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Development and Characterization of a Sucrose Microneedle Neural Electrode Delivery System

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Abstract

Stable brain-machine interfaces present extraordinary therapeutic and scientific promise.

However, the electrode-tissue interface is susceptible to instability and damage during long-term implantation. Soft, flexible electrodes demonstrate improved longevity, but pose a new challenge with regard to simple and accurate surgical implantation. We have developed a high aspect ratio water-soluble microneedle based on sucrose which permits straightforward surgical implantation of soft, flexible microelectrodes. Here, we present a description of the microneedle manufacturing process, along with in vitro and in vivo safety and efficacy assessments.

Successful fabrication required control of the glass transition temperature of aqueous sucrose solutions. The insertion force of 5 different microneedle electrode vehicles was studied in agarose brain phantoms, with the sucrose microneedle eliciting the lowest insertion force and strain energy transfer. Short- and long-term assessments of the pathological response to sucrose microneedle implantations in the brain suggested minimal tissue reactions, comparable to those observed following stainless-steel hypodermic needle punctures. Finally, microelectrodes fabricated from graphene, carbon nanotubes, or platinum were embedded in sucrose microneedles and implanted into an epileptic rat model for 22-days. All electrodes were functional throughout the implantation period, with the graphene electrode exhibiting the largest seizure signal-to-noise ratio and only modest changes in impedance.

1. Introduction

Reliable communication between brain and machine generates a plethora of opportunities, ranging from fundamental neuroscience investigation to clinical intervention for otherwise incurable or untreatable neurological disorders.^[1-4] A stable neural recording interface is a conduit via which electrophysiological data is obtained. Such data can be relevant to clinical diagnostics,^[5, 6] as well as provide neural control signals for thought-controlled prosthetic devices.^[7, 8] Different to the passive acquisition of electrophysiological information, stimulation pulses can be delivered to modulate brain activity for the treatment of neurological disorders such as Parkinsonian tremor, epilepsy, and depression,^[9-12] as well as to return sensory information to either blind^[13-16] or deaf patients.^[17-21] Though the advantages of sustainable neural communication are now well-known, the challenge remains to minimize the disruptive processes that lead to degradation of the neural interface and loss of these communication channels long-term.^[22] While there are a number of mechanisms by which a brain-machine interface might fail, the physical and biochemical properties of the implanted electrode and the resultant biological response, to a large extent, dictate how long the device will remain functional *in vivo*.^[23] Several recent studies have identified the properties of a brain-machine interface electrode that improve longevity, including small size and mechanical compliance,^[24, 25] low-density,^[26] and a lack of lead wire tethering to the skull.^[27, 28]

Graphene-based materials, given their flexibility, low density, biocompatibility, and chemical sensitivity are gaining significant traction in neural interfacing.^[29, 30] Previously, our group investigated flexible wet-spun reduced graphene oxide yarns for use as neural stimulation and recording electrodes.^[31, 32] These electrodes were capable of recording single unit intracortical neural responses *in vivo* and also exhibited high electrochemical capacitance, leading to safe and efficacious stimulation in an *ex vivo* retina model. Although small diameter, mechanically-

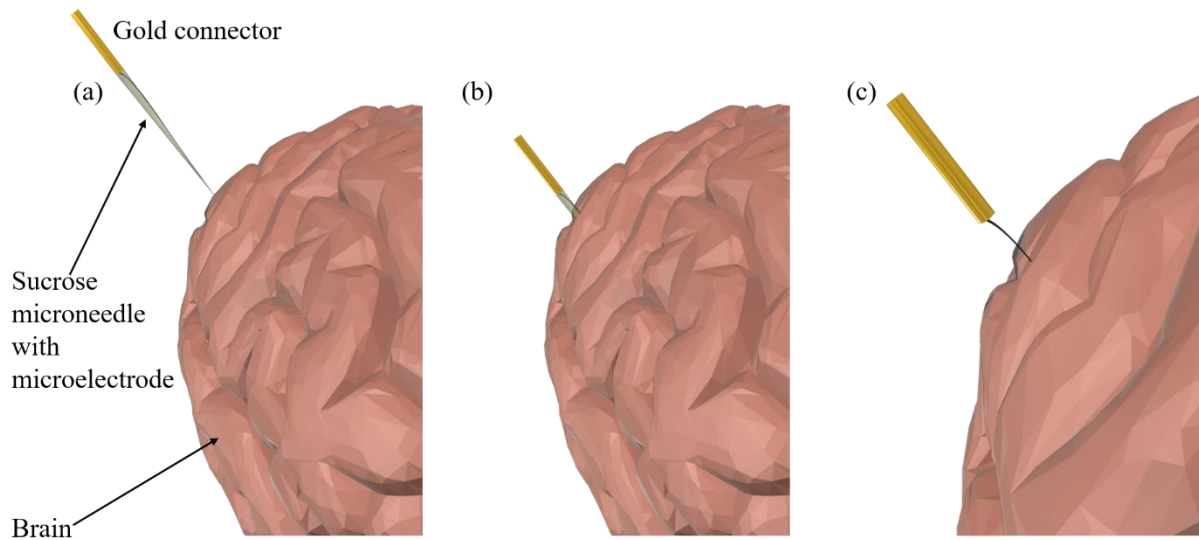


Figure 1. Illustration of surgical delivery of a flexible microelectrode using a water-soluble microneedle. (a) Microyarn electrode is embedded in a sucrose microneedle and (b) implanted into brain tissue. (c) After approximately 15 seconds, the microneedle dissolves, leaving behind the flexible microelectrode.

compliant electrodes potentially elicit less glial scarring and neuronal death proximal to the electrode site, their use gives rise to another challenge: facile and targeted surgical implantation. Herein, we describe a low insertion force technique to implant soft, flexible microelectrodes into neural tissue using a water-soluble sucrose microneedle (**Figure 1**). Originally, water-soluble microneedles were developed as a transdermal drug and vaccine delivery method.^[33, 34] Microneedles offer several advantages compared with hypodermic needles, including reduced microbial invasion^[35] and reduced perceived pain.^[36] Other groups have implanted microelectrodes using materials such as carboxymethyl cellulose (CMC),^[37] maltose,^[38] gelatin,^[39, 40] silk,^[41] and tyrosine-based polymers.^[42] Jeon *et al* recently presented a method to stiffen a MEMS-based planar probe based on polyimide using sucrose gels.^[43] Furthermore, neuronal axons have been implanted using hydrogel columns to create “living” electrodes.^[44] However, there still remains a need for a method to encapsulate flexible, yarn-like microelectrodes without introducing excessive size to the total implant whilst maintaining a high aspect ratio if needed. In this work,

water-soluble microneedles were fabricated using amorphous sucrose. Sucrose was chosen for a number of reasons, including its global ubiquity and low cost,^[45] relative chemical simplicity,^[46] investigation as a cell culture substrate,^[47] and extensive knowledge of its glass transition and rheological properties due to its widespread use in the pharmaceutical industry.^[48]

2. Results and Discussion

2.1 Drawing Sucrose Glass Microneedles.

In order to draw polymeric materials into needles suitable for implantation, careful control and monitoring of the glass transition temperature (T_g) is required during processing as described by Lee *et al.*^[49] In the case of aqueous sucrose, water has a plasticizing effect and its presence shifts the T_g according to the Gordon-Taylor model (**Equation 1**):

$$T_{g,mix} = \frac{w_{Water}T_{g,Water} + Kw_{Sucrose}T_{g,Sucrose}}{w_{Water} + Kw_{Sucrose}}, \quad (1)$$

Where ‘ w ’ is the weight fraction of the component and ‘ K ’ is a constant used to reflect the ratio of the free volumes of the components and is calculated as shown in **Equation 2**:^[48, 50]

$$K = \frac{\rho_{Water}T_{g,Water}}{\rho_{Sucrose}T_{g,Sucrose}} = 0.2721, \quad (2)$$

Where ‘ ρ ’ is the true density and ‘ T_g ’ is the glass transition temperature, which is 347 K for sucrose and 135 K for water.^[51] Samples of sucrose solutions, acquired at several temperatures, were weighed before and after baking in a dry oven at 80 °C. This method enabled the estimation of the water content of the aqueous sucrose solution as a function of processing temperature (**Figure 2a**), thereby guiding the drawing the process. Following the Gordon-Taylor model (Equation 1), the theoretical T_g temperature was estimated (**Figure 2b**). In this work, the water content was controlled by heating the solution to temperatures as high as 150-165 °C. At these temperatures,

the water content of the melt steadily decreases. Moisture content data as a function of processing temperature were fit with a logistic function (R-square=0.9718) having the form shown in

