

Purpose:

Primary open angle glaucoma (POAG), a blinding optic neuropathy, often results from elevated intraocular pressure due to obstruction of aqueous humor outflow via the trabecular meshwork (TM). Reduced TM cellularity in POAG has been reported to contribute to the elevated pressure. A common treatment for glaucoma is laser treatment, which lowers the intraocular pressure. Our previous work showed that the medium from laser-treated human cadaver eyes (conditioned medium) increased TM insert stem cell division and migration to untreated fresh eyes. This suggested that there was a factor causing the cell division and migration which was secreted into the medium from the laser-treated TM cells. Previous studies also suggested that the "laser factor" could be cytokine molecules TNF-alpha (TNF-a) or IL-1 alpha or IL-1 beta (IL-1a or IL-1b), or some combination of these, but this is unclear. Our purpose is to isolate and identify this "laser factor" to increase the TM cell number and restore TM intraocular pressure to normal. This "laser factor" could later be chemically synthesized and placed into eye drops to use as a novel alternative treatment for glaucoma.

Methods:

Laser Treatment: Human cadaver eyes were subjected to 360 degree treatment of the TM by Selective Laser Trabeculoplasty (SLT) using the Lumenis Selecta II laser. There were 70 spots administered per eye, with a 0.8mJ setting per spot. The sham-treated cadaver eyes had the same exact treatment, except that the laser was on standby, so no actual laser light reached the TM.

Cell and Organ Culture: TM cell culture was conducted according to standard procedures using Dulbecco's Modified Eagle's medium (DMEM) with medium glucose, 10% FBS and 1% antibiotic/antimycotic. Whole globes were dissected to anterior segment preparations consisting of the cornea, TM, Schlemm's canal, and a rim of sclera. These were maintained in stationary organ culture for up to 1 week prior to laser or sham laser treatment in serum-free DMEM (medium glucose) with 1% antibiotic/ antimycotic. Conditioned medium is the standard medium for TM cell culture (this paragraph) that has been already on cultured TM cells. These cells secrete substances into the medium that may be growth factors, cytokines, or other molecules. This media then may cause various responses in fresh TM cell cultures.

ELISA Assay: (Enzyme-linked immunosorbent assay) This assay was used to detect the presence of TNF-alpha and IL-1-beta after SLT laser treatment.

Cell Division: The cell division assessment subsequent to laser treatment was performed with *Click-iT Edu (Invitrogen). This method visualized dividing TM insert stem cells by intercalating into the DNA of dividing cells, when combined with confocal microscopy. Edu molecules are visible 24 hours after SLT laser treatment as they are stained bright green. We are counting hundreds of cells to get good statistical analysis (Poisson distribution). This will be a comparison of two sets of groups, either lasered and the sham-lasered anterior segments, or the conditioned media that is either laser-treated or sham-lasered in paired eyes, and we will use the paired t-test for significance analysis. This analysis is part of this ongoing investigation.

References:
* Bradley, Kelley, et. al., IOVS, 2000

Fig. 1. Structures of the anterior segment of the eye near the trabecular meshwork (TM) and Schlemm's Canal.

