



*Suberoylanilide Hydroxamic Acid Versus Mitomycin C:
Comparative Study
of Toxicity in Adjunctive Treatment of Corneal Haze After PRK
in Rabbit Model*

Michael Waggoner DO, Ajay Sharma PhD, Rajiv Mohan PhD

The authors have no financial interest in the subject matter of this e-poster.

hypothesis:

When compared to Mitomycin C (MMC), Suberoylanilide Hydroxamic Acid (SAHA) provides similar efficacy for inhibiting post PRK corneal haze while producing less endothelial toxicity.

potential impact:

Provide safer long-term results for PRK patients with high risk for corneal haze

Study Design

Pre-op evaluation	Specular microscopy performed on all rabbits (n=18)		
Surgery	Manual epithelial scrape, followed by -9 D PRK (Laser: Summit Technologies, Inc.)		
Antifibrotic Treatment groups	None (6 rabbits)	MMC (0.02% for 2min) (6 rabbits)	SAHA (25uM for 5min) (6 rabbits)
Post-op evaluation	Specular microscopy performed at 5 days, 2 weeks, 1 month		
Tissue harvest			
1 month	3 eyes	3 eyes	3 eyes
4 months	3 eyes	3 eyes	3 eyes

Result: haze formation

PRK only

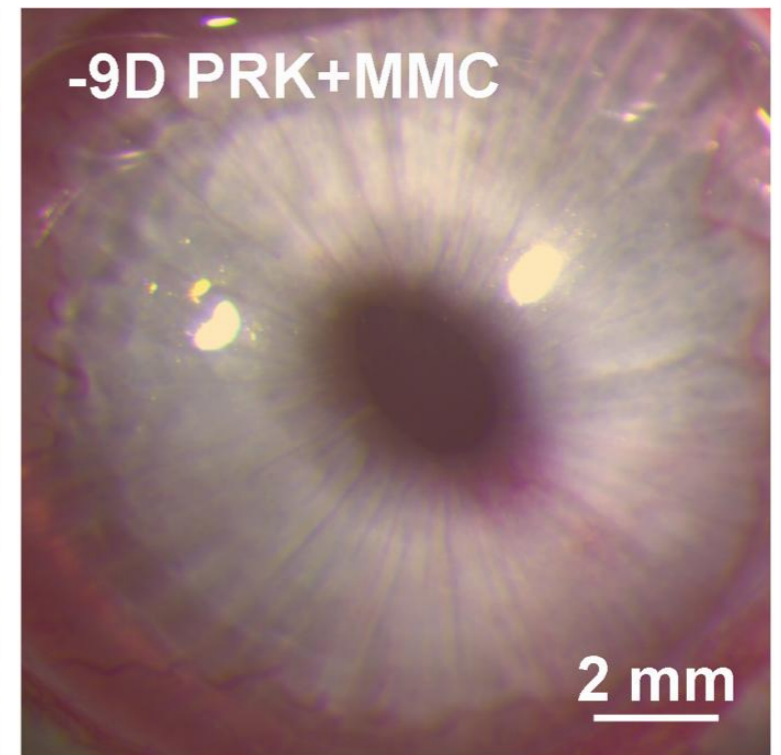
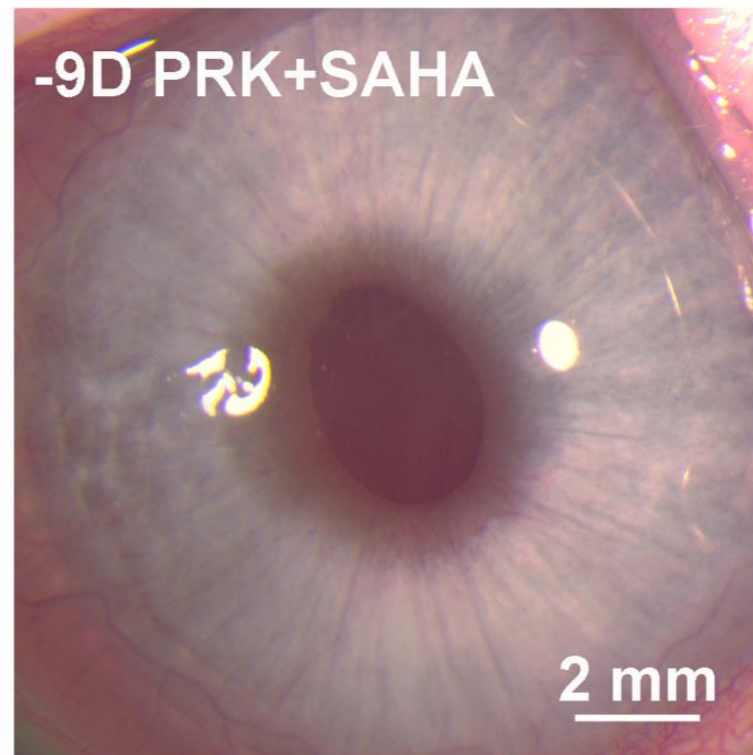
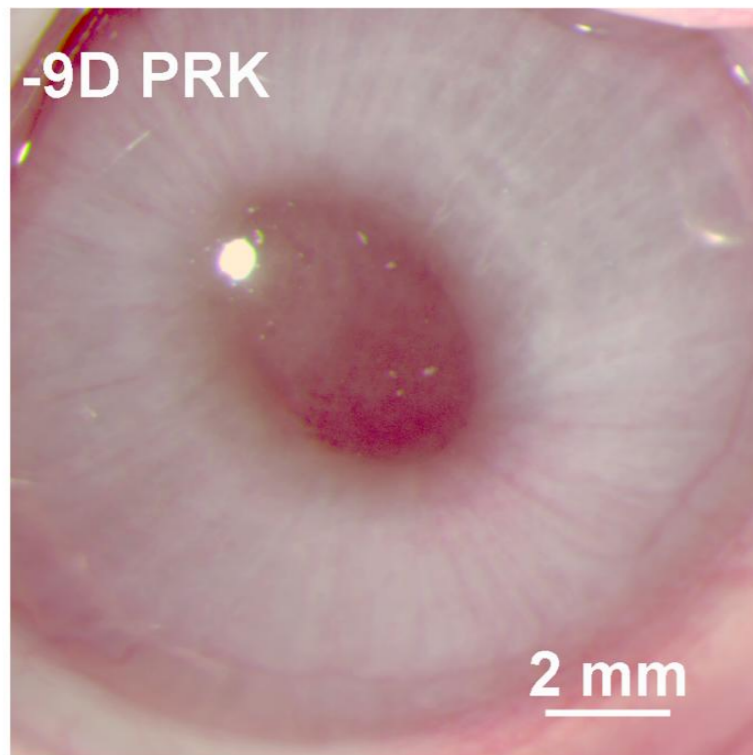
SAHA

