



MODELING DIRECTED-ENERGY LASER INJURY USING HUMAN iPSC-DERIVED RETINAL PIGMENT EPITHELIUM

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Combat Casualty Care & Operational Medicine

READINESS THROUGH RESEARCH & DEVELOPMENT



DISCLAIMER

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NON-DISCLOSURE

- Hope Newcomer-Neeson, MSc has no conflicts to report.



MILITARY RELEVANCE

- Q-switched Neodymium-doped Yttrium Aluminum Garnet (Nd:YAG) lasers are some of the most widely used and critical solid-state lasers in modern military applications
- Directed-energy systems and high-intensity pulsed lasers present an increased risk of ocular injury
- A review of the Defense and Veterans Eye Injury and Vision Registry found 132 U.S. service members with documented laser exposure from 2006-2018

Harris et al.; JTS CPG ID 79, 2020



RETINAL PIGMENTED EPITHELIUM

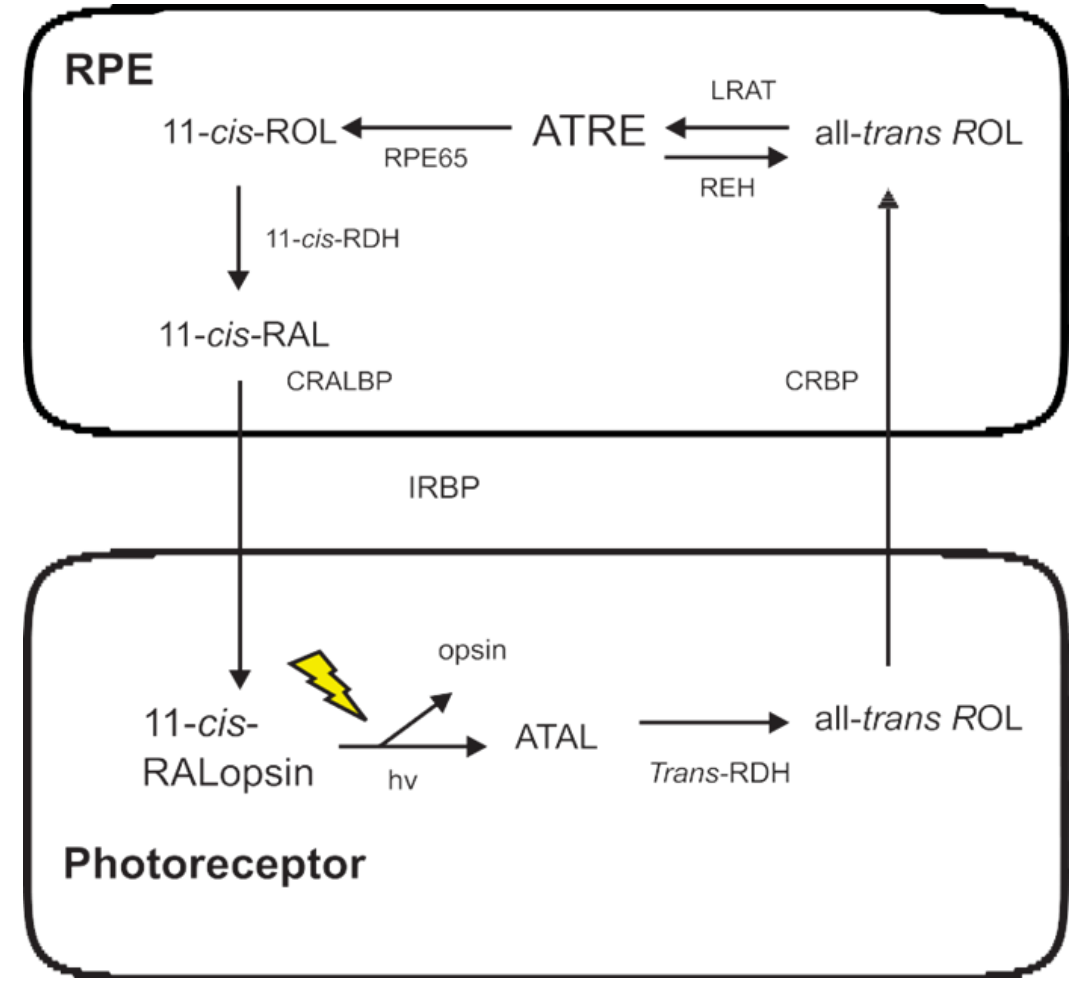
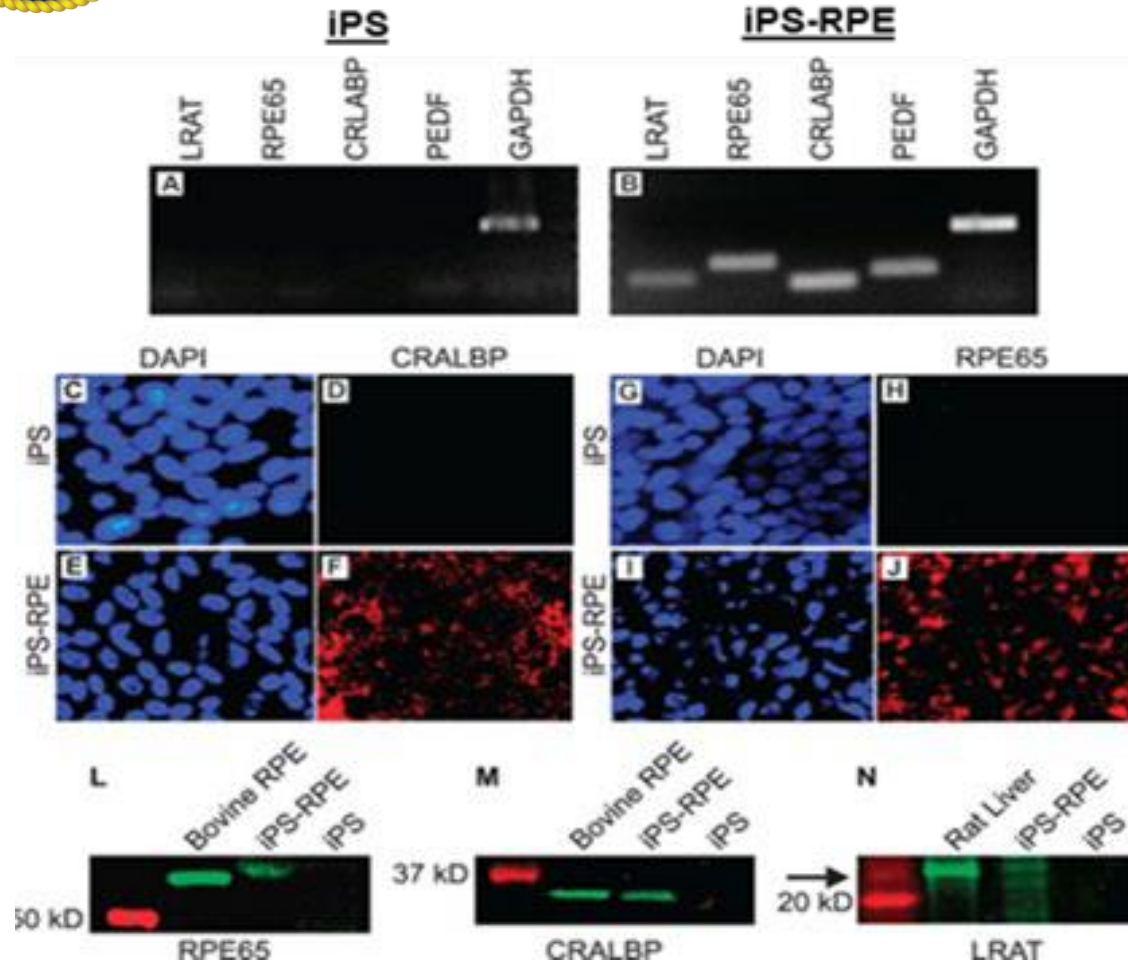
- Retinal Pigmented Epithelium (RPE) supports photoreceptor survival and visual function
 - Maintains the outer blood-retina barrier
 - Phagocytoses photoreceptor outer segments
 - Regulates fluid, ion, and metabolic exchange

Induced pluripotent stem cell-derived RPE (iPS-RPE) offers a highly physiological, human-relevant alternative to animal models and immortalized cell lines, which often lack key functional and structural characteristics.

