17TH INTERNATIONAL CONFERENCE OF THE RESEARCH INSTITUTE OF OPHTHALMOLOGY
RIO 2025
Anterior Segment Eye Diseases
Current Status & Future Directions

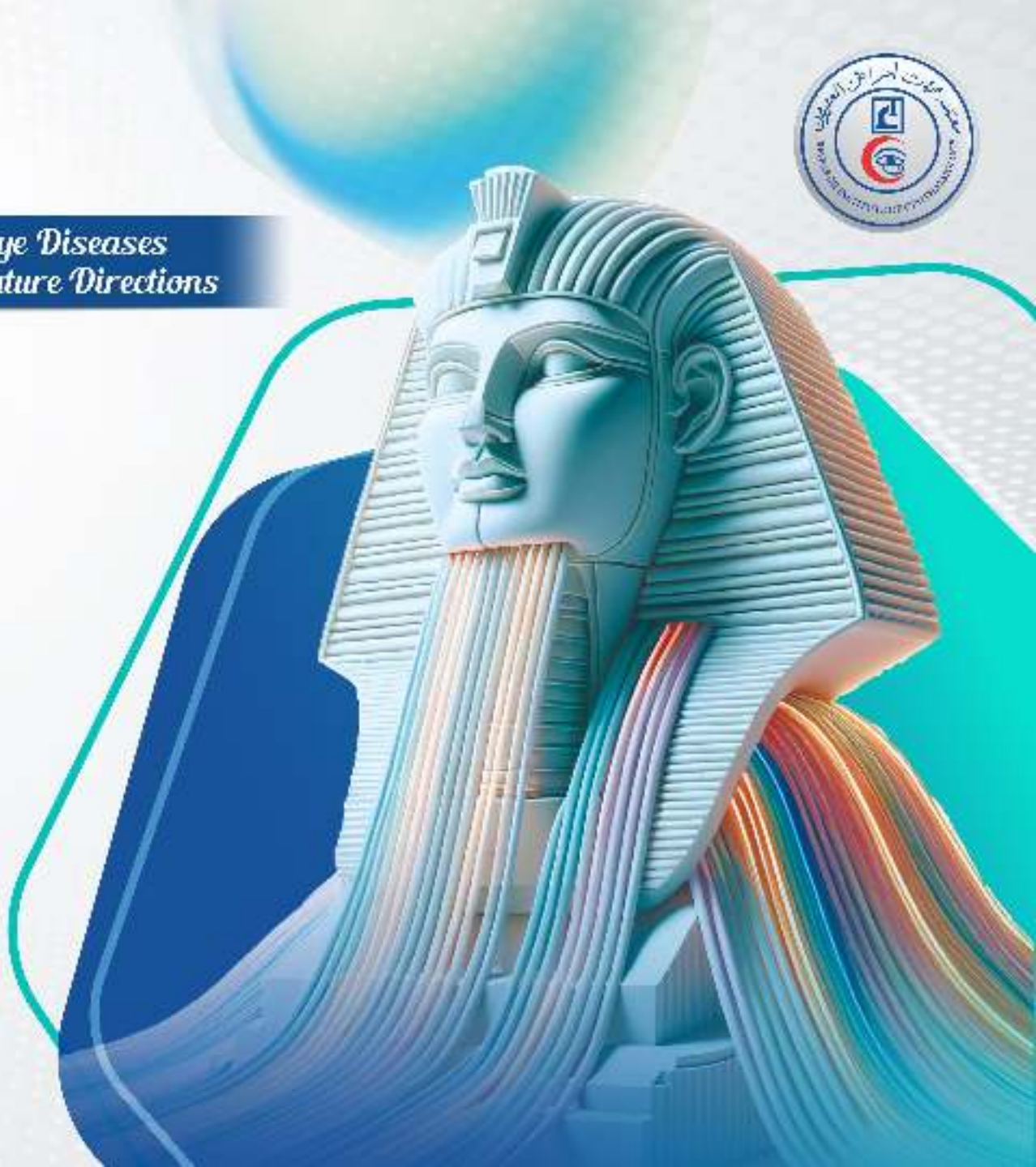
Role of Mesenchymal Stem Cells in Treating Limbal Stem Cell Deficiency in Experimental Rats

A PhD research by

Ahmed Mostafa Ata, MSc.

Research Assistant/ Biochemist

Research Institute of Ophthalmology-Egypt



What is Limbal stem cell deficiency?

- Recognized by the lack of stem cells in the limbus
- “which has a vital role in corneal epithelium re-population and maintains the barrier function of the limbus”

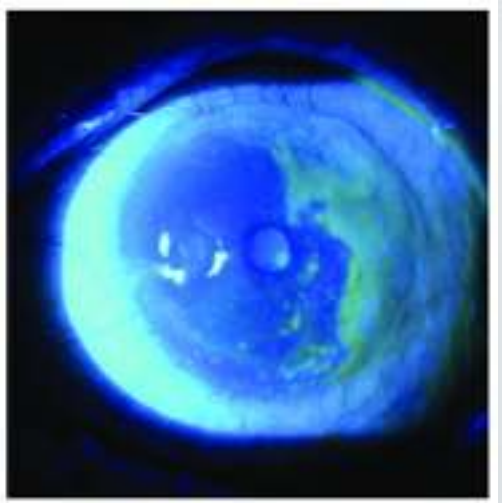
- Partial or complete loss of the regenerative ability of the cornea
- Corneal scarring
- Loss of normal corneal luster
- Conjunctivalization

- Corneal opacification, chronic inflammation, neovascularization, and eventually to complete blindness.