MMC

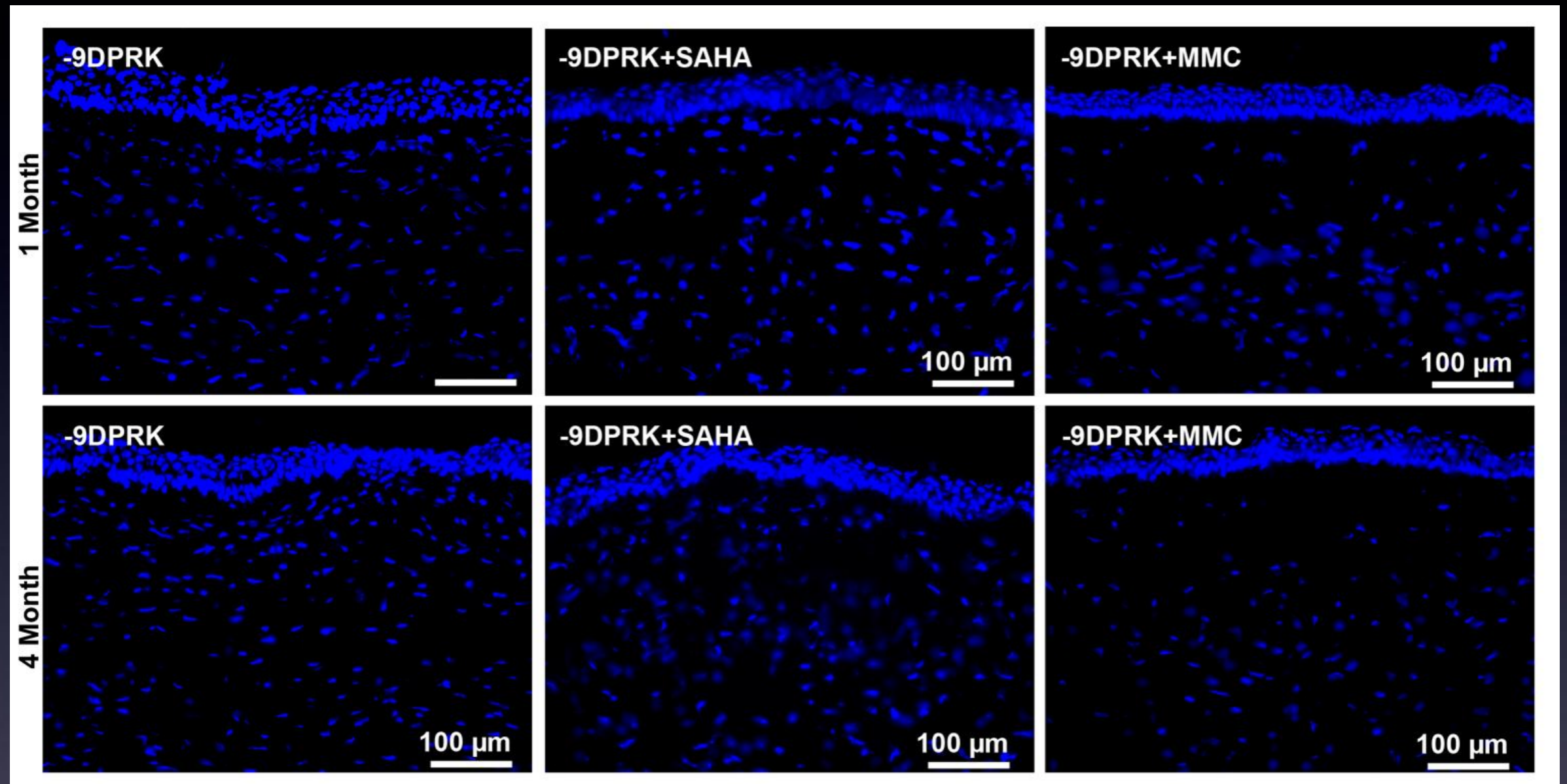
Haze: 4+

Trace

None

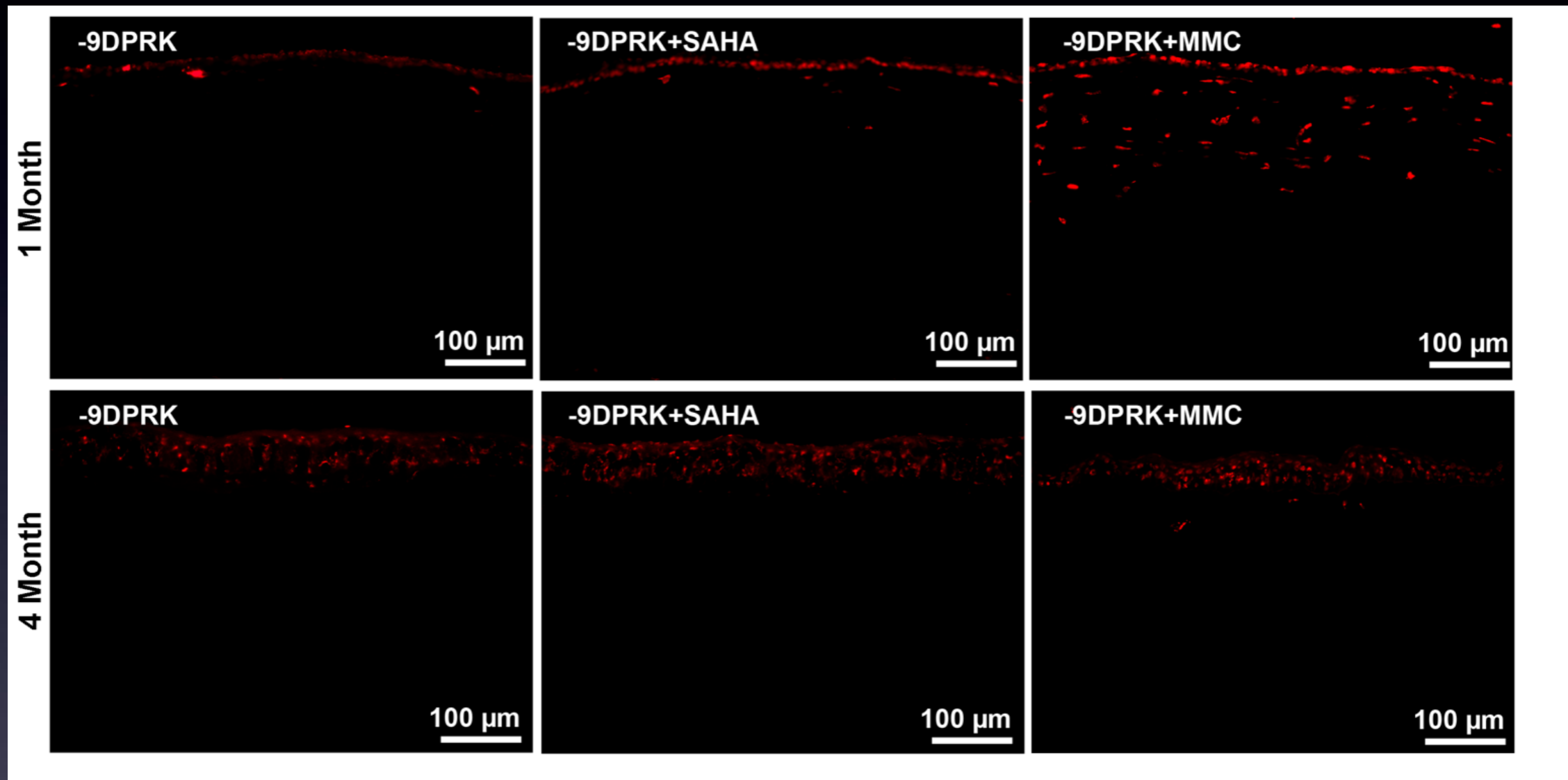


Result: keratocyte cell density



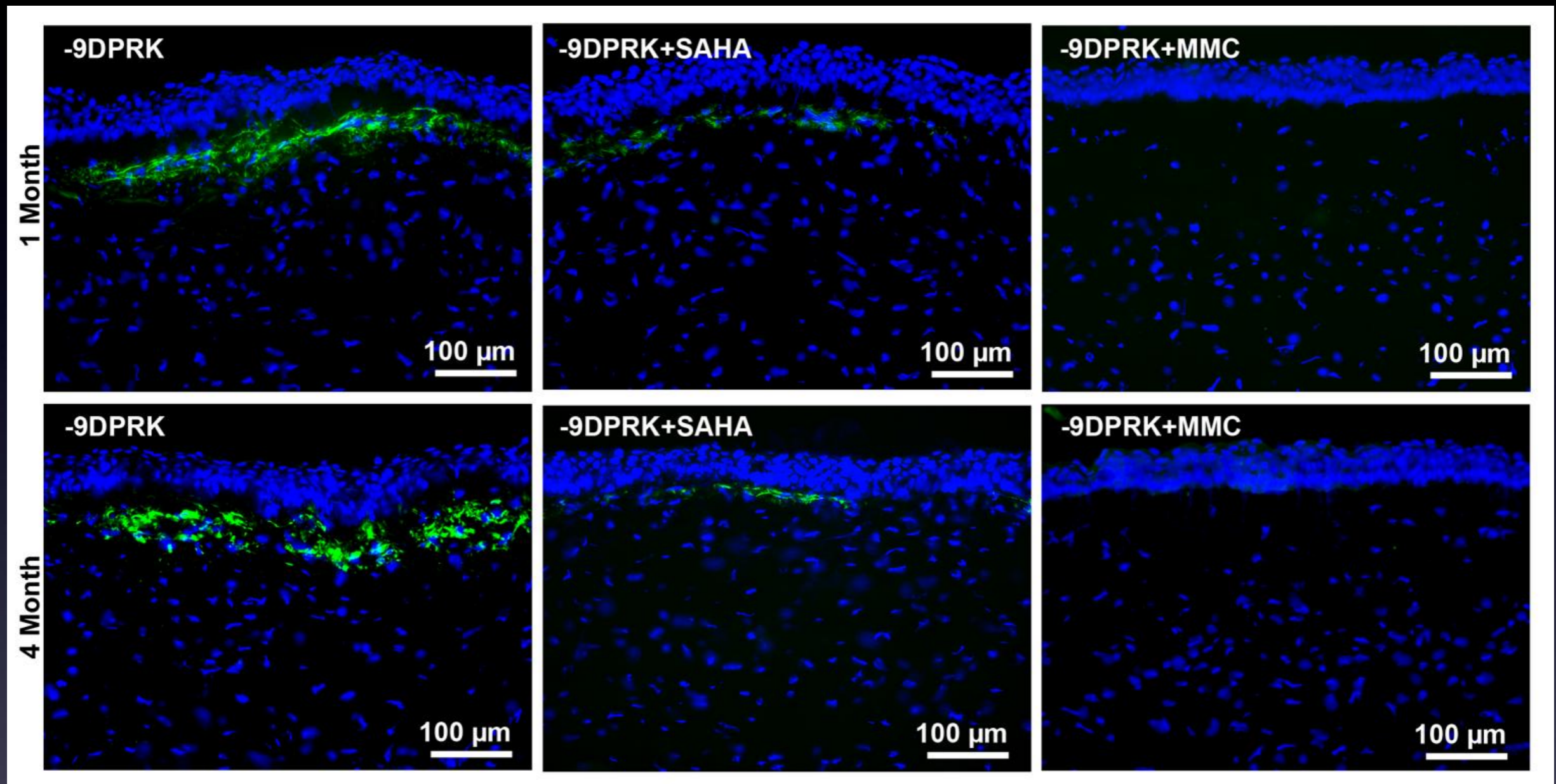
Immunohistochemistry. Blue (DAPI) staining labels individual cell nuclei. The epithelium (top of photograph) is healed with normal structure in all treatment groups. The anterior stromal keratocytes are absent in the MMC group, but are preserved in the SAHA and control groups.

Result: keratocyte apoptosis



TUNEL assay. The red stain highlights apoptotic cell nuclei. A normal amount of apoptosis is demonstrated in the epithelium of all treatment groups. There are no apoptotic cells seen in the control or SAHA groups. The MMC-treated group shows a high number of apoptotic cells at 1-month post treatment. By 4 months, these apoptotic cells have totally degenerated and are no longer seen.