Feature	Biological Relevance
Authentic Pigmentation	High intracellular melanin content, mirroring <i>in vivo</i> RPE.
Functional Visual Cycle	Expresses visual cycle proteins and actively participates in retinoid processing
In Vivo-like Morphology	Classic, tightly packed hexagonal cobblestone morphology with tight junctions



RETINAL PIGMENTED EPITHELIUM



Invest Ophthalmol Vis Sci, 2014 Jan 9;55(1):198-209



COMPLICATION

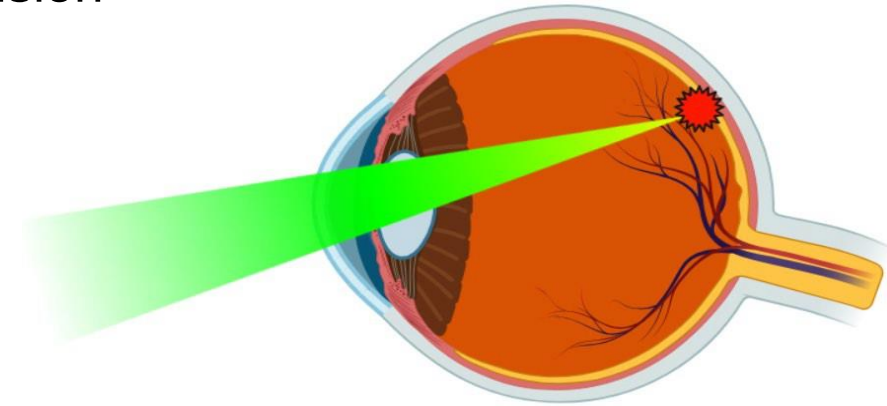
Short duration laser exposures can rapidly damage the retinal pigmented epithelium (RPE) leading to permanent visual impairment

The lens can focus laser light onto the retina, increasing the energy density up to 100,000 times compared to the incident beam

The retina contains photoreceptors and neural tissue needed for vision

Mechanisms of Damage

- Thermal damage
- Photochemical damage
- Acoustic/ photodisruptive damage



Laser-induced retinal injury occurs as ocular focusing concentrates energy at the retina. Created with BioRender.com



WHY THE CELL RESPONSE MATTERS

- Laser exposure can create localized ocular injury
- Surviving cells may activate repair-like responses such as an increased expression of TGF- β 2
- Proliferation, migration, and Epithelial-Mesenchymal Transition (EMT) can contribute to intraocular fibrosis
- Modeling these responses *in vitro* allows controlled study of post-injury mechanisms



NEED FOR A CONTROLLED IN VITRO LASER INJURY PLATFORM

Why is this model necessary?

- Human injury data are limited and variable
- *In vivo* models capture complexity but are lower throughput
- Early mechanistic studies require controlled, reproducible systems
- An *in vitro* Nd:YAG model can isolate cellular wound-response mechanisms

Approach	Strength	Limitation
Human Data	Clinically Relevant	Variable, retrospective
Animal Models	Whole-eye response	Cost, ethics, lower throughput
<i>In vitro</i> Model	Controlled, repeatable	Reduced biological complexity



OBJECTIVE & HYPOTHESIS

Objective

Develop an *in vitro* Nd:YAG laser injury model to characterize post-exposure ocular cell responses.

