Inhibition of dihydrotestosterone (DHT) formation via dual wavelength orange-red light production of nitric oxide

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INTRODUCTION

- Androgenetic alopecia (AGA) causes balding in approximately 50% of men and women. One of the main causes of AGA is the conversion of testosterone to dihydrotestosterone (DHT) by 5-α reductase.
- Local administration of red or near-infrared light via low level light therapy (LLLT) has emerged as a safe and effective alternative to systemic medications for AGA.
- Despite multiple FDA-cleared LLLT devices, the mechanisms of action, including DHT inhibition, for LLLT remain unknown.
- Orange-red wavelengths of light have been shown to exert biological effects that may lead to the inhibition of 5-α reductase conversion of testosterone to DHT.
- REVIAN RED® uses light-emitting diodes (LEDs) emitting dual wavelengths from the orange (620 nm) and red (660 nm) portion of the visible light spectrum.
- In this study, we evaluated how red/orange wavelengths of light:
    1. Stimulate nitric oxide (NO) release
    2. Inhibit testosterone conversion to DHT

RESULTS

Red and orange wavelengths of light induce release of NO from nitrosothiols and nitrosylated proteins

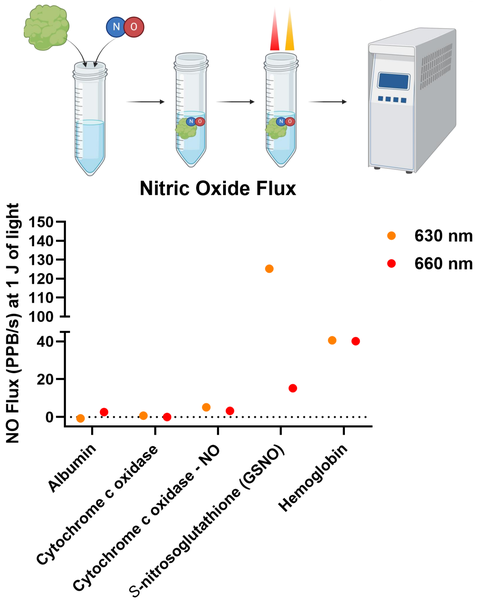


Figure 1. Orange and red wavelengths of LED light induce NO release from nitrosothiols and nitrosylated proteins. Proteins and the low molecular weight glutathione known to store NO *in vivo* were nitrosated/nitrosylated with commercially available NO-donating compounds. Following nitrosation/nitrosylation, the compounds were illuminated with orange (630 nm) or red (660 nm) wavelengths of light for 20 minutes. The amount of NO release was measured via chemiluminescence with a nitric oxide analyzer. Data plotted is NO Flux at 1 J of light energy. Methods figure created with Biorender.com.

Nitric oxide inhibits 5-α reductase conversion of testosterone to DHT

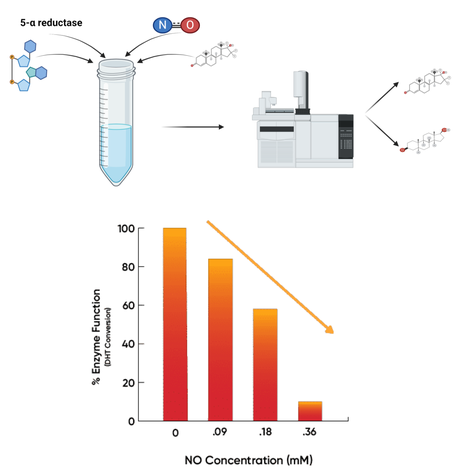


Figure 2. Nitric oxide inhibits conversion of testosterone to DHT by 5-α reductase in a dose-dependent manner. The 5-α reductase enzyme was isolated from human prostate tissue and incubated at 37ºC with an excess of testosterone, NADPH, and S-nitrosoglutathione. The reaction was terminated with acetonitrile. Testosterone and DHT were extracted and concentrations determined via gas chromatography-mass spectrometry. Methods figure created with Biorender.com.

