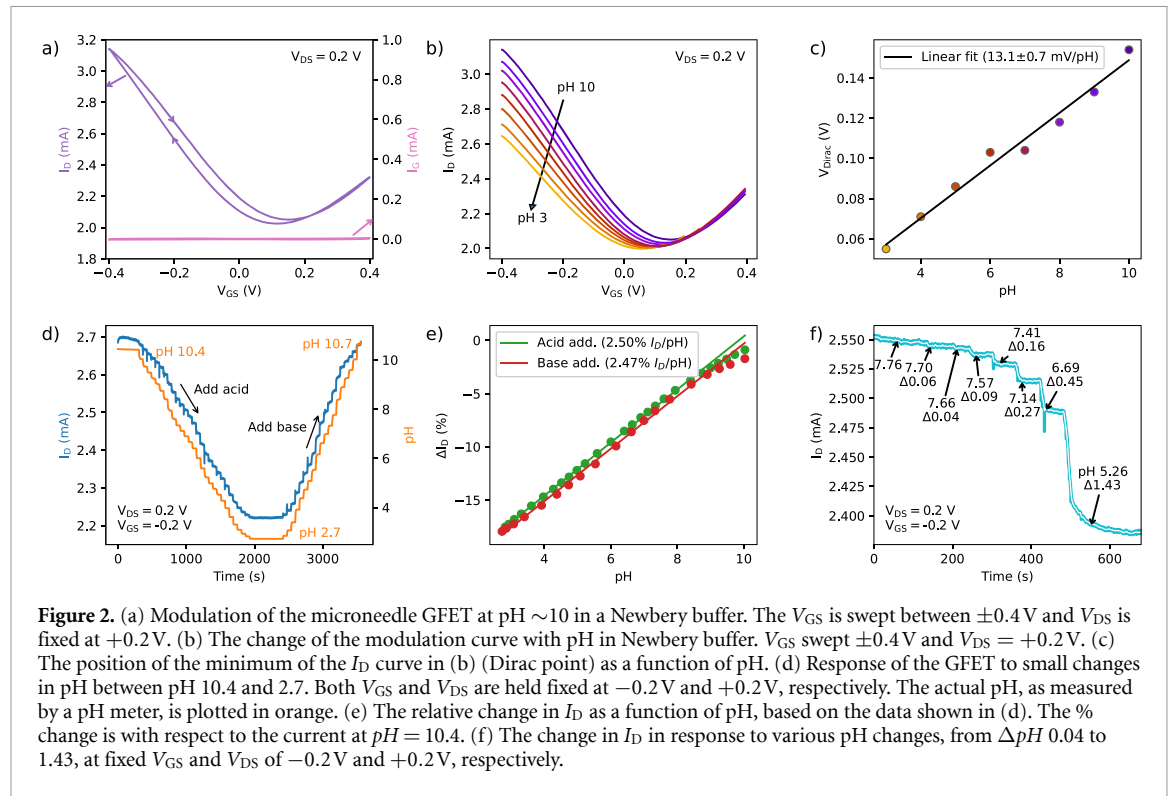
$$\mu_{FE} = \frac{g_{mt} L_{ch}}{W_{ch} C_G V_{DS}} \quad (1)$$

where g_{mt} is the transconductance ($g_{mt} = \delta I_D / \delta V_{GS}$), L_{ch} the channel length, W_{ch} the channel width and C_G the gate capacitance. The channel geometry for the present device was $L_{ch} \sim 67 \mu\text{m}$ (determined from electron micrographs) and $W_{ch} \sim 43.8$ mm (determined from the designed geometry). From the modulation measurement in figure 2(a), at $V_{GS} = -0.35$ V and $V_{DS} = 0.2$ V, we extracted the transconductance $g_{mt} = -2912 \mu\text{S}$, and using electrochemical impedance spectroscopy (SI section S2) we measured $C_G = 5.34 \mu\text{F} \cdot \text{cm}^{-2}$. While the observed mobility of $4.10 \text{ cm}^2 \cdot \text{V}^{-1} \text{s}^{-1}$ is lower than the values typically observed for LPE graphene ($\sim 100 \text{ cm}^2 \cdot \text{V}^{-1} \text{s}^{-1}$) [40], the devices here are highly unique in their 3D shape.