LSCD signs and symptoms

- Chronic conjunctival redness
- Poor epithelial wound healing
- Foreign body sensation, photophobia, tearing, and decreased vision

- Corneal epithelium shows an irregular surface
- Thick fibrovascular pannus formation
- Chronic keratitis and scarring.
- +ve fluorescein



LSCD etiology

Primary factors	Secondary factors
• **Aniridia** • **congenital epidermal dysplasia** • **Turner syndrome**	• **External stimuli (damage the stem cell niche and destroy LSCs)** - chemical injuries - chronic inflammation • **Stevens-Johnson syndrome** • **Injury caused by surgeries in the limbic region**

Problem facing LSCD patients

One of the main causes of blindness in Egypt is corneal blindness, that caused by chemical injuries because of exposure to alkalis and acids in some industries.

“Prof. Mostafa Salah – RIO president”

So, it can be treated by Corneal transplant surgeries “keratoplasty”

Problem facing LSCD patients

- We are facing a significant challenge in our work at Research Institute of Ophthalmology as the number of patients on the waiting list for corneal transplant surgeries continues to grow every year.

- As the healthy corneal graft is imported from international cornea banks, because of the local cornea banks' legal issues

- So, this takes a long time for the healthy corneal graft to be available for the patient.

Aim of Work

To explore the potential therapeutic impact of bone marrow-derived MSCs (BM-MSCs) in treating LSCD induced in experimental rats

by investigating the influence of such treatment in mitigating the inflammatory response, angiogenesis and apoptosis associated with LSCD

Methods

Experimental Animals

- Sixty-Six mature female Wistar rats with a weight range from 200 to 220 g were acquired from the animal house facility of the Research Institute of Ophthalmology, Giza, Egypt.

- The rats were accommodated individually in Ventilated Caging System at a temperature of 25 °C, and supplied with tap water and rodent chow

Experimental Protocol

66 rat

- Control group
- Alkali burn group
- Stem cells group

	Day Zero	Day Two	Day Seven
Control group	Subconjunctival injection of 0.1ml PBS in each eye	Subconjunctival injection of 0.1ml PBS in each eye	All rats were sacrificed under general anesthesia with an overdose of 10% chloral hydrate
Alkali burn group	Alkali burn with NaOH + Subconjunctival injection of 0.1ml PBS	Subconjunctival injection of 0.1ml PBS in each eye	
Stem cells group	Alkali burn with NaOH + Subconjunctival injection of $2x10^6$ MSCs /rat eye in 0.1ml PBS	Subconjunctival injection of $2x10^6$ MSCs /rat eye in 0.1ml PBS	

Ophthalmology examination

- All rats were subjected to clinical follow-up using a slit lamp to determine the score of opacity, neovascularization grade.
- Moreover, it subjected to topical fluorescence staining using fluorescein strips to determine the ulcer development.
- The clinical outcome was evaluated by a masked investigator

Biochemical and Molecular investigations

	ELISA	Rt-PCR
Inflammatory markers	TNF-α	TNF-α
	IL-8	NF-kB
		COX-2
Angiogenic markers	VEGF	
	MMP-2	
Apoptotic markers		Caspase-3