Equation 3:

$$M(T) = \frac{a}{1 + bc^{-T}}, \quad (3)$$

Where ' M ' is water content, ' T ' is temperature, and ' a ' (0.7009), ' b ' (2.647×10^{-8}), and ' c ' (0.8358) are empirical constants yielded by logistic regression. Interpretation of Equation 3 is difficult without further experimentation, but the logistic relationship between processing temperature and moisture content is likely explained by both the temperature dependence of water activity in sucrose solutions^[51] as well as the temperature dependence of water's diffusion coefficient in aqueous sucrose glasses.^[52] Simplistically, greater energy is needed to remove water from the melt as the solution becomes more concentrated due to water's significantly decreased diffusion coefficient at high sucrose concentrations.^[52]

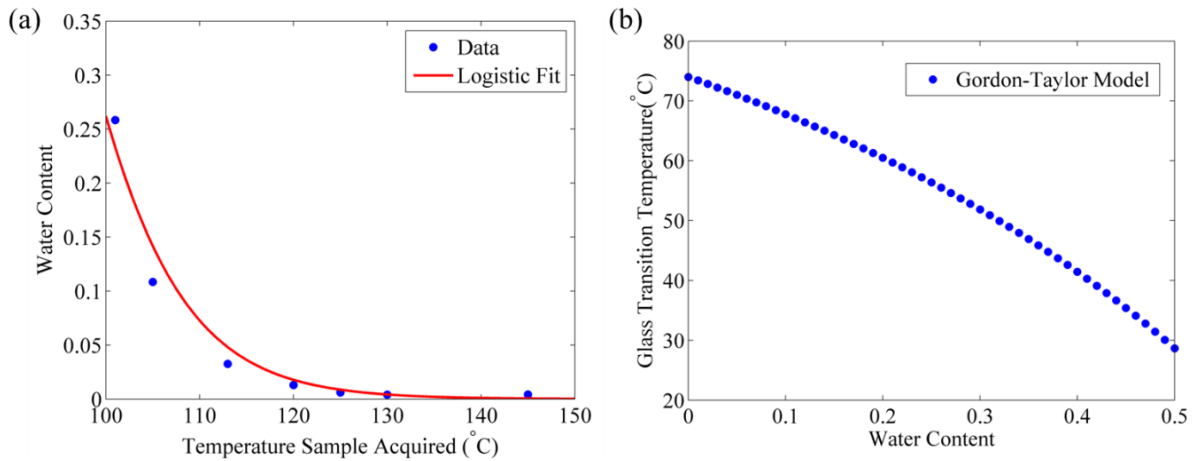


Figure 2. Tuning T_g of molten sucrose. (a) Water content by mass ($\text{mass}_{\text{water}} / \text{mass}_{\text{sucrose}}$) as a function of processing temperature. (b) Theoretical estimation of T_g based on the water content ($\text{mass}_{\text{water}} / \text{mass}_{\text{sucrose}}$) of the melt.

Knowledge of the T_g was then used to determine the optimal time for microneedle drawing to begin. The sucrose solutions were found to cool slowly ($2.85 \times 10^{-3} \text{ s}^{-1}$ from fitting with Newton's

law of cooling, which enabled a “drawing range” (**Supporting Information; Figure S1**). The heat transfer was assumed primarily convective from a surface area of $9.62 \times 10^{-4} \text{ m}^2$, determined from the 3.5 cm diameter silicone cup sucrose solutions were poured into prior to microneedle formation. The sucrose solution cooled slowly due to the known low thermal conductivity of concentrated sucrose solutions (i.e., $0.402 \text{ W m}^{-1} \text{ K}^{-1}$ for 60% sucrose by weight at 20°C),^[53] in addition to the diminished thermal conductivity that amorphous materials experience as a consequence of phonon scattering.^[54] The mechanical properties of the cooled sucrose as a function of processing temperature were studied using a force indentation set-up (**Supporting Information; Figure S2**). The spring constant of sucrose acquired after processing to 130°C was $0.229 \text{ mN } \mu\text{m}^{-1}$ compared with $8.05 \text{ mN } \mu\text{m}^{-1}$ for sucrose processed to 140°C . The latter value represents the spring constant of the silicone holder used to prevent damage to the load cell. Since water-soluble microneedles were initially developed as a method to deliver drugs across the skin, the buckling force requirements were great. In the case of brain tissue, the rigidity is significantly lower than skin but stable mechanical properties can prevent vehicle (or needle) deflection which leads to inaccuracies during anatomical targeting.^[55]

Several sucrose microneedles with and without embedded electrodes were produced using drawing lithography (**Figure 3**). Force insertion mechanics were studied using sucrose microneedles without embedded electrodes. Microneedle geometry was measured using ImageJ (<https://imagej.nih.gov/ij/>) (Figure 3 a,b) and diameter was plotted as a function of length (Figure 3 c). The average diameter increase per length of microneedle of $n=18$ microneedles was found to be $65 \text{ } \mu\text{m mm}^{-1}$ ($R^2=0.9928$) up to 5 mm from the end at which point the diameter value saturates to $0.5293 \pm 0.011 \text{ mm}$. The tip geometry of hypodermic needles is currently under investigation as a means to mitigate insertion force and pain. The geometries of various types of

hypodermic needles have been modelled and studied in detail in an attempt to reduce the forces experienced by the tissues into which they are implanted.^[56]