Dual wavelength LEDs reduce the conversion of testosterone to DHT by epithelial cells *in vitro*

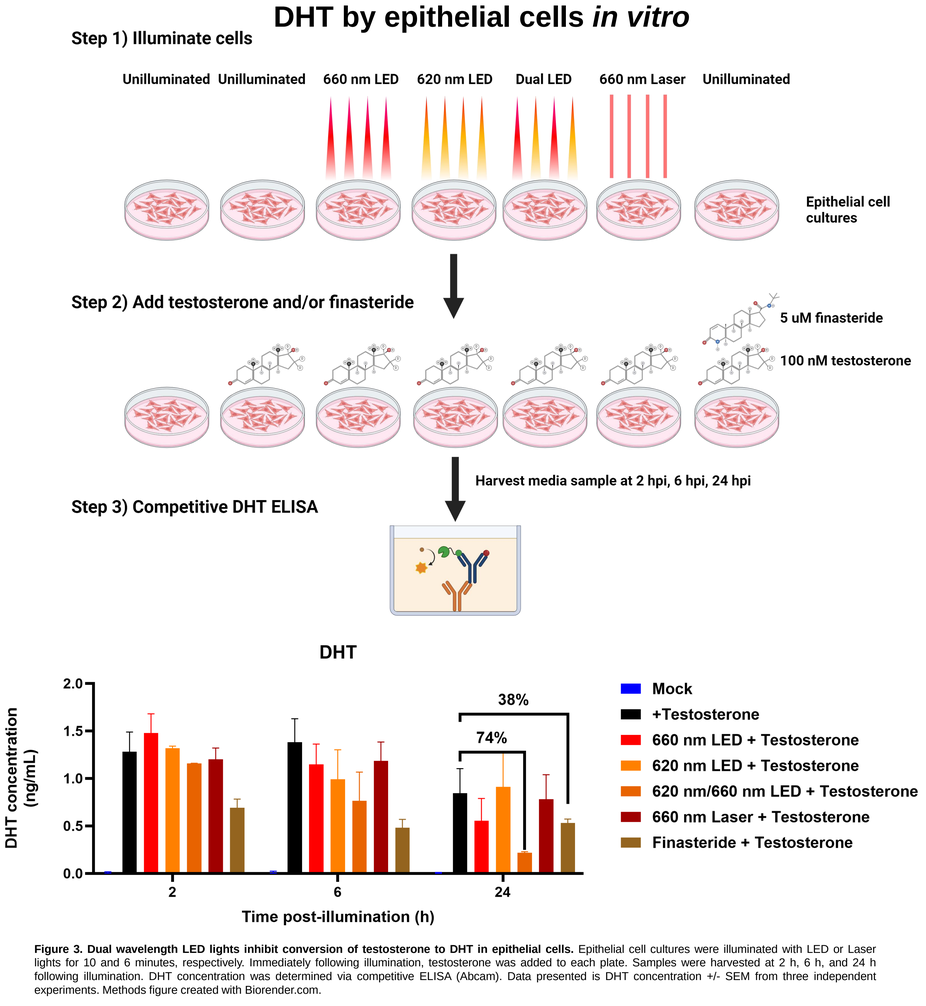


Figure 3. Dual wavelength LED lights inhibit conversion of testosterone to DHT in epithelial cells. Epithelial cell cultures were illuminated with LED or Laser lights for 10 and 6 minutes, respectively. Immediately following illumination, testosterone was added to each plate. Samples were harvested at 2 h, 6 h, and 24 h following illumination. DHT concentration was determined via competitive ELISA (Abcam). Data presented is DHT concentration +/- SEM from three independent experiments. Methods figure created with Biorender.com.