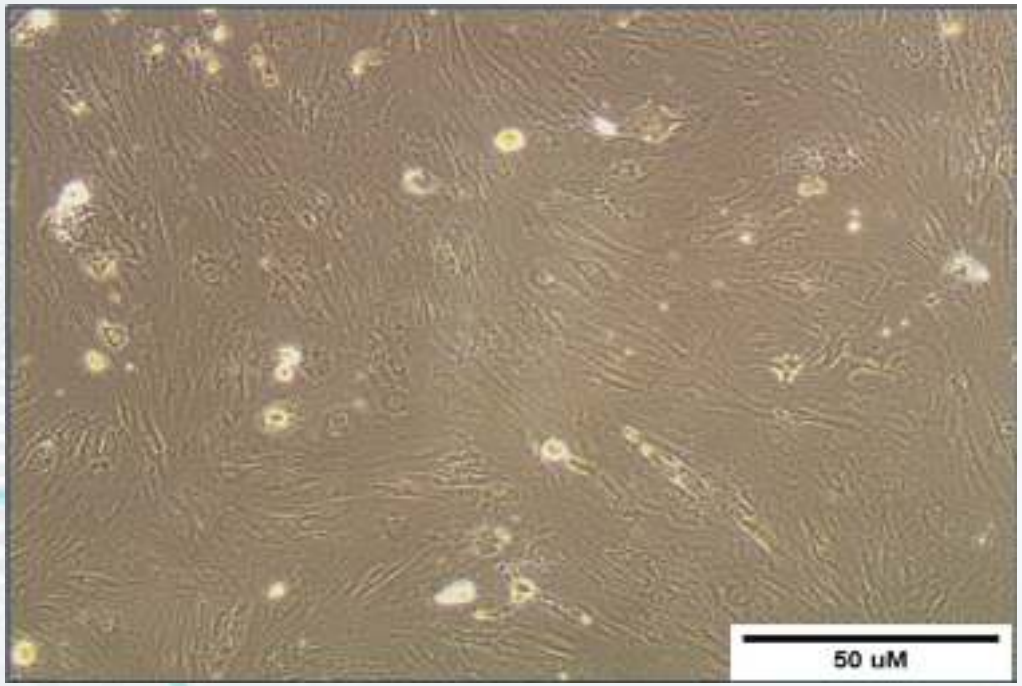
Histological examination

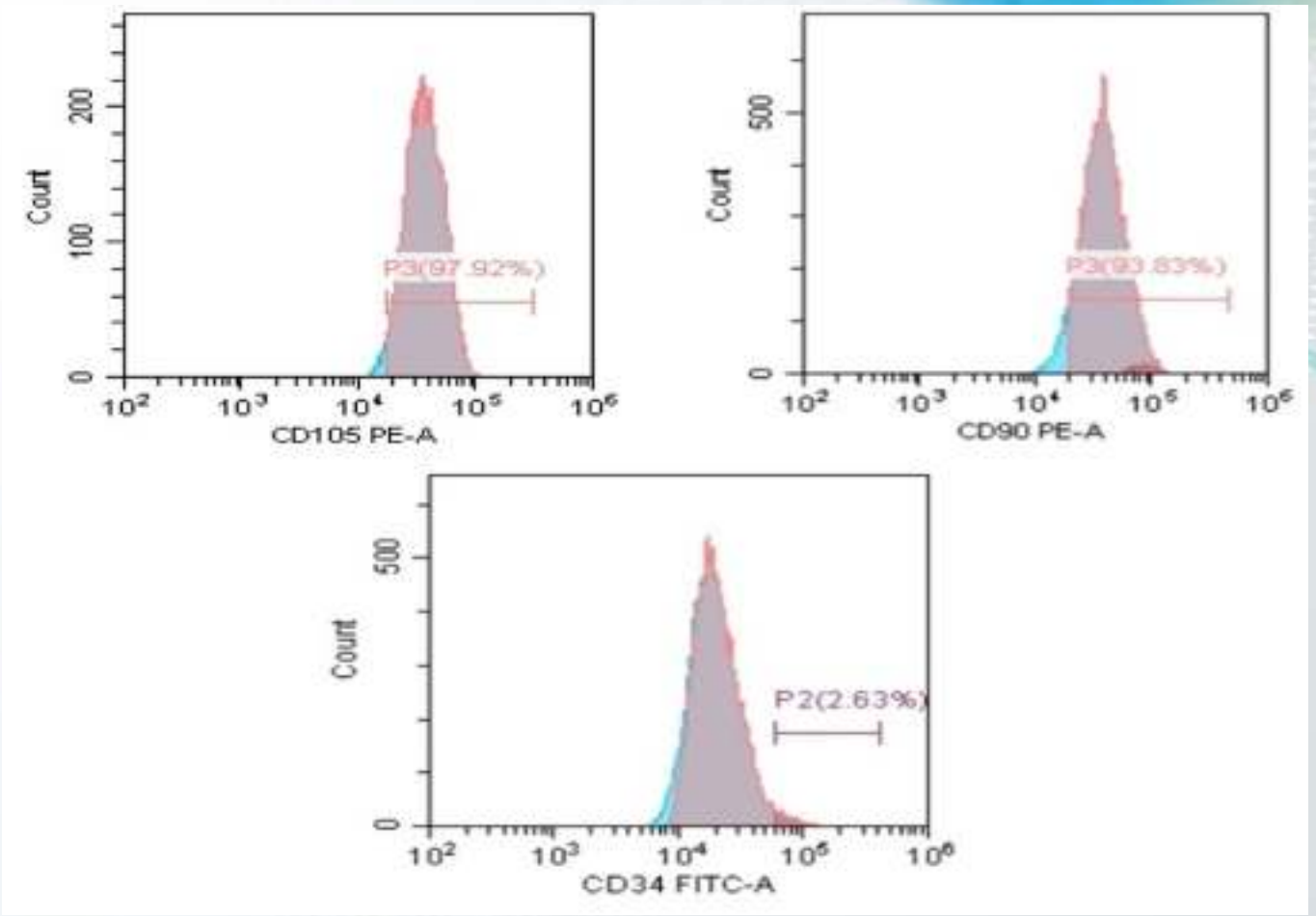
- Histological examinations were performed by hematoxylin & eosin (H&E) stain method for light microscopic observation.

- For determination of the effect of alkali burn on the cornea layers and the tracking the therapeutic impact of BM-MSCs.

