Graphene-based Electrodes for Neural Stimulation and Recording

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Abstract

There are multiple methods in fabricating graphene-based electrodes for neural stimulation and recording. In this project, we aim to fabricate graphene-based electrodes using inkjet printing. Numerous graphene ink types are explored in this paper to find the suitable ink to be used for inkjet printing. Conductive graphene inks can be both bought commercially (Anexus Ink, BareConductive Graphene paste) and self-made using graphite flakes with different solvents such as SDS solution and ethylene glycol solution. Also, graphene can be obtained by reducing graphene derivatives such as graphene oxide.

The project consists of mainly 3 parts: 1) Preparation of graphene-based inks; 2) Ink-jet printing of the conductive inks using the Dimatix Materials Printer, DMP 2800 on flexible substrates and lastly; 3) Characterising them via conductivity tests, and linking back their application to neural Science.

In the preparation of inks, sonication and centrifugation processes are crucial in creating suitable inks. Also, the addition of surfactants and bases are crucial for a better dispersion and in preventing agglomeration. The inkjet-printed samples then undergo conductivity tests to test their conductance and resistance. In general, it is observed that the unfiltered inks and those of higher concentrations tend to form better printouts with continuous layers and higher conductance.
Acknowledgements

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Lastly and most importantly, I would like to thank my family members who were with me through thick and thin throughout my entire student life.
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1. Introduction

Neural interfaces and its electrodes are becoming an increasingly powerful tool for clinical interventions requiring stimulation and/or recording of the electrical activity of the neural systems. The human neural systems are able to generate, transmit and process electrochemical signals in different parts of the body. To read and interpret these signals, it relies on the ability of electrodes to transduce and record biological signals from the body’s central or peripheral nervous system. Developing proper interface electrodes is important in today’s society as it helps to restore sensory and motor functions after spinal cord or peripheral nerve injuries, or to interface with artificial limbs.

In general, electrodes have four main uses, namely stimulation, recording, controlled drug delivery and in situ bio-sensing. Here, we focus on fabricating electrodes for stimulation and recording purposes. Stimulation electrodes are usually used in the deep brain, spinal cord stimulation, cochlear and retinal implants and in peripheral nervous systems. Recording electrodes requires recording signals of individual neurons over large areas.

*Figure 1-1: Clinically developed neural electrodes/interfaces and their uses.* [2]
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There is an increasing interest in the applications of such an electrode in health issues such as bladder dysfunction, motor-related disorders like Parkinson’s diseases and even cochlear implants. Therefore, it is important that an efficient and a cost-effective way is to create to fabricate these electrodes.

1.1 Advantages of Graphene

In this project, we focused on creating these electrodes with graphene. Graphene is an atomically thin aromatic carbon sheet, with excellent mechanical stability and electrical conductivity. It has excellent biocompatibility, it is chemically inert and integrates well with flexible substrates; all which could make it an ideal neuro-interfacing electrode. 2D graphene substrates have been found to exhibit enhanced adhesion, good viability, improved neurite sprouting and outgrowth.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Graphene</th>
<th>Gold</th>
<th>PEDOT</th>
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</thead>
<tbody>
<tr>
<td>Biocompatibility</td>
<td>✓ ✓</td>
<td>✓</td>
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<tr>
<td>Long-term Stability</td>
<td>✓ ✓</td>
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<td>Adhesion [1]</td>
<td>✓ ✓</td>
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<td>Impedance [2]</td>
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<td>SNR [2]</td>
<td>✓</td>
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Table 1-1: Characteristics of electrodes made from different materials.

Graphene has the best ability to improve the electrical interface between neuronal cells and electrodes. Poly (3, 4-ethylene dioxythiophene) substrates degrade with aqueous solutions overtime while gold on electrodes is usually sputtered with Iridium oxide which is corrosive in the long term. [2]

1.2 Objectives

The objective of this project is to develop graphene-based electrodes, and test their efficacy by interfacing them with the nervous system. In this project, we focus on the fabrication of graphene-based electrodes via inkjet printing. We aim to use this newer, scalable and more efficient method to create an alternative method to fabricate neural electrodes.
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To do so, we first need to prepare various conductive inks based on graphene, or graphene derivatives (such as chemically or physically modified graphene) that are suitable for inkjet printing. After which, ink-jet printing of such materials as electrodes on flexible substrates. Lastly, characterisation is carried out through conductance and resistance measurements before drawing a link back to neural science and application.

Developing proper electrodes for bidirectional interfacing – with low impedance, biocompatible, with long-term chemical stability – remains a challenge in creating viable neural electrodes.
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2. Methodology

2.1 Method of Fabrication

The Dimatix Material Printer, model DMP 2800, was used to fabricate these electrodes. The DMP 2800 is a laboratory and limited production tool that enables one to evaluate the use of ink jetting technology for new manufacturing and analytical processes.\[^3\] The printer cartridges are filled up with the respective conductive ink and placed into the printer. After which, it is patterned and inkjet onto electrode substrates. The DMP 2800 is connected to a desktop, where the program enables us to depict the pattern that is printed on a selected point. This gives us the flexibility to create different patterns and print in only the needed locations. These tasks can be achieved through the Dimatix drop manager interface as shown below.

