SURGICALLY IMPLANTED MICRO-PLATFORMS IN *MANDUCA SEXTA* MOTHS

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ABSTRACT

We demonstrate for the first time *surgically* implanted platforms and substrates for attachment of microsystems in insects without adverse effect to adult lifespan. The technology demonstrates the insertion of biocompatible balsa-titanium anchor in the late pupal stage of a moth, *Manduca sexta*, resulting in a permanently anchored base in the adult. We also present results on inserting silicon chips (4mm X 2mm X 0.5mm) in the late pupal stage. The inserted substrates become permanently incorporated into the resulting adult moth as the wound is healed and the anchor site reinforced during the process of metamorphosis. This technology could open a wide venue to use insects with surgically incorporated MEMS devices as flying machines, to deploy payloads at a target of interest.

INTRODUCTION

Over the past four decades, tremendous interest has developed in creating cm-scale autonomous micro aerial vehicles (MAV) for applications ranging from reconnaissance in the battlefield to environmental monitoring. Significant advances made by microfabrication technology have made it possible to realize mm-scale low-power microsystems capable of carrying out a wide array of sensing and actuation tasks. However, the power sources for the flight of a mechanical MAV have not scaled down well. Hence attempts by aerodynamic and robotic engineers to create mechanical MAVs have only met with limited success.

In this paper, we employ nature's best flyers, insects, to realize "biobot" MAVs. Insects are autonomous flying machines and have much better aerodynamic performance than any existing mechanical flying machine. Insects also exist in diverse shapes and sizes. Large insects like the hawkmoth, *Manduca sexta*, have powerful flight muscles and can be equipped to carry telemetric payloads weighing up to 1 gram. Insects provide models for robotics as "biobots" [1] [2], where an intact biological system is harnessed using applied artifacts like MEMS.

Several research groups have successfully glued telemetric and electronic devices onto insects to track the insect's movement and migratory path [3] and attached miniature "backpacks" for environmental monitoring [4] [5], wireless communication or biobotic manipulation of behavior [6]. However, attaching backpacks at the adult stage leads to unreliable connections and displacement of the payload, and leads to trauma for the insect as it perceives the load as foreign weight (Table 1). Glued-on telemetric devices are temporary attachments and have been reported to fall off when the insect is in confined spaces or when foraging in vegetation. Moreover, handling a mobile and active adult to attach payloads is difficult for mass production. And with the typical adult lifespan of 2-3 weeks, delivery to the site of deployment at the correct time is challenging.

Figure 1. Schematic illustration of the working principle on surgical insertion of the micro-platform at different stages during the life cycle of the moth.

With the increasing interest in developing biobots that can be harnessed as MAVs with MEMS sensors and actuators, we present a Hybrid Insect with micro cargo platform surgically implanted at different life stages in *Manduca sexta*, without adverse effect to the insects life span or flight capacity. We take advantage of the metamorphosis process during the growth of the insects, when they transform from a feeding and foraging larva to an adult, to successfully implant platforms for microsystems without traumatizing the adult insects.

THEORY

Insects survive extreme surgery and this has been proved by parabiosis experiments [7], where anatomical and physiological union of a thorax with head to an abdomen from two individual silk moths has been demonstrated. These experiments were performed on silk moths at the pupal stage of development. This encouraged us to attempt to permanently attach a payload to the insect by (a) surgically inserting the micro-system into the insect for permanent attachment and (b) using the pupa, a relatively immobile stage of development. Insects grow by regenerating their outer exoskeleton (cuticle) with every molt. Hence, it is feasible to incorporate biocompatible foreign material in a life stage before emergence as adult, therefore allowing healing of the wound around the inserted material during the development of the new cuticle that occurs during the molt. This new tissue interface may ensure permanent attachment of the insert to the insect. This resource could eventually be tapped to develop devices that could be integrated into the insect tissue system like muscles or nerves to control the insect's behavior (movement). It is known that insects respond to pheromones, changes in wavelength of light,

and to various frequencies of ultrasound by altering their movement pattern either away or towards the source of stimuli. Hence one can potentially control the insects' flight path by utilizing the intrinsic natural responses to various stimuli like light, ultrasound and micro-discharge of pheromone from microsystems attached in addition to the payload.

METHODS

The moths, *Manduca sexta*, larvae and pupae were purchased from Carolina Biological Supply Company (Burlington, NC) and were reared at 26°C, 80% relative humidity and a 12:12h light and dark photoperiod. Upon emergence, adults were transferred to a larger wire cage for proper expansion of the wings. We used the 5th instar larva, pupa and adult stage (Fig 1) of *Manduca sexta* (moths) to develop the surgical protocol.

The experiments were aimed at evaluating (a) mortality rates (b) successful integration of the insert at adult emergence and (c) longevity of the adult compared to an unaltered control adult after the surgical procedure was performed at various stages of the life cycle of the moth. The first experiments involved insertion of surgical thread into the three stages of development (last instar larvae, pupal, and adult). The second experiment involved insertion of biocompatible titanium wire as anchors into these three life stages. The titanium wire post design was later modified to support a balsa wood block as the platform before the wire was inserted into the abdomen of the insect. The third experiment comprised of silicon chip surgically inserted into the larva, pupa, and adult on the ventral side of the abdominal body cavity. SEM photographs were taken to observe any tissue growth forming an interface between the titanium wires and the silicon insert, reinforcing the implants.

Table 1: List of advantages and disadvantages of using robotic MAV and insects as payload carriers.

