Implantable Light Source Development

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UW BME Design

Abstract

The Sandor lab is investigating immune cell trafficking using photoconversion and optogenetic activation of immune cells utilizing photoactivation. KikGR mouse cells can be photoconverted from green to red when exposed to a 405 nm wavelength light and Ai32 mouse cells can be photoactivated when exposed to a 450-490 nm wavelength range. The current method for photoconversion/photoactivation involves a fiber optic cable with a needle attachment which lacks surface area exposure necessary for efficient photoconversion/activation. A previous semester design that utilized LEDs was improved with a printed circuit board (PCB). LEDs on a breakout board were used for ease of use, testing, and debugging. The LEDs successfully photoconverted KikGR mouse cells and was found to be within the photoactivation range of the Ai32 mouse cells. Temperature changes of LEDs stayed well below tissue coagulation temperatures over time.

Background

- Tuberculosis(TB) is the deadliest single infectious agent [1]
- Antibiotic resistant TB strains are increasing prompting the need for alternative therapies [1]
- The Sandor Lab is investigating immune cell trafficking into granuloma sites and immune cell activation [2]

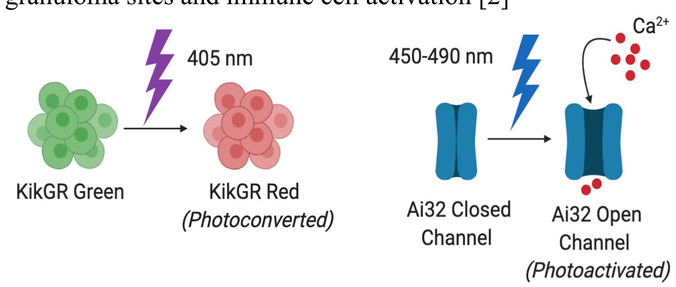

Figure 1. Sandor Lab uses two mouse models: KikGR which has photoconvertible cells when exposed to 405 nm wavelength and Ai32 which has photoactivatable cells that undergo calcium influx by channel rhodopsins after exposure to 450-490 nm light (*Biorender*).

- Their current method utilizes a laser and fiber optic cable which lacks tissue penetration and illumination area

Motivation

Current photoconverting devices are expensive and fail to efficiently photoconvert or photoactivate areas larger than 1 cm^2. Alternatives such as confocal or multiphoton microscopy are expensive and inefficient. A novel, inexpensive design is needed to improve photoconversion and photoactivation to identify immune trafficking and activation.

Design Specifications

- Biocompatible material, emit minimal heat, and non phototoxic
- Temperature below 50-60 °C to prevent tissue coagulation [3]
- 405 nm must photoconvert >1 cm^2 of cell tissue
- 450-490 nm with 1 min 50% duty cycle pulse width modulation
- Minimum intensity of 95 mW/cm^2 for photoconversion and 400 mW/cm^2 for photoactivation
- Simplify previous LED design onto PCB with only 3 wires
- Ideal PCB size: 10mm x 10mm x 1mm (L x W x H)

Final Design

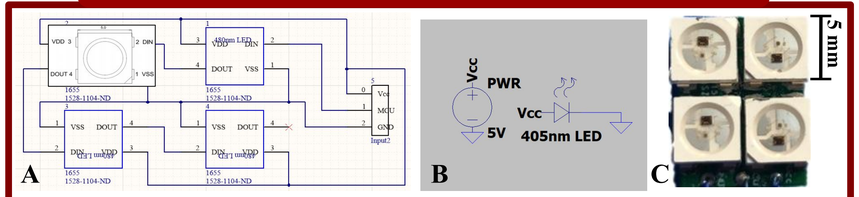

Figure 2. A, PCB schematic of 480 nm LEDs. **B,** 405 nm LED schematic. **C,** 480 nm LEDs on a PCB.

Testing

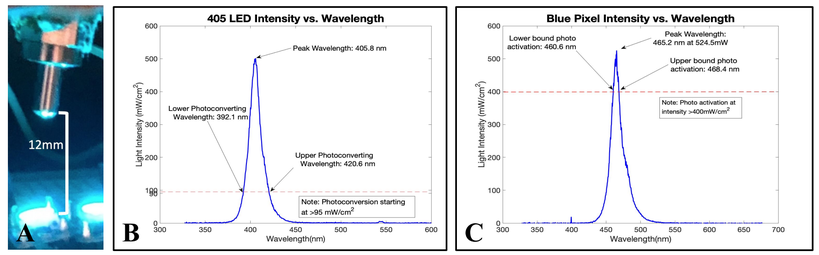

Figure 3. Identifying photoconverting/activating wavelength range of LEDs. A, Device was tested in triplicates using Ocean Optics Spectrometer (USB2000+). **B,** The photoconvertible range of the 405 nm LED is on average from 392.2 nm and 420.6 nm with a peak wavelength at 405.8 nm and intensity of 501.9 mW/cm^2. **C,** The blue LED photoactivating range is on average from 449.9 nm and 486.9 nm with a peak wavelength at 465.2 nm and intensity of 520.73 mW/cm^2 (*MATLAB)*.