2.3. pH sensing

The measurement of the pH of the ISF provides helpful diagnostic information, for instance, in detecting metabolic acidosis [41] and insulin resistance diagnostics [42]. Furthermore, several enzymatic reactions result in a pH change, such as β -lactamase processing penicillin [3] or acetylcholine esterase hydrolysing acetylcholine [21]. By immobilising a suitable enzyme on the microneedles and measuring pH changes, biosensors specific to the enzyme's substrate can be constructed [3, 21]. Therefore, the ability to measure pH was investigated as the next step in the microneedle GFET characterisation.

The graphene flakes in the GFET channel contain residual oxygen on their surface (as shown by x-ray



photoelectron spectroscopy in figure S5 in SI), which renders the channel sensitive to pH changes [19, 43, 44]. To investigate the use of the microneedle GFETs as pH sensors, they were submerged in a Newbery buffer solution, and the modulation curve was measured between pH 10 and pH 3 (figure 2(b)). Newbery buffer, based on phosphate, acetate and metaborate ions, has a linear pH response to added acid or base between pH 2 and 12, making it suitable for pH characterisation studies [45]. We noticed that the minimum current in the modulation curve (Dirac point) shifted linearly with pH, at a rate of ~ 13.1 mV/pH (figure 2(c)). This is lower than previously published GFET pH sensors, showing a response of 26 mV/pH [20], 29 mV/pH [46] and 21 mV/pH [47], where the GFET configuration was planar.

The GFET can be used to measure changes in pH as a function of time (figure 2(d)), by fixing V_{DS} at $+0.2$ V and V_{GS} at -0.2 V. $V_{GS} = -0.2$ V was chosen as it avoids the Dirac point where a change in V_{GS} would yield a negligible change in I_D . At the same time, it is low enough not to observe significant I_G due to Faradaic currents. As shown in figure 2(d), the sensor responded linearly to pH changes within the pH range from 2.7 to 10.7, more than covering the typical physiological ISF pH range of 6.6–7.6 [42]. The slope of I_D change with pH was $\sim 2.5\%$ I_D /pH, similar for both acid and base addition (2.50% and 2.47% I_D /pH, respectively). This responsivity is similar to 3.05% I_D /pH previously observed for planar devices [20].

The detection of various magnitudes of pH changes at physiologically relevant pH values [42]

was investigated in figure 2(f). Changes as small as 0.04 pH could be recognised, with the response time (to settle within 95% of the final value) of only 11 s. The settling for a larger pH change of 1.43 pH takes longer, at ~ 140 s. The raw data in figure 2(f) are shown as output from the custom SMU without smoothing or digital filtering. The root-mean-square (RMS) noise of the drain current ($I_{D,RMS} = \sqrt{n^{-1} \sum (I_D - \bar{I}_D)^2}$, where n is the number of measured points, I_D is the drain current at a point in time and $\bar{I}_D$ is the mean I_D) was 0.65 μ A. It can be seen that the I_D noise has a direct impact on the resolution and limits of detection of the sensor, and at responsivity of $\sim 2.5\%$ I_D /pH, the equivalent noise in pH is ~ 0.01 pH. Applying a 5th-order, infinite-impulse-response (IIR) digital Butterworth low-pass filter [48] with a cut-off frequency of $f_C = 0.04$ Hz (white line in figure 2(f)), decreases the $I_{D,RMS}$ to ~ 0.25 μ A, improving the in pH resolution to ~ 0.004 pH.

As demonstrated in figure 2, the microneedles GFETs can act as pH sensors, with comparable properties to planar GFET sensors. These results, therefore, pave the way for the development of biosensors with immobilised enzymes that produce a pH change as well as for direct measurements of the ISF pH.