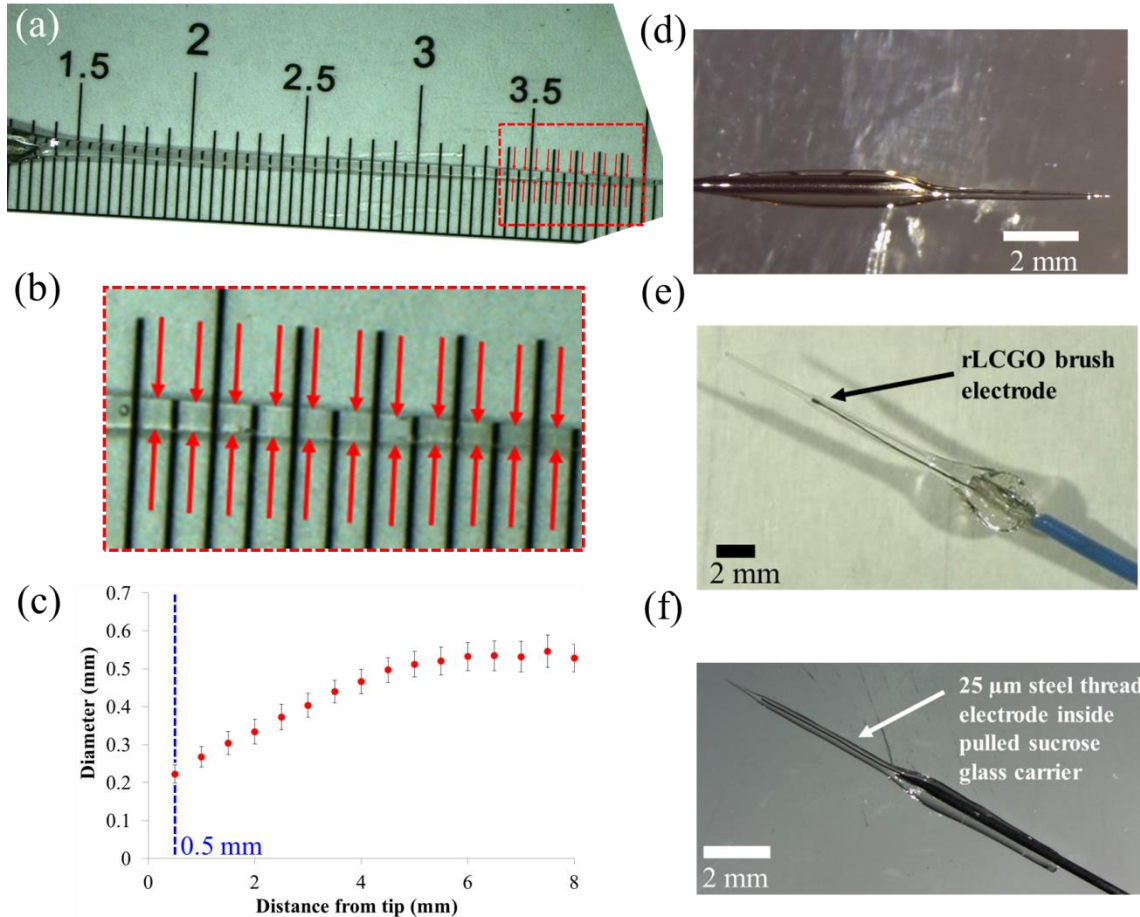


Figure 3. Production of water-soluble sucrose microneedles. (a) Example of high aspect ratio sucrose microneedle without electrode embedded. (b) Increased magnification of microneedle dimensions indicating locations where diameter was measured. (c) Diameter as a function of distance from the tip of the microneedle ($n=18$ microneedles). The broken blue line indicates a point 0.5 mm from the tip of the microneedle. Measurements were made using ImageJ (<https://imagej.nih.gov/ij/>) and error bars represent standard error of the mean. (d) a dummy sucrose microneedle, and sucrose microneedles containing (e) reduced liquid crystal graphene oxide (rLCGO) electrodes and (f) stainless steel wire electrodes.

One study found that a hypodermic needle with 5 bevels, rather than 3, reduced penetration force by 23% and significantly reduced the pain experienced by patients.^[57] By introducing additional beveled edges, the authors were effectively approaching a conical shape in which more edges lead to a smoother transition between the tip and cannula. Given that the probes studied here had

roughly the same cannula diameter, the major difference was the tip shape. Other groups have correlated the steepness of the transition from needle tip to shaft with axial force experienced, but this effect is largely dominated by the needle tip geometry.^[58]

2.2 Insertion Force in Agarose Brain Phantom.

A hydrogel model of brain tissue based on agarose (0.6 wt%) was used to characterize the insertion mechanics of various electrode shuttles: (i) hypodermic needles, (ii) occluded hypodermic needles, (iii) blunt spherical probe, (iv) sucrose coated hypodermic needle, and (v) sucrose microneedles. Agarose gels (0.6 wt%) have been used by several groups as a brain phantom to study the insertion mechanics of hypodermic needles.^[59, 60] As applicable to insertion force characterization, the most important differences between agarose and brain tissue arise due to the heterogeneity of the latter. Brain is comprised of both grey and white matter, each of which has their own respective mechanical material properties. Sucrose microneedle geometry was modelled as the combination of a cone and a cylindrical shank. The average surface area (SA) for the first 8 mm of the sucrose microneedles was found to be 5.039 mm². The SA of the hypodermic needle was calculated by modelling the first 8 mm (from sharp end) as a cylindrical segment or “truncated cylinder” with a cylindrical segment added to represent the inner lumen for the non-occluded sample. The occluded hypodermic had a SA of 15.36 mm² and the standard (hollow) hypodermic had an SA of 25.31 mm², in which the SA enhancement is due to the surface area present within the lumen. The spherical probe was modelled as a hemisphere atop a cylinder and had a calculated SA of 15.09 mm². The sucrose coated hypodermic needle was assumed to have the same geometry as the occluded hypodermic needle except 0.3 mm was added to the cylindrical diameter to represent the sucrose sheath (SA=18.18 mm²). Values for SA were computed to determine whether SA had a significant impact on insertion force. Average

insertion force transients for all probes studied were plotted as a function of displacement into the agarose (Figure 4).

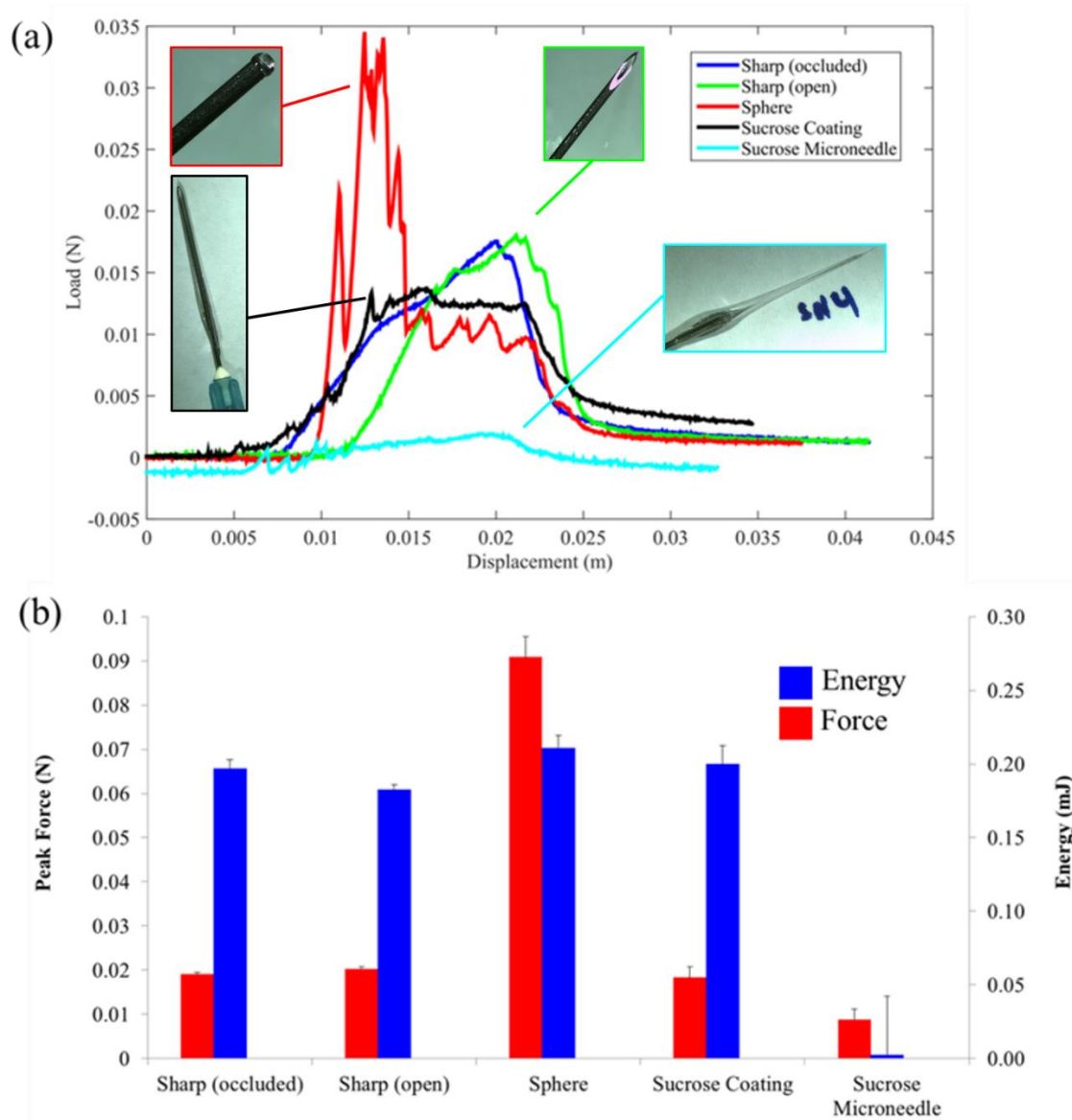


Figure 4. Insertion mechanics for 5 different probes. (a) Averaged insertion force profiles as a function of displacement into the agarose brain phantom for all 5 probe types (inset, excluding the occluded hypodermic needle). All insertions were performed $n=12$ times except for the sucrose microneedles which were performed for $n=18$ samples and sucrose coated 23G hypodermic needles which were performed with $n=10$ samples. (b) Comparison of peak force and energy transfer for 5 different probes. Error bars represent standard deviation.