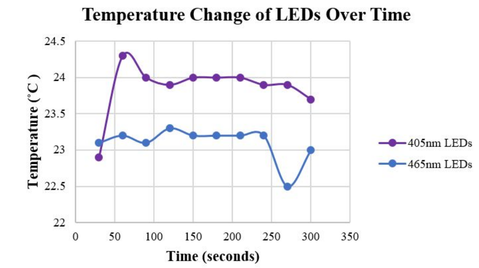

Figure 4. LED Temperature Testing. Temperature was measured from the back of the LEDs for 5 minutes and analyzed (*VassarStats)*. Change in temperature is neither statistically significant nor correlated to time for both the 405 nm and 465 nm LEDs (regression analysis, p=0.565 and p=0.187).

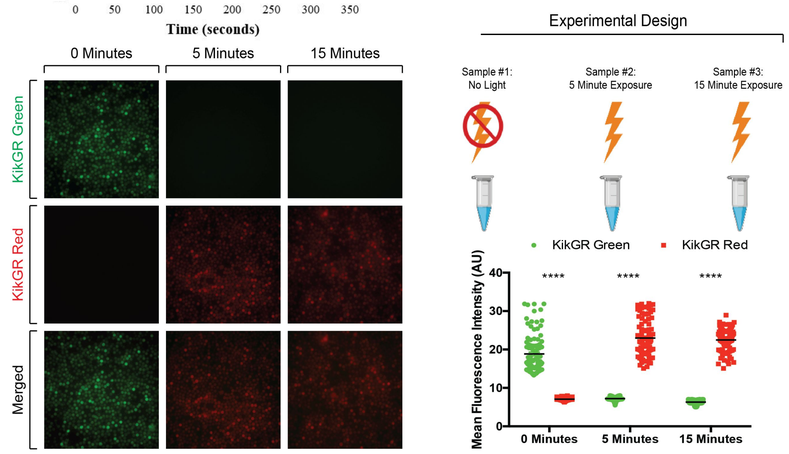

Figure 5. Successful photoconversion of KikGR cells with 405 nm LED. The average mean fluorescence intensity in arbitrary units (AU) of 100 cells were measured using *ImageJ*, and quantified using *Graphpad Prism*. Two-way ANOVA, mean +/- s.e.m., $n = 100$ cells per group, **** $p < 0.0001$. No significant differences between 5 minute/15 minute groups. Cell viability, assessed with trypan blue, showed the LED had no immediate impact on cell viability.

Material

Material	Quantity		Cost
Printed Circuit Board (PCB)	10		$43
DotStar 5050 RGB LED	20		$47.10
5050 LED Breakout PCB	10		$15.97
Microcontroller and Circuitries	N/A		$0.00
Ocean Optics Spectrometer	1	1036 ECB	$0.00
		Total	$106.07

Discussion

Accomplishments:

- Developed user-friendly Arduino code to control LED wavelength, brightness, and pulse width modulation (Fig. 6)
- Designed a printed circuit board to streamline design
- Tested *in vitro* an effective prototype that photoconverted a large area of mice immune cells
- Determined that an LED exposure of 5 minutes is sufficient to photoconvert all cells and will not affect cell viability

Constraints:

- Design restriction → size limits footprint space availability
- Fabrication → solder paste difficulties due to small SMD footprints

Future Work:

- Coat the prototype with PDMS for *in vivo* testing
- Test blue LEDs' ability to photoactivate 450 - 490 nm cells
- Test the prototype *in vivo* and collect data for analysis
- Identify tissue penetration properties (Fig. 7)

```
[<pix_index>/c?<wavelenght(nm)>]
[<pix_index>/b?<brightness>]
[<pix_index>/f?<period(ms)>:<duty_cycle>:<cycles>]
```

Figure 6. The format of the command input for Arduino.

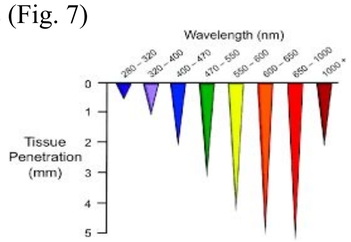

Figure 7. Tissue penetration at different wavelengths [4].

Acknowledgements

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References

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