33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Bioelectrical Enhancement in Tissue-Electrode Coupling with Metamorphic-Stage Insertions for Insect Machine Interfaces

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Abstract— **Implanting microtechnologies into insects with an aim of domesticating its locomotion poses certain challenges, however, performing surgical implantation during the early stages of metamorphic growth was shown to mitigate some of the related detriments. This study reports the bioelectrical enhancement at the tissue-electrode interface allowed with these metamorphic stage insertions, where the electrodes implanted in the insect during the early pupal stages and right after emergence were compared. An average 1 kHz impedance of 8.9 kΩ was obtained with pupal stage inserted electrodes, ten days after the emergence, as compared to 12.1 kΩ observed when electrodes were implanted in the adult state. Charge storage capacity also increased to 52 mC/cm^2 from 38 mC/cm^2 with the early metamorphic insertions. The performed voltage excursion studies also confirmed the enhancement demonstrating an increase from 3.5 mC/cm^2 to 5.1 mC/cm^2 in the injectable amount of charge in the water window.**

I. Introduction

THE domestication of insect locomotion has been under investigation through tapping into its neuromuscular system via implanted microelectrode based systems [1,2]. Recent studies have also highlighted that it is possible to benefit from metamorphic development to minimize or eliminate some of the drawbacks that come with surgical implantation of neuromuscular stimulation systems on insects [1, 3]. The goal for such a neuromuscular stimulation system is to induce local potentials in the muscular tissue in order to evoke a biomechanical outcome towards controlling locomotion. To achieve this objective, pulse streams containing collection of electrons are injected into the tissue through coupled metal electrodes. At the interface where electrodes meet tissue, the charge carrier is transduced from electrons in the metal electrode to the ions in the tissue electrolyte. This conversion is a sensitive electrochemical process that limits the ability to form a stable tissue-electrode interface. Therefore, it is necessary to assess the efficacy of implanting electrodes during early metamorphic stages in terms of their bioelectrical performance.

Manuscript received March 25, 2011. This work was fully supported by DARPA HI-MEMS Program. Asterisk indicates corresponding author.

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Electrochemical measurements allow the capability of real time monitoring of the implanted electrodes to follow the tissue-electrode interface *in vivo* at multiple time-points [5]. This technique has the potential to shed light on the mechanisms at the insect tissue-electrode interface so that the effect of any manipulation could be characterized. Here, we present our work on applying these measurement methods to quantify the improvement in charge injection capability of the tissue-electrode interface formed with the moth's flight muscle, especially when implantations were done following Early Metamorphosis Insertion Technology (EMIT) [3].

II. Anatomical Changes during metamorphic growth

This section summarizes the anatomical changes during the metamorphic development of flight muscles [1,3] in moths for a better understanding of the process. Neuromuscular tissue of moths undergoes extensive change during metamorphic growth. Larval neurons required for adult behavior are retained and respecified by forming new dendritic morphologies, whereas the neurons lacking adult functions are removed by programmed cell death [6]. During this intermediate stage between the larval and adult stages, an aerodynamic body shape and appropriate sensory equipment are also formed to support adult aerial behaviors. Specifically, the metamorphosis of flight muscles starts with the molting process, which is triggered by the secretion of ecdysteroids by the larval prothoracic glands. This in turn retracts the terminals of motoneurons from the larval thoracic body wall muscles that will form specialized flight muscles after complete degeneration. The fibers of these degenerated muscles form the templates for thoracic flight muscles and myoblasts start to migrate to these templates. The flight muscles continue to develop by accumulating myoblasts on the fiber templates, where the number of accumulated myoblasts determines the eventual size of the adult muscle.

After the onset of muscle formation, motoneuron terminals start to develop first by forming the central dendrites, followed by the allometric growth of finer branches on the periphery to build new synaptic contacts with predetermined muscle fibers [7]. The proliferation of flight muscle motoneuron dendrites occurs during the last two thirds of the pupal stage [8].When the neurostimulating electrodes are implanted in the insect during the pupal stages of metamorphosis, this change is also hypothetically projected to the interface formed between the tissue and electrode, as presented in the "Results" section below.

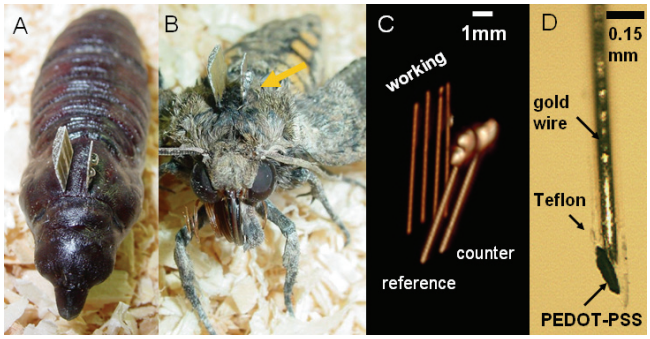

Fig. 1. (A) Wire electrodes inserted to the pupae with exposed PCBs. Left-hand side PCB holds the four working electrodes, whereas right-hand side PCB is for platinum reference and counter electrodes, (B) The post-metamorphosis emerged adult insect with the electrodes, (C) Relative positions of the isolated electrodes with electrode types as indicated (X-ray micro-CT), (D) Tip of the gold working electrodes are coated with PEDOT-PSS.