2.4. Testing in human skin

To investigate the behaviour of the microneedle GFETs inside human skin, abdominal *ex-vivo* human skin samples were obtained as a by-product of cosmetic surgeries. The microneedles were inserted into the skin by pushing them in with thumb pressure.

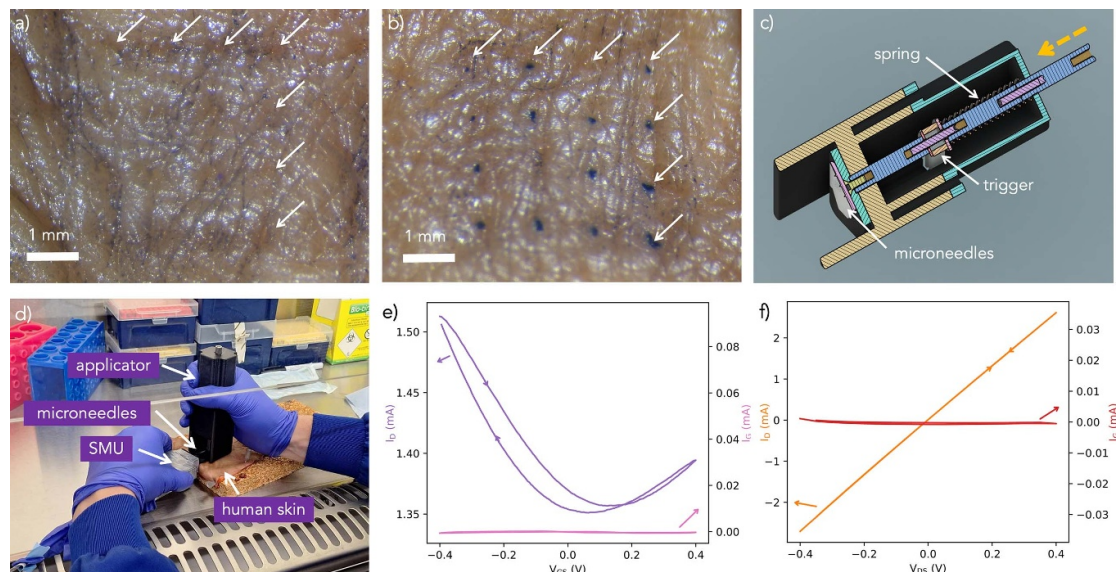


Figure 3. (a) Indentation marks in an *ex-vivo* human skin sample remaining after the microneedle array was pushed in, removed after 60 s, and the skin stained with trypan blue. No skin penetration (staining) is visible. The arrows indicate the rows and columns of the 4×4 microneedle array. (b) Stained skin after microneedles were inserted with a spring-loaded applicator with the stained penetration spots clearly visible. (c) A cross-section of the spring-loaded applicator used for microneedle skin insertion. (d) Measurement setup for in-skin GFET measurements. (e) V_{GS} sweep of the microneedles array inserted in the skin, showing GFET modulation. A constant $V_{DS} = 0.2$ V was used. (f) V_{DS} sweep between $V_{DS} = -0.4$ V and $+0.4$ V whilst the microneedles were inserted in the skin. The V_{GS} was held fixed at -0.2 V.

After 60 s, the microneedles were removed, and the spots were immediately stained with a trypan blue solution, which stains any pierced dermis tissue blue [49, 50]. However, after the excess trypan blue was removed, no staining was observed (figure 3(a)), implying no epidermis penetration. Furthermore, increasing the insertion pressure or time yielded identical results.

It has been previously observed that the microneedle penetration success rate can be considerably improved by using a spring-loaded or electromagnetic applicator. That is, not only the insertion force but also the insertion speed determine the success rate [51–53]. Therefore, a 3D-printed spring-loaded applicator was designed, as shown in figures 3(c) and (d). Applying the microneedles with the applicator yielded considerably better results, with the number of needles penetrating increasing from 0% to 81% (figure 3(b)).