Result: myofibroblast differentiation

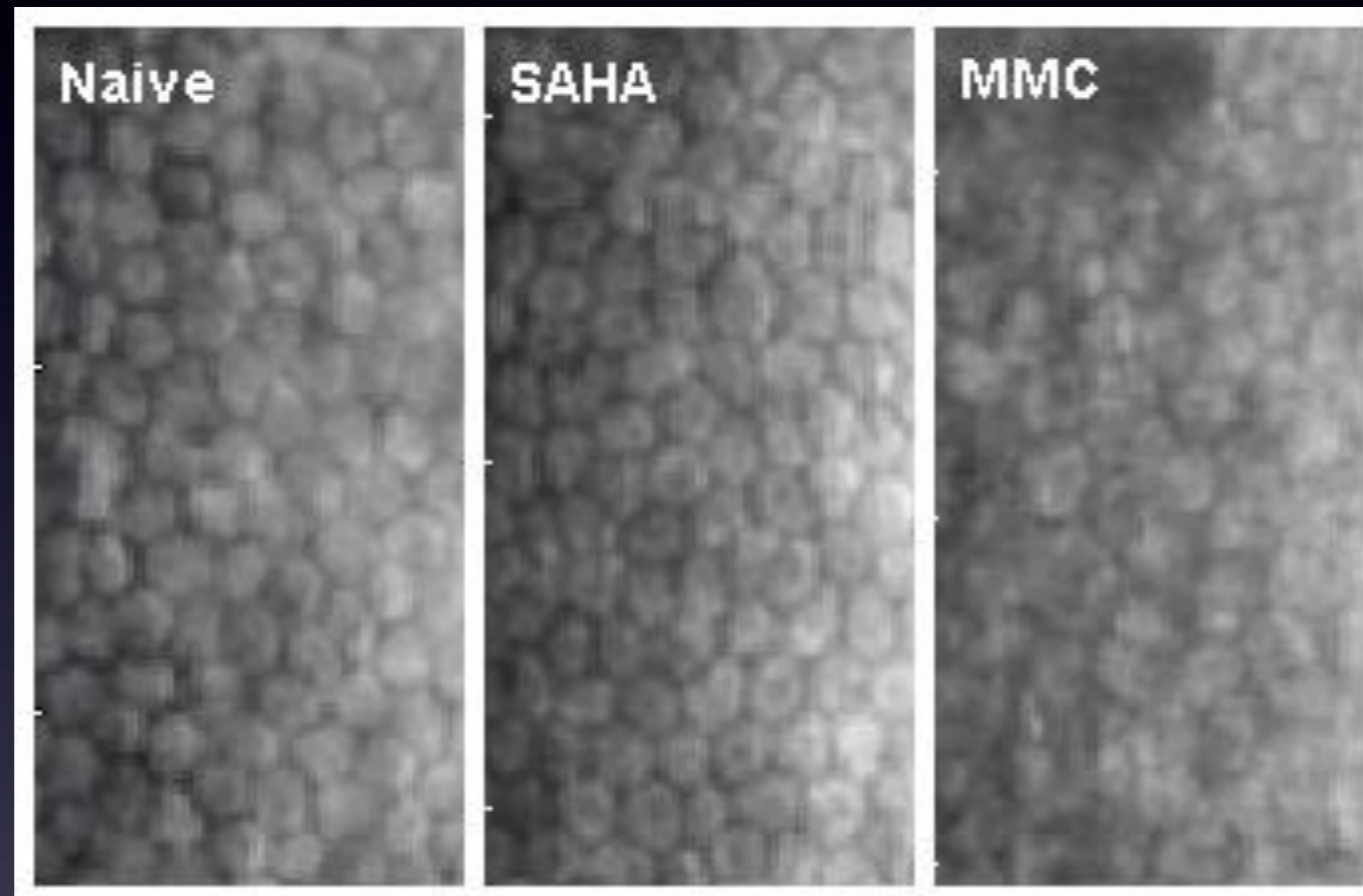


Immunohistochemistry.

Staining shows alpha-smooth muscle actin (α-SMA) in green.

α-SMA is a cell marker for myofibroblasts. Their presence signifies a scarring/haze transformation in the keratocytes. This myofibroblast formation is absent in the MMC treated group and greatly reduced in the SAHA treated corneas when compared to control.