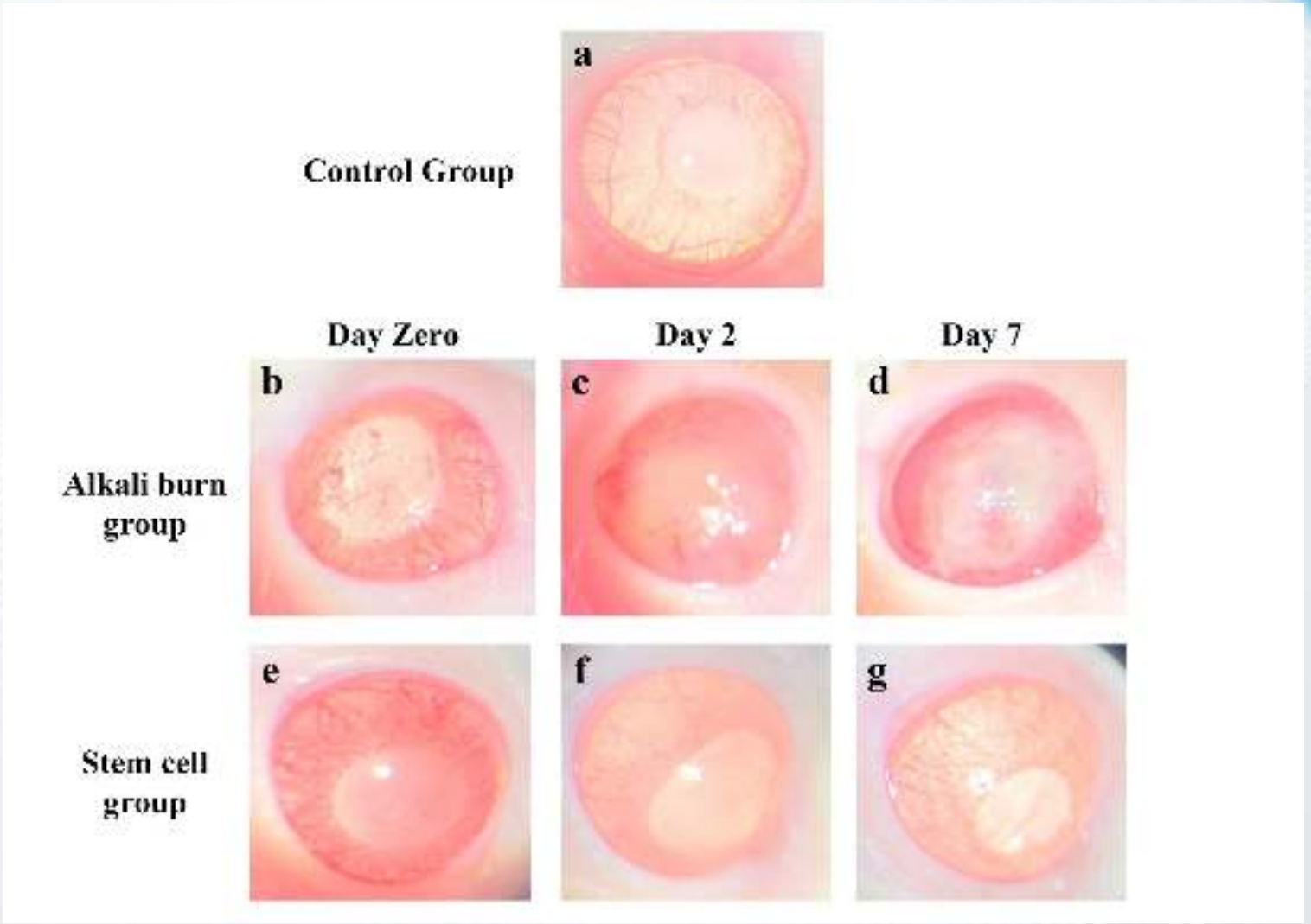
Results & Discussion

BM-MSCs identification

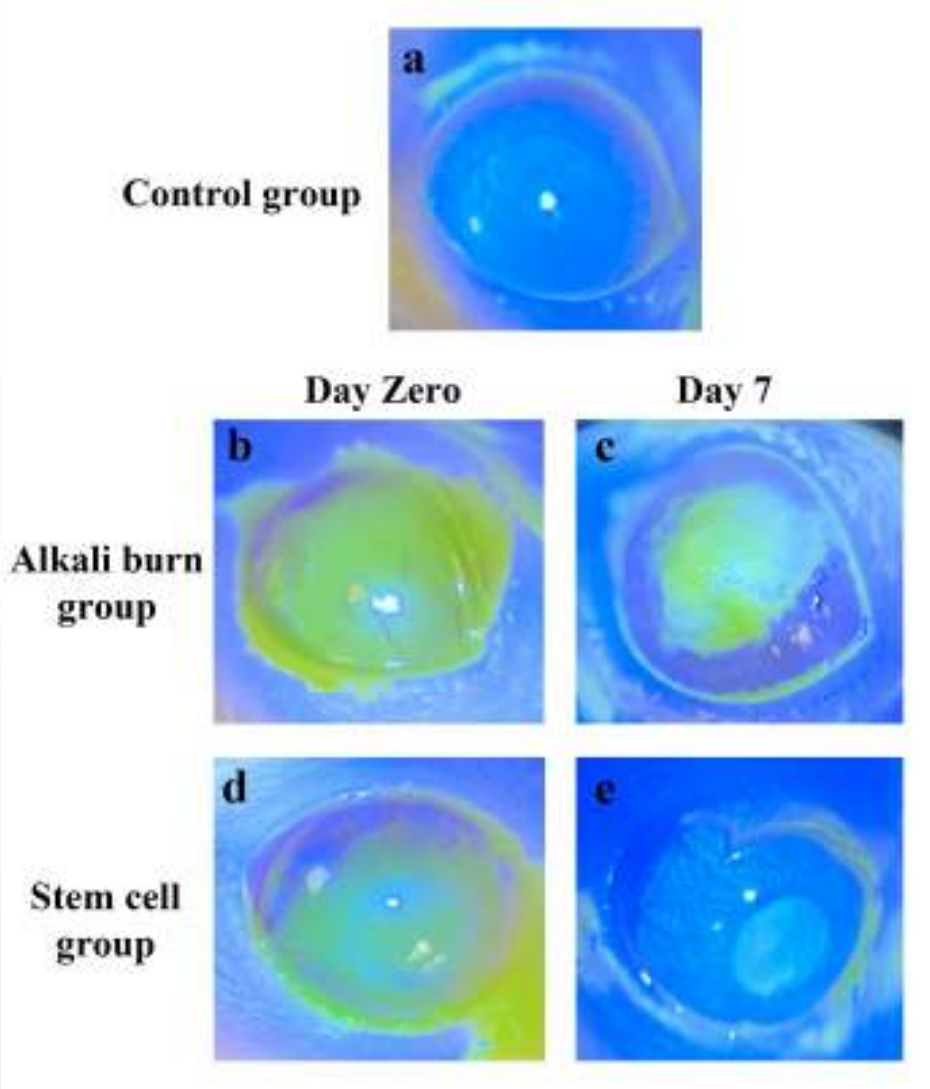




Clinical Ophthalmology outcomes