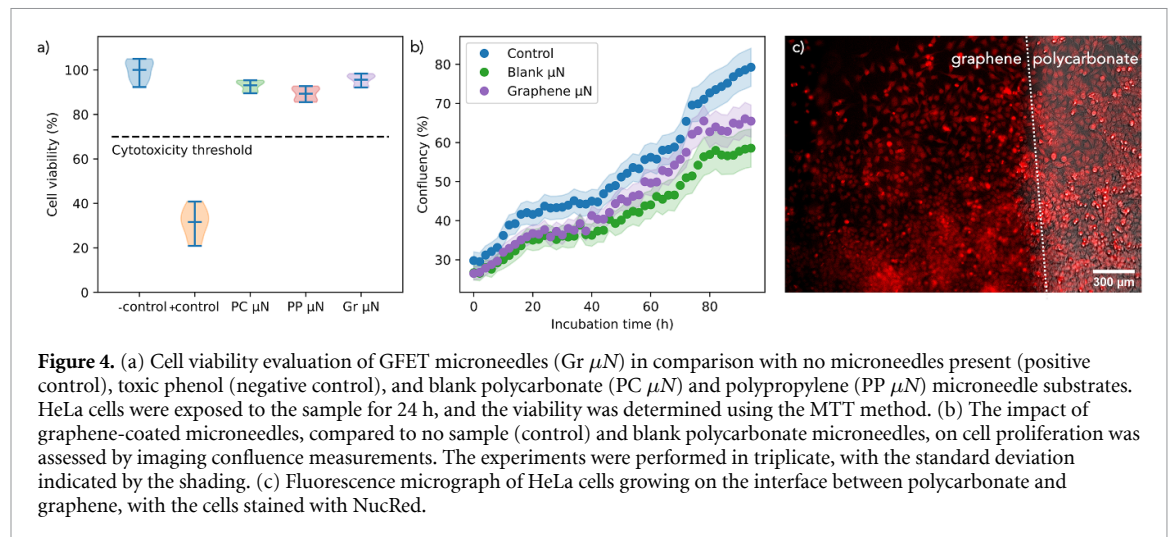
After inserting the microneedles into the skin with the applicator, the SMU was connected (figure 3(d)). Then, V_{GS} was swept ± 0.4 V at a constant drain-source bias ($V_{DS} = 0.2$ V), obtaining characteristic GFET modulation (figure 3(e)). The I_G remained negligible, at $\sim 1/100$ th of the change in I_D due to modulation. Additionally, a V_{DS} sweep is shown in figure 3(f), also showing negligible I_G . The observed modulation in figure 3(e) was weaker than in buffer solution previously (figure 2(a)), where applying V_{GS} of -0.4 V increased I_D from the minimum at the Dirac point by a factor of 1.51 times (buffer solution) vs. 1.11 times (skin). Crucially, these experiments demonstrate that the microneedle GFETs can operate

inside the human skin, with the GFET gating occurring through the skin.

2.5. Cytotoxicity testing

The safety of the sensing system, especially as it penetrates the epidermis, is critical to its real-world deployment and future human trials. As there is mixed evidence and contradictory conclusions on the safety of graphene and related materials [54], cytotoxicity studies of the microneedle GFETs were performed. The investigation followed the procedure in the standard ISO 10993-5:2009 [55] which uses the viability of cell cultures as a metric for cytotoxicity. If the viability decreases below 70%, the material is considered cytotoxic [55].

Small pieces (4×4 mm) of microneedle GFETs were placed in a multi-well plate, and human HeLa cells were seeded in the same wells. For comparison, uncoated polycarbonate and polypropylene microneedles were also tested to determine if any effects originated from the substrate. After the incubation, an MTT cell viability assay was performed [56]. A solution of the MTT dye was added, which is processed by viable cells into a purple formazan [56]. After extracting the formazan, its concentration was determined spectrophotometrically, and the cell viability was calculated as the ratio of the formazan concentration with respect to the control (figure 4(a)). All microneedle samples tested were found to have a viability significantly above the 70% cytotoxicity threshold, and therefore were found to be non-toxic. A positive control sample of phenol, which



is known to be toxic [57], was the only sample to show a low viability of $31 \pm 6\%$.