![Pattern editor (Left) and Fiducial Camera (Right).](image)

The pattern editor allows parameters such as the substrate size, the type of pattern and the drop spacing when printing. Drop spacing is the distance (in microns) between each drop when printed to form the pattern feature. The lower the drop spacing value, the more each printed drop will overlap and the feature will have a greater resolution. It also allows us to depict where each drop is printed with the aid of coordinates. The fiducial camera allows us to view the substrate and pinpoint where we want the feature to be printed on.
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In the DMP 2800, a plastic 2 ml cartridge is used and attached to a print head. The printhead has 16 jetting nozzles, with each nozzle 10 μm in diameter and approximately 255 μm apart in a single row. Each droplet produced is about 10 picolitre in volume. This enables us to print in high resolution. Also, we have attached a heating plate onto the main plateau of the printer. The original plateau could only heat up to 60°C. With this temperature, the ink that has been printed does not dry fast enough and spreads across the sample, not giving a precise shape. The external heater is able to heat up to 90 °C, which enables a faster drying process.

To fabricate a good electrode, we first have to create a good conductive ink that is suitable for ink jet printing. The ink particles need to have a size smaller than 9 μm \(^3\) as well as viscosity between 10 to 12 centipoise. These are important parameters that we need to follow throughout the project, and it is essential to do so that the ink can be precisely printed without clogging. With the formulated ink, we can then proceed to use the DMP to inkjet on a substrate.

There are also other deposition methods that have been widely used and researched on. Such methods include physical vapour deposition (PVD) and chemical vapour deposition (CVD). PVD involves a material transfer from a condensed-phase evaporate, sputtering techniques and other non-chemical methods.\(^4\) Evaporation refers to the heating of the material until it vaporises and
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condensed onto the intended substrate, while sputtering is the process of physical impacts transferring kinetic energy to atoms in the target. CVD, on the other hand, involves a process of chemically reacting a volatile compound of the material intended to be deposited, with other gases, producing a non-volatile solid that deposits on a substrate.\[^5\]

However, both of these above techniques are usually operated in a high vacuum and high-temperature systems for the best results.\[^5\] These hence result in a high capital cost. Also, these methods were time-consuming to operate and are not as flexible as compared to the more efficient process of inkjet printing.

With the appropriate fabrication method, we can then characterise its structural and biocompatibility properties and to assess feasibility for its application to Neural Science.

### 2.2 Imaging

In order to do comparisons and documentation of results obtained, it is important that we do imaging of the samples printed on a micro scale. In the following report, the images shown were taken by the optical microscope and the Scanning Electron Microscope (SEM).

Majority of the images as seen in this report is taken using an optical microscope. The microscope used here is from Leica Microsystems. The microscope is able to take up to x100 magnification.

A digital microscope camera is attached and images are captured.

Higher magnitude images were taken using a Scanning Electron Microscope, SEM. It scans a focused electron beam over the substrate’s surface. The electrons then interact with the sample to produce various signals that can be used to create an image, showing the surface topography and the composition of the sample.\[^6\]
2.3 Characterization

2.3.1 Electrode Requirements

The crucial and key conditions that an electrode needs to have are that it is minimally invasive and is biocompatible with the human body with minimal inflammatory responses. It is also important that the electrode has mechanical compliance with neural tissues, so as there will be no damage or scaring to the neural tissue. For the electrode to be effective and efficient, the electrode needs to be conductive and maintain a low impedance towards the interfacing tissue for the duration of its functional lifetime.

2.3.2 Conductivity Test

It is crucial to ensure that the inkjet-printed graphene to be conductive, In order to do so, we performed a conductivity test on the printout. It is important that we printed on a non-conductive substrate so that the measurement is reliable and accurate. The conductivity test is performed using the TSP Express. It is a utility from the Keithley Instruments SourceMeter 2634B series that creates runnable scripts written in the TSP express environment for current-voltage recording. Scripts for a single or multiple voltage sweeps can be created, alongside multiple bias channels. A single sweep contains one or more sweep channels where the output and the input for each channel can be independently specified. Each point of the sweep is synchronized using the trigger layer of the first, or master, sweep channel and the arm layer of the other channels. Source values can be specified as start and stop values with linear or logarithmic steps, or the source values can be specified in a custom list. A typical use for a single sweep is to characterize the voltage-current behaviour of a two terminal device set-up, which is what we used in this project.
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Here, the voltage is being swept in a single sweep across the sample from -0.1 V to 0.1 V in steps of 0.001 V. The script can be edited using the graphical user interface as shown in the figure below.

![Image of script](image.png)

**Figure 2-5: Script used for the conductivity test.**

The range of the voltage sweep and the steps taken can be customized using this program with TSP Express.

The program plots a graph of current against voltage. This shows the current-voltage (I-V) characteristic between the current that is passing through the sample and the corresponding potential difference across it. There are many types of graph that can be obtained. For graphene, a linear I-V curve should be obtained with significant current if the sample is conductive \(^7\). Graphene is known to obey Ohm’s law, where the voltage (V) and current (I) are directly proportional to each other.

With these parameters, we can then calculate the conductance and resistance of the graphene that is printed. Electrical resistance is the measure of the difficulty faced for a current to pass through the conducting sample. Electrical conductance, on the other hand, is the inverse of that of electrical resistance, where it the ease in which the current passes through.
Resistance, \( R = \frac{V}{I} \quad - \) (1), \quad Conductance, \( G = \frac{I}{V} = \frac{1}{R} \quad - \) (2)

*Equation 1 & Equation 2: Resistance, \( R \) and Conductance, \( G \).*

They have the units of \( \Omega \) (Ohms) and \( S \) (Siemens) respectively.

