This work presents crystalline SiC-on-glass as a transparent, robust, and optically stable electrode for simultaneous electrochemical characterization and optical microscope imaging. Experimental results show a large potential window, as well as excellent stability and repeatability over multiple cyclic voltammetric scans in common redox biomarkers such as ruthenium hexaaamine and methylene blue. The high optical transmittance and biocompatibility of SiC-on-glass were also observed, enabling cell culture, electrical stimulation, and high resolution fluorescence imaging. This new platform opens exciting opportunities in multi-functional biosensing-probes and observation.

There has been significant interest in transparent semiconductor based electrochemical electrodes for biosensing applications.\textsuperscript{5-4} The transparency of the electrode provides attractive functionalities such as it allows the simultaneous observation of biological samples along with electrical stimulation and electrochemical measurement.\textsuperscript{5-8} In addition, the use of semiconductor electrodes also enables the integration of sensing devices with signal amplification circuits on a single chip that can reduce or eliminate parasitic noises.\textsuperscript{5,10} Ultrathin Si films on ITO (Indium Tin Oxide) have been used as transparent semiconducting heterojunctions for simultaneous light activated electrochemistry and optical microscopy.\textsuperscript{11-13} Nevertheless, since Si experiences a relatively fast hydrolysis and oxidation process inside aqueous mediums, the long term stability (e.g., cell culturing for several days at 37 °C and high temperature thermal lysis) of Si-based functional devices is questionable.\textsuperscript{14-16} Furthermore, due to its small band gap, Si is sensitive to visible wavelength, making its electrical conductivity markedly affected under light illumination. This property leads to another challenging issue to compensate for photo-generated signals.\textsuperscript{17} As a consequence, semiconducting systems that can simultaneously offer (i) excellent optical transparency, (ii) low thermal oxidation and hydrolysis rates, (iii) high conductivity, (iv) large electrochemical windows, (v) electrically visible-light blind, and (vi) biocompatibility are desirable for in vitro biosensing applications. Additionally, the capability of wafer-scale-level fabrication, and device miniaturization would be of significant interest for reducing device cost and scaling up mass-production.

This work introduces crystalline silicon carbide (SiC) on glass as a new electrode system that satisfies all the above-mentioned requirements. Owing to its wide band gap, SiC is blind to visible wavelengths, making its electrical properties stable under light illumination. The work also explores the electrochemical behavior of SiC, its biocompatibility and the capability for simultaneous optical characterization along with electrical stimulation under light illuminating environments.

Our material of choice for visible-light-insensitive, biofluid stable, and transparent semiconducting electrodes is cubic silicon carbide (i.e., 3C-SiC).\textsuperscript{18} The films were epitaxially grown on a Si substrate using low pressure chemical vapor deposition (ESI). To form the highly conductive electrode, we employed ammonia (NH\textsubscript{3}) as the in situ dopant to form n-type 3C-SiC with a carrier concentration of 5 \times 10^{19} \text{ cm}^{-3}. It is worth noting that the bulk Si substrate typically blocks the visible wavelengths.\textsuperscript{19-21} Therefore, to develop transparent electrodes, we transferred the as-grown SiC films onto a borofloat substrate using anodic bonding, Fig. 1(A).\textsuperscript{22} The initial Si handling layer was completely removed using KOH etching, leaving a transparent SiC-on-glass wafer with a diameter of 6 inches, Fig. 1(B). Since micro-machining...
of SiC thin films is compatible with conventional Micro Electro Mechanical Systems (MEMS) processes, thousands of SiC-on-glass electrodes can be fabricated from a full 6-inch wafer. As a proof of concept, we fabricated a multiplexed SiC-on-glass device, with eight working electrodes and one reference electrode integrated on a single chip, Fig. 1(C). The Atomic Force Microscopy (AFM) data shows excellent surface smoothness of the bonded SiC films, with root mean square (RMS) roughness of less than 4 nm, Fig. 1(D). The optical transmittance was measured using a spectrometer (Nanospec AFT210), showing that more than 60% of the visible light transmitted through the SiC/glass bilayer. The large optical transmittance of SiC resulted from its wide energy band corresponding to absorption wavelengths in the UV range. This indicates the excellent transparency for opto-biological sensing applications. It should also be pointed out that the 700 nm thick SiC film on glass has a much higher transmittance than a 20 nm amorphous Si-on-ITO bilayer (transmittance ≈ 5% to 40% in the 350 nm to 600 nm range), Fig. 1(E). The transparency of SiC/glass can be further improved by thinning down the SiC layers. To form the electrical contact to the electrode, 300 nm thick aluminium (Al) film was deposited using sputtering and then wet-etched. The current–voltage curve shows excellent ohmic behavior between the metal/semiconductor (i.e. Al/SiC) interface. The sheet resistance of the SiC films was found to be 14 Ω cm⁻¹, corresponding to a resistivity of 0.001 Ω cm, which is higher than amorphous and nanocrystalline SiC. The stability of the output current under dark and illumination conditions indicates the potential of the new electrode platform for simultaneous and real-time bio-electrical stimulation and optical observation with standard microscopes, Fig. 1(F).