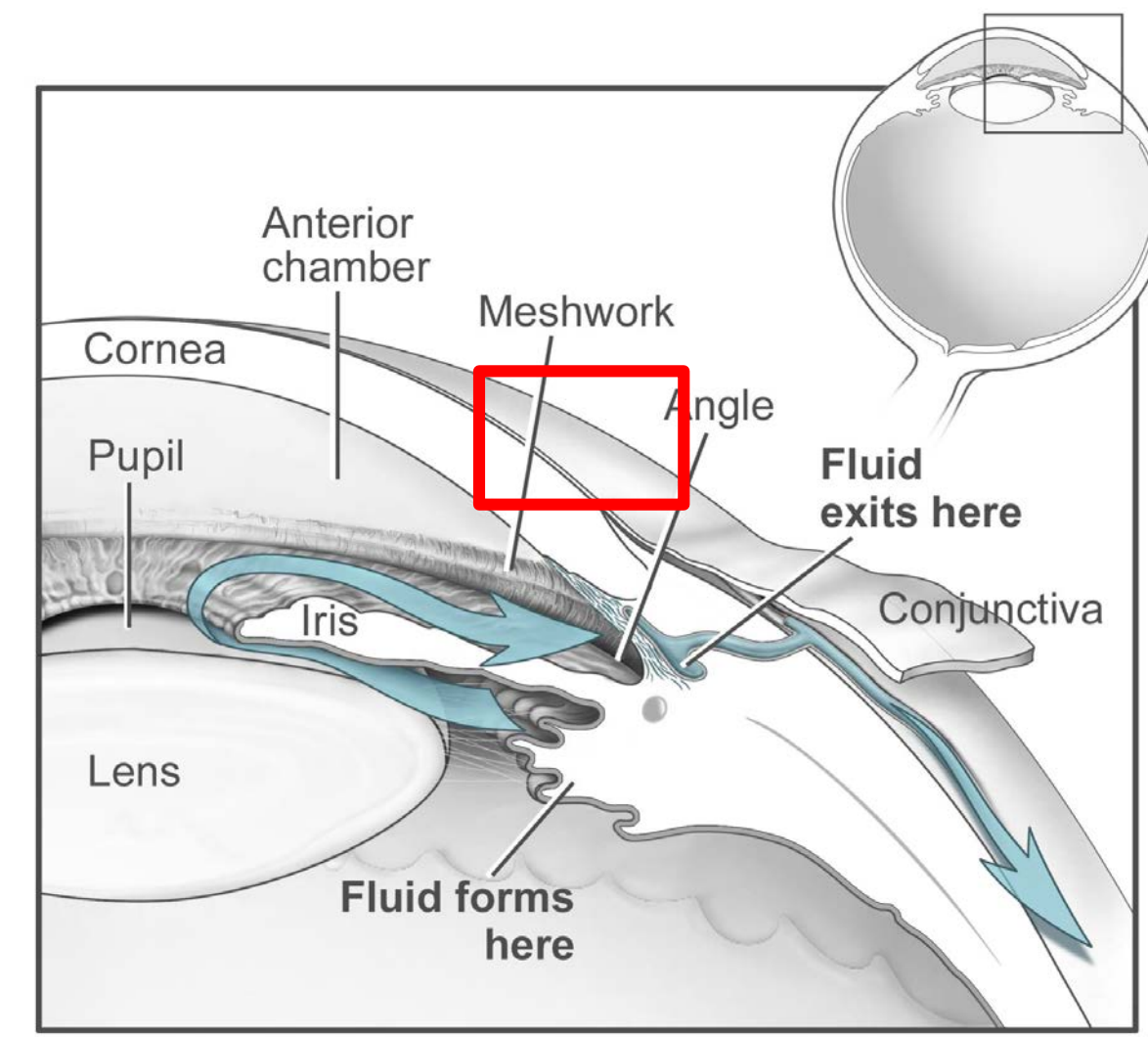


Fig. 2. TM anatomy showing the aqueous humor flow, TM beams (extracellular matrix), attached TM cells, JCT layer of TM, Schlemm's Canal inner wall cells (blue), TM insert stem cells (left in purple).