III. Materials And Methods

A. Preparation of Electrodes

Gold wires (75 μm) with Teflon (65 μm) coating were used to construct the electrodes. The wires were cut at the tip at an angle to expose a 75x140 μm^2 elliptical gold surface. Then, a single row was formed with an array of four wires by soldering onto a PCB with a 2.5 mm pitch (Figure 1). Next, these wires were coated with PEDOT-PSS for improved charge injection properties. For the electropolymerization of PEDOT-PSS over gold electrodes, a monomer solution was prepared by stirring 35 mg of 3,4-ethylenedioxythiophene (EDOT) with 250 mg polystyrenesulfonic acid (sodium salt) in 25 ml of deionized water for 2 hours until all the formed globules of EDOT were dissolved [9]. The gold wire electrodes were immersed in the solution to act as anodic working electrodes. The cathodic counter electrode was a 2.5×2.5 cm^2 platinum sheet. A galvonastatic charge of 100 $\mu A/mm^2$ was applied for 120 seconds. The electrodes were rinsed with DI water and nitrogen-dried. To assure the efficacy and stability of the electrodes and the recording system, control measurements in saline solutions were performed in parallel to the *in vivo* experiments. The nominal 1 kHz impedance of the control electrodes in saline solution was approximately 8 kΩ (with a 0.75 kΩ of standard error) and was stable during the course of the measurement.

B. Surgical Implantation Protocol

Manduca sexta (tobacco hawkmoth) were obtained for surgery from the Boyce Thompson Institute, where they were reared on an artificial diet under a 17:7 hr light/dark cycle regimen at 26ºC and ~60% humidity. Both chronological and morphological criteria were used to determine the developmental stage for the probe insertion. Pupae with seven days to eclosion were selected for the surgery. For the insertion location, the dorsolongitudinal flight muscles in the dorsal thorax were selected, which provided a large and uniform volume of muscle tissue [2]. After anesthetizing the insect pupae and the adult insect with cold treatment (4ºC) for 10–15 minutes, four mesothoracic holes were created with a scalpel both on the epicuticle and exocuticle of each insect, matching the pitches of the electrodes. Two additional holes were made 4 mm away from the first set of holes for the reference and counter electrode insertions. For adult insects, the scales covering the thorax were cleaned under anesthesia before the incisions were made. The wire electrodes were inserted into the muscle tissue through these incisions. The pupae with inserted wire electrodes were supported from the left and right body sides for at least 36 hours to prevent pupae rotary movement, which could shift the probes before sealing occurred. No adhesive was used to seal the incisions for pupal insertions. Instead, the cuticle was allowed to heal naturally, creating a biological seal. For the adult insects, dental wax and superglue were applied to the insertion points for mechanical fixing.

C. Electrochemical Measurement

The body of the PCB, where wire electrodes were soldered, was shaped to fit into an FFC (Flat Flex Connector), enabling an external electrical connection for electrochemical characterization. For all the electrochemical characterizations, the Gamry Femtostat (FAS2) System was used. A second PCB was prepared holding reference and counter electrodes to obtain a three-electrode measurement system (Figure 1). 75 μm platinum wires were used to construct both the electrodes. The counter electrode was an uncoated bare wire. The reference electrode was coated with 65 μm Teflon with 500 μm exposed at the tip. The impedance amplitude and phase were recorded through the electrochemical impedance spectroscopy (EIS) to characterize the charge transport mechanism. An alternating sinusoidal waveform with 25 mV amplitude was used as the input signal. The current density was monitored and ensured to stay below 0.4 mA/cm^2 in order to have the interface components to behave stably. The impedance was recorded between 10 Hz and 100 kHz at 10 discrete frequencies per decade.

Cyclic voltammetry (CV) analysis was also performed to characterize the ability of the interface to store charge. A scan rate of 500mV/s was used to sweep the range between -0.6 and 0.7 V vs. Pt to remain within the safe potential limits associated with hydrogen and oxygen evolution through electrolysis of water (water window). The area under the CV curve was calculated as a qualitative indication of the amount of stored charge in the water window without any gas evolution.