The viability for the blank polycarbonate microneedles was $93 \pm 2\%$. To determine whether the $\sim 7\%$ viability decrease, as compared to the control, was due to the effects of the polycarbonate material or due to the presence of a foreign body (microneedles), polypropylene microneedles were also measured. The viability of the PP microneedles was observed as $89 \pm 3\%$, close to the polycarbonate microneedles. Indeed, it has been observed before that the substrate geometry and roughness can negatively affect cell growth [58], which can offer an explanation for the observed decrease. The viability of the GFET microneedles used for measurements throughout this work was $95 \pm 2\%$, suggesting that the graphene coating is non-cytotoxic under the investigated conditions, and any decrease in viability likely comes from the geometry of the substrates rather than the materials.

The cell viability assays can be affected by interactions between the assay components, such as the dye, and the material investigated, leading to false positive or false negative results [59–61]. Therefore, to further validate the results above, a different method to assess the toxicity was used. The cells were imaged every 2 h using a IncuCyte Live-Cell analysis system and the confluency (% of the surface area covered by cells) was determined from the images. A key advantage of this method is that no reagents needed to be added, minimising possible interference [62].

Figure 4(b) shows the confluency increase over time. After incubating for 96 h, the confluency was $79 \pm 5\%$ for negative control, $59 \pm 5\%$ (75% of control) for blank PC microneedles, and $65 \pm 4\%$ (82% of control) for GFET microneedles. Continued cell proliferation was observed despite the presence of the graphene microneedles, confirming the non-cytotoxic nature of the sensors developed in this work.

Finally, figure 4(c) shows a fluorescent micrograph of the HeLa cells stained with a far-red nuclear stain, growing on the graphene microneedle substrate. Overall, all cytotoxicity tests performed on the sensors developed here returned a negative result, that is, the sensors are not cytotoxic—a prerequisite for future human trials.

3. Methods

Microneedle substrates.

Following reference [34], microneedles were injection-moulded out of polycarbonate (Makrolon OD2015). To form the microneedle tips, moulds made out of aluminium with electric discharge milling were used.

Gold coating. The microneedles were sputter-coated with Korvus HEX dual-source physical vapour deposition system. A 5 nm Ti adhesion layer was followed by a 100 nm gold layer.

Photoresist coating. A 5 μ m thick photoresist film (Microchemicals AZ4533) was deposited by spray-coating using a custom spray-coating setup described in [32]. An atomisation pressure of 275790 Pa was used, with the spray head at 45 mm distance and moving at 4000 mm·min⁻¹. To lower the viscosity of the resist [63], it was diluted 1:1:1 with butanone (TCI, >99%) and propylene glycol methyl ether acetate (TCI, >98%). The film was soft-baked in an oven at 100°C for 10 min.

Lithographic patterning. A custom laser lithography system developed in [32], based on a 1 mW 405 nm laser, was used to pattern the photoresist, forming the electrodes for the GFET. Following the laser exposure, the photoresist was developed in the AZ400 developer (Microchemicals), diluted 1:3 with deionised (DI) water, for 60 s. Then, the gold film was selectively

etched with a mixture of 2 g KI 99%, Sigma-Aldrich), 0.5 g I_2 (>99.8%, Sigma-Aldrich), and 40 mL DI water for 120 s. Finally, the photoresist was removed by immersion in ethanol for 60 s and subsequent rinsing with further ethanol.

Graphene ink preparation. Graphene LPE ink was prepared following the procedure described in [20]. A mixture of 50 mg·mL⁻¹ graphite (Ceylon Graphite K1) and 0.4 mg·mL⁻¹ PVP (Merck, Mw = 40 000) in 2-propanol was exfoliated in a bath sonicator (Elmasonic S150) for 9 h. Then, unexfoliated graphite was removed by centrifugation at 13 000 g for 1 h (Allegra 64 R centrifuge, F06050 rotor). Finally, the top 80% supernatant was collected as the graphene ink.