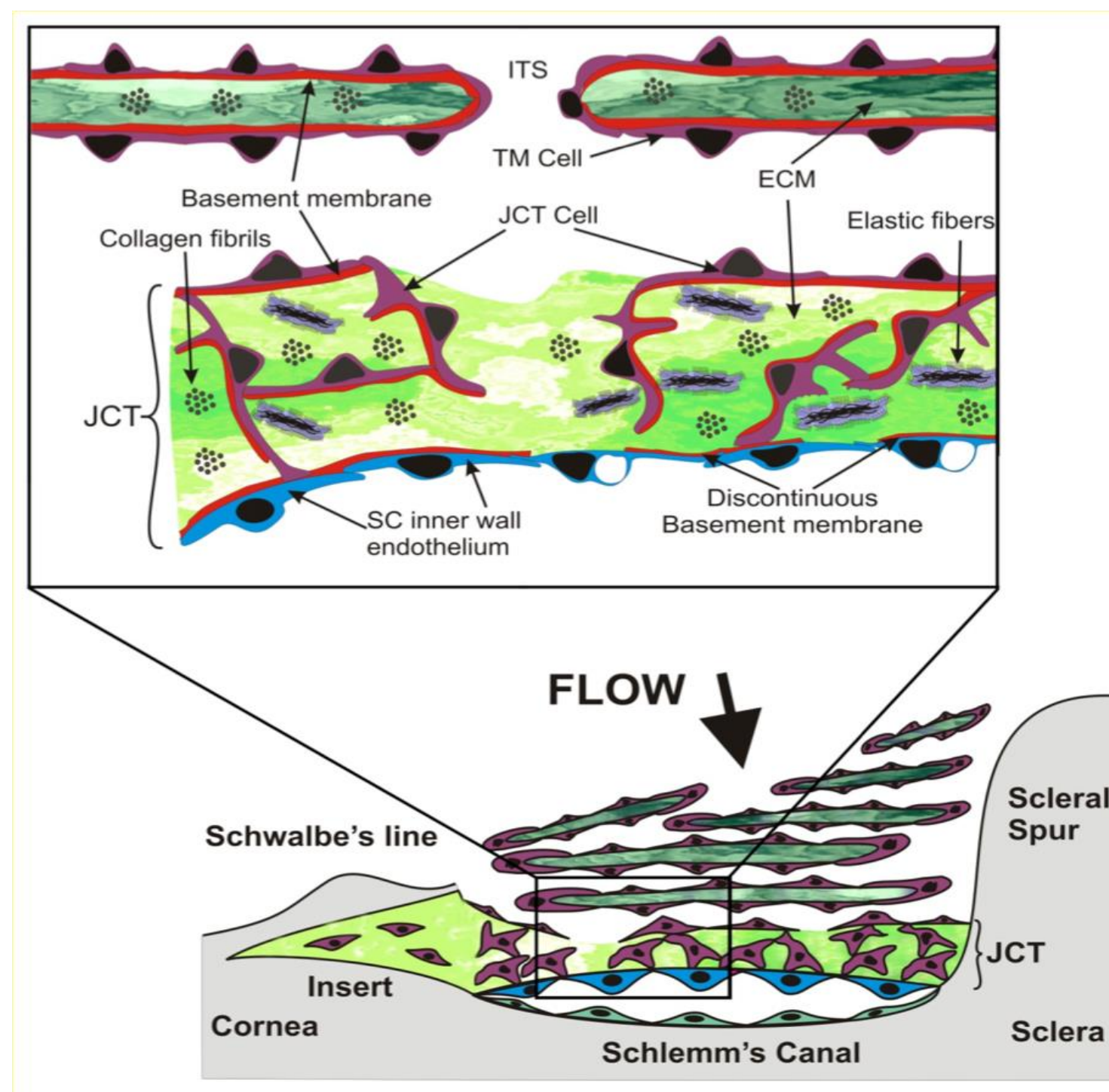


Fig. 3. Conditioned media from laser-treated eyes 8 hrs post-treatment stimulated TM insert stem cells to increase cell division when placed in stationary organ culture post-laser treatment. *