Hypothesis

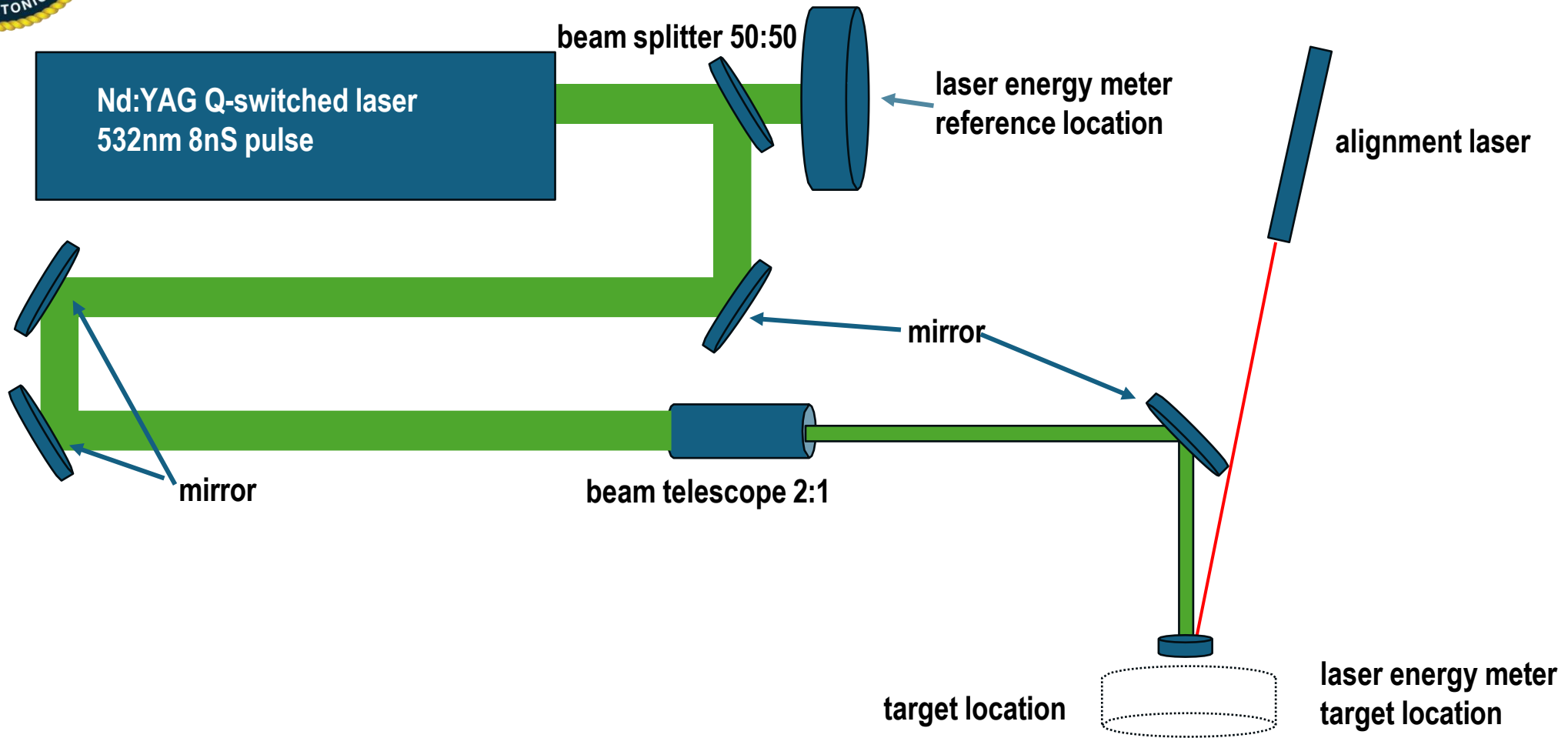
Nd:YAG laser exposure will produce a consistent localized injury site, followed by cell migration and proliferation, while TGF- β 2 treatment will enhance fibrotic marker expression within the injury region.