Insertion force is an important variable to examine in terms of electrode vehicle design because it has been correlated both with mechanical injury and pain^[61] as well as with probe or needle

deflection which limits the accuracy of anatomical targeting.^[62] From examination of the force-displacement graph it is possible to characterize the type of opening or “crack” formed in the tissue upon insertion of different probe types.^[56] Generally, two types of tissue cracks are identifiable from the force-displacement curves obtained from insertion experiments: (i) Mode I (planar) cracks and (ii) Mode II (ring) cracks.^[56] The major distinguishing features of the crack types are the way that the cracks propagate and the geometry of the probes used to initiate them. The penetration of a sharp conical probe into tissue initiates a planar crack which propagates through the tissue and the load on the probe increases steadily and linearly as the probe is driven through the tissue, such as that observed for the 23G hypodermic needles in Figure 4a (*blue* and *green* traces). For dull- or blunt-tipped probes, a ring crack is typically initiated and characterized by an oscillating load as the probe is driven into the tissue such as was observed in this study for the hemispherical probe (Figure 4a, *red* trace). To examine whether sucrose – a hygroscopic material - might provide lubrication and reduce insertion force in a hydrogel, hypodermic needles were dip-coated with molten sucrose solutions. The insertion force profile of a sucrose-coated needle may experience a lubrication effect depicted as unchanged force occurring between 0.015 and 0.025 m on the x-axis (Figure 4a, *black* trace) where uncoated needles were experiencing increasing forces (Figure 4a, *blue* and *green* trace).

The highest peak force, surface area normalized force (SANF), and energy transfer were observed for the blunt, hemispherical probe (‘Sphere’). The peak insertion force was significantly greater for the ‘Sphere’ probe compared with the other probes, all of which were sharp or pointed ($P < 0.001$; Kruskal-Wallis comparison test). A similar effect has been observed previously in which vehicle sharpness, which is inversely correlated with the force required to drive through tissue, was shown to decrease placement error of radioactive seeds into prostate tissue for cancer

treatment.^[63] For this study, “energy” was taken as the strain energy – a value positively correlated with the size of a growing crack in soft tissue.^[56, 58, 61] The sucrose microneedle (‘Sucrose MN’) experienced the lowest peak force and energy transfer, but dividing by SA makes the Sucrose MN peak SANF second highest of the group, trailing only the Sphere probe. The SANF was examined to determine whether the SA had a significant impact on the peak forces observed. Two geometrical features of the sucrose MN were examined to determine whether they were significant predictors of the peak insertion force or total energy transfer: (i) average diameter of the first 8 mm (total insertion depth) of the sucrose MN as measured from the tip and (ii) average linear slope of the first 5 mm (point where MN geometry appears to stabilize) of the sucrose MN as measured from the tip. The average diameter of the microneedle was not significantly correlated with either peak force (Pearson coefficient = -0.1911, P=0.4474) or energy transferred (Pearson coefficient = 0.4475, P=0.0905) to the agarose brain phantom. Furthermore, the average slope from the tip of the needle to a point 5 mm from the tip was also not significantly correlated with peak force (Pearson coefficient=0.1957, P=0.4363) or energy transfer (Pearson coefficient = -0.1595, P=0.5271). Peak force and energy transfer were, however, significantly correlated (Pearson coefficient=0.4978, P<0.05). The dominant factor affecting force and energy transfer is not simply surface area considering that the largest surface area probe in this study, the hollow hypodermic needle, did not experience the highest insertion forces nor did it transfer the most energy to the agarose brain. The effects are likely due to a combination of friction, geometry, and surface area, but further work is required to answer this question.

2.3 Histopathological Assessment of Sucrose Microneedle Implantation

Successful tissue implantation is an important proof-of-concept, but it is equally important to characterize the damage and trauma done to the brain tissue following insertion, especially for novel vehicle materials and geometries. In the acute study, 2 dummy sucrose microneedle implantations were made into the feline visual cortex as described in the *Methods* section below. The histopathological responses to insertions were investigated using hematoxylin and eosin (H&E) staining (**Figure 5**). Within 1 hour of implantation, there was an area of bleeding and a hole in the tissue that was approximately 100 μm in diameter (Figure 5a; black arrow), which is similar in size to brain implantation wounds observed by other groups using hypodermic needles.^[60, 64] The 3-day wound appears to have healed with minor gliosis and residual blood cells remaining at the implantation site (Figure 5b; black arrows). The 3-week wound appears to have healed with minor gliosis and residual blood cells remaining at the implantation site (Figure 5c; black arrows). (d) A stab wound from a 30G hypodermic needle (diameter = 300 μm) in the same animal, 4 mm posterior to wound shown in (c). Magnification

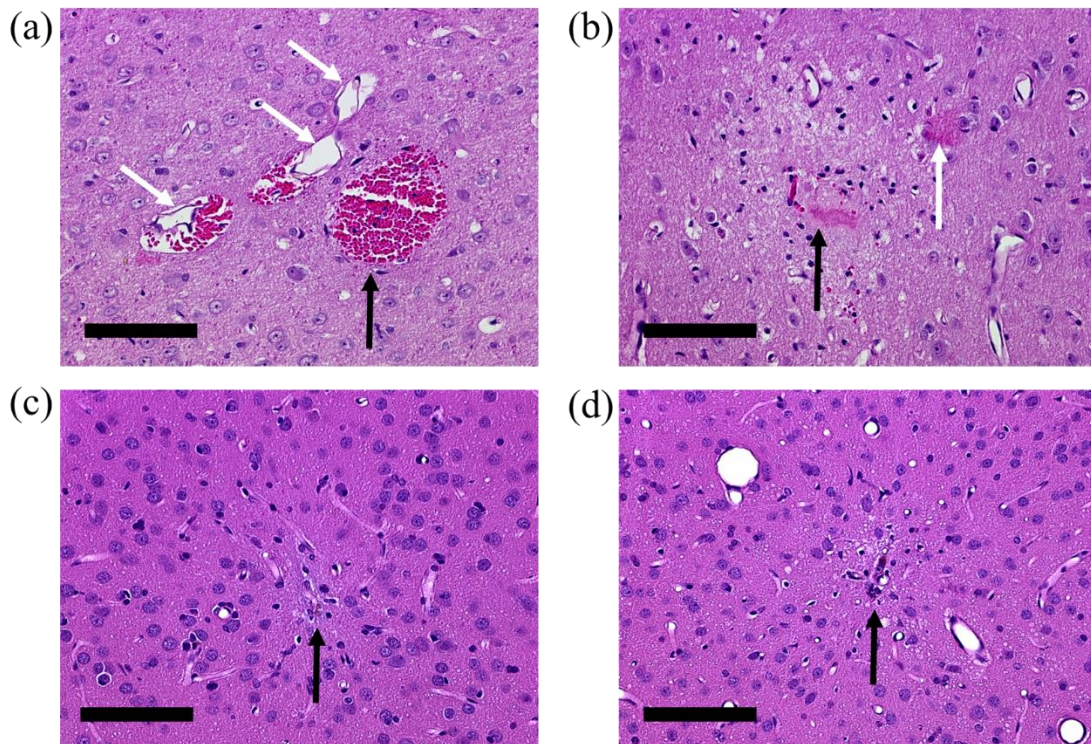


Figure 5. Histology from acute and chronic sucrose microneedle insertions at (a) 1 hour, (b) 3 days, and (c) 3 weeks post-implantation. (d) A stab wound from a 30G hypodermic needle (diameter = 300 μm) in the same animal, 4 mm posterior to wound shown in (c). Magnification

is 20X and scale bar is 100 μm for all images. *Note:* (a,b) are from a feline model and (c,d) are from a rat model. *Note:* black arrows in (a-d) indicate the insertion sites and white arrows indicate blood vessels and processing artifacts in (a) and (b), respectively.

To examine the chronic response to a dummy sucrose glass microneedle and compare it to a “stab” insertion wound from a 30G hypodermic needle, implantations were made into both hemispheres of a rat model and the rat was euthanized following a 3-week implantation period. Damage from the stab wound and the sugar needle had healed over the 3-week duration (Figure 5 c,d), though there is evidence of both insertions, including scarring and increased density of vessels and vessel diameter closer to the injury site. From this pilot study, there was no evidence of any significant tissue necrosis, nor a sustained inflammatory response.

2.4 Intracortical Seizure Monitoring in an Epileptic Rat Model

The full electrode delivery system was tested using a tetanus toxin epilepsy rat model, whereby the seizure functioned as a clinically-relevant signal.^[65] Considering the relatively low interfacial impedance and surface structure of the carbonaceous materials investigated here, the hypothesis was that a higher quality neural signal might be acquired over a longer duration, as well as provide the opportunity to deliver large stimulation pulses in future studies if desired.

