Paper: An Efficient Production Process for Extracting Salivary Glands from Mosquitoes

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Advanced Computer Integrated Surgery II: Literature Review

Group 1: Automation of Mosquito Dissection for Malaria Vaccine Production
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Project Summary

Currently malaria is one of the leading causes of mortality in many developing countries. Our groups goal to is assist in the development of an automated mosquito processing robot for Sanaria to help improve their throughput of processed mosquitoes glands which are used to produce a malaria vaccine. The entire system as it is currently being designed consists of three main units. The frist is a feeder system that is designed to take the mosquitoes from an aqueous solution and present them in a manner that is sufficient for a robot to then grasp. The robot, the second unit of the system, uses a small grasper to pick up the oriented mosquito and places it into the third unit of the system. This final unit is the focus of our groups work. This unit takes in a mosquito and processes it to extract its salivary glands which contain *Plasmodium falciparum* sporozoites (PfSPZ). The PfSPZ is then used to help manufacture a vaccine that has the potential to save millions.

Selection Motivation

Due to the level of confidentiality associated with this work few papers if any have been published on this process. Sanaria is interested in maintaining its intellectual property and would like to details of the system being developed to be kept confidential. This paper however serves as one of only a few good references on the work being done in this area. The paper discusses the semi-automated gland dissection that was used to help increase throughput from the manual method used prior to LCSR's involvement on the project, with the final goal of implementing an automated system. Since this work has directly informed the current design of the system to this date, it is believed to be a useful paper to look back to help improve the final design.

Summary and Background

Sanaria has made significant progress in developing PfSPZ vaccines from the salivary glands of infected mosquitoes. However, they are severely limited in their ability to manufacture this vaccine at scale with their current collection method of infected glands. Automating this process would help to increase the rate and volume at which mosquitoes could be processed so that they could produce the vaccine in quantities that would meet expected demand. However, the automation of this process has significant challenges so a semi-automated procedure was introduced first to provide improvements on the current method to Sanaria in a shorter time frame. Prior to the introduction of the semi-automated system technicians would have to manually dissect each mosquito one by one with an average of about 5-6 mosquitoes per minute. This task is difficult and requires substantial training to become sufficient at and is clearly quite slow.

The main focus of this paper was on improving the harvesting method of PfSPZ from the salivary glands of Anopheles sp. Mosquitoes through a semi-automated processing method. The goal was to take the very tedious and labor intensive method currently implemented by trained technicians at Sanaria and deskill the task while also increasing the rate of mosquito dissection through the development of a mechanical system. The device designed consists of several modular components: a sorting stage in which mosquitoes are placed into slots, a decapitation stage, and a squeezing and gland collecting stage. The tested system was designed for only twenty mosquito at a time but it was suggested that the number of slots could be increased to further increase throughput. This design effectively allowed for several downstream steps to be done concurrently by a single technician in a shorter time. This system provides valuable design lessons that can help to inform engineers developing the automated system while also providing short term improvements on the existing method of collection.

Significance

The results of this paper are significant to the research in that they have directly informed current design concepts that are currently being utilized. Though the current research has built on top of what was done in this paper it has served as a strong educational guide as to why certain design avenues where pursed.

Design and Experimental Setup

The current fixture design, as stated earlier, consists of several modular components (see Fig 1). The sorting cartridges, blade assembly, squeezer, and staging area. The sorting cartridge is designed to hold twenty mosquito in slots designed specifically to allow for just enough room for the mosquitoes body to fit in to keep them well aligned during the squeezing stage of the dissection process. The staging area is designed to be a smooth surface so that the mosquitoes are not damaged as they are dragged to the slots, the area is also sufficiently large enough for 30-40 mosquitoes to be placed on at once which is more than is needed to fill the slots allowing for more efficient filling time. The sorting cartridge is also removable so that extracted glands do not become trapped behind the blades. The design of the blades are like that of clippers with notches for each slot, each notch is about 0.6mm wide in which the neck of the mosquito fits. The blades are 50μm thick, made of stainless steel, and are easily removable to be cleaned or replaced. The squeezer is designed to fit directly over each slot with a matching but negative geometry such that each slot is fully contacted by a squeezer. This ensures all glands are dissected from each mosquito. To ensure proper user interaction with the system two alignment pegs constrain the system so that the squeezer aligns properly with the slots each time. The 'teeth' of the squeezer are designed with the goal of having a very close tolerance to the slot so that all of the gland is forced out of the front of the slot through the incision at the neck.

The work flow for this semi-automated mosquito microdissection system (SAMMS) is fairly simple. First a cartridge is inserted into the apparatus (Fig. 1) so that the slots align with the blades, several mosquitos are placed onto the the staging area with a small amount of aqueous solution and then individually each mosquito is placed into the slot. Using tweezers the technician graps the mosquito by the proboscis and drags them into the slot so the neck is between the blades. The orientation of the mosquito is key and the process of dragging them into place helps to align them properly in the slot. With all the slots filled the technician then manually actuates the blades severing the heads of each mosquito. After the technician removes the decapitated heads the squeezer is then used to force the glands from the body onto the flat surfacer behind the blades to be collected by a pipettor.