Outcome measures

- Proliferation
- Migration
- EMT marker expression/ morphology



MODEL

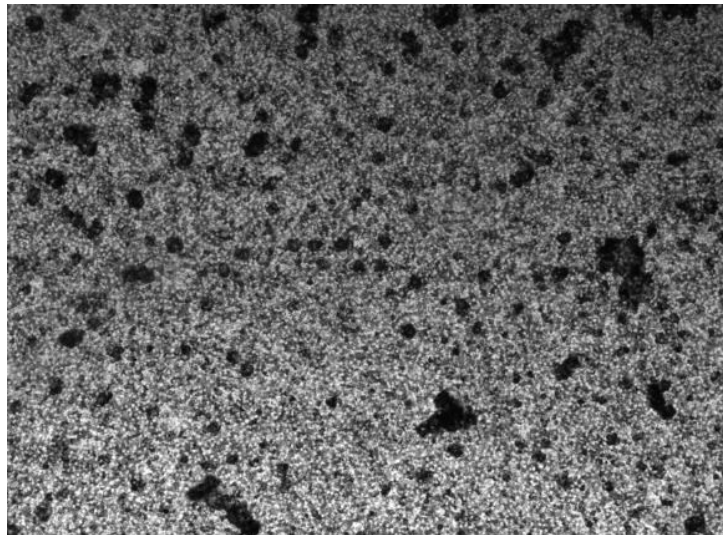


Optical schematic for laser exposure system

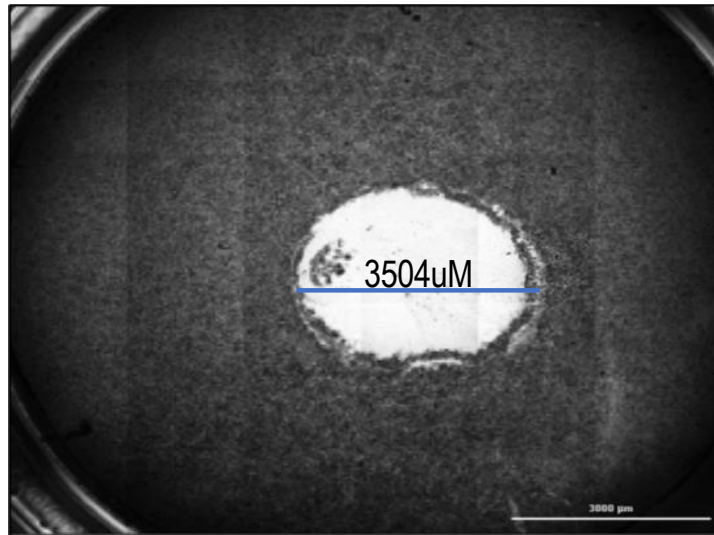


Nd:YAG EXPOSURE PRODUCES A DEFINED INJURY REGION

- Laser exposure generated a localized injury/stress region
- Cells surrounding the exposure site showed altered morphology
- A defined injury area allowed assessment of post-exposure cellular behavior



Control



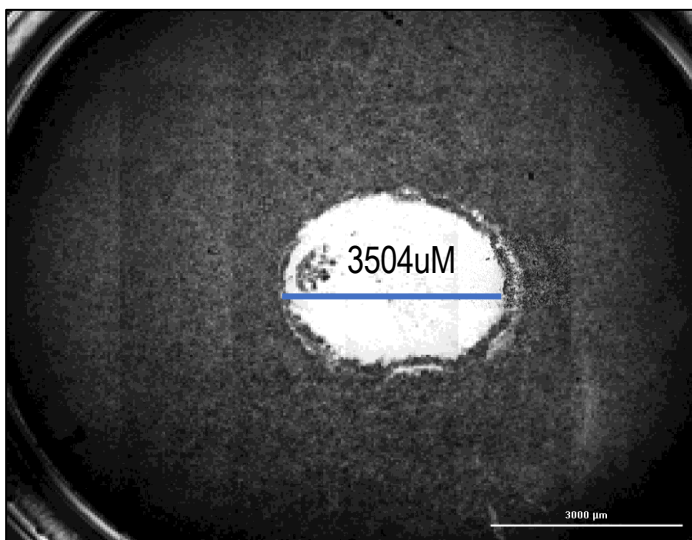
Nd:YAG Exposed

Representative control and Nd:YAG-exposed cultures showing localized exposure region and surrounding cellular response.