With the aid of the equations above, we are able to measure both the resistance and the conductance which are the inverse of the I-V curve slope and the gradient respectively of the I-V graph obtained. Also, for more specific resistance and conductance based on the material size and type, the equations below can be used, where \( \ell \) is the length of the material measured in metres and \( A \) is the cross-sectional area \((m^2)\).

\[
\text{Resistance, } R = \frac{\rho \ell}{A} \quad - \) (3), \quad Conductance, \( G = \frac{\sigma A}{\ell} \quad - \) (4)

*Equation 3 & Equation 4: Resistivity, \( \rho \) and Conductivity, \( \sigma \).*

They have the units of \( \Omega \text{ m} \) (Ohm-metre) and \( S \text{ m}^{-1} \) (Siemens per metre) respectively.

The resistivity and conductivity are proportionality constants which solely depend on the material type that is being measured. The resistivity and conductivity are reciprocals of each other. Here, the current density is assumed to be constant throughout the material measured, as the area and length measured is small, approximately 0.02 mm\(^2\) and 1 mm respectively.
3. Preparation of Inks and Samples

Through the specifications of the DMP 2800, we were able to narrow down on the parameters required to make a good electrode for neural stimulation and recording, as well as to find the suitable inks fitting for the Dimatix printer that was being used here.

3.1 Ink Preparation

To ensure that the inks were homogeneously mixed before printing, the sonicator is also used. Here, the Sonica Q700 was used. Sonication is a process where sound energy is applied to the sample which agitates the particles in the sample. This is done by generating a 20 KHz high voltage electrical signal that drives a piezoelectric converter. This electrical signal is then converted to mechanical vibrations that are transmitted by a horn. The rapid vibration in the liquid inks breaks up the aggregates and intermolecular interactions found in the sample. This ensures that the graphene flakes found in the ink are mixed well and broken down to a small enough size to be able to pass through the nozzles of the printhead.

3.1.1 Commercial Inks

One commercial ink used was the Anexus Ink. This ink is a multilayer graphene ink, where its concentration is 0.4 milligram per millilitre in Ethyl glycol solution. Before printing, the ink was sonicated for 30 minutes at an amplitude of 45% with a power of approximately 20 W.

Another commercial ink that was purchased is a graphene-based electric paint from Bare Conductive. The electric paint is soluble in water, hence it is mixed with distilled water to dilute it to the needed viscosity. It was also sonicated for the same period of time as above.
**3.1.2 Homemade Graphene Ink**

Also, I made our own Graphene ink, from home-synthesized graphite flakes. To create this ink, we vary the concentration of graphene flakes in different solvents to create different variations of inks. Solvents such as SDS solution and Ethylene glycol solution were used. SDS solution is made from dissolving sodium dodecyl sulfate in 20% ethanol, which is a synthetic organic compound. It is commonly used as a surfactant in many cleaning and hygiene products, hence ensuring it is safe to be used in these neural electrodes. Ethylene glycol solution is an organic compound which is commonly used in anti-freeze products and polymers. For mixing, the selected solvent and graphite flakes were sonicated for 2 hours at an amplitude of 45% and a power of approximately 25 W.

**3.1.3 Homemade Graphene Oxide Ink**

Graphene Oxide ink can be made in a similar way to the Graphene ink above. Graphene Oxide (GO) powder, from BGT Materials, was mixed with distilled water and sonicated at an amplitude of 45% for 2 hours. After which, it is centrifuged at 4000 rpm for 30 minutes. Only the top 10% of the ink was used for printing.

To reduce the GO ink after printing, the printout was submerged into C₆H₈O₆, more commonly known as ascorbic acid, for a day at room temperature. Ascorbic acid is a commonly used reductant, known to have a mild reductive ability and non-toxic property, which naturally act as a reducing agent without extreme conditions[^8]. The reduction of GO causes the print out to change its colour from yellow-brown to greyish black[^9]. Reducing GO removes the oxygen molecules present on the surface.
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Figure 3-1: Reduction of GO to Graphene with the aid of a reducing agent.\textsuperscript{[12]}

The reduction of GO removes the oxygen from the surface of each flake, forming reduced GO (rGO), which is a graphene layer with numerous defects.

It is important to note that the reduced graphene obtained is one with numerous structural defects like edge planar defects. As defect density of the graphene layer increases, the thermal conductivity of the material will increase when it is at room temperature.\textsuperscript{[13]} The thermal conductivity relation with the defect density of the material can be proven through the Boltzmann transport equation as well as molecular dynamics simulations\textsuperscript{[13]}.

GO is chosen to be used and reduced as it is an important and relatively new material which has properties alike to those of pristine graphene, giving rise to countless of potential applications.\textsuperscript{[14]} There are numerous other ways to reduce GO, but this method was chosen as it is less toxic. Reducing GO a less toxic way does not affect the conductivity of the reduced GO, and the attained conductivity is comparable to other more well-known methods such as the Hydrazine or hydrothermal method\textsuperscript{[9]}. However, hydrazine is highly poisonous and volatile, and it is not biocompatible with the human body\textsuperscript{[8]}.

3.2 Sample Preparation

Besides changing the ink type, we can also control the cleanliness of the substrate before and after being printed on. Before printing, the substrate surface was cleaned by Piranha solution. Piranha solution is prepared by mixing 7 parts sulfuric acid and 3 parts hydrogen peroxide. This makes the
cleaning agent a very strong oxidizing agent which removes most of the organic contaminants from the surface, making it highly hydrophilic. This can be proven by the following printing results.