Additionally, an epilepsy model offers a large, spontaneous signal that is easily distinguishable from background noise. Additionally, the seizure waveform is easily confirmed by video of animal behavior. Electrodes were implanted into 5 distinct anatomical locations and seizures were recorded continuously for 22 days (**Figure 6**). Additionally, electrode impedance was monitored over the implantation period (Figure 6b). Of the intracortical electrodes, the graphene electrode exhibited the lowest impedance and least amount of change over the implantation period (36.9 ± 7.91 kOhms) compared with the Pt/Ir electrode (54.2 ± 25.9 kOhms) and CNT/G electrode (114 ± 25.6 kOhms). The graphene electrode (Figure 6c, *blue trace*) was implanted

into the hippocampus which was also the site of the tetanus toxin injection. Given that electrodes implanted into this anatomical location might experience larger electrical fields at the time of seizure onset, SNR was calculated 1 second after the seizure waveform generalized across the cortex in an attempt to normalize the signal amplitude detected by all electrodes. For both timepoints examined, SNR for the rLCGO electrode was significantly larger following filtering (see *Experimental Methods* for filter characteristics) (Figure 6 d).

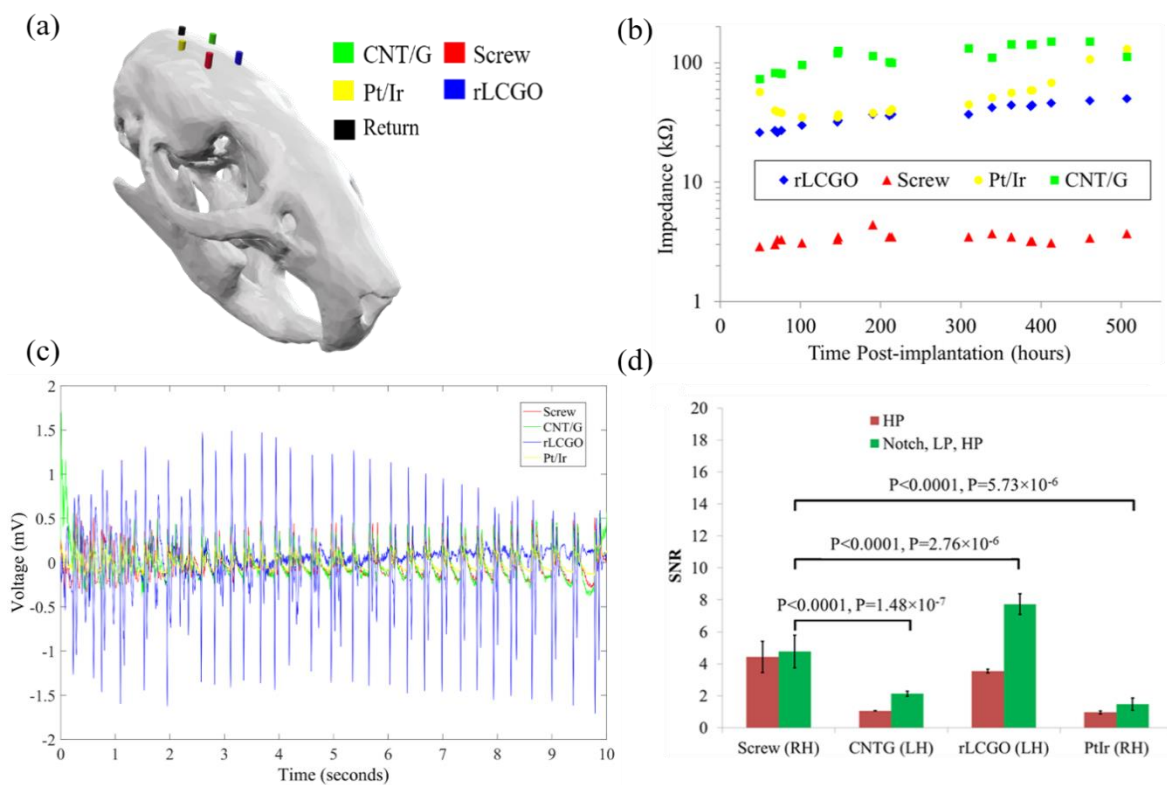


Figure 6. Intracortical seizure monitoring with electrodes implanted using sucrose microneedles. (a) Illustration of rat skull depicting electrode implantation sites. (b) impedance monitoring of all electrodes over the 22-day implantation period. (c) example of filtered seizure recording from Day 16 timepoint, and (d) comparison of seizure signal-to-noise ratio (SNR) for 21 days post-implantation. Rat skull illustration adapted from Doney *et al.*^[66] HP (High Pass), Notch, and LP (Low Pass) correspond to data filtering routines, the details of which are included in the *Experimental Methods* section. The statistical comparison in (d) is a 1-tailed t-test assuming unequal variances for n=21 seizures. Note: 'rLCGO' refers to the graphene electrode and 'Screw' electrodes are stainless steel screws (3 mm diameter) in an ECoG-like configuration.

While enhanced SNR is possibly explained by the reduced impedance and intracortical location, further work is required to determine whether rLCGO graphene is a superior electrode material for long-term intracortical neural recordings. The aim of this pilot study was to demonstrate a method for implantation of a microelectrode leading to a stable interface capable of acquiring clinically-relevant neural signals for up to a 3-week period.

3. Conclusions

In an attempt to **minimize** the foreign body response to invasive intracortical electrodes, a number of new materials and electrode geometries are under investigation, including flexible yarns based on nanocarbon and conductive polymer composites. However, the move to more compliant electrodes leads to a new challenge: surgical implantation. In our previous work, we developed a high charge injection capacity microelectrode capable of acute (15 minute) neural recordings.^[32] In this work, we present microelectrode implantations into brain tissue and demonstrate recordings of epileptic neural signals for a 3-week time period. In brain phantoms, sucrose microneedles elicited minimal force and energy transfer compared with other types of probes which might be used to deliver flexible microelectrodes into target tissue. Minimizing the insertion force leads to improvements in anatomical placement and minimizing strain energy transfer reduces the amount of tissue trauma. Additional work is required to validate the anatomical accuracy of electrodes implanted using water-soluble sucrose microneedles, likely in the form of *in situ* medical imaging. The ability to acquire neural signals long-term with an electrode also capable of stimulation, **introduces** the possibility for closed-loop neuromodulation for epilepsy, Parkinson's disease, and also for a feedback system relevant to neural prosthetic limb control. It is also worth noting that this technique may be utilized to implant electrodes into bundles of, or very near, peripheral nerves for both stimulation and recording applications.

Considering that the electrodes investigated by our group are carbon-based and can be manufactured at a range of diameters (μm - mm), their implementation in a range of neural engineering applications is possible, perhaps with the use of image-guided technologies^[67] and the insertion method described in the present work.

4. Experimental Methods

4.1 Preparation and Characterization of Sucrose Glasses

Samples of aqueous sucrose were prepared in a 50 mL beaker using 10 mL of deionized water (MilliQ; nominal resistance of $18\text{ M}\Omega\text{ cm}$) and 10 grams of sucrose (Sigma; S9378). The solutions were heated on a temperature-controlled hotplate containing a Teflon probe (Stuart; CD162). A K-type thermocouple was placed into the solution and temperature readings were acquired at a rate of 0.5 Hz with a data logger (MicroDAQ; EL-GFX-DTC). Data were imported using the EasyLog USB software (Lascar electronics) and processed using Matlab (Mathworks). To measure the water content of aqueous sucrose, samples were acquired at the following temperatures: 101, 105, 113, 120, 125, 130, 145 °C during a typical heating run. The samples were stored in glass specimen vials with plastic lids (ProSciTech) and immediately measured on a digital balance. Next, the samples were baked, with lids ajar, at 100 °C in a convection oven for 42 hours and then re-massed. The change in mass was used to determine the sucrose content (%wt) of the samples acquired from the melt. The stiffness (spring constant) of sucrose glass sheets as a function of processing temperature were studied using a custom-built force indentation set-up. The glasses were poured into silicone moulds (PinkySil), cooled, and then a probe was driven into the sucrose to estimate stiffness.