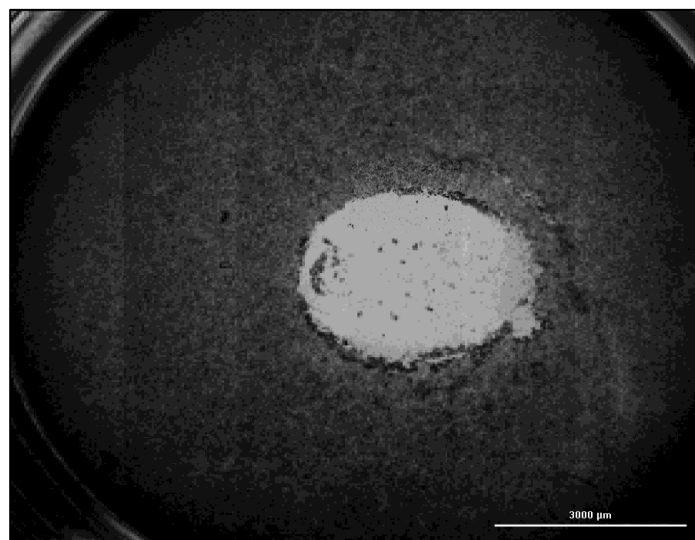


CELLS MIGRATE INTO THE INJURY SITE BY DAY 7

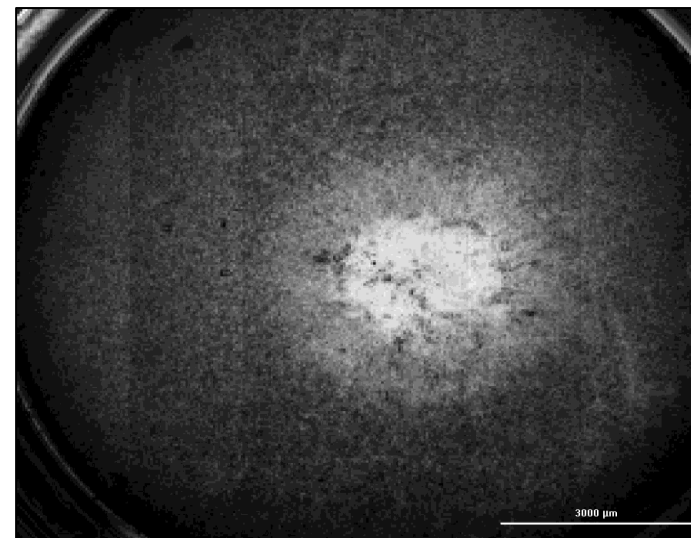
- Light microscopy revealed cell migration into the injury site by day 7
- Migrating cells showed low to negligible pigmentation
- Early repopulation suggests activation of a wound-healing response
- The injury site remained visible but showed evidence of cellular movement inwards