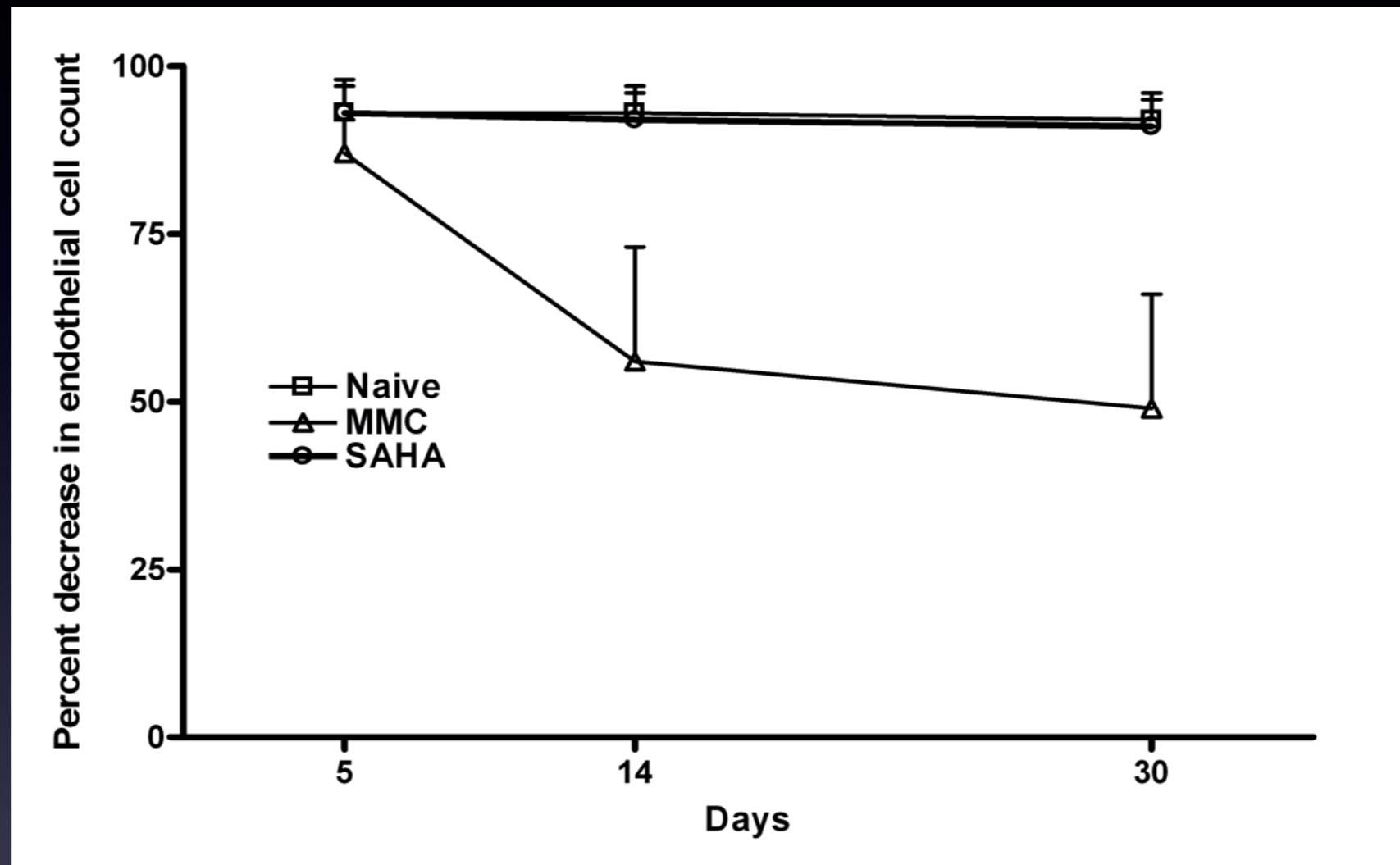
Result: endothelial density and morphology



Specular microscopy at one month after treatment.

Cell density and morphology is similar between Naïve and SAHA groups. MMC treated corneas show reduced endothelial cell density and abnormal endothelial cell morphology.

Result: endothelial cell counts



Endothelial cell count performed by specular microscopy.

The control and SAHA treated groups show a slight decrease in endothelial cell density from pre-operative measurement. The MMC treated eyes show a decrease to 50% of their pre-operative endothelial density.

Conclusions

Compared to MMC treated corneas in this study, SAHA demonstrated:

- Reduced PRK-induced keratocyte apoptosis
- Improved endothelial cell survival
- Improved endothelial cell viability
- A similar reduction in corneal haze

Limitations

- Study size
- Nonhuman study
- Protocol application
 - This study used a broad beam Summit laser which is more likely to induce haze
 - Duration and concentration of MMC is variable among clinicians