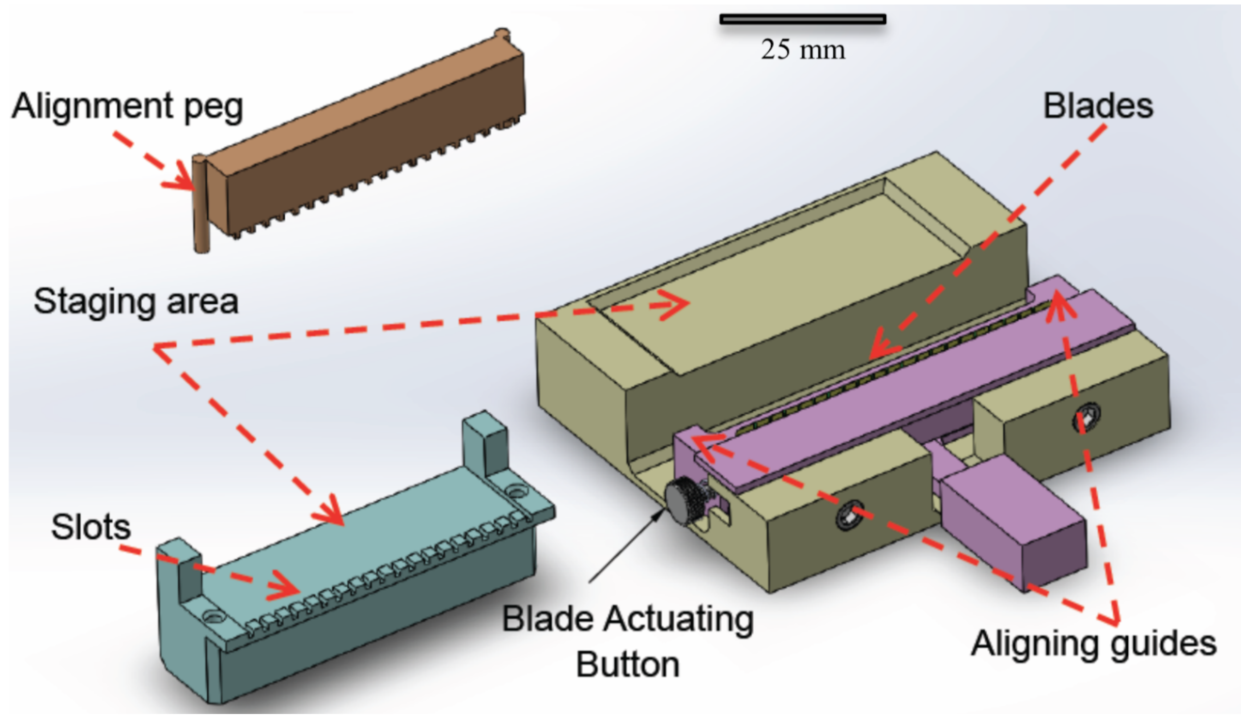

Fig 1. Design of mosquito gland extraction apparatus, including the sorting cartridge (blue), the blade assembly (pink), squeezer (brown) and staging area (tan). [1]

Results and Discussion

The paper looked at three criteria when evaluating the system: the overall quality of the salivary gland SPZ, the impact on training time, and increased throughput as compared to prior manual method. When using SAMMS the overall quality of the glands was found to be nearly identical those to that of the manual method. SAMMS also showed an overall reduction in training time required to qualify technicians for mosquito dissection the limiting factor was the time it took to load mosquitoes into the slots. Operators were quickly able to use SAMMS effectively with a test of 8 operators extracting glands from 338-649 mosquitoes per hour with an average of 470 (see Table 1). SAMMS shows that there is potential for at least a two fold increase over the manual method, targeting 600 mosquitoes per hour, as compared to the manual method. Improvements to the manual method from 2015 to 2016 showed an increase in manual dissection rate but spread is quite large and even at its maximum the rate of dissections per hour is less than 600 (see Fig. 2).

The system developed in the paper demonstrated the effectiveness of a high level prototype for semi-automating the current task of gland extraction. This system is capable of batch-processing serval mosquitoes at a time, which the researchers suggesting a maximum of 40 do to specimen drying issues. Gland extraction can be done at scales from tens to hundreds of mosquitoes at once. As a result the team expects SAMMS to allow for single operator throughput to be increased two to even three fold as compared to the manual method. SAMMS greatest advantage is its radical reduction in training time, 15-20 fold, as compared to the manual method. The semi-automated nature of the design also reduced operator fatigue which can help. SAMMS also showed that the glands extracted are comparable, if not better then, that extracted by the manual method.