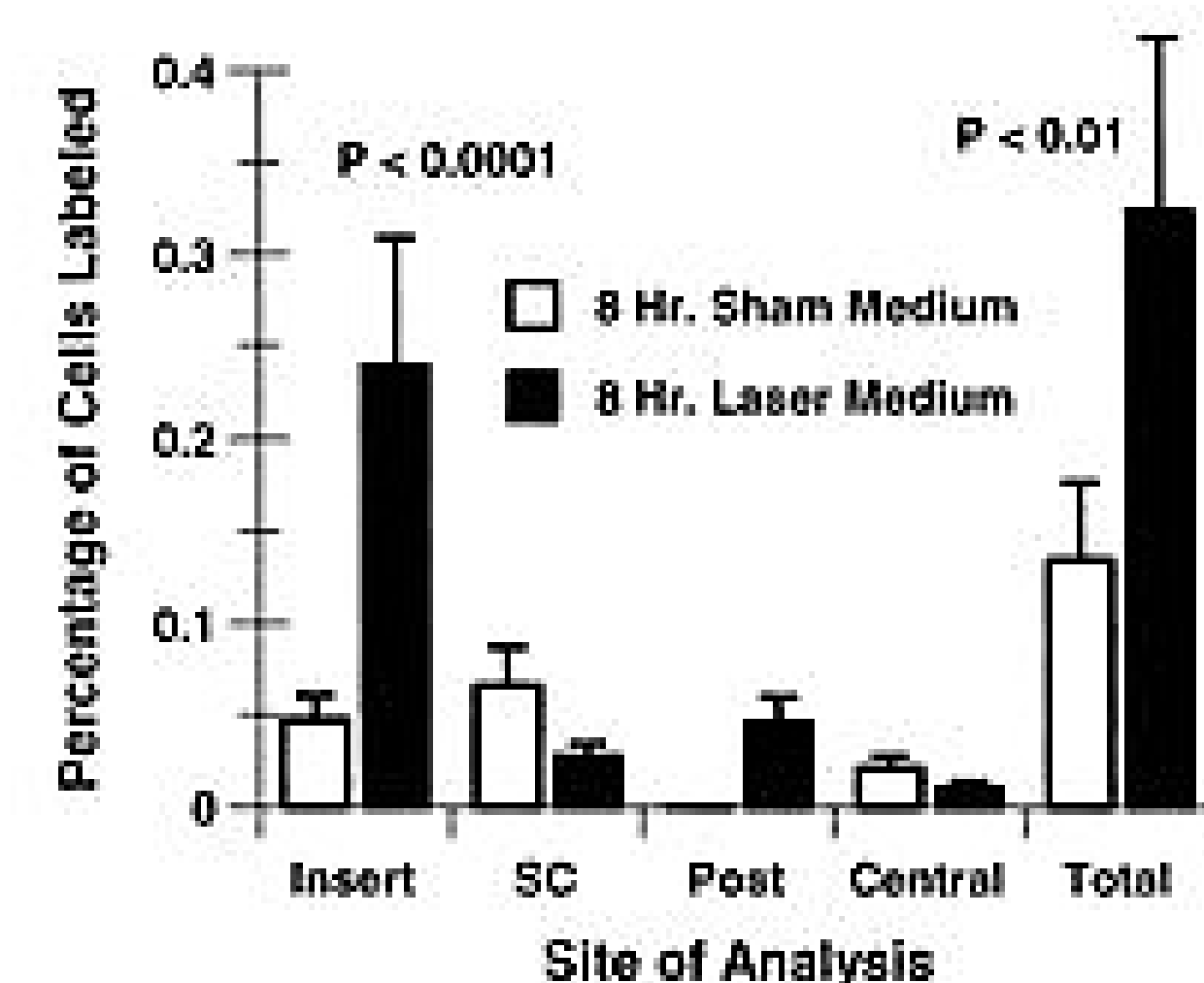


Fig. 4-Plan of Action

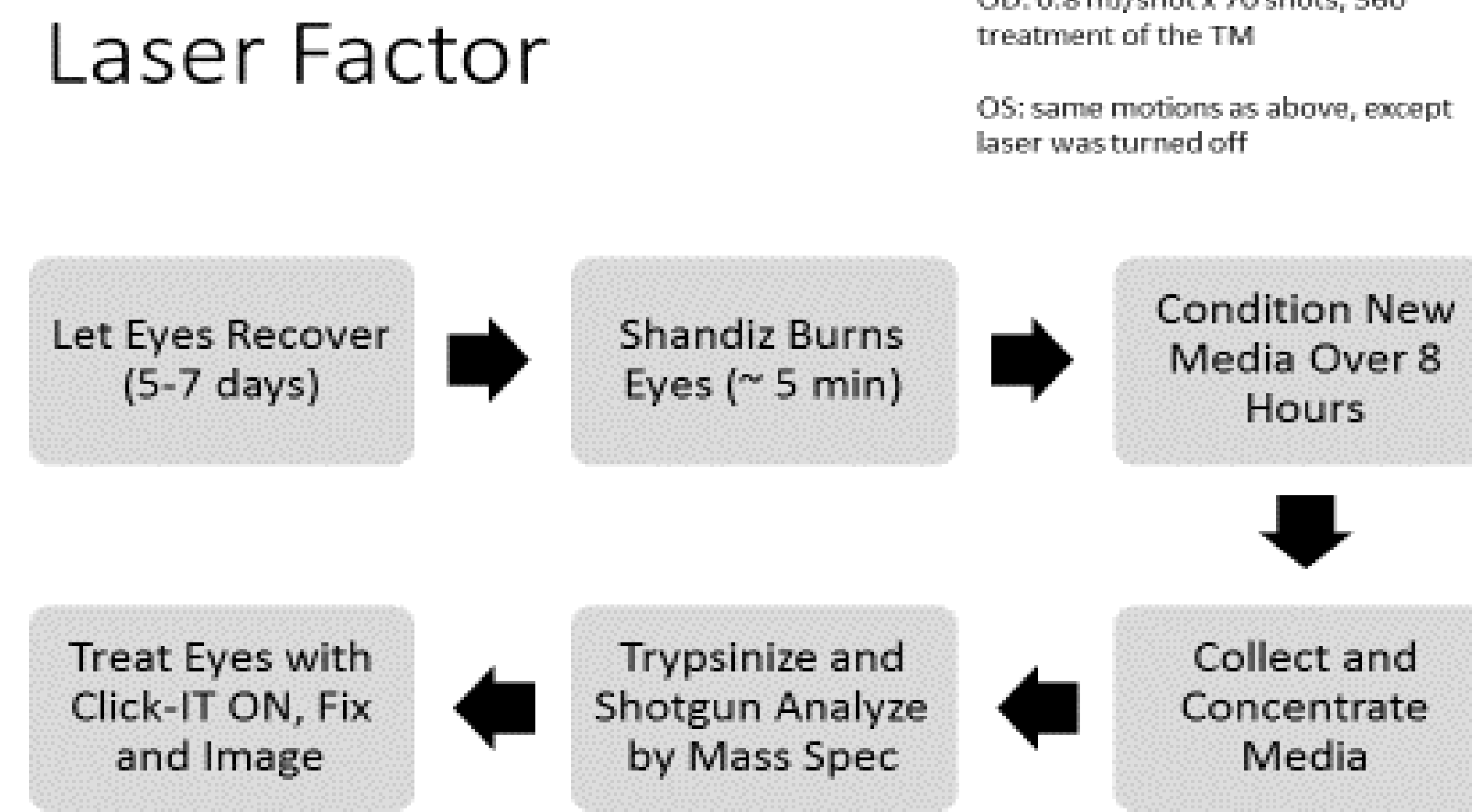


Fig. 5. Dose-Response Curve for TNF alpha in porcine TM cell culture (48 hr. treatment)