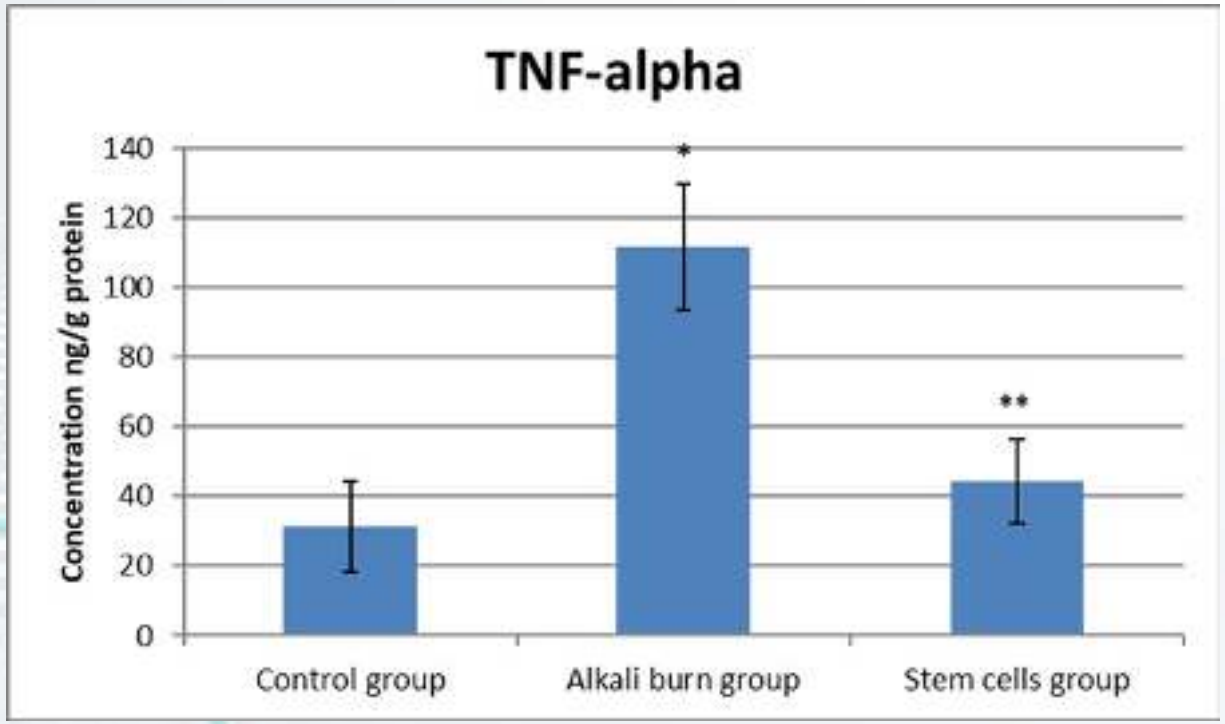


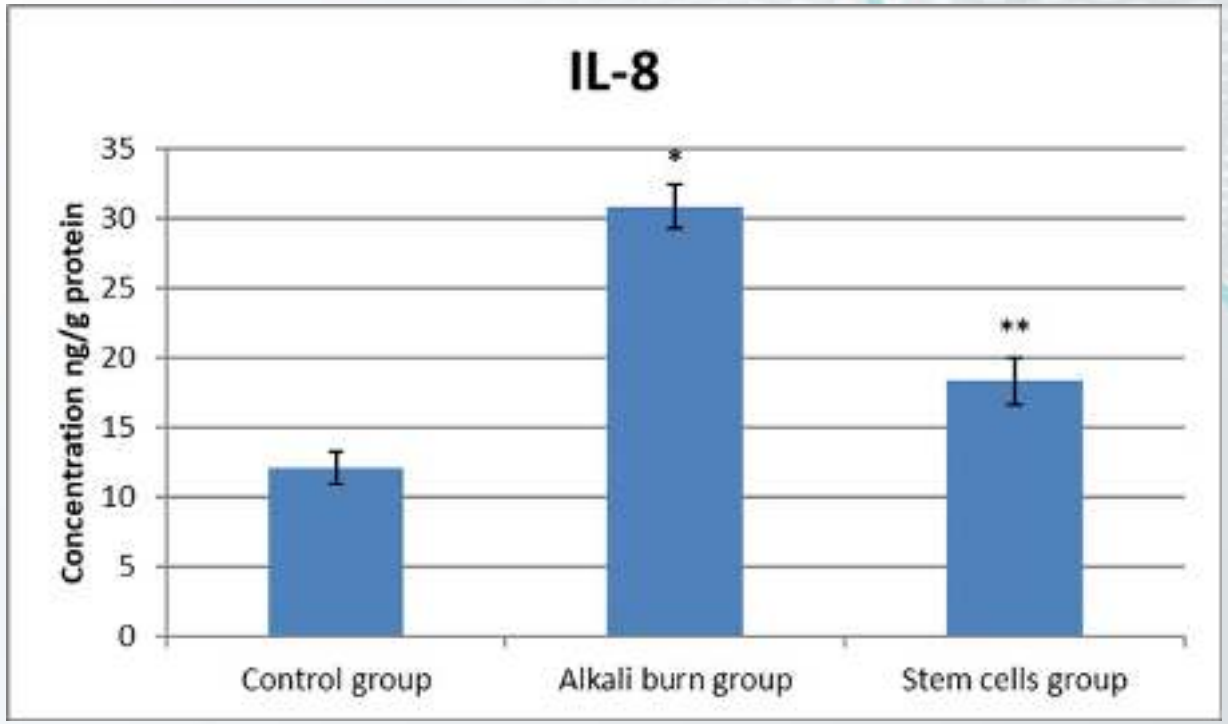
Clinical Ophthalmology outcomes



Biochemical Findings

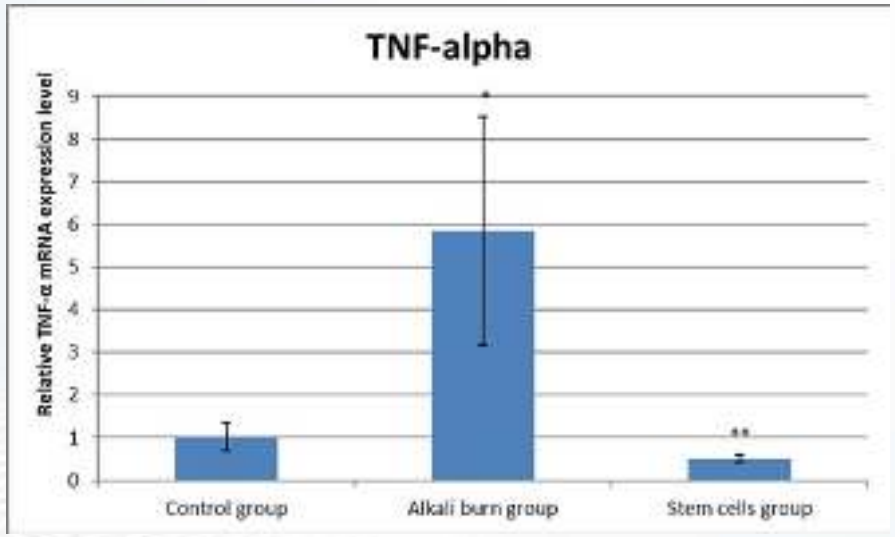