After printing, the surface of the printout can be annealed, using the planarGROW-2S CVD system by planarTECH. Annealing is the heat treatment process of the sample, which alters the microstructure of the material, which in turn changes its mechanical or electrical properties [10]. Annealing also helps to reduce impurities such as residual organic surfactants used and hydrocarbon contaminants, which can alter graphene’s electronic and chemical properties [10]. It is crucial that the surface of the printed graphene is kept clean for proper functioning in the electrodes. The annealing parameters here were set to 500 °C for 120 minutes.
4. Results and Analysis

4.1 Commercial Inks

4.1.1 Anexus Ink

We first printed with a commercial ink, also known as Anexus ink. The first test was to inkjet print a set of horizontal lines on a silicon wafer. 3 layers were printed, with each line measuring up to 1 cm in length and 0.01 mm in thickness. The results are as seen in the microscopic images below.

For filtering, the ink is pushed through a 0.2 μm filter. From the figure above, it is clear that the graphene flakes are very sparse and insufficient to cover the lines fully when it is filtered, and that there are more flakes in the latter. For the next few processes of inkjet test, the unfiltered ink was used.

The sonication timings for the Anexus ink is also varied. It is observed that the ink that has been sonicated for longer periods of time has deposited a greater number of graphene flakes as compared to the other. The graphene flakes are denser here and form a somewhat interleaved pattern. This can be seen from the Figure 4-2.
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![Image of Graphene-based Electrodes for Neural Stimulation and Recording](image1)

**Figure 4-2 (a), (b):** Sonicated unfiltered Anexus ink for 45 mins at 40% amplitude (Left); Sonicated unfiltered Anexus Ink for 3 hours at 40% amplitude (Right).

A 3mm by 3mm block is being inkjet printed on to a Si wafer. A total of 4 layers were printed.

Based on the previous 2 tests, we can justify that the Anexus ink is better suited when it is unfiltered and sonicated for longer periods of time. This could be due to the presence of more graphene flakes in the unfiltered ink and that the flakes are smaller and can be ink-jetted with greater ease, without clogging the nozzles of the printhead.

The conductivity of the Anexus ink can also be measured. Given that the printouts were segmented and discontinuous as seen as the above pictures, we opted for the conductivity measurement to be done on an ink droplet instead. The graph is obtained as shown.

![Graph of Current against Voltage for the Anexus ink droplet](image2)

**Graph 1: Graph of Current against Voltage for the Anexus ink droplet.**

2 drops of Anexus ink were dispensed on a SiO₂ wafer, which was placed on a heated plateau of 90 °C to aid the drying process.
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From the slope of the graph, the conductance and resistance of the printout can be obtained. There is only a small conductance present, approximately 0.00008 S.

4.1.2 Conductive Electric Paint

Another commercial ink that was used is the electric paint from Bare Conductive. For the electric graphene-based paint obtained from Bare conductive, 2 inches of the paste was mixed and sonicated with 10 ml of water at an amplitude of 50% for 30 minutes. After which, the ink is filtered through a 0.45 μm filter.

Figure 4-3 (a), (b), (c): Unfiltered diluted Graphene paste paint in droplet form (Left); Inkjet-printed horizontal lines with filtered diluted Graphene paste paint (Middle); Inkjet-printed horizontal lines with unfiltered diluted Graphene paste paint (Right). Horizontal lines were inkjet-printed onto a SiO₂ substrate. All samples were dried on a heated plate.

Graph 2: Conductivity test for the Graphene paste from BareConductive. All of the inks were dispensed on a SiO₂ wafer in droplet form.
The electric paint (from Bare conductive) was very conductive in its original paste form as promised. However, upon dilution and filtration, the conductivity of the ink started to decrease. The conductivity graph of current against voltage can be seen in Graph 2. It can be observed that the conductivity decreases when it is diluted and filtered. The filtered diluted ink was not conductive at all. The conductance can be obtained from the slope of the I-V graph, where the conductance of the paste and diluted ink are 0.0006 S and 0.0004 S respectively.

### 4.2 Homemade Graphene Ink

Besides using the commercial Anexus ink, making my own graphene ink is also possible. The ink is made by adding graphene flakes to SDS solution. We first started out with an ink that has similar concentration as that of the commercial Anexus ink, 0.4 mg/ml to ensure a fair comparison.

To do a comparison, we had both inks to print a set of horizontal lines as shown below. The results were clear that the homemade graphene ink was a better and more preferred choice. There is a greater number of graphene particles being printed in the left picture as compared to the other, and that there is a better precision in the printer when the homemade ink was used. More distinct lines can be seen.

*Figure 4-4 (a), (b): Sonicated unfiltered Anexus ink, ink jetted into horizontal lines (Left); Sonicated unfiltered Homemade Ink, ink jetted into horizontal lines (Right).* Sets of 20 horizontal lines were inkjet printed on a Si substrate. A total of 3 layers each were printed.
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The homemade graphene ink can be filtered using the 0.22 μm filter. As seen from the microscopic photos below, there is a large difference between the filtered and unfiltered homemade ink. The diagrams show the filtered and unfiltered ink dispensed in a droplet form on a Si wafer. It is clear that there are more graphene particles found in the homemade and unfiltered ink.

![Figure 4-5 (a), (b): Sonicated filtered Homemade Ink droplet (Left); Sonicated unfiltered Homemade Ink droplet (Right). An ink droplet of each respective ink was dispensed onto a Si wafer and left on a heated plateau to dry.](image)

4.2.1 Increasing Concentrations

Since the Homemade ink had a better performance, we moved on in changing the parameters of this ink. The concentration of graphene flakes in SDS solution was changed accordingly, making inks of concentration 0.7 mg/ml and 0.8 mg/ml.