The research team learned several important design criteria while developing the system. The method of grasping the mosquito by the proboscis and dragging across a lubricated surface into the cartridge slots proved to be very successful. After many design paths the clipper style blade design was seen as the most promising. The comb squeezer and pipet collection approach also showed superiority over other designs, with particular importance placed on the need for a tight fit between the teeth of the squeezer and the slots of the cartridge. This tolerancing was key in ensuring that the glands were forced only out of the front of the cartridge.

	1	2	3	4	5	6	7	8	Avg.
(A)	2.4	2.5	2.3	2.5	1.5	1.3	1.1	1.2	**1.9**
(B)	0.7	0.8	0.8	1.1	1.2	0.7	1.1	0.7	**0.8**
Total	3.1	3.3	2.8	3.6	2.7	2.0	2.2	1.9	**2.7**
Rate	393	364	429	338	444	600	545	649	**470**

Table 2: Operation times and production rates for 8 operators using a proposed apparatus. (A) Minutes to align 20 mosquitoes; **(B)** Minutes for gland extrusion and collection. Total time for 20 mosquitoes is in minutes, and rate is mosquito throughput per hour. [1]

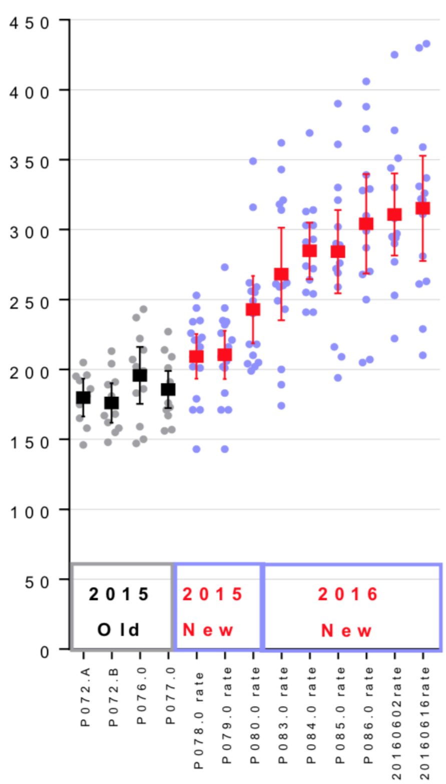

Fig. 2: **Rate of dissection of mosquito salivary glands by manual dissection.** The rate is calculated as the number of Mosquitoes Dissected Per Hour (Mdph). Individual scatter points represent a single dissector's average recorded for one day of production. The mean (black or red bars) of all participating dissectors in a specific production day is plotted with 95% confidence intervals. In an earlier iteration all steps in dissection used to be performed by trained dissectors (grey scatter points, black bars). In a new modified configuration (blue scatter points, red bars) only the task of decapitation and separation of glands from mosquito bodies was conducted by highly trained dissectors and glands were pooled and collected by separate less- specialized personnel. [1]

Conclusion and Future Work

The teams work after the development of this prototype has two main design avenues; the first is further refinement and implementation of a cGMP-compliant version of SAMMS, the second is to use the lesson learned to develop a fully automated version of the system replacing the operator with a robot grasper and vision system. They leave off on the fact that the development of such a robotic system is being developed along with an advanced feeder system.

Assessment

This paper is a useful insight into why our current automated design exists the way it does. Without the research conducted by this team in developing the semi-automated system our design would not be as well informed as they are today. It provided important lessons on key designs such as the squeezer geometry and decapitation mechanisms. As an individual who joined this project at a later date it served as an important tool for understanding why certain designs exists as they do in their current form.

Critique

It might be unfair to make these claims not knowing the full timeline or scope of the prototype in this paper but I think a higher level design could have been manufactured for testing by the technicians at Sanaria. The product given to test with was made of 3D printed components and acrylic parts. Though this is good for lab testing and basic refinement I believe that having manufactured a more final version from machined components would have been more true to a final product. Having used these manufacturing techniques in the past I am aware of the issues with tolerancing and warping that can occur. Having components professionally manufactured would have provided a product that would be closer to what Sanaria would eventually get, and would have shown results more true to the final production series. However, this might not have been necessary for the scope of the project.

It should be noted that the design was given to Keytech to make a manufactured version of the system after the team finalized the design of the prototype.

Key Lessons

The following are all key design lessons that have informed our current automated design and have proven to be essential to the success of the system:

- Method for placing the mosquito into the cartridge
  - The method of dragging the mosquito by the proboscis into the slots is a task we have been able to automate with a pick and place robot and vision system.
- Squeezer geometry
  - In our automated version we have taken the same
- Decapitation mechnaism
  - The same method of cutter is used and has been shown to still be effective once properly aligned within the automated system.

Reference

1. "An Efficient Production Process for Extracting Salivary Glands from Mosquitoes" Mariah Schrum, Amanda Canezin, Sumana Chakravarty, Michelle Laskowski, Suat Coemert, Yunuscan Sevimli, Greg Chirikjian, Stephen L. Hoffman, and Russell H. Taylor. *arXiv:1903.02532.* 2019.