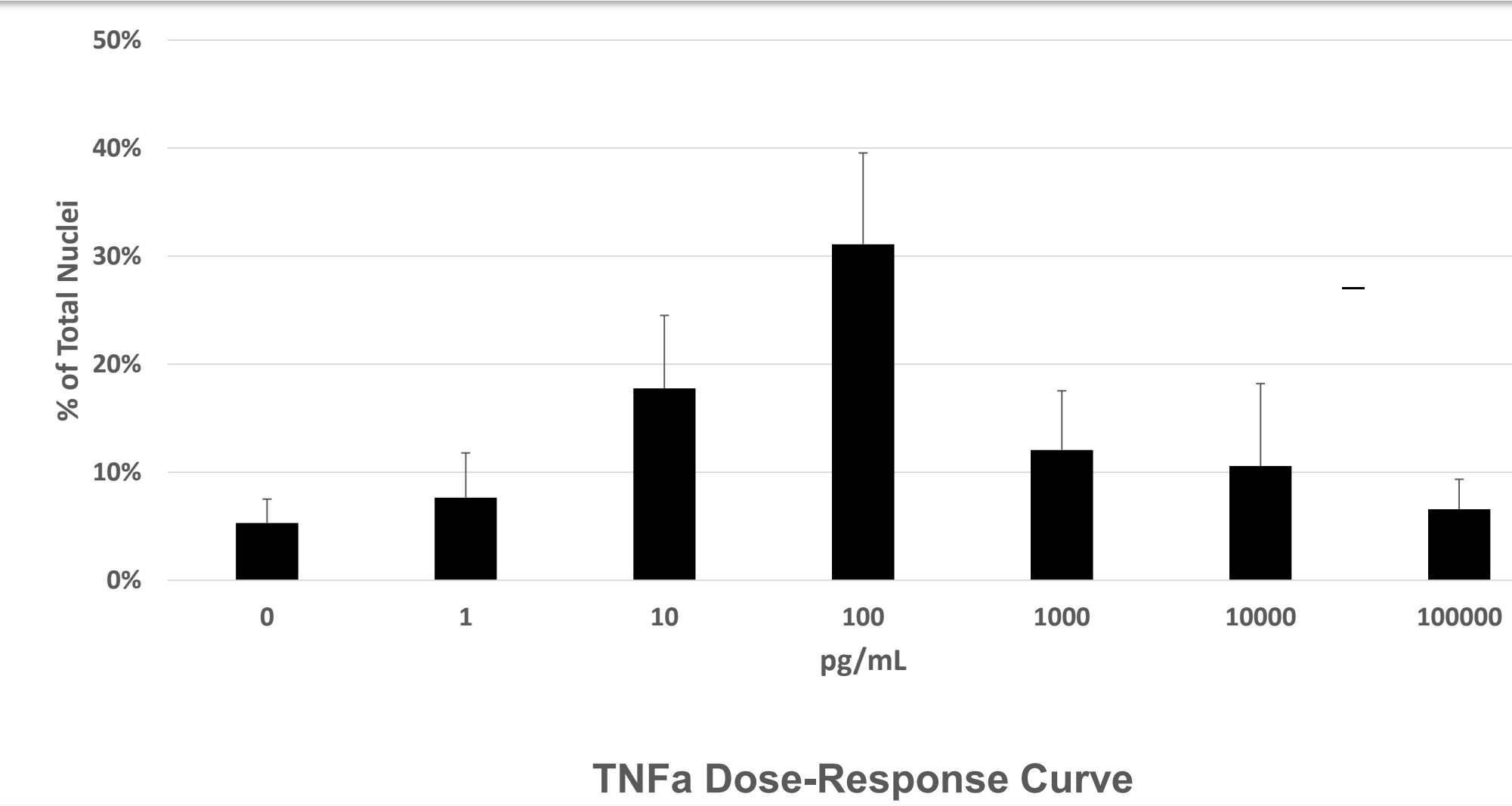


Fig. 6. Dose-Response Curve for IL-1 alpha in porcine TM cell culture (48 hr. treatment)