![Figure 4-6 (a), (b), (c): Sonicated unfiltered 0.4mg/ml Homemade Ink (Left); Sonicated unfiltered 0.7mg/ml Homemade Ink (Middle); Sonicated unfiltered 0.8mg/ml Homemade Ink (Right). There are 3 layers of ink of are being printed in a horizontal line pattern for each.](image)
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One drastic difference observed between the original 0.4 mg/ml and the 0.7 mg/ml, 0.8 mg/ml concentration is the number of the graphene flakes that is being ink jetted. The ink that is being jetted at higher concentration has more graphene flakes, as more flakes are able to pass through the nozzles. The precision of the pattern printed also increases, where the 0.8 mg/ml concentration print out shows more distinct horizontal lines as compared to the other 2.

Higher concentrations of graphene ink above 1 mg/ml were also prepared, but they produced unsatisfactory results. This is because as graphene concentration increases, agglomeration becomes more likely.[11]

To test the conductivity of the different concentrations of ink, a droplet of each concentration was dispensed onto a glass slide and it was set aside to dry on a heated plateau of 90 °C. The inks used here were unfiltered and re-sonicated for another 30 mins after the preparation process. The graph below shows a plot of the current against voltage, obtained using the TSP Express mentioned in Chapter 2.

*Graph 3: Graph of Current against Voltage for graphene ink in SDS solvent.*

The concentration of the ink was increased from 0.4 mg / ml, 0.7 mg / ml and 0.8 mg / ml. A droplet of each ink concentration was dispensed onto a glass slide and dried on a heated plateau at 90 °C.
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It is observed that as the concentration of the ink increases, the conductance of the inks also increases.

### 4.2.2 Changing Solvents used

Another solvent such as Ethylene glycol (EG) solution can be used to dissolve the graphite flakes. Here, 20 mg of graphite flakes were placed into 40 ml of EG solution. The suspension was sonicated for approximately 2 hours at an amplitude of 50 %, which has a power of approximately 39 W. An attempt was made to filter the ink at this stage, but the filter was rapidly clogged and the filtrate was clear. We then centrifuged the ink for 30 minutes at 4000rpm, and the top 5 ml of the ink was used and filtered again. The filtered ink still remains clear.

As EG solution is not a surfactant, it is unable to disperse the graphene effectively. The graphene flakes are inclined to aggregate due to strong Van der Waals interaction. Most of the graphene agglomeration settles to the bottom due to gravity, which causes the ink to be less conductive than it should be [11].

*Figure 4-7 and Figure 4-8: Agglomeration of Unfiltered Homemade Graphene ink in EG solvent (Left); Agglomeration of graphene flakes in EG solvent when inkjet-printed onto a Si wafer (Right).* From the left picture, there is a clear separation between the graphene flakes and the solvent as most of the flakes have sunk to the bottom. Similarly, when printed, the graphene flakes then to clump together and is unable to form a continuous layer.
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Comparing both graphene inks made using SDS solvent and EG solvent, there is a drastic difference between the dispersion of the inks. As seen from Figures 4-9 and 4-10, it is observed that there is a better dispersion of graphene when the SDS solvent is used during preparation. In the SEM images, the print-out with the SDS solvent ink on the SiO₂ wafer has better coverage of the substrate as compared to that with the EG solvent one. It has a continuous layers graphene flakes with a minimal number of voids.

![Figure 4-9 & Figure 4-10: SEM images of Graphene ink in SDS solvent (Left) and Graphene ink with EG solvent (Right). There is a better dispersion of the thin film graphene when the SDS solvent is used. It is observed that there are more 3D islands formed when the EG solvent is used.](image)

However, in the print-out using the EG solvent ink, there are large 3D islands of graphene can be seen on the surface, and there are large voids present. This occurs because there is a strain induced due to the deposition of atoms with a different covalent radius than that of the substrate used. In such a strained system, the normal growth mode would involve the formation of 3D islands which helps relieve the strain by relaxation at the island edges \(^{15}\).

This does not occur when a surfactant, for example, SDS solution, is used. Surfactants induce layer-by-layer growth in strained systems by avoiding the formation of 3D islands for film thicknesses much beyond what is obtained under normal conditions \(^{15}\).
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Therefore to improve the performance of the EG solvent ink, one of the recommended ways was to add a surfactant to the solvent\textsuperscript{[11]}. Surfactants are organic compounds that are amphiphilic, as they have both a hydrophobic group and hydrophilic group. This allows them to be adsorbed at the interfaces of these carbon-based particles and prevents agglomeration.

\textbf{Figure 4-11: Schematic illustration of surfactant-assisted dispersion of Graphite flakes.} The polar part of the surfactant used, SDS (Sodium dodecyl sulfate and ethanol solution), coats each graphite flake to prevent them from agglomerating together.\textsuperscript{[16]}

\textbf{Figure 4-12 (a), (b), (c), (d): Unfiltered and Filtered Graphene ink with EG and distilled water (Top Left & Right): Unfiltered and Filtered Graphene ink with SDS and EG (Bottom Left & Right).} Both ink suspensions were dispensed as a droplet onto a SiO\textsubscript{2} wafer and dried on a heating plate.
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The solvent in which the graphite flakes were suspended in was further altered. With the aid of further research, a solvent of EG and a surfactant (SDS) or distilled water, with ratio 1:1 was created. It was suggested that by doing so, graphite flakes are able to form stable dispersions in such a solvent \[1\]. The results are as seen in the microscopic images in Figure 4-12. However, the addition of surfactants such as SDS solution can greatly affect the inkjet printing and drying of the graphene ink. The addition of surfactants can cover the surface of the substrate and graphene flakes, which influences the physical properties of adhesiveness and conductivity. The ink with the added SDS solution does not dispense into a continuous layer as intended. The difference in the conductivity between the inks can be seen in the graphs below.