To investigate the suitability of the single crystal SiC electrode for electrochemical measurements, the potential window and the double-layer capacitance were measured using cyclic voltammetry (CV) in 0.1 M PBS (0.01 M phosphate buffer, 0.0027 M KCl and 0.137 M NaCl, pH 7.4 at 25 °C). As can be seen from Fig. 2(A), the SiC electrode possesses a wide potential window ranging from −1.5 to 1.5 V, presenting their superiority for interrogating towards a wide range of electrolyte and redox molecules. The double-layer capacitance (C, μF cm⁻²) was found to be 20.6 μF cm⁻² using the typical formula of \( C = j/v \), where \( j \) is background current density (μA cm⁻²) and \( v \) is scan rate (V s⁻¹). This value is higher than typical boron-doped diamond (2.1–4.5 μF cm⁻²) suggesting its applicability in electro-analytical and capacitance based sensor uses. The electrocatalytic activity of the single crystal SiC electrode towards biosensing was examined using a common redox biomarker ruthenium hexaammine (i.e., RuHex). RuHex is a redox label which stoichiometrically binds with the phosphate backbone of nucleic acid (DNA and RNA) absorbed onto the electrode surface for chronocoulometry (CC).

A well-distinct CV wave of RuHex was obtained with anodic and cathodic peaks at −57 mV and −111 mV (versus Ag/AgCl) respectively (Fig. 2). The ΔE [difference between anodic and cathodic peak potentials] of 54 mV (≈ 60 mV) indicates the one-electron reversible process of RuHex (\( Ru^{2+} + e \rightarrow Ru^{3+} \)). To understand the charge transport mechanism, we recorded the CV of the SiC electrode using RuHex as a function of scan rate ranging from 10 to 1500 mV s⁻¹. As shown in Fig. 2(C), both anodic and cathodic currents, \( i_{pa} \) and \( i_{pc} \), increase with increasing scan rate and both currents are proportional to the square-root of the scan rate (inset, Fig. 2(C)). This finding clearly indicates the RuHex redox process at the single crystal SiC electrode occurred through a diffusion-controlled process and the process is quasi-reversible. The linear curve for the cathodic process (\( i_{pc} \) versus square root of scan rate \( v^{1/2} \)) is much steeper than that of the anodic process (slope; 9.64 versus 7.30), demonstrating the rate for reduction of RuHex is greater than the oxidation process. Moreover, both \( i_{pa} \) and \( i_{pc} \) increase with increasing concentration of RuHex (Fig. 2(D)). The high current density in our SiC electrodes (in comparison to nanocrystalline SiC and Br-doped-diamond) resulted from the high conductivity of our materials. Besides the excellent...
electrocatalytic activity, the electrode shows good CV stability and repeatability over multiple runs (Fig. S3, ESI†), which is one of the remarkable features for achieving durable and repetitive sensors for multi-step measurements. In addition, the CV scan showed identical characteristics under dark and illumination conditions owing to the wide band gap of SiC. These intrinsic properties of single crystal SiC electrodes make them highly promising for electrochemical sensing for clinical applications. The versatility of the SiC-on-glass electrode system was also verified by testing the electrocatalytic activity towards the redox process of another promising redox marker methylene blue (MB). Two well-defined peaks with potential differences of 31 mV were obtained representing the characteristics of the two-electron redox process of MB (Fig. S4, ESI†). Similar to RuHex, MB also resulted in increasing trends of both currents, $i_{pa}$ and $i_{pc}$, with increasing scan rate (Fig. S5 and S6, ESI†). Moreover, with successive additions of MB, the current density increased until reaching a plateau around a 10 μM concentration of MB (Fig. S7 and S8, ESI†).

