Longitudinal Migration Tracking of Fluorescent Stem Cells in Vivo in the Mouse Brain (BME 200/3001:Lab 310)

Product Design Specifications | 24th, September 2021

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Client Requirements:

- Design a biocompatible implantable device
- The device should incorporate three lenses
- Include a laser that shines into the cavity of the brain
- Design the cannula to hold the endoscope

Function:

Current setups for imaging intact neural networks only allow for imaging through a field of view in a single lens. This setup only allows for imaging in a restricted area of the tissue. An endoscope with multiple fields of view is required for accurate tracking of cell migration dynamics in mice that are awake and functioning normally. The design of this endoscope must be small in size and weight and must allow the mouse to maintain normal function and behavior. The endoscope must be able to function in the fluid region of the brain. The endoscope will be powered through a connection port and must be compatible with a Windows OS computer where images will be uploaded using basic software to communicate.

Design requirements:

1. Physical and Operational Characteristics

a. Performance requirements: This device must be able to run without interruption while attached to the mouse. Additionally, due to the nature of the test, the device must be fluid resistant as the lenses will be inserted into the brain where interstitial and cerebrospinal fluid is abundant. This implies that the lens will also need to compensate for any refractions in the fluid while maintaining accuracy [1]. This device must also resist wear and tear over the course of many experiments since it will be reused among different mice.

b. Safety: The device will be accompanied by a laser light source. The intention of this laser is to excite cells within the ventricle to obtain visible information. The strength of this laser may be strong enough to irritate sensitive tissues, so safety precautions must be taken when handling the laser outside of experimental use. All small parts will be encased within the tube of the device, leaving little to no harmful imperfections on the device's surface. Additionally, sterilizing between usages is recommended to prevent any unwanted contamination.

c. Accuracy and Reliability: The fourth ventricle of the mouse brain has a maximum width of around 1700 microns. Given this restriction, the device must be able to clearly record imaging of fluorescent stem cells within 1700 microns from the lens [2]. As requested by the client, the device will also contain multiple lenses, increasing its field of view and depth. In order to maintain consistency between tests, the lenses must be set securely into the device to minimize any lens shifting.

d. Life in Service: This device must be durable enough to withstand months of use. The device must be able to operate on a continuous basis for periods of time extending from two days at the lowest, to three months at the highest. In order to reduce built up residue in between parts of the device, after use soak the implant for approximately twelve hours in a mixture of distilled water and soap.

e. Shelf Life: While not in use, the device will be stored at a standard laboratory environment. The temperature will be around 20°C or 68°F [3]. The device will be sterilized, reprocessed, and flushed with alcohol before being hung in storage. The standard shelf life should be no longer than approximately twelve weeks, any longer could result in an increase in microbial growth.

f. Operating Environment: The device will be used in a standard laboratory environment of about 20°C and be able to withstand 30% relative humidity. Before use, the device must go through extensive cleaning to ensure a sterile environment.

g. Ergonomics: The device must be easily installed, cement is the current standard for attachment. While a platform may be used for additional stability, it would be ideal to avoid this

since it could have an impact on how the mice behave. The portion of the endoscope that will be outside the mouse brain should have an easily accessible connection port to export the images. The device as a whole should be as user friendly as possible and be practical for regular laboratory use.

h. Size: This design must be small enough that its size will not impact the normal behavior and movement of the mouse it is placed in. This device must not exceed the dimensions of 25mm x 25mm x 30mm. Staying within these specified dimensions will ensure the mice are not affected by the size. Since the lenses are the only portion of this device that will be installed into the mice these should be as small as possible without impacting the image quality. The diameter of the lens to be used is 1 mm with a length of 1.883 mm [4].

i. Weight: This device must be lightweight enough to be able to be supported by a mouse head, preferably this device will be as lightweight as possible. Ideally this mini endoscope will not weigh more than 7 grams, this weight is an estimate based on other devices currently available for lab use [5].

j. Materials: As a requirement of the client, GRIN lenses will be used. The materials implanted into the brain should be free of contaminants [6].

k. Aesthetics, Appearance, and Finish: The array of lenses should be prism shaped since this reduces the amount of damage caused to the brain tissue and fits well in the lateral ventricle [7].

2. Production Characteristics

a. Quantity: The client has requested one functional implantable endoscope utilizing three GRIN lenses.

b. Target Product Cost: The budget for this project is $750, however it is a flexible budget that can be moved accordingly. The GRIN lenses we will be using cost approximately $150. The final product will ideally cost less than $400 to allow for prototyping as well. Only one final implantable endoscope needs to be made.

3. Miscellaneous

a. Standards and Specifications: Currently, there is a neurological endoscope listed by the FDA, as well as many similar endoscopes, but none are specifically implantable endoscopes. We would like to adhere to the standards listed for these devices, and will need to meet Class II FDA standards that require a Premarket Notification 510(k) [8]. Class II FDA classification places this device under general controls and special controls.

b. Customer: The client would like for the final product to have three GRIN lenses incorporated, instead of just one. The client also requests that a cannula be created to attach to

the implantable endoscope, and that software to analyze the findings is created as well. The main priority for the semester is to develop a working endoscope first in order to meet the client's primary request.

c. Patient-related concerns: Since this device will be made for mice, one will have to consider the experimental ethics that comes with working with animals. As we design the device, we will have to be conscious of the rules and limitations when it comes to what is safe and proper for animal experimentation. Since the device will be used on multiple mice it will have to be properly cleaned in between subject changes.

d. Competition: There have been studies conducted ex vivo in mice lung tissue that utilized two-photon microscopy in addition to GRIN lenses. Such experiments yielded conclusive results about how three-photon microscopy, which has its advantages, is possible [9]. In 2007, researcher Murayama and his team created a one-photon endoscope with a GRIN lense. Their probe was quite invasive but it did utilize a microprism which allowed for a 90 degree angle to film the dendrites they were studying [10]. Other researchers took simpler, less invasive approaches and were semi-successful in imaging the brain. Such studies, like done by Osanai, were able to capture cellular fluorescence images and the detection of calcium ions. But their setup couldn't track the single cell activity of the calcium ions [11]. Essentially, many groups have attempted to capture brain activity in vivo but not many have been completely successful.

Works Cited

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