![Graph 4 & Graph 5: Plots of Current, I, against Voltage, V, for graphene ink with EG and distilled water as a solvent (Left); graphene ink with SDS and EG solvent (Right). For both inks and their solvents, droplets of unfiltered and unfiltered ink are dispensed onto a glass slide and dried on a heated plateau.](image-url)

From both graphs above, it shows that the unfiltered ink is more conductive in comparison to those that were filtered. Also, the ink that has the addition of distilled water has a higher conductance as compared to the other. SDS solution has indeed affected the conductance of the samples printed, as the flakes adhere less readily to the substrate.
4.3 Homemade Graphene Oxide Ink

When the first batch of ink was made, we were unable to filter the ink through the 0.45 μm filter. The filter got clogged quickly and the filtered ink was clear with little to no graphene oxide particles left after filtration.

This is due to the graphene oxide particles being agglomerate together and unable to disperse properly \[17\]. To tackle this, ammonium hydroxide (NH$_4$OH) base was added to the ink before sonicating and centrifuging it. This increases the pH value and prevents agglomerations from happening. At higher pH values (pH ≈ 14), the GO flakes do not experience a change in its molecular structure and are not easily absorb others in the water. This is due to the electrostatic repulsions between the carboxyl groups creates a barrier which hinders GO agglomeration. At lower pH, the GO flakes become less hydrophilic and result in the formation of suspended GO aggregates in water \[17\].

![Figure 4-13(a), (b): Inkjet-printed 3mm by 3mm block using GO ink without a base (Left); Inkjet-printed 3mm by 3mm block using GO ink with base (Right). Both inks are filtered using the 0.45 μm and 5 layers of ink are printed on a Si substrate. The graphene oxide flakes then to agglomerate together when there is no addition of a base, causing it to be less evenly spread out.](image)
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After the printout is dried, it can be submerged in ascorbic acid, commonly known as vitamin C for a day. This reduces the graphene oxide printed. There should be a colour change in the printout from brownish yellow to grey.

![Image](image1.png)

**Figure 4-14 (a), (b): Unreduced Graphene oxide print (Left); Reduced Graphene oxide print (Right).** Both images show the same set of horizontal lines printed on a SiO2 substrate, with 10 layers printed with inter-delays in between layers to facilitate drying. The wafer was soaked in ascorbic acid for 1.5 days.

However, evident from Figures 4-13, there is no obvious colour difference seen before and after. Another sample was inkjet-printed and it was submerged in the same reducing agent for a longer period of time, for approximately 5 days. There is a more obvious colour difference as seen from Figures 4-14 (a) and (b).

![Image](image2.png)

**Figure 4-15 (a), (b): Unreduced Graphene oxide print (Left); Reduced Graphene oxide print (Right).** Both images show the same set of 3 mm by 3 mm block printed on a SiO2 substrate, with 15 layers printed with inter-delays in between layers to facilitate drying. The wafer was soaked in ascorbic acid for approximately 5 days.
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**Graph 6 & Graph 7:** Both graphs show a plot of current against voltage for both the unreduced graphene oxide and the reduced graphene oxide after being submerged in ascorbic acid for a different amount of time. The inkjet-printed substrates were placed in ascorbic acid for 1.5 days for it to be reduced (Left); The inkjet-printed substrates where left in ascorbic acid for approximately 5 days for reduction to occur (Right).

The conductivity test was also conducted on the homemade graphene ink sample. When the sample was not fully reduced (no change in colour), there was little conductance measured and the sample was not conductive. On the other hand, when the sample is properly reduced, there is a visible difference in the conductance between the unreduced and reduced sample. The conductance values for the unreduced and reduced graphene oxide are 3E-9 S and 2E-7 S respectively.
4.4 Overall Results

4.4.1 Conductance

With the aid of Equations (1) and (2) as mentioned earlier, the conductance and resistance of each printout can be calculated from the I-V characteristic graphs obtained. Conductance and resistance can be plotted against the varying ink types as seen in the graphs below.

![Graph 8 and Graph 9: Graph of Conductance and Resistance for varying ink types measured using TSP Express.](image_url)

The values obtained through calculation are as seen in Table 3 (Appendix).

From both graphs 8 and 9, it can be seen that the conductance and resistance are inversely proportional to each other. The higher the conductance, the lower the resistance. It is also observed that the filtered inks, in general, have a lower conductance as compared the unfiltered ones. Conductivity also increases with the concentration of the ink.
Comparing all the inks used, both the conductive paste and the homemade graphene ink, in SDS solvent, with a concentration of 0.8 mg/ml has the highest conductance of 0.0006 S and 0.0005 S respectively. However, the resistivity is still too large as compared to literature values of 0.81 ± 0.2 kΩ \(^{21}\). The magnitude of the resistivity values, in general, are larger than the conductance values.

4.4.2 Varying ink-jetted layers and Conductance

It is also observed that the number of layers does affect the conductance of the sample. From the previous section, given that the conductance of the graphene ink in SDS solvent with a concentration of 0.8 mg/ml is one of the highest, it was used to see the relationship between the number of passes printed and its conductance.

**Graph 10: Conductance values obtained against an increasing number of layers being printed.**

Here, the graphene ink with SDS solvent [0.8 mg/ml] is being used and inkjet-printed on a SiO2 substrate as a 3 mm by 3 mm block. Values are in Table 4 (Appendix).