Post Laser Exposure



24hr

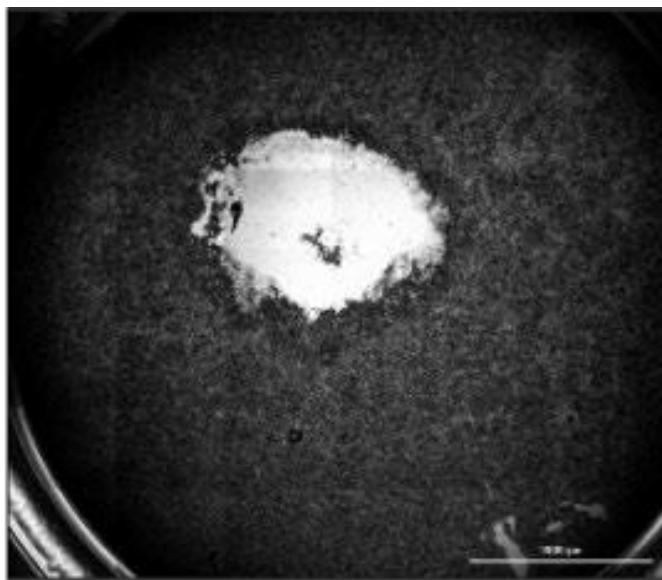


Day 7

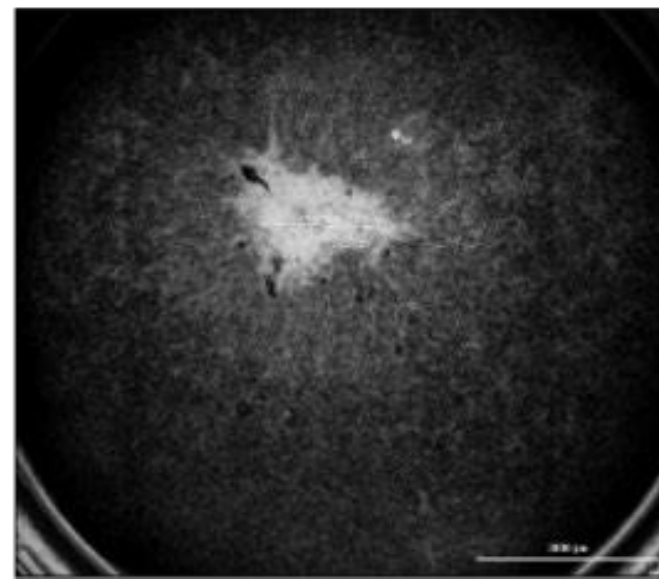


BY DAY 14, THE INJURY SITE IS REDUCED AND REPOPULATED

- Injury site decreased to approximately 1.59 ± 0.1 mm
- The site was populated by marginally pigmented cells
- DAPI staining indicated increased cellular presence within the injury region
- Findings support both migration and proliferation during repopulation



Post Laser Exposure (Immediate)



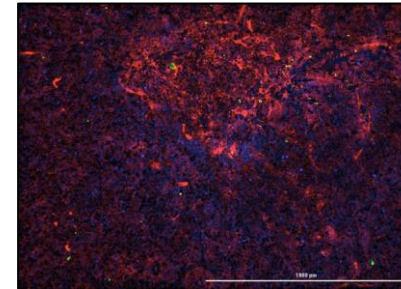
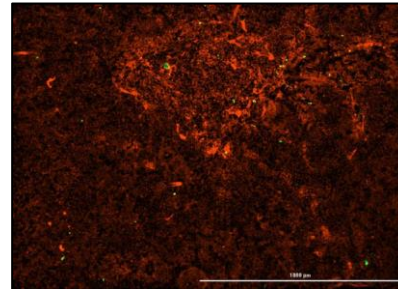
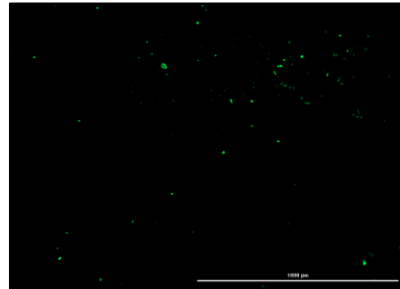
Day 14



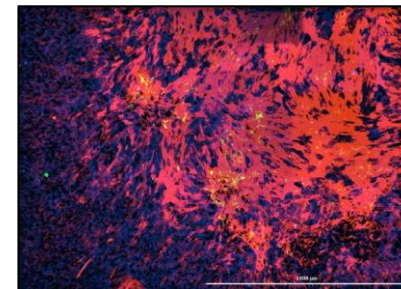
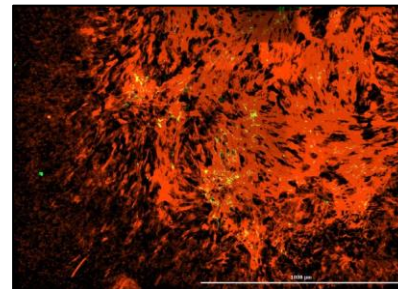
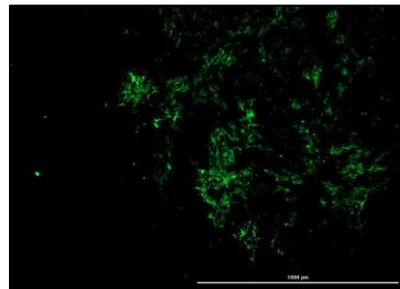
TGF- β 2 ENHANCES FIBROTIC RESPONSES AT THE INJURY SITE

- TGF- β 2 treatment significantly increased α -SMA expression at the injury site
- TGF- β 2 treatment significantly increased fibronectin expression at the injury site
- Minimal positive staining was observed at the periphery
- Fibrotic marker expression was spatially concentrated in the injured region

Day 14: CM



CM plus TGF β 2



Fibronectin (FN)