Dual wavelength LED light induces NO and ROS production in epithelial cells *in vitro*

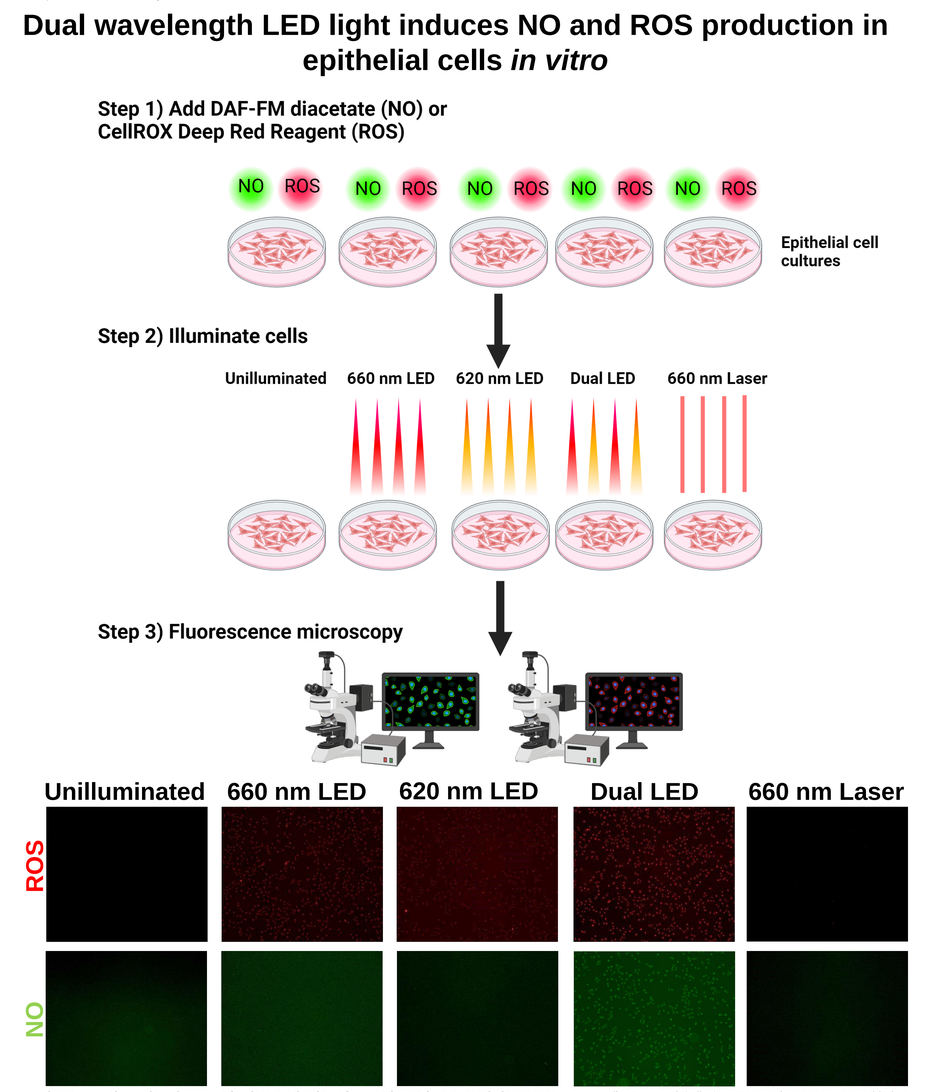


Figure 4. Dual wavelength LEDs stimulate production of NO and ROS in a synergistic manner. DAF-FM diacetate and CellROX Deep Red Reagent were added to determine production of NO and ROS, respectively. Cells were illuminated with LEDs and lasers for 10 and 6 minutes, respectively. ROS and NO production were measured immediately following illumination (0 hours post-illumination). Representative images of two independent experiments. Methods figure created with Biorender.com.