4.2 Drawing Sucrose Glass Microneedles

A custom-built drawing lithography apparatus was constructed to perform microneedle fabrication. The apparatus was comprised of a solid wooden base, 4 steel rods, a 3D-printed stage to hold the electrodes or hypodermic needles, fishing line, a pulley system, linear actuator bearings (Action Bearings), and a Lego Mindstorms motor and motor controller (**Supporting Information; Figure S3**). The 3D-printed stage was designed for compatibility with hypodermic needles and hypodermic systems due to their ubiquity in our laboratory, relatively low cost, consistent geometries, and range of available diameters and lengths. An illustration of the drawing lithography process as applied to electrode encapsulation is depicted in **Figure 7**.

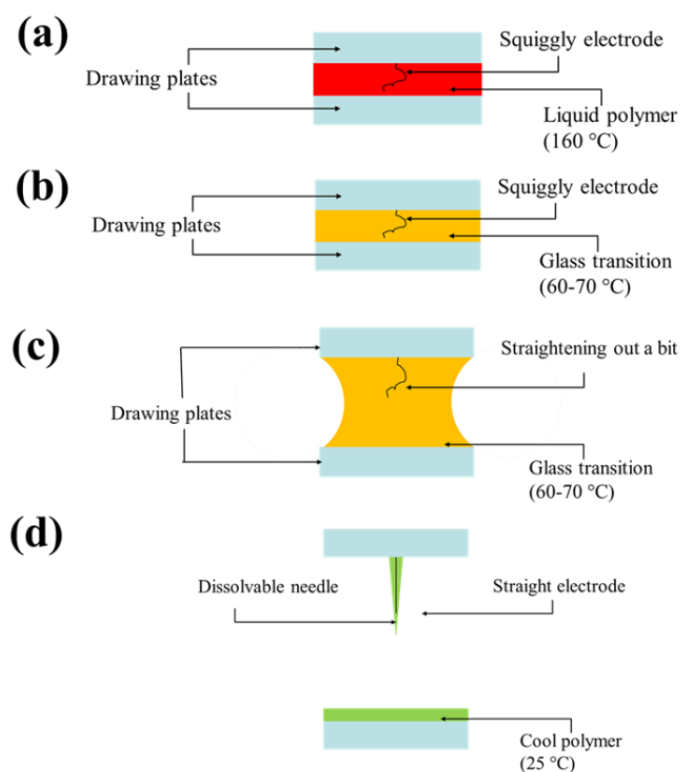


Figure 7. Method of using drawing lithography to encapsulate microelectrodes in sucrose microneedles.

Once the sucrose solutions were heated to approximately 155-165 °C, the beaker was taken from the hotplate and left to cool for 2 minutes. After 2 minutes, the solution was poured into a

silicone cup (diameter = 3.5 cm) for drawing lithography. Electrodes were submerged into the sucrose melt while temperature was monitored. Once the temperature reached the theoretical T_g (70-75 °C), the motor was used to drive the stage upwards to form microneedles.

4.3 Insertion Force in Agarose Brain Phantom

Agarose “brains” were prepared in 12-well (4×3) tissue culture plates using 0.6% agarose powder (Sigma; A5093-100G) in deionized water (MilliQ; nominal resistance of 18 M Ω cm) according to a previously published protocol.^[64] The agarose brains were covered and stored at 4 °C when not in use. For measurement of insertion force, a load cell (MARK-10) and Microdrive (Sutter) were used. Several probe geometries were trialed, including blunt, sharp, and sucrose coated cannulas, as well as sucrose microneedles. Blunt cannulas had hemispherical tips and were fashioned from 18G (1.27 mm outer diameter) blunt drawing up needles and 2-part epoxy (Araldite). All probes were advanced into agarose brain phantoms at an average rate of 2 mm s⁻¹ to a depth of 8 mm. The probes were placed 4 mm above the agarose phantoms and an acceleration of 1 mm s⁻² was used for implantation. Insertion force (Newtons) and time (seconds) were monitored using MESURgauge (MARK-10) software at a sampling rate of 50 Hz. The time was converted into a displacement by multiplying it by the advancement velocity (2 mm s⁻¹). The area under the force-displacement curve was determined using numerical integration in Matlab (Mathworks) and reported as “energy transferred” to the brain phantom.

4.4 Electrode Fabrication and Electrochemical Characterization

Carbon nanotube/graphene hybrid yarns and reduced liquid crystal graphene oxide fibers were produced according to previously published methods.^[31, 68, 69] Conductive yarns (rLCGO and CNT/G) were attached to Au connectors (Plastics One; E363/0) with conductive epoxy (circuit works), coated with Parylene-C, laser excised, and then cleaned electrochemically as described

previously.^[32] Throughout the *in vivo* implantation period, impedances were interrogated using a 60 Hz, 200 nA sinusoidal waveform.

4.5 Surgical Implantation and Epileptic Rat Model

For acute implantation timepoints, a feline model was utilized in conjunction with another research group to minimize animal use. The surgical implantation and anaesthesia methods were performed as described previously and care was taken to prevent any experimental interactions between studies.^[14, 32] Sprague-Dawley rats (Male; 200-500 g) were anaesthetized with an i.p. injection of ketamine (70 mg/kg) and xylazine (10 mg/kg) immediately prior to surgery. Once anaesthetized, the rats were placed in a stereotaxic apparatus and administered isoflurane (0.5% in oxygen, 2 L/min) via a nose-cone anaesthesia mask. Carprofen (5 mg/kg) was administered for pain relief and 2mL of 0.9% NaCl for cardiovascular support. As needed, postoperative treatment included subcutaneous buprenorphine (0.03 mg/kg BID), 0.9% NaCl (2 mL daily), and carprofen (5 mg/kg, daily) for a maximum of 3 days. A surgical drill (KaVo dental) with a 3 mm burr was used to drill 6 craniotomy holes (**Figure 8**). Screw electrodes were implanted and adjusted prior to insertion of depth electrodes to avoid disturbing the fragile electrode implantation site. To stabilize the site and protect the brain, the electrode was first cushioned with a layer of polyvinyl siloxane impression materials (3M ESPE AG) around the craniotomy and then structurally supported by a micro-hybrid light-cured flowable Resin Composite (Aeliteflo, Bisco).

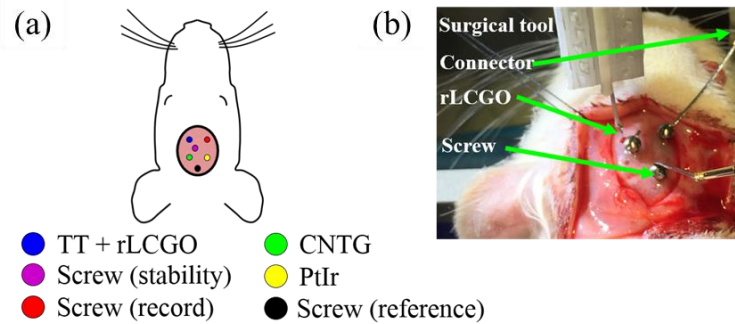


Figure 8. Surgical implantation sites for rat model. (a) Schematic of surgical implantation site and (b) image of surgical site and surgical stabilization tool.

The epilepsy model was created with a tetanus toxin injection (125 nL/100 g bodyweight) into the right hippocampal CA3 area of the rat.^[65] Tetanus toxin solution contained 25 µg of tetanus toxin dissolved in 250 µL of 0.05 M phosphate buffered saline (Sigma-Aldrich) and 2% bovine serum. A Hamilton microhypodermic and infusion pump (KD Scientific Inc., USA) were used to administer the tetanus toxin at a rate of 100 nL/min. The hypodermic was left *in-situ* for 5 minutes before it was slowly retracted to avoid back flow through the injection track. When necessary, the tissue surrounding the skull-mounted connector was cleaned with iodine and irrigated with 0.9% NaCl. The animals were filmed continuously to capture video evidence of seizure events.

4.6 Histopathological Assessment

The animals were deeply anaesthetized using Ketamine (60 mg/kg) and Xylazine (4 mg/kg), then given 1.5 ml sodium pentobarbitone (at 325 mg/ml; Virbac) followed by cardiac perfusion of warm (37 °C) saline and finally cold (4 °C) 10% Neutral Buffered Formalin (NBF; equivalent 4% paraformaldehyde). The heads were removed and post-fixed in 10% NBF for 6-7 days at 4 °C. The skull was removed and the head placed in a stereotactic frame for coronal sectioning. Next, dissected samples were blocked and trimmed to cassette size, dye spotted for identification and orientation and post-fixed for a further 1-2 days at 4 °C before processing for paraffin embedding

and cutting. Following overnight processing, tissue samples were embedded in paraffin wax, with cortical surface down (i.e. to be cut first). After trimming to the region of interest, sections from the block were cut with a microtome (Leica) into 5- μ m sections in 100- μ m levels and mounted on Superfrost+ glass slides (Thermo Scientific).