Voltage excursion studies were conducted in addition to the impedance and potentiodynamic studies described above, to compare how much of the stored charge was actually injected into the tissue through biphasic current pulses. Studying the voltage transients through the balanced biphasic pulse excursions allows one, in principle, to address the electrically safe charge injection limitations to keep the operation in the range in which no harmful reactant is released to the tissue. A programmable stimulator was used to generate charge

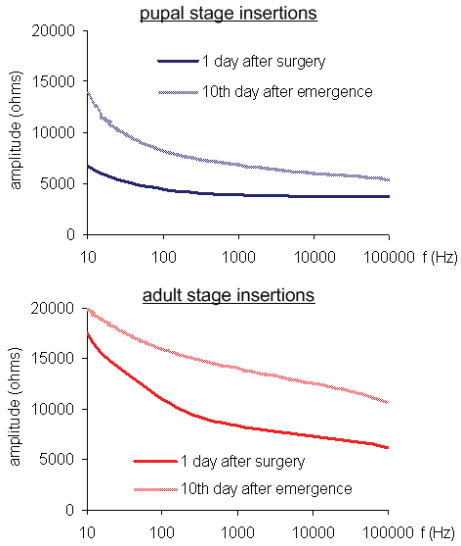

Fig. 2. Representative electrochemical impedance spectroscopy data comparing pupal and adult stage inserted electrodes one day after the surgery and 10th day after emergence.

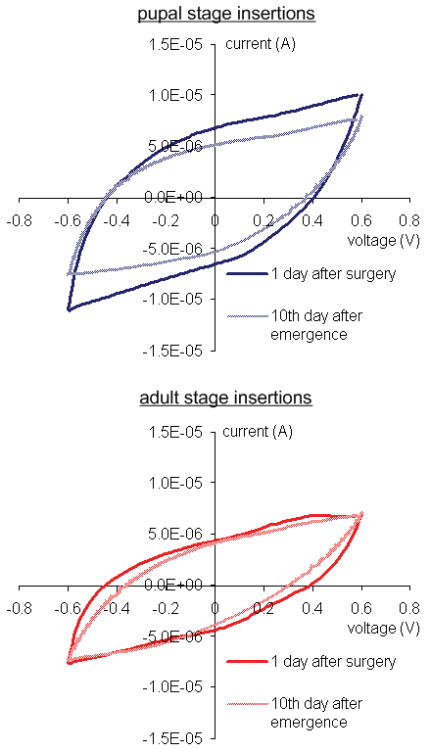

Fig. 3. Representative cyclic voltammetry data comparing pupal and adult stage inserted electrodes one day after the surgery and 10th day after emergence.

balanced, cathodic first, biphasic pulse currents (Multichannel Systems STG2008). Pulses of 2 ms pulse width, 2% duty cycle and varying degrees of charges (between 0.2 and 20 mC/cm^2) were sent to the electrodes *in vivo*. The resulting waveforms were recorded using an oscilloscope and then post-processed to calculate the actual voltage drop across the interface to determine the amount of charge that could be safely injected. The voltage drop across the interface to compare different electrodes and charge densities was accepted to be -0.6 V and 0.7V.

The data were collected from three insects for both pupal and adult stage insertion, resulting in n=12 electrode sites for each surgical method. Presented values represent the average values with standard deviations provided when available. The error-bar regions indicate the standard error of the mean.

IV. Results and Discussion

The pupae implanted and emerged electrodes can be seen in Figure 1. The same figure also demonstrates the X-ray micro-computed-tomography of the three-electrode measurement system. A representative electrochemical data set for one electrode is provided in Figure 2, 3 and 4. Day-by-day changes of these electrochemical parameters for all the electrodes are presented in Figure 5, starting 6 days before emergence until the 10th day after emergence.

The impedance curves immediately after the implantation changed noticeably over time for both pupal and adult stage insertions (Figure 2). An upward shift was observed over time while the general shapes of the curves look similar qualitatively. The adult stage insertions caused an overall higher plot soon after insertion, resulting in an average impedance magnitude of 9.6 kΩ at 1 kHz, compared to 4.1 kΩ for pupal stage insertion. With these adult stage implanted electrodes, a larger magnitude shift over time was also observed, especially at the higher frequencies, which is indicative of a larger resistive change with the adult tissue formation. The nonlinear trend of the change over frequency for the pupal stage inserted electrodes also indicates a resistive-capacitive (RC) effect introduced to the system due to tissue formation, which manifests itself over the time course of development.

In neurophysiological experiments, it is a tradition to measure the impedance magnitude at 1 kHz in order to set a quantitative standard for comparison across different electrodes and subjects, as well as published literature because 1 kHz is almost the fundamental frequency for action potentials. Therefore, the average 1 kHz electrochemical impedance magnitude change during this time course can be seen in Figure 5. A monotonic increase was observed over the course of the pupal stage development where the degree of increase was much less than that after the insect emerged. Implanting the electrode during the adult stage caused higher average 1 kHz impedance, which followed a similar trend of change with pupal stage electrodes after the emergence of the insect.