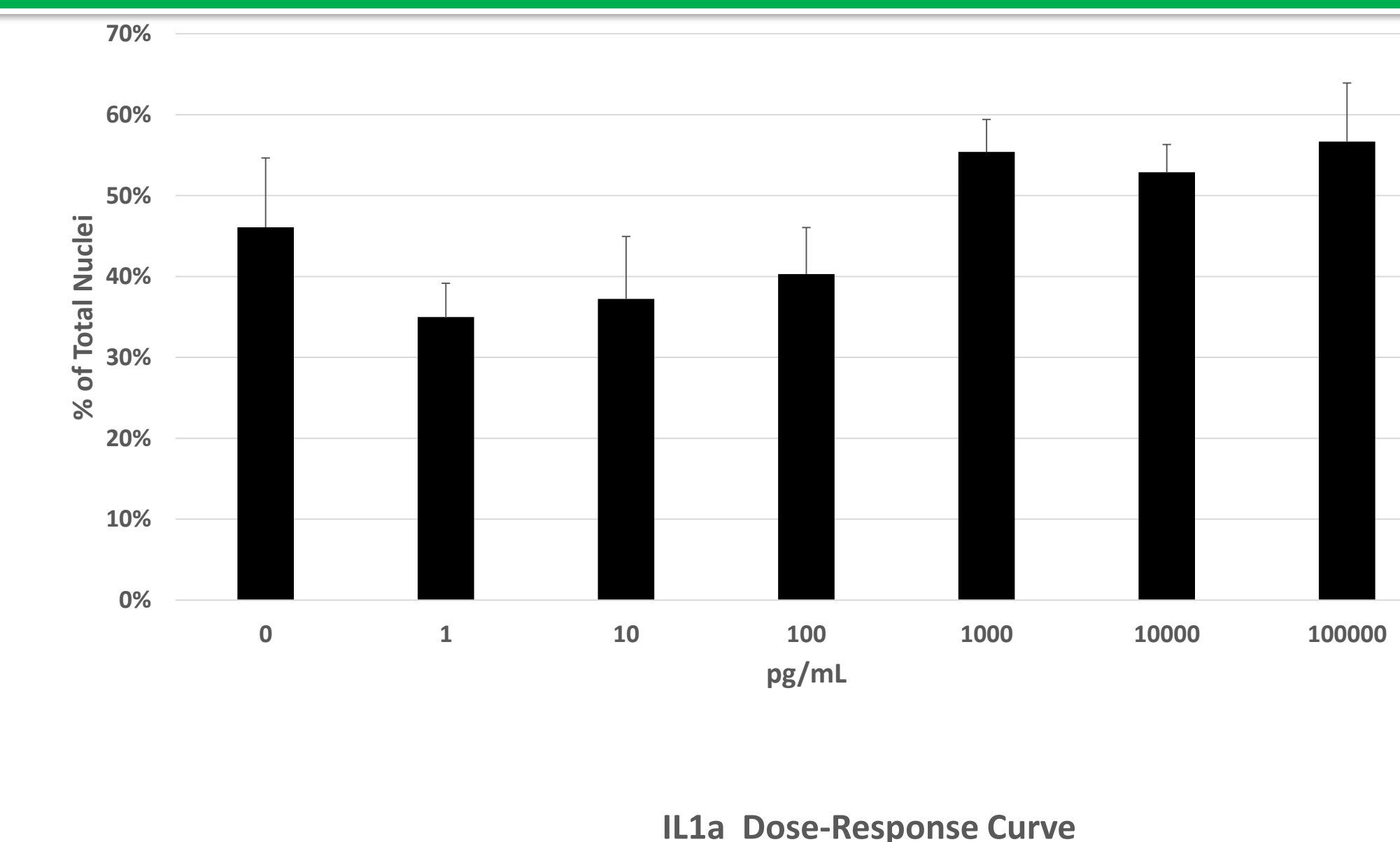


Fig.7. Increased TNF-alpha and IL-1beta Concentration after Laser Treatment (ELISA assay)