The greater the number of passes being ink-jetted, the higher the conductance value obtained.
5. Applications

5.1 Current Study

Currently, Singapore Institute of Neuro-technology, SINAPSE is fabricating a flexible neural clip (FNC), which allows it to be interfaced with a wide variety of peripheral nerves. The main goal of this technology is to have a wireless bioelectric neural interface that is scalable and can be attached to nerves located deep within the body without any neural damage. Hence, there is a crucial need for the clip electrode to be flexible to adapt and attach to the nerve, biocompatible, and be easily implanted to send signals to the nerve.

The FNC uses a clip strip and clip spring process, to increase the ease of implantation. Such a technology helps to aid patients with bladder dysfunction. It is to be clipped onto the pelvic nerve of the bladder, to stimulate the nerve for bladder function. The electrode that was fabricated has a polyimide-gold-polyimide sandwiched structure, which was grown using the PVD technique.

![Figure 5-1: Flexible Neural Clip (FNC).](image)

The fabricated FNC with its gold active electrodes and its connectors. The figure on the bottom right also shows how the clip is implanted onto the nerve in a clip spring manner. The nerve is inserted between the clip-strip and clip springs after bending the clip springs. [18]
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There were numerous challenges faced in this study. The main challenge faced is the high impedance and high signal-to-noise (SNR) faced. Having high impedance results in the electrodes needing high voltages to inject desired amplitudes of signals. This gives rise to undesirable electrochemical reactions which in turn may cause damage to the nerve and tissue. Other limitations include the risk of scar tissue formation, which is a long-term effect for an electrode that is interfacing with the neural system as the gold electrode is not flexible. Also, the current electrode that is fabricated is unable to do recording.

5.2 Linking to Neural Science

We were able to obtain some sample electrodes from SINAPSE to have a closer look at them and view the conductivity and flexibility of them. The figures below show the microscopic image on the electrodes that we obtained from SINAPSE.

![Figure 5-2: The head of the FNC electrode where the active electrodes are (Left); Connecting patterns of the electrode which connects the active electrodes to the source (Middle); A closer look at the wires on the electrode revealing a break in the circuit (Right). This is the FNC electrode obtained from SINAPSE, which has already been lifted off its substrate, and it is on a very flexible polyamide cut out.]

With reference to the microscopic images above, it is observed that due to the flexible nature of the electrode, the gold on the electrode breaks off easily. Gold is not as flexible as the electrode substrate hence it easily cracks up.
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To rectify the problem faced, graphene can be used instead. The initial plan was to inkjet print the original gold parts with graphene. Graphene is known to be conductive and one is able to obtain results with low impedance and low signal-to-noise ratio as compared to the original gold contacts used. \[18\] However, based on the results obtained in this project, the graphene ink itself is not sufficient to fabricate an electrode. Hence, we opted for a second approach – to print graphene over the already present gold wires on the electrodes. In this way, graphene will be able to span over gold and hold the gold together. Graphene is very flexible, therefore it is feasible to do so.

With the aid of Figure 5-1, we were able to map out the wire shape found in the electrode. The image can be drawn and saved as a bitmap image and loaded into the DMP for printing.

![Figure 5-3: Bitmap Image of the scalable image to be printed.](image)

The image was first constructed with reference to Figure 6-1, following the measurements as stated \[18\]. The image is then converted to bitmap image before uploading it into the DMP 2800 software to be printed.

![Figure 5-4 (a), (b): Inkjet-printed graphene ink printed on Kapton tape before (Left) and after (Right) bending and stretching.](image)

15 layers of Graphene in SDS solvent [0.8 mg/ml], is being printed and dried on a heated plateau at 90 °C.
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To test the flexibility of graphene, we inkjet-printed graphene onto Kapton tape. Kapton is a polyamide material which has similar flexibility to the polyamide used to fabricate the electrode. After printing onto the Kapton substrate, it is then bent and stretched to test its flexibility.

With the aid of microscopic images as seen in Figure 5-4, the graphene layer has little to no damage done after the bending test. The only difference that can be seen is the hairline cracks as seen in the right image, but the graphene layer still remains consistent across the surface. The conductivity of the printout has little to no change after bending.
6. Future Works and Research

Apart from the characterisations such as conductivity and viscosity of the ink as mentioned above, there are other characterisation tools that can be used.

One such example is cyclic voltammetry. Cyclic voltammetry is an electrochemical technique where it allows us to measure the amount of current that passes through an electrochemical cell when excess voltage is applied \[19\]. Using this, we will be able to measure the specific charge and capacitance that the fabricated electrode has. For instance, the capacitive current response of the electrode measured is directly proportional to the potential change in time/scan rate. These parameters are important in characterising the electrodes to its different functions. Different electrode functions need different parameters as seen in the table below.

<table>
<thead>
<tr>
<th>Electrode Function</th>
<th>Parameters needed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stimulation</td>
<td>Min level of charge injection to depolarize the membrane of excitable cells in the vicinity (order of hundreds of (\mu)C cm(^{-2}) to few mC cm(^{-2}), in pulses between 100 (\mu)s and 1 ms) [2]</td>
</tr>
<tr>
<td>Recording</td>
<td>Recording signals of individual neurons (~(\mu)V to mV) over large areas (~10 cm(^2)) [2]</td>
</tr>
</tbody>
</table>

*Table 2: Needed parameters for each specific electrode functions.*

The parameters do change when it is interfacing with a different neural interface. In general, for a stimulation electrode, high charge injection capacity provides a better focal stimulation. Also, for recording electrodes, the smaller they are, the higher the impedance they will experience, resulting in a larger signal to noise ratio. \[2\]

Another idea that we can look into is linking these graphene-based electrodes with a recent advancement in the field – Optical stimulation. Optical stimulation, also known as optogenetics, is a biological technique that uses light to control cells in living tissues, usually neurons, which have been modified genetically to express light-sensitive ion channels. Unlike previous electrical stimulation, optical stimulation is able to provide more selective stimulation, higher spatial resolution and reduced invasiveness of the device. \[20\] For graphene to stay relevant in this field, it
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is crucial for it to integrate itself into the current research. One could look into creating graphene-based bio-interfaces which can be used for optical stimulation.