The average stored charge values (Q_{stored}), the area under the CV curve (Figure 3), also exhibited a dramatic decrease over time during metamorphic development and emergence for electrodes implanted in both pupal and adult stages (Figure 5). It decreased by time from 81 mC/cm^2 to 51 mC/cm^2 for pupal stage insertions, and from 50 mC/cm^2 to 38 mC/cm^2 for adult stage insertions. The increase in the low-frequency impedance (EIS) was correlated with change in CV curve, for the

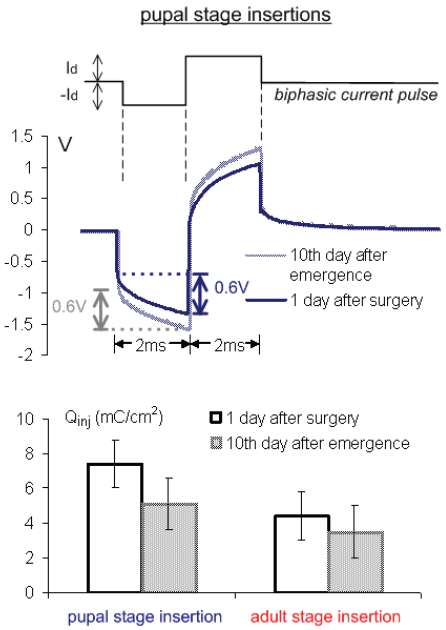

Fig. 4. Representative voltage excursion data obtained by sending biphasic current pulses to the pupal stage inserted electrodes. A similar waveform was obtained for adult stage insertions (not displayed). The 0.6 V polarization voltage was selected as the level to compare the safely injectable amount of charge (Q_{inj}) as provided in the bottom for both insertions.

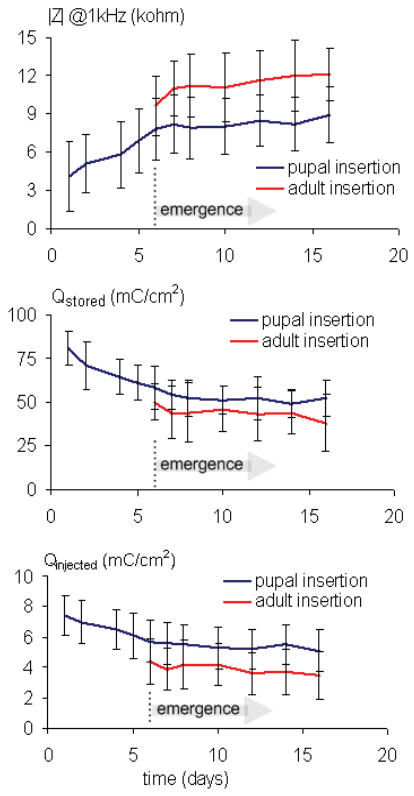

Fig. 5. The averaged day by day data indicating the changes in the 1kHz impedance acquired through EIS (top), the amount of charge stored at the interface (Q_{stored}) from CV plots and amount of safely injected charges ($Q_{injected}$) with biphasic pulses obtained via voltage excursion studies. For all the graphs, the emergence occurs at the 6th day for both pupal and adult stage insertions.

"slower" ramp waveform (500 mV/s sweeping -0.6 to 0.7 range) of CV concentrates more on this range.

The CV data was supplemented by the voltage excursion studies (Figure 4). Similar to the stored charge, the degree of charge injected through the charge-balanced biphasic pulses in the water window also decreased over time for both kinds of implantations, while the pupal stage inserted electrodes were able to inject more charge into the tissue (Figure 5).

A clear correlation was observed between stored charge at the interface, an injected charge with biphasic pulses and the 1 kHz impedance magnitude.

V. Conclusion

The objective of this study was to characterize the bioelectrical properties of the tissue-electrode interface formed with metamorphic insects to control their locomotion. In particular, we investigated the enhancement in the charge injection properties when the implantations were done following Early Metamorphosis Insertion Technology (EMIT) [3]. The electrochemical measurements (electrochemical impedance spectroscopy, cyclic voltammetry and voltage excursion studies) performed *in vivo* allowed us to follow the tissue-electrode coupling noninvasively at multiple time points. The results of these preliminary measurements suggest that EMIT improved the impedance of the formed interface with the insect in the sense that more charge could be stored at the interface and could be injected to the insect tissue through biphasic current pulses in the water window.

Acknowledgment

Authors would like to thank Mark Riccio for technical support with the X-Ray Computed Tomography, Janice Beal and Katherine Renwick for supplying insects. This work was fully supported by DARPA HI-MEMS Program.

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