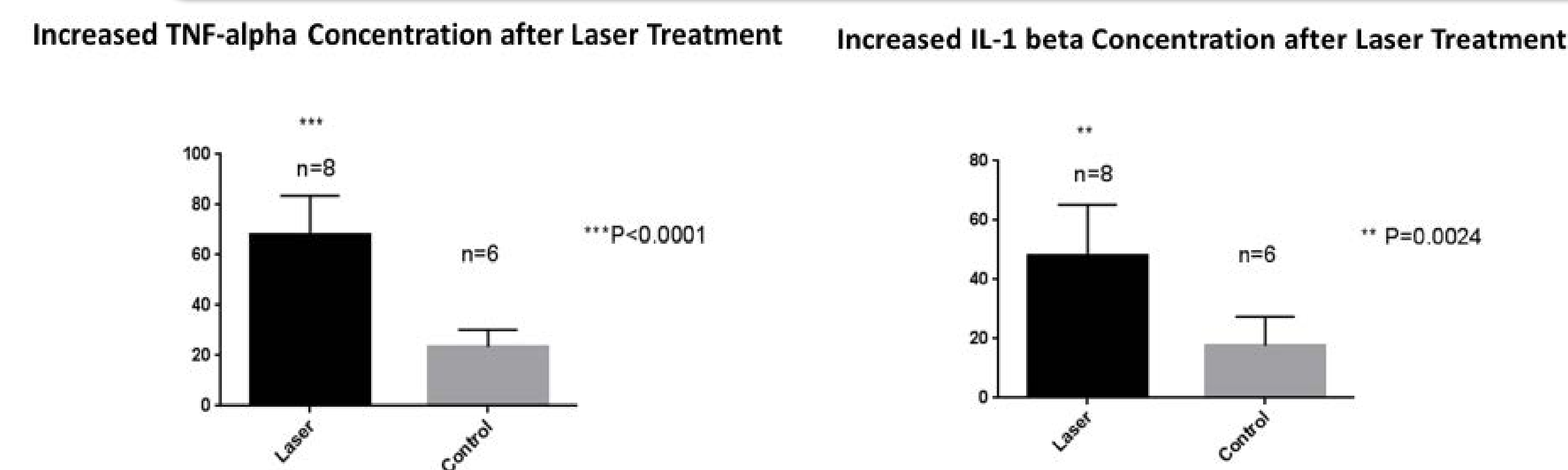


Fig.8. Porcine TM Cell Division Responses to Various Treatments N=2 with internal replicate.

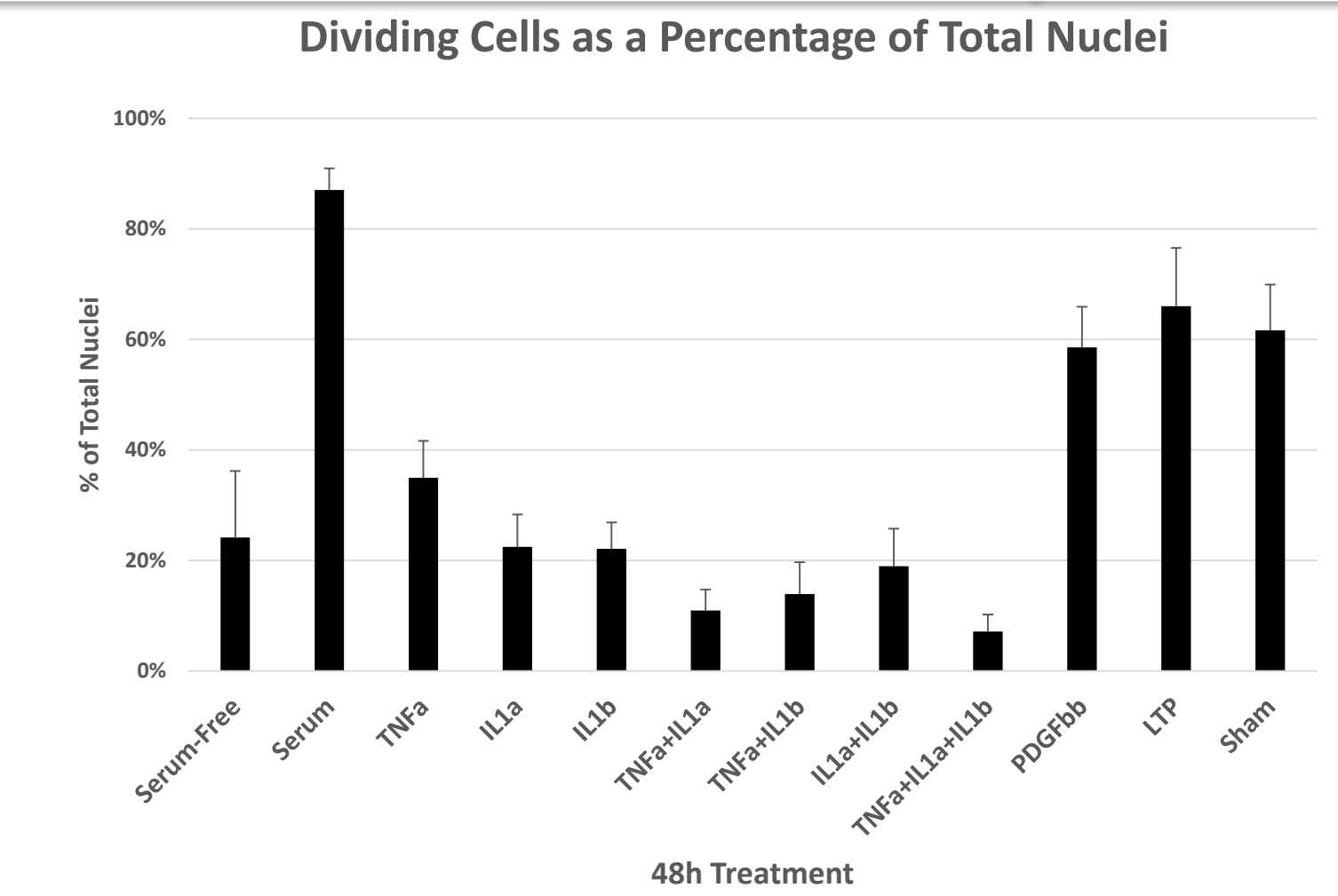
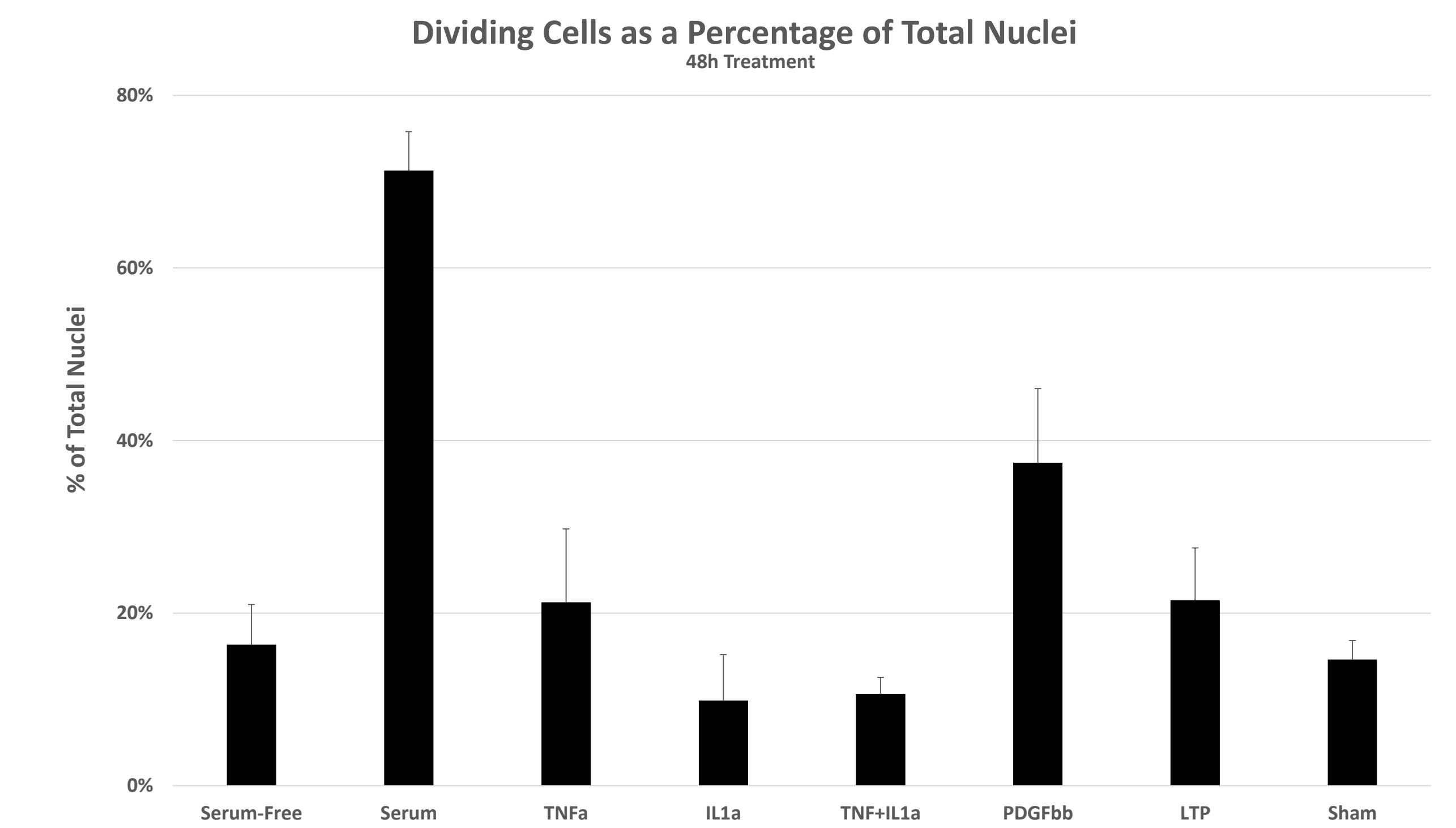


Fig.9. Human TM Cell Division Responses to Various Treatments

All Human Cell Culture Data Combined: N=6



Results & Conclusions:

- Optimized the use of Click-iT Edu with confocal microscopy to monitor cell division.
- Used the ELISA assay to determine that TNF-alpha and IL-1b increased with SLT laser treatment.
- Established optimum doses of TNF-alpha and IL-1alpha to add to determine increased cell division.
- Found that PDGFbb is a major contributor to TM cell division. Although serum causes even more cell division, the TM in a healthy eye does not have serum, this is used for TM cell culture to propagate cultured cells.
- It no longer appears as though mass spectrometry will be needed.

Future Directions:

- Establish optimum doses for PDGFbb growth factor.
- Investigate cytokine combinations of TNF-alpha, IL-1alpha, IL-1 beta and in combination with PDGFbb and other potential factors causing cell division.
- Use cell division blocking agents to deplete the factor(s) from the SLT-treated conditioned media and further verify the participation of these molecules in stimulating cell division in cell culture with the Click-iT Edu kit. Use this same methodology to determine cell migration.

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