7. Conclusion

In conclusion, it is feasible to fabricate graphene-based electrodes for neural stimulation and recording using the inkjet printing method. Graphene inks can be both bought commercially and self-made. For the preparation of homemade inks, it is important that we include a surfactant, for example, SDS solution, into the ink so as to allow the graphene to be dispersed properly on the substrate. Bases such as NH₄OH can also be added to provide an alkaline environment and prevent agglomeration of graphene flakes from occurring. Other graphene-based derivatives such as graphene oxide can also be used. Graphene oxide can be reduced by soaking the samples in ascorbic acid until the colour of the printout changes from yellowish brown to a greyish colour.

Sonication and centrifugation processes can aid the preparation of the graphene-based inks. Through the results obtained, it is observed that unfiltered inks and those of higher concentrations tend to form better printouts and have a higher conductance. Comparing all the inks fabricated, the best performing ink is graphene ink made from graphite flakes with SDS solution as the solvent, with a concentration of 0.8 mg/ml. With a conductance of 0.5 mS, its conductivity is comparable to that of a commercial electric paste.

All-in-all, the inkjet printing of graphene possesses suitable characteristics not only to fabricate electrodes but can also play a crucial role in other forms of neural technology today.
References


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Appendix

<table>
<thead>
<tr>
<th>Ink Type</th>
<th>Gradient</th>
<th>Conductance, G / S</th>
<th>Resistivity, R / Ω</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anexus Ink</td>
<td>8.00E-05</td>
<td>8.00E-05</td>
<td>1.25E+04</td>
</tr>
<tr>
<td>Conductive Paste</td>
<td>6.00E-04</td>
<td>6.00E-04</td>
<td>1.67E+03</td>
</tr>
<tr>
<td>Conductive Paste (Diluted)</td>
<td>4.00E-04</td>
<td>4.00E-04</td>
<td>2.50E+03</td>
</tr>
<tr>
<td>Conductive Paste (Diluted, Filtered)</td>
<td>6.00E-09</td>
<td>6.00E-09</td>
<td>1.67E+08</td>
</tr>
<tr>
<td>EG DI Graphene (Filtered)</td>
<td>1.00E-08</td>
<td>1.00E-08</td>
<td>1.00E+08</td>
</tr>
<tr>
<td>EG DI Graphene (Unfiltered)</td>
<td>1.00E-04</td>
<td>1.00E-04</td>
<td>1.00E+04</td>
</tr>
<tr>
<td>EG SDS Graphene (Filtered)</td>
<td>1.00E-09</td>
<td>1.00E-09</td>
<td>1.00E+09</td>
</tr>
<tr>
<td>EG SDS Graphene (Unfiltered)</td>
<td>3.00E-06</td>
<td>3.00E-06</td>
<td>3.33E+05</td>
</tr>
<tr>
<td>SDS Graphene [0.4 mg / ml]</td>
<td>1.00E-07</td>
<td>1.00E-07</td>
<td>1.00E+07</td>
</tr>
<tr>
<td>SDS Graphene [0.7 mg / ml]</td>
<td>2.00E-04</td>
<td>2.00E-04</td>
<td>5.00E+03</td>
</tr>
<tr>
<td>SDS Graphene [0.8 mg / ml]</td>
<td>5.00E-04</td>
<td>5.00E-04</td>
<td>2.00E+03</td>
</tr>
<tr>
<td>Graphene Oxide Ink</td>
<td>3.00E-09</td>
<td>3.00E-09</td>
<td>3.33E+08</td>
</tr>
<tr>
<td>Reduced Graphene Oxide</td>
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<td>2.00E-07</td>
<td>5.00E+06</td>
</tr>
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</table>

*Table 3: Conductance and Resistivity of the Inks used.* The values are obtained from the conductivity test conducted using Keithley Instruments, TSP Express. I-V graphs are plotted and the gradient is taken.

<table>
<thead>
<tr>
<th>No. of layers printed</th>
<th>Gradient</th>
<th>Conductance, G / S</th>
<th>Resistivity, R / Ω</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>1.00E-07</td>
<td>1.00E-07</td>
<td>1.00E+07</td>
</tr>
<tr>
<td>10</td>
<td>1.00E-08</td>
<td>1.00E-08</td>
<td>1.00E+08</td>
</tr>
<tr>
<td>8</td>
<td>7.00E-09</td>
<td>7.00E-09</td>
<td>1.43E+08</td>
</tr>
<tr>
<td>5</td>
<td>7.00E-09</td>
<td>7.00E-09</td>
<td>1.43E+08</td>
</tr>
<tr>
<td>4</td>
<td>7.00E-09</td>
<td>7.00E-09</td>
<td>1.43E+08</td>
</tr>
</tbody>
</table>

*Table 4: Conductance and Resistivity values for varying ink jetted passes printed.* The values are obtained from the conductivity test conducted using Keithley Instruments, TSP Express. I-V graphs are plotted and the gradient is taken.