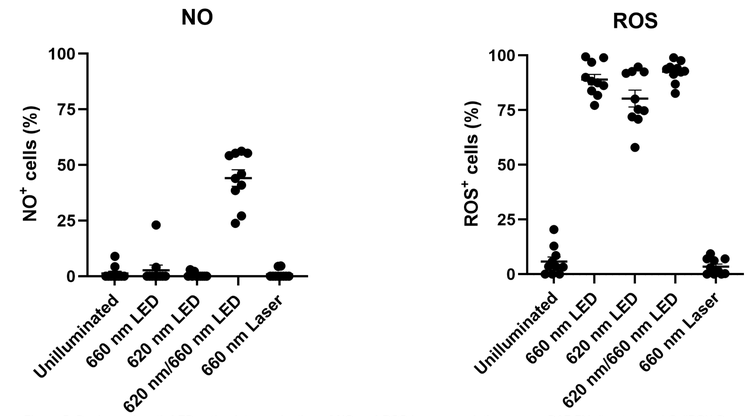


Figure 5. Dual wavelength LEDs stimulate production of NO and ROS in a synergistic manner. DAF-FM diacetate and CellROX Deep Red Reagent were added to determine production of NO and ROS, respectively. Cells were illuminated with LEDs and lasers for 10 and 6 minutes, respectively, to mimic delivery of laser-based and LED-based LLLT medical caps. ROS and NO production were measured immediately following illumination (0 hours post-illumination). Data presented are percent positive cells +/- SEM relative to nuclear fluorescence (DAPI). Data acquired across two independent experiments. Methods figure created with Biorender.com.

CONCLUSIONS

Dual wavelengths of 620 nm and 660 nm visible light maximally stimulate the production of reactive oxygen species and nitric oxide, decreasing the production of DHT *in vitro*.

The results of our study provide evidence of the ability of LED-based LLLT therapies to stop hair loss by decreasing production of DHT.

This conclusion is supported by the following data:

1. Orange-red wavelengths of light can induce the release of NO from nitrosothiols and nitrosylated proteins.
2. Nitric oxide inhibits 5-α reductase enzymatic conversion of testosterone to DHT *in vitro*.
3. Using a translational optics platform, we demonstrated the production of NO and ROS by dual wavelength LEDs in epithelial cells *in vitro*.
4. Using the same translational optics platform, we demonstrated that dual wavelength LEDs inhibit DHT formation in epithelial cells *in vitro*.
5. Dual wavelength-based LED illumination induced stronger biological effects *in vitro* than individual wavelength LED-based illumination, suggesting a synergistic effect between the two wavelengths.
6. However, 660 nm laser-based illumination did not have any impact on the production of DHT in epithelial cell cultures.

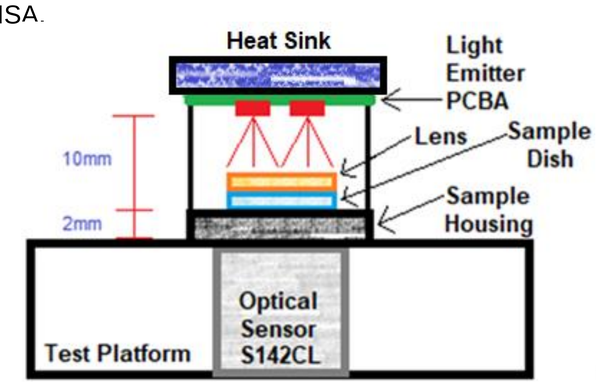
Translational Optics Platform

We designed a translational optics platform that:

- Simulates the distance from emitter to scalp (~10 mm).
- Mimics end product design for LLLT devices, including power input, irradiance, and fluence.

Using this platform, we:

1. Detected the stimulation of NO in epithelial cells at the time of illumination using DAF-FM diacetate.
2. Detected the stimulation of ROS in epithelial cells at the time of illumination using CellROX Deep Red Reagent.
3. Determined the conversion of testosterone to DHT by epithelial cells following illumination using competitive ELISA.



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