Inflammatory markers

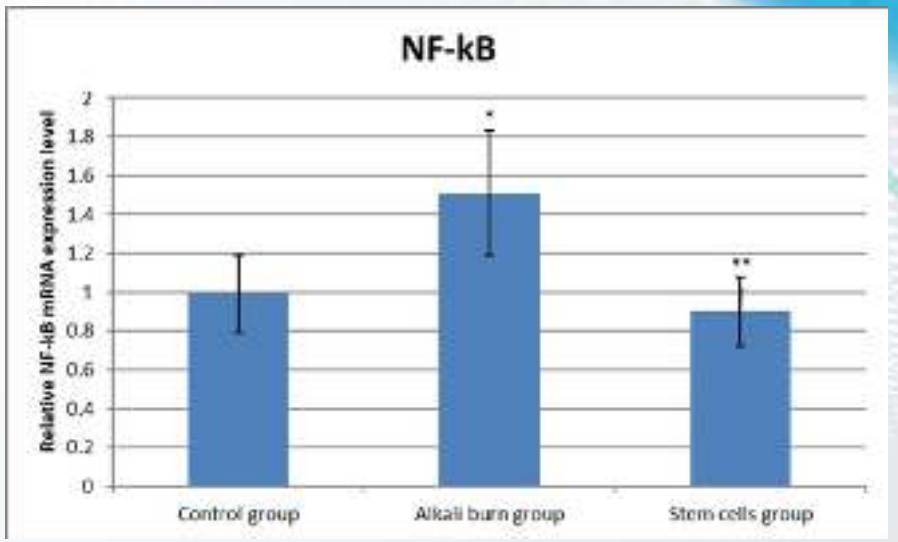


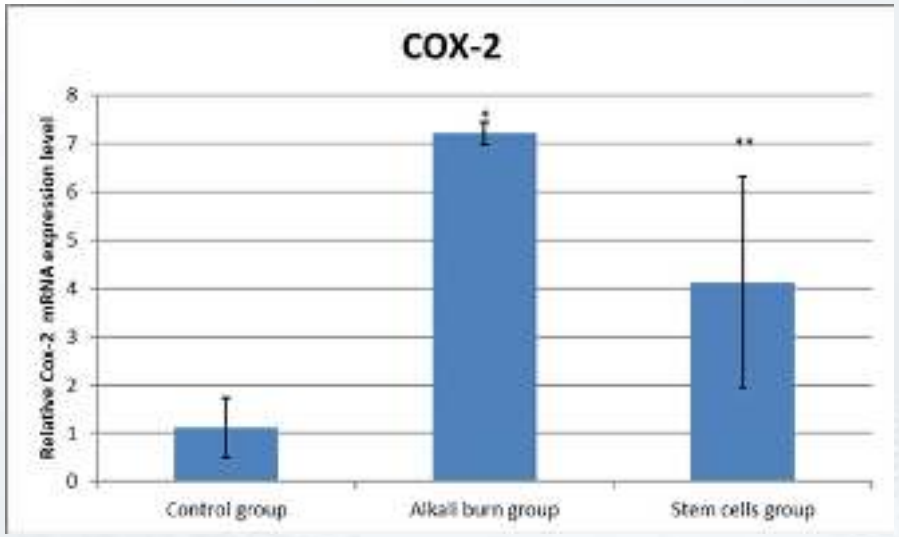


Molecular Findings

Inflammatory markers