Slide staining and grading/selection criteria. Slides were stained with H&E, which revealed nuclei (purple) and cytoplasm/protein (pink) respectively, using an automated slide processing system (St. Vincent's Hospital Melbourne, Department of Anatomical Pathology). Representative sections were examined microscopically by a clinical pathologist.

4.7 Seizure Recordings and Determination of Signal-to-Noise Ratio

Clinical EEG monitoring software (Profusion EEG; Compumedics) was used to acquire continuous recordings through a Graef pre-amplifier at a sampling rate of 2048 Hz. EEG data were exported from Profusion software (Compumedics) using Matlab (Mathworks). A series of notch, low-pass, and band-pass filters were used to process EEG signals where noted. Signal-to-noise ratio (SNR) was calculated using background, pre-seizure EEG as the “noise” and seizure activity as “signal”. Seizures were detected and marked with a Matlab program using the following criteria: (1) minimum line-width amplification of 2-times baseline and (2) minimum seizure duration of 10 seconds. The SNR was calculated twice for the same data to determine the effects of filtering on the SNR value. This procedure was performed for all electrodes for n=21 seizures for two timepoints: (i) 10 days post-surgery and (ii) 22 days post-surgery. Finally, power spectrograms for both seizure and non-seizure periods were produced in Matlab. Guided by the aforementioned “marking”, seizure activity was determined by manual investigation of trace data and confirmed with video depicting behaviours as characterized by the Racine scale.^[70] Often,

the seizure was readily observable from the EEG trace up to 30 seconds before the animal showed any physical manifestation.

4.8 Statistical Analysis

Distinct statistical analyses were performed for insertion forces and electrophysiological recordings. As has been observed by other groups,^[64] the data for insertion forces in both agarose and brain tissue do not follow a normal distribution, but instead are described more accurately with a 3-parameter Weibull. As a result, a non-parametric statistical comparison (Kruskal-Wallis)^[71] was used to compare 3 different features of the insertion transient for the 5 different probes: peak force, surface area normalized force (SANF), and energy transfer to the agarose during insertions (**Supporting Information; Table S1**). Sample sizes for probes were n=18 for sucrose microneedles, n=10 for sucrose-coated hypodermic needles, and n=12 for all other probes. A Pearson coefficient was computed to examine correlations between insertion force, insertion energy transfer, and physical microneedle features. These analyses were performed using Minitab Express 1.2 (Minitab, Inc.). Significance was taken as P-values less 0.05.

For electrophysiology, data were acquired with a sampling rate of 2048 Hz and filtered in Matlab (R2016b; Mathworks, Inc.). Data were either (a) DC-corrected (High-pass filtered at 1 Hz) or (b) processed using the following digital filters in Matlab: (i) High-pass at 1 Hz, (ii) Bandpass between 49-51 Hz, and (iii) low-pass at 70 Hz. A 1-tailed t-test assuming unequal variances was utilized to determine statistically significant ($P < 0.05$) differences in SNR of seizures (n=21 seizures for each timepoint) between electrode positions and materials. All statistical tests were performed in Excel using the Data Analysis toolbox.

AUTHOR INFORMATION

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Competing Financial Interests

SP is a shareholder and director of iBIONICS.

Supporting Information Available:

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ToC Entry

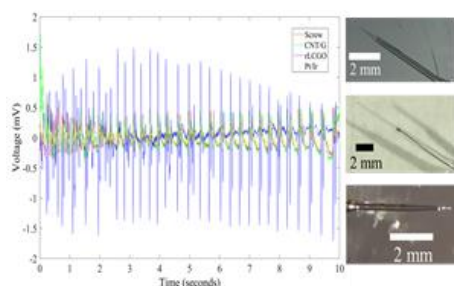
Brain-machine interfaces revolutionize the way we study, and treat disorders of, the nervous system. However, hardware issues limit the long-term utility of these devices due, in part, to their stiffness and size. Soft, flexible electrodes have shown great promise, but they present a surgical challenge. This work describes a system to implant soft, flexible microelectrodes can using water-soluble sucrose microneedles.

The table of contents entry should be 50–60 words long, and the first phrase should be bold. The entry should be written in the present tense and impersonal style. The text should be different from the abstract text.

Keyword

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Development and Characterization of a Sucrose Microneedle Neural Electrode Delivery System



Supporting Information

Development and Characterization of a Sucrose Microneedle Neural Electrode Delivery System

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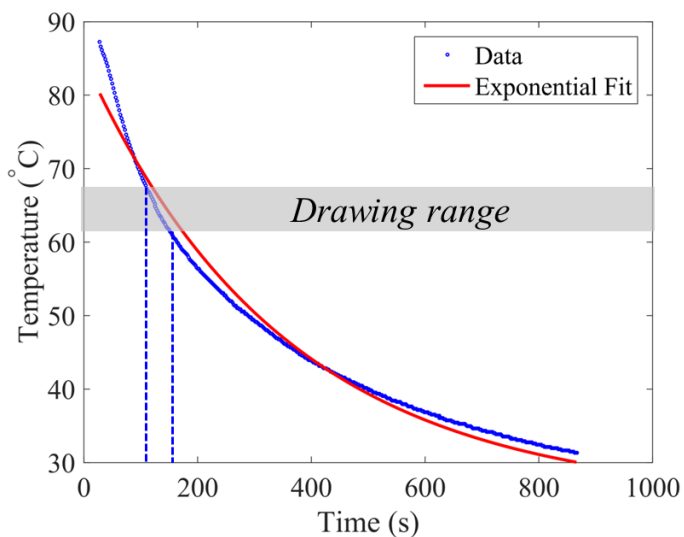


Figure S1. Example cooling profile of sucrose solution and optimal drawing range.

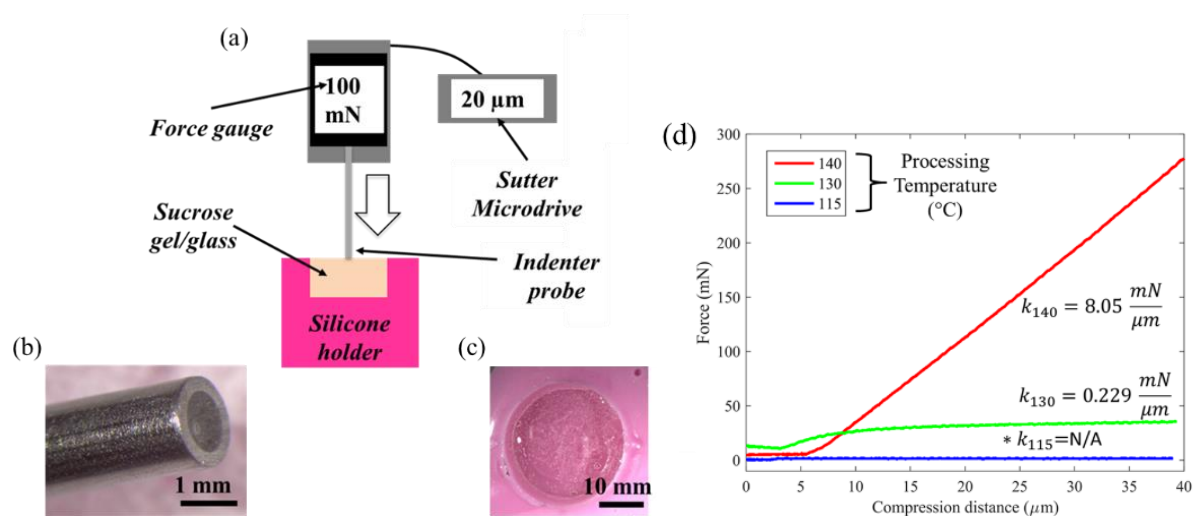


Figure S2. Rigidity of sucrose solution as effected by processing temperature. (a) A sensitive force gauge attached to a microdrive was used to drive polished smooth probes (b) into sucrose gels (c). (d) A stress-strain relationship was identified for sucrose solutions processed at 3 different temperatures: 115, 130, and 140 °C after they had cooled to room temperature.