To explore the biocompatibility and the possibility of using high-resolution transmission microscopy for imaging purposes on the SiC/glass electrode, we utilized Human Mammary Fibroblasts (HMF) as the test cell line (ESI†). Fig. 3(A) represents cell growth and adhesion on the SiC surface after 24 hours and

![Fig. 3](image-url)
72 hours. The phase contrast images clearly indicate the good proliferation of the grown cells. The HMF cells were then stained with actin and DAPI (4′,6-diamidino-2-phenylindole) and imaged using an inverted fluorescence microscope. The cytoskeleton of the HMF cells was clearly visualized after stained with actin, while the cell nucleus was also well imaged with DAPI. Evidently, the HMF cells extensively spread their cytoplasmic projection, meaning that the cell can anchor to the SiC surface. These results not only indicate the biocompatibility of the SiC surface but also imply that SiC-on-glass is compatible with high resolution transmission microscope due to its excellent transparency. We further demonstrate the possibility of simultaneous transmission microscope imaging with electrical stimulation. A SiC micro heater was formed using laser micromachining to define the conducting path followed by HMF cell culture. A DC voltage of 6 V was applied to the heater which results in an increase in the surface temperature of SiC of approximately 80 °C. Thermal heating eventually leads to early disintegration of membrane integrity, thereby facilitating lysis of cells which possibly causes cell death, Fig. 3(B). The real-time quantification of cell lysis was examined by adding 5 μL of (10 μg mL⁻¹) propidium iodide (PI) per 1 mL of cells of the samples prior to heating. Upon binding to DNA, the PI fluorescence was enhanced 20- to 30-fold, which indicates the characteristics of cell death associated with necrosis. The cells without red fluorescence appeared to be healthy and continued establishing contact/adherence to neighboring cells. Furthermore, increasing the applied voltage could result in complete cell lysis (Fig. S10, ESI†).

In conclusion, we have reported a new platform of SiC-on-glass electrochemical system, which provided simultaneous electrochemistry, optical observation, and electrical stimulation. With its high transmittance, biocompatibility, excellent electrical stability under light illumination as well as large potential windows, our crystal SiC-on-glass platform is a good candidate for cell culture, stimulating, imaging and bio-electrochemical sensors.

H.P.P., M.K.M., M.J.A.S. and N.T.N. planned and designed this work. H.P.P., M.K.M., K.N., T.K.N., and T.D. prepared the samples. H.P.P., M.K.M. and R.K.V. conducted the electrochemical and hydrolysis experiments. T.D. and T.K.N. performed the transmittance measurements. All authors discussed the results, analyzed the data, and wrote the manuscript.

This work was partially funded by the Australian Research Council grants LP150100153 and LP160101553. This work was performed in part at the Queensland node of the Australian National Fabrication Facility, a company established under the National Collaborative Research Infrastructure Strategy to provide nano and micro-fabrication facilities for Australia’s researchers.

Conflicts of interest

There are no conflicts to declare.

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