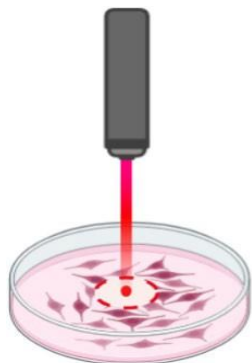
Fn + α SMA

Fn + α SMA + DAPI

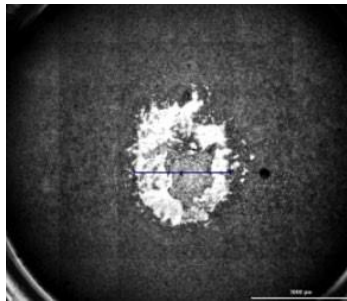


INJURY MODEL CAPTURES POST-INJURY REPAIR AND FIBROTIC REMODELING

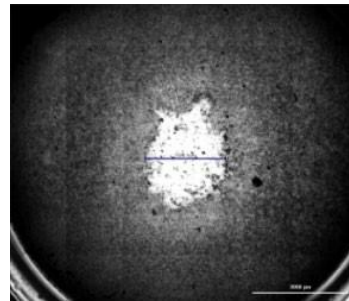
- Laser exposure produced a reproducible ablation site
- Cells migrated into the injury region by day 7
- Injury site size decrease by day 14 with evidence of repopulation
- TGF β -2 amplified injury-site fibrotic marker expression
- The model captures both repair-like and fibrosis-associated responses



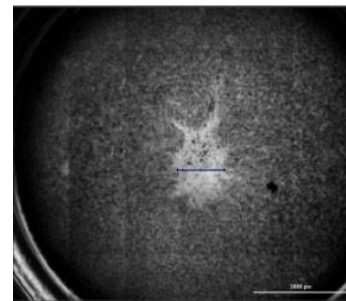
Nd:YAG Laser
Exposure:
Defined 2.5 mm
target region



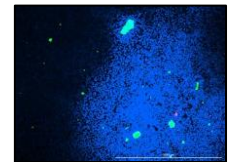
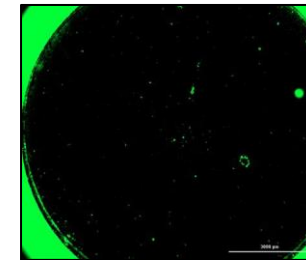
Localized cell ablation
Injury Site:
 2.88 ± 0.05 mm



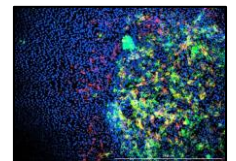
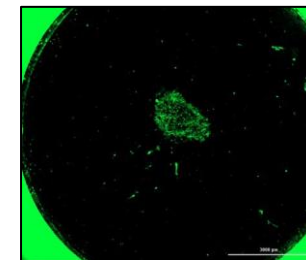
Day 7 migration
Low pigmented cells
migrate into injury site



Day 14 repopulation
Measure of low pigmented
cells: 1.59 ± 0.05 mm



Inset Image at
wound site



Inset Image at
wound site



A PLATFORM FOR STUDYING LASER-INDUCED OCULAR INJURY AND THERAPEUTIC SCREENING

- Produces consistent, reproducible injury in targeted regions
- Allows measurement of injury size, migration, repopulation, and fibrosis
- TGF- β 2 enhances severity and fibrotic marker expression
- Provides a controlled platform for studying cellular and molecular responses to laser irradiation
- Can be used to screen therapeutics targeting migration, proliferation, EMT, or fibrosis



LIMITATIONS & FUTURE DIRECTIONS

Limitations

- *In-vitro* model does not fully replicate the intact eye
- Lacks complete immune, vascular, and mechanical environment
- Pigmentation changes require further phenotypic characterization
- Additional molecular markers are needed to confirm EMT/fibrotic pathways

Future Directions

- Validate additional EMT and fibrosis markers
- Quantify migration and proliferation over more time points
- Optimize laser parameters and TGF- β 2 dosing
- Screen candidate anti-fibrotic or wound-modulating therapeutics



CONCLUSIONS

- An *in vitro* Nd:YAG laser injury model was established
- Laser exposure produced localized, reproducible cell ablation in targeted regions
- Cells migrated into the injury site by day 7
- By day 14, the injury site was reduced and repopulated by marginally pigmented cells with DAPI-stained nuclei
- TGF- β 2 significantly enhanced α -SMA and fibronectin expression at the injury site
- This model provides a robust platform for studying cellular and molecular responses to laser irradiation and screening potential therapeutics



ACKNOWLEDGEMENTS

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- **Naval Medical Research Unit- San Antonio**
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