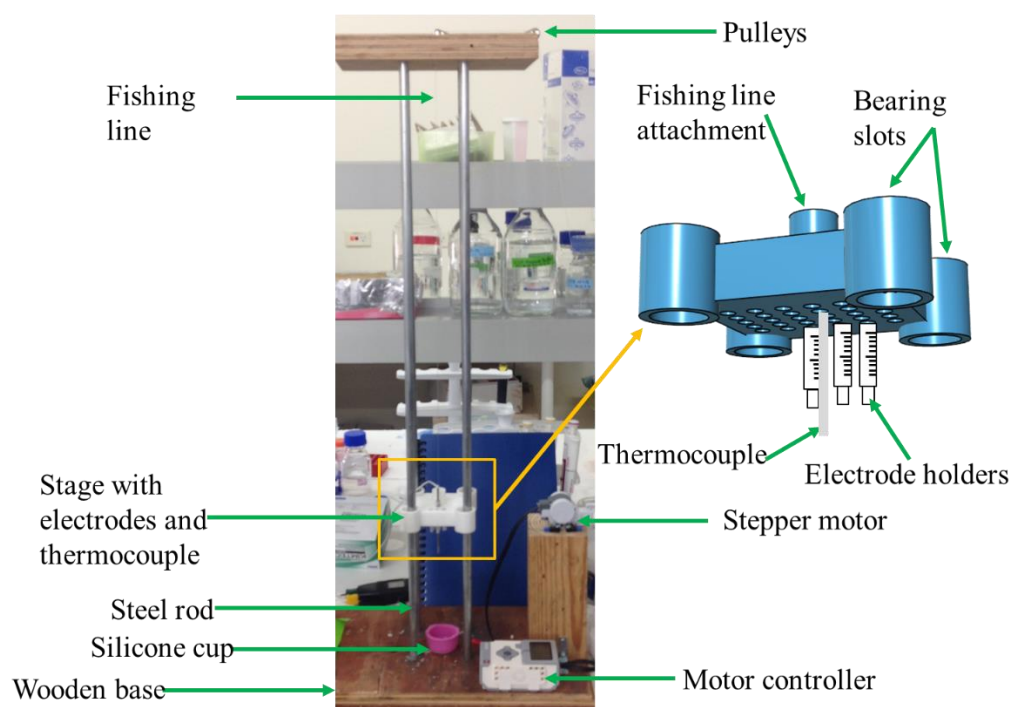
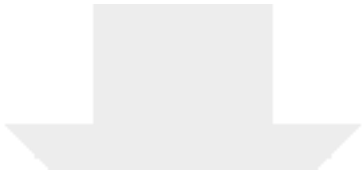


Figure S3. Set-up used for sucrose microneedle drawing, including a 3D printed stage controlled by a Lego Mindstorms motor. Microelectrodes, along with a thermocouple, were embedded in the stage and lowered into the silicone cup containing molten sucrose. Once the drawing range was reached, the motor was driven upward to create sucrose microneedles.

Table S1. Statistical table from insertion force experiments (n=18 for sucrose MNs, n=10 for sucrose-coated hypodermic needles, and n=12 for all others) in which peak force (Newtons), strain energy transfer (mJ), and surface area normalized force (N mm⁻²) were compared using Kruskal-Wallis comparison tests. Peak force is depicted in **red**, strain energy transfer in **blue**, and surface area normalized force in **green**.

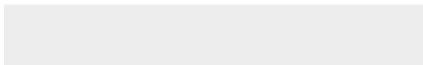
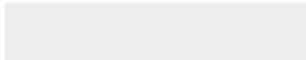
	Occluded 23G	Open 23G	Sphere	Sucrose coat	Sucrose MN
Open 23G	0.410 0.179 **				
Sphere	**** 0.098 ****	*** *** ****			
Sucrose Coat	0.259 0.552 0.063	0.056 0.061 0.463	**** 0.324 ****		
Sucrose MN	0.070 **** 0.118	** ** ****	**** **** 0.079	0.549 **** ****	

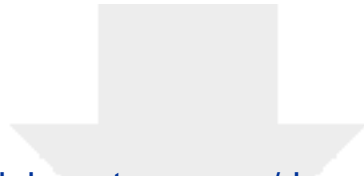
*P<0.05, **P<0.01, ***P<0.005, ****P<0.001



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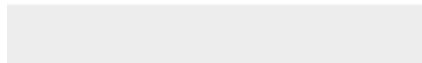


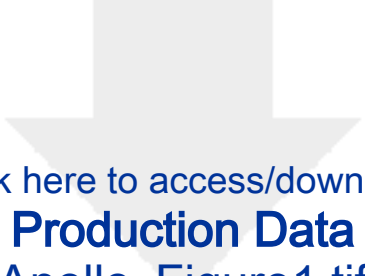


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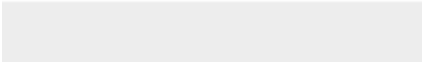
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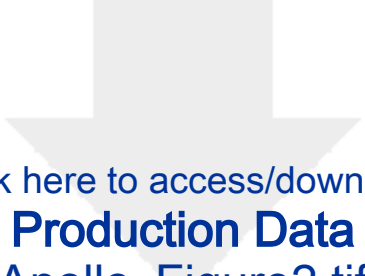




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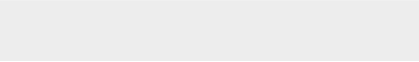
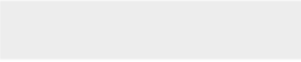
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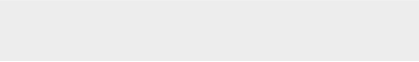
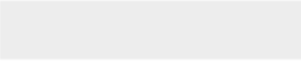
Production Data
Apollo_Figure2.tif





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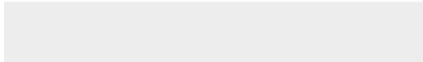
Production Data
Apollo_Figure3.tif





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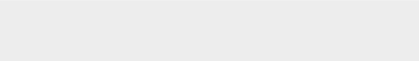
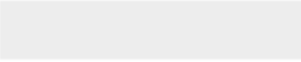
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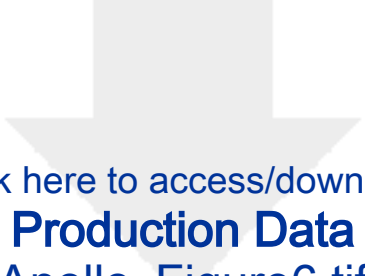




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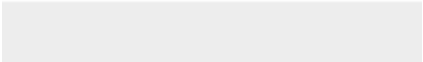
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Apollo_Figure5.tif





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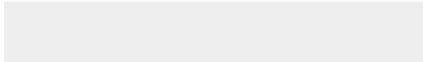
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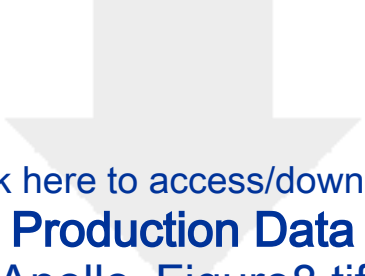




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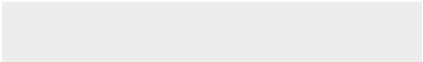
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Apollo_Figure7.tif





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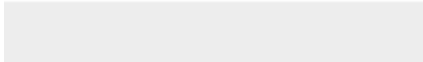
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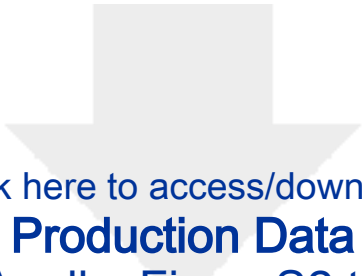




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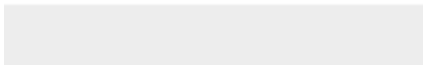
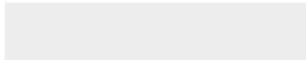
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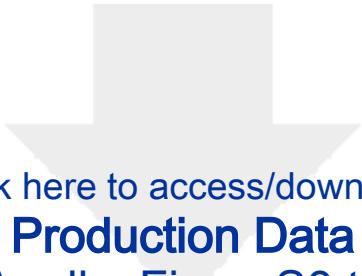




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