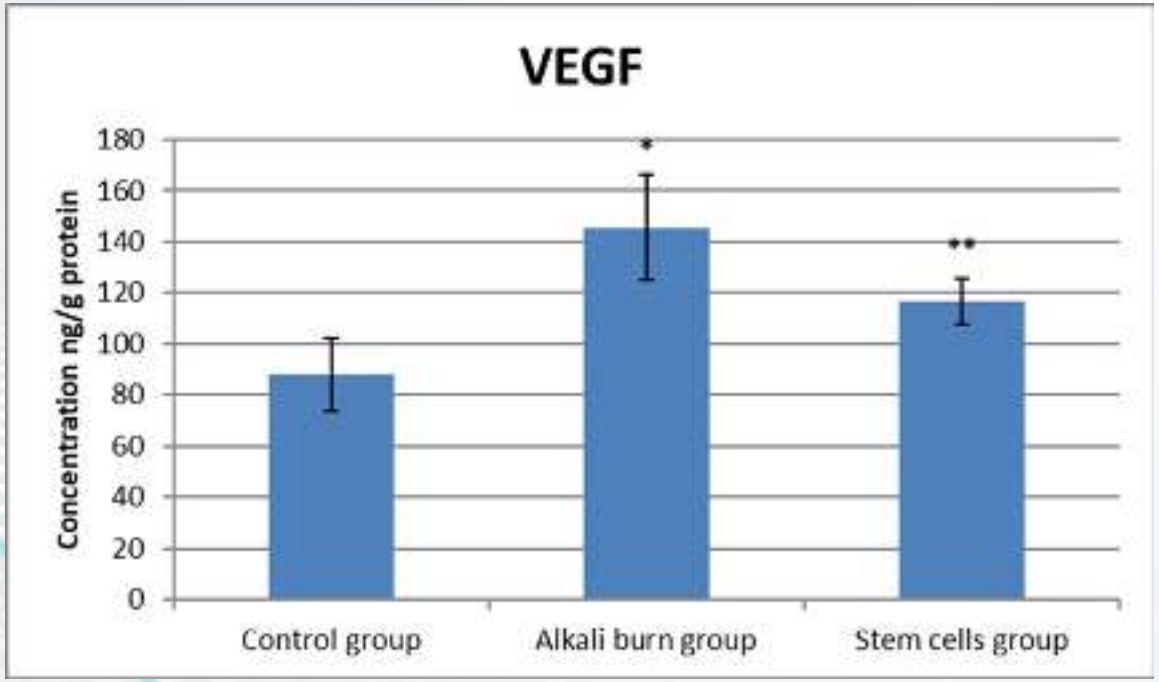


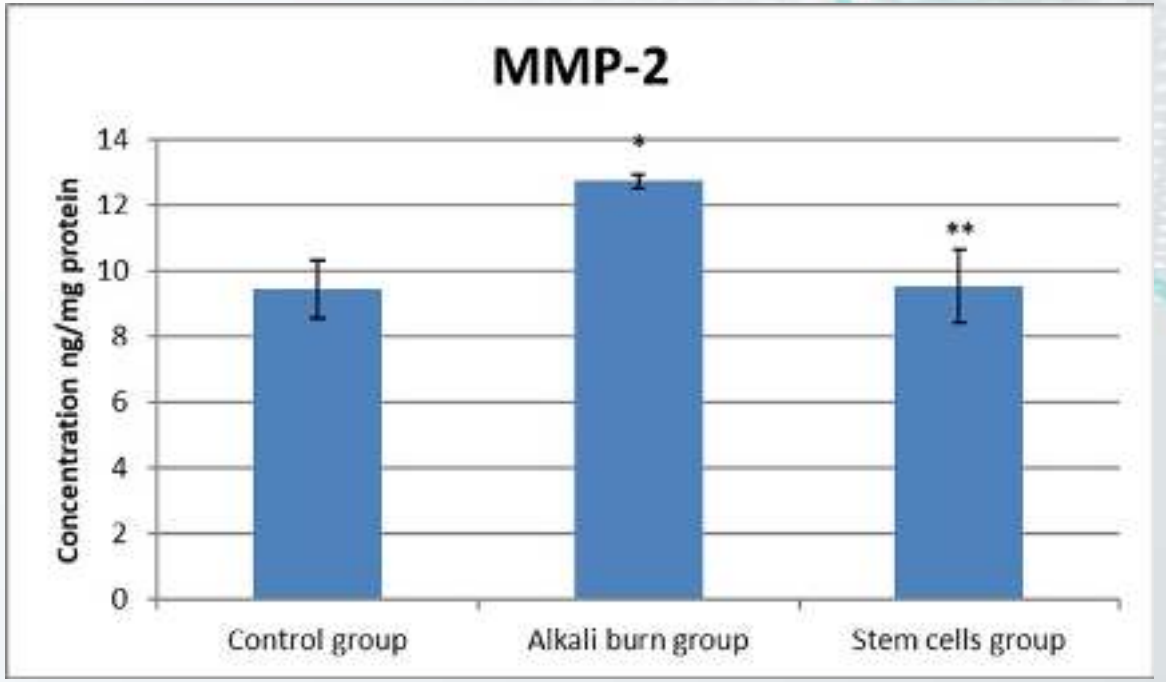




Biochemical Findings

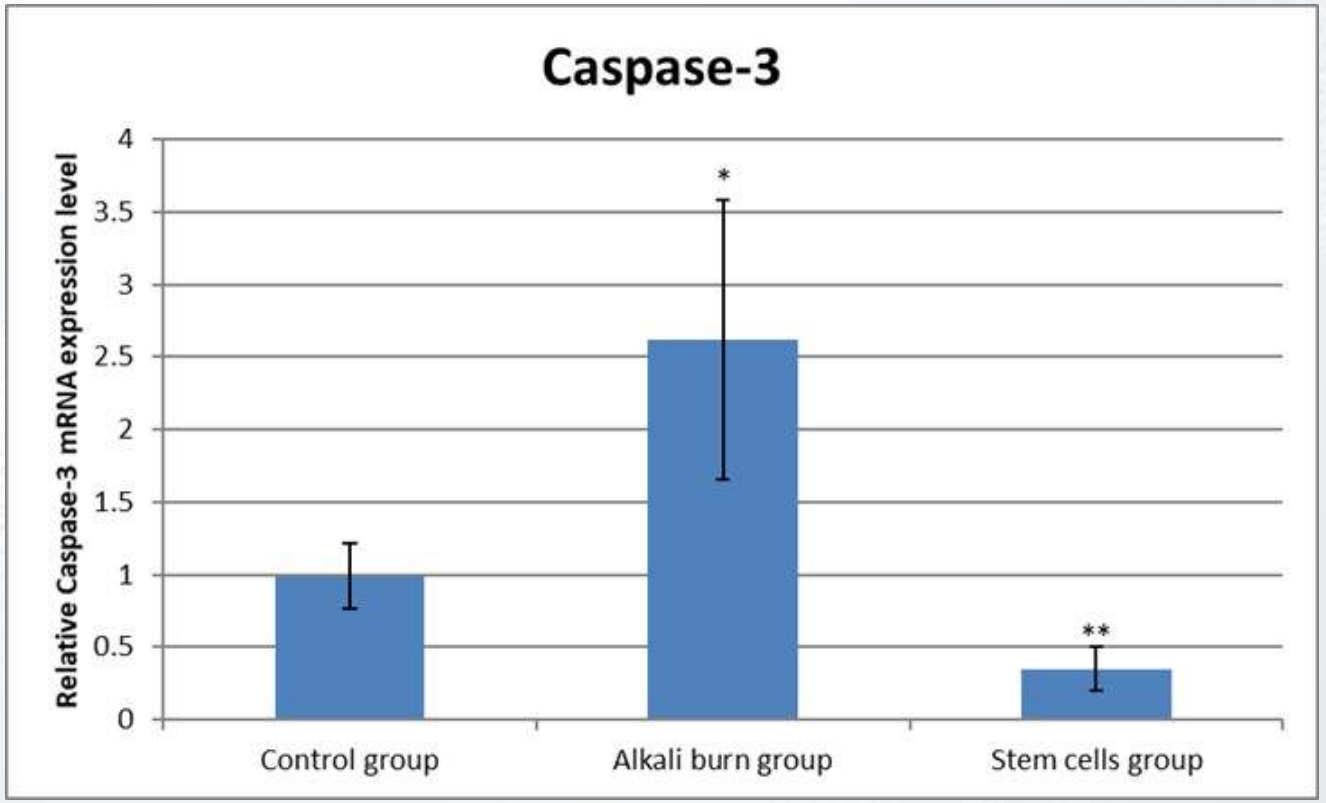
Angiogenesis markers