Graphene deposition. The ink was spray-coated with the same setup and deposition conditions as the photoresist. The channel resistance during the spray was continuously monitored by applying a 0.1 V bias with a Biologic SP-150 potentiostat, to reach $\sim 160 \Omega$ [32]. Then, intense pulse light annealing with a Xe flash lamp (2 pulses, 2.5 J·cm⁻² was used to lower the channel resistance.

Electrode insulation. To insulate the parts of microneedle bases and traces on the microneedles and only leave the microneedle tips exposed, insulating varnish (Gelab Cosmetics LLC) was manually deposited with a brush and cured.

Electrode insulation. Commercial Ag/AgCl paste (Sun Chemical, C2130809D5) was manually applied with a brush and dried in an oven at 60 °C for 30 min.

Scanning electron microscopy. The devices were imaged with a LEO Gemini 1525 FEG-SEM system. No surface coating was used, only the electrodes were grounded with conductive carbon tape.

GFET measurements. The custom SMU, described in SI section S1, was connected to the microneedle substrate. Then, the sensor was submerged into Newbery buffer, prepared following [45]. 1.36 g of $CH_3COONa \cdot 3H_2O$ (99%, Thermo Scientific), 1.42 g of Na_2HPO_4 (99%, Sigma Aldrich) and 1.38 g of $Na_2BO_2 \cdot 4H_2O$ (99%, Sigma Aldrich) were dissolved in 1 l of deionised water. The buffer solution had a pH of 10.45 as prepared. To obtain lower or higher pH values, the pH was adjusted with 0.4 M H_2SO_4 or 0.8 M NaOH, respectively. The pH was measured using a Hanna Instruments HI-5521 pH meter with the HI-1131B pH electrode, which was reporting the measurements in real-time to the custom GFET characterisation software.

Measurements in skin. This study was performed in accordance with the Declaration of Helsinki. Human skin samples were obtained from patients undergoing abdominoplasties at The Plastic Surgery

Group after receiving their informed written consent using Imperial College Research Ethics Committee-approved consent forms. Fat was removed, and the skin was cleaned using iodine and ethanol wipes and stored at $-20^\circ C$ in a subcollection approved by the Imperial College Healthcare Tissue Bank (ICHTB). ICHTB is approved by Wales REC3 to release human material for research (17/WA/0161). Skin was used in an ICHTB-approved research project. The skin was allowed to thaw completely before use, stretched on a corkboard and pinned in place for microneedle application. Excessive moisture from the skin surface was removed using a paper towel.

Skin penetration testing. The microneedles were first pushed into the skin with firm thumb pressure. Then, 0.5 ml of trypan blue stain (0.4%, Gibco) was pipetted onto the skin and left on for 10 seconds. After wiping off excessive stain, the skin was imaged using a microscope (Leica M80). To test the penetration with an applicator, a spring-loaded applicator was designed and 3D printed, as shown in figure 3(c). This contained a carbon steel spring (110 N·mm⁻¹) with an effective travel path of 30 mm.

Measuring modulation in skin. The microneedles were applied into the ex vivo human skin using the spring-loaded applicator described above. Then, the microneedle patch was connected to the custom SMU and the SMU measurements were performed. The skin sample was used as received with no modifications except drying its surface with a paper towel.

Cell viability using MTT assay. HeLa cells were purchased from ATCC. Typically, 3×10^4 cells/well were seeded in 48-well plates and cultured in Dulbecco's Modified Eagle Medium (DMEM), high glucose, GlutaMAX™, supplemented with 10% foetal bovine serum and 1% penicillin/streptomycin at 37 °C under 5% CO₂. After 24 h, the cells were washed with 100 μ L of serum-free DMEM twice and incubated with the test samples for 24 h. Afterwards, the cells were washed twice with 100 μ L of serum-free DMEM and incubated with 50 μ L of 0.5 mg/ml MTT stock solution (Cayman Chemical MTT Assay Kit, 10 009 365) at 37 °C for 4 h, according to the manufacturer's instructions. Subsequently, 150 μ L of the solubilising solution (containing sodium dodecyl sulphate, Cayman Chemical MTT kit) was added to the wells overnight. Then, 150 μ L of the solution was transferred to a new 96-well plate to prevent any interference with the absorbance values at 570 nm. The optical density of the produced stain was monitored at 570 nm using a SpectraMax ABS microplate reader. Untreated cells (without any substrates added) were used as a negative control, and phenol (Sigma-Aldrich, ACS reagent) as a positive control. Cell viability was calculated as $viability(\%) =$

$A_{\text{sample}}/A_{\text{control}} \times 100$ where A is the optical absorbance at 570 nm.