		Advantage	Disadvantage
MAV manual robotics		1. Manipulation of flight is possible. 2. Loss of MAV not due to external natural stimuli (pheromone, ultrasound)	1. Expensive to design and manufacture versatile models. 2. Useful load disappears with decrease in MAV size.
	Superficial Attachment	1. Versatile MAV models. 2. Can carry a load of half its muscle weight	1. Lack of control of flight direction. 2. Unreliable and prone to dislodge. 3. Light weight power source not available.
Insect as payload carriers	Surgical Attachment	1. Permanent structure for attachment of payload. 2. Versatile MAV models. 3. Can carry a load of half its muscle weight. 4. Acclimatization to the extra load is easier when load is attached at a pre-adult stage.	1. Lack of control of flight direction. 2. Light weight power source not available

Figure 2. Micro-cargo platform on adult *Manduca sexta* (a) photograph showing the balsa- titanium platform surgically inserted on the abdomen, (b) SEM photograph shows tissue growth on the titanium wire inside the abdomen resulting in a stronger and reliable attachment due to tissue support.

Light was the stimuli/ method that was used for the control of locomotion or direction of flight. A miniature LED-based optical cue generator (0.7cm X 1.8cm, 0.5g) was designed (Fig 5) to manipulate flight direction. The device was designed to turn the LED on either the left or right side of the moth to bias flight either towards or away from the light stimulus. The experiment was designed to be conducted in a wind tunnel under red light.

RESULTS AND DISCUSSION

The results for the first set of experiments with silk tethers inserted in the late pupa showed successful adult emergence and no significant change in survival rates, mobility or behavior (Table 2) when compared to controls. The silk inserts in the larval stage (late 5th instar) showed only partial success: When the silk thread was inserted just below the surface it was sometimes shed during the molt to the pupa, whereas deep insertions sometimes fatally punctured the insect's gut.

In the second set of experiments, titanium wires to be used as anchors were inserted into the abdominal cavity. Insertion of wires into the larval stage was not successful as the larvae failed to molt. However, insertion in the late pupal stage resulted in successful emergence of the adult with firmly anchored titanium wire posts (Fig 2 a,b). The healed wound around the wire anchors showed tissue growth when analyzed under a scanning electron microscope.

In the third set of experiments, silicon chips (4mm X 4mm) inserted in ventral abdominal cavity of the late pupal stage allowed for the successful emergence of the adult (Fig 3) with the chip firmly embedded in the body cavity. Prying the chip out of the body cavity caused the removal of a large piece of body wall, demonstrating the permanent attachment of the insert (Fig 5). Inserts into the pupae caused the formation of a tissue interface, thereby anchoring the insert firmly inside the body cavity. Initial test with the LED device mounted on the moth showed no direct response to light stimuli. These tests were not done in a wind tunnel.

The technology demonstrated here opens up the possibility to integrate either MEMS sensors and actuators (or rigid platforms to attach the Microsystems) in the various life stages in an insect's lifecycle. The cm³ scale microsystems are a near perfect fit to the cm-dimensions of flying insects such as moths and butterflies. This method of implanting not only creates a venue to use the different life stages of an insect, it also results in healing of the insect around the insert forming a permanent

Figure 3. Silicon chip inserted into (a) late pupal stage resulting in (b) emergence of adult with the silicon insert and (c) a SEM photograph of the silicon in the adult body.

Figure 4. Integration of the silicon chip within the abdominal cavity is demonstrated by the tissue loss while prying the insert out of the body cavity of the adult. This is due to tissue growth around the silicon chip.

Figure 5. Circuit board with a miniature LED based device to regulate direction of flight in the adult moth.

Table 2. Survival rates of *Manduca sexta* with inserts at all the developmental life stages (larvae, pupae and adult).

Procedure	Life-stage (insert)	Number of replicates	Survived life stages with the insert.			Total% survived.
			Larva	Pupa	Adult	
Silk thread (CS insert)	Larvae	20	0	0	0	0
	Pupae	10	NA	8	8	80
	Adult	5	NA	NA	5	100
Silk thread (LS insert)	Larvae	20	19	18	18	90
	Pupae	10	NA	10	9	90
	Adult	5	NA	NA	5	100
Titanium Wire	Larvae	10	0	0	0	0
	Pupae	30	NA	30	30	100
	Adult	15	NA	NA	14	93.33
Silicon chip	Larvae	5	0	0	0	0
	Pupae	8	NA	7	7	87.5
	Adult	5	NA	NA	3	60
Total = 153						

structure yielding a more reliable bio-microsystem interface between the insect and the inserts. Once the platforms are integrated into the physiological system of the insect body at a stage earlier than the adult, the foreign load can be perceived as the insect's own body weight on emergence. This could result in higher payload carrying capacity of the insect. Moreover, the possibility of integrating the payload at the (relatively) immobile pupal life stage of an insect, whose emergence time into the adult stage can be manipulated (lengthened) with temperature and/or other environmental stimuli, opens the possibility of mass producing hybrid insect biobots and on time deployment of the insect at the target site, respectively.

Additionally, the tissue growth around the microsystems in the insect body could eventually present a bio-electromechanical interface that could be used to control locomotion by stimulating nerves and/or muscles to bias insect behavior like flight. Locomotion could also be controlled by manipulating the sensory organs to external stimulus like ultrasound, pheromones, and light wavelength and patterns. These external stimuli, if generated from a MEMS sized actuator, can be positioned on/in the insect. This might override the insect's instinctive behavior and result in more accurate control of its locomotion towards the target.

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