Optical confluency monitoring. After culturing HeLa cells in a T-75 flask, the cells were trypsinised and prepared for seeding in a 12-well plate at a seeding density of 10^5 cells/well. The microneedle chips were sterilised by UV treatment and 70% ethanol treatment and placed in the 12-well plate before cell seeding. The well plate was then placed in the IncuCyte instrument and kept in a normal humidified incubator at 37 °C with 5% CO₂. The automated phase-contrast imaging was then carried out by scans taken every 2 h for 72 h, with a picture taken for the whole well (1.22 μm /pixel resolution) to monitor cell growth on the chips. Data obtained was first optimised using the control, untreated cells. The IncuCyte software allowed adjustments in the processing definitions for analyses, so we optimised the confluence masks for the phase-contrast images. We adjusted the default settings to account for cell debris. Once a suitable setting was identified, this was used across all wells.

Widefield fluorescence microscopy HeLa cells were seeded at a density of 10^5 cells/well in glass-bottomed 8-well chamber slides (Ibidi, GmbH). The microneedle chips were sterilised by UV treatment and 70% ethanol treatment and placed in the chambered slides before cell seeding. After 24 h, the media was removed, and cells were washed gently with 100 μL of phosphate-buffered saline (PBS). The cells were then incubated with NucRed Live 647 ReadyProbes Reagent for 15 min and then washed twice with PBS. The cells were then imaged on a widefield microscope (Leica DMRB).

4. Conclusions

By patterning graphene channels on the tips of microneedles, we have created arrays of graphene field-effect transistor microneedle sensors. The microneedle GFETs show characteristic modulation in solution with an observed mobility of $4.10 \text{ cm}^2 \cdot \text{V}^{-1} \text{ s}^{-1}$. Without any functionalisation, they respond to pH, with a sensitivity of $\sim 2.5\% I_D/\text{pH}$, which is comparable to planar devices. Furthermore, we demonstrated that GFET modulation inside the human skin is possible, with the sensor showing a characteristic response while inserted into *ex-vivo* human skin. To assess the sensor biocompatibility, a series of cytotoxicity evaluation experiments were performed, all showing the GFET microneedle as not cytotoxic, paving the way towards future human trials of this system. These results unlock the power of microneedles by enabling multi-analyte detection via individual interrogation of each GFET and by offering direct sensing of the ISF with GFETs. Furthermore, our demonstration of GFET sensing

on 3D objects, such as the microneedle tips, could be directly translated to other non-planar surfaces, such as contact lenses for biosensing in tears.

Data availability statement

All data that support the findings of this study are included within the article (and any supplementary files).

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Conflict of interest

The authors have no conflicts to disclose.

Author contributions

Martin Holicky: conceptualisation, methodology, investigation, writing—original draft; **Benji Fenech-Salerno:** investigation, writing—review & editing; **Rupali Dabas:** investigation, writing—original draft; **Oliver Teenan:** investigation, writing—review & editing; **Xinran Li:** investigation; **Isobel V. Steer:** investigation; **Claire A. Higgins:** supervision, writing—review & editing, funding acquisition; **Mo Akhavan:** resources (skin samples); **Nikolaj Gadegaard:** resources (microneedles); **Nazila Kamaly:** supervision, writing—review & editing, funding acquisition; **Anthony E. G. Cass:** supervision, writing—review & editing, funding acquisition; and **Felice Torrisi:** supervision, writing—original draft, funding acquisition.

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