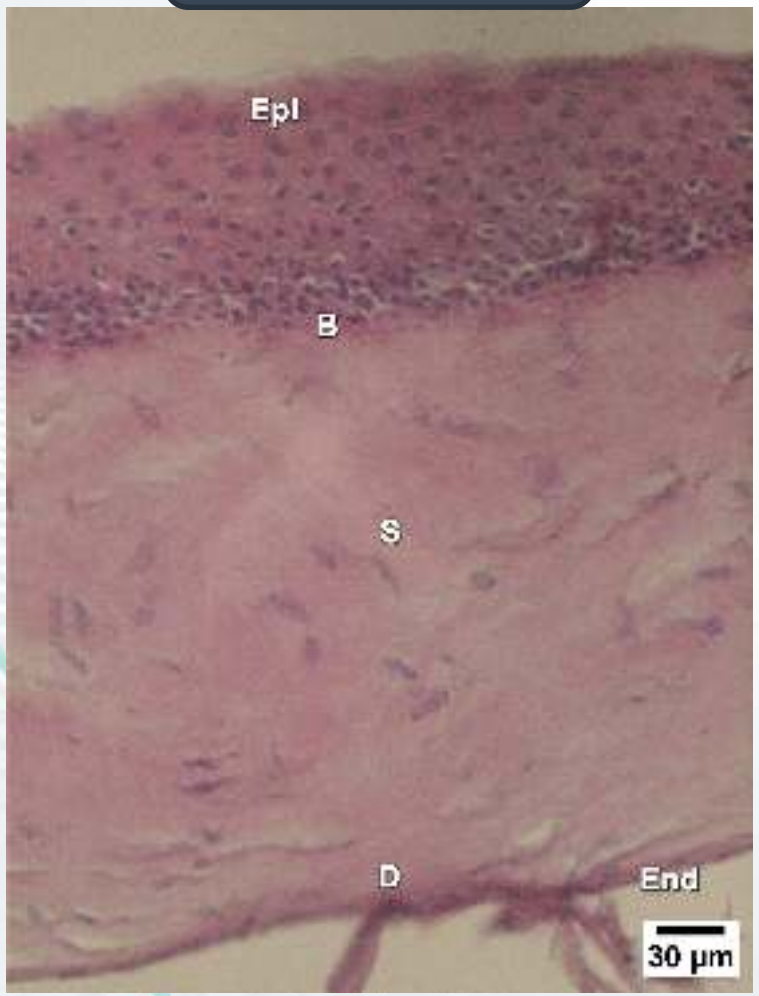
Molecular Findings

Apoptotic marker

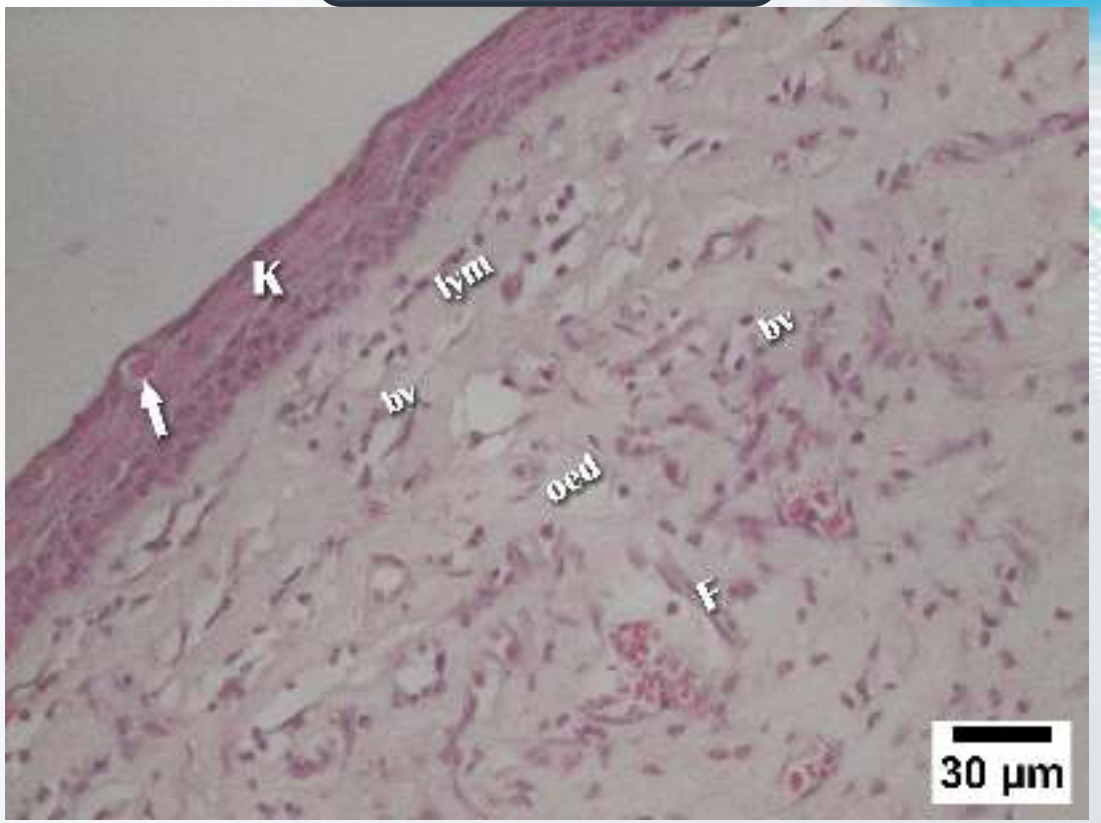


Histological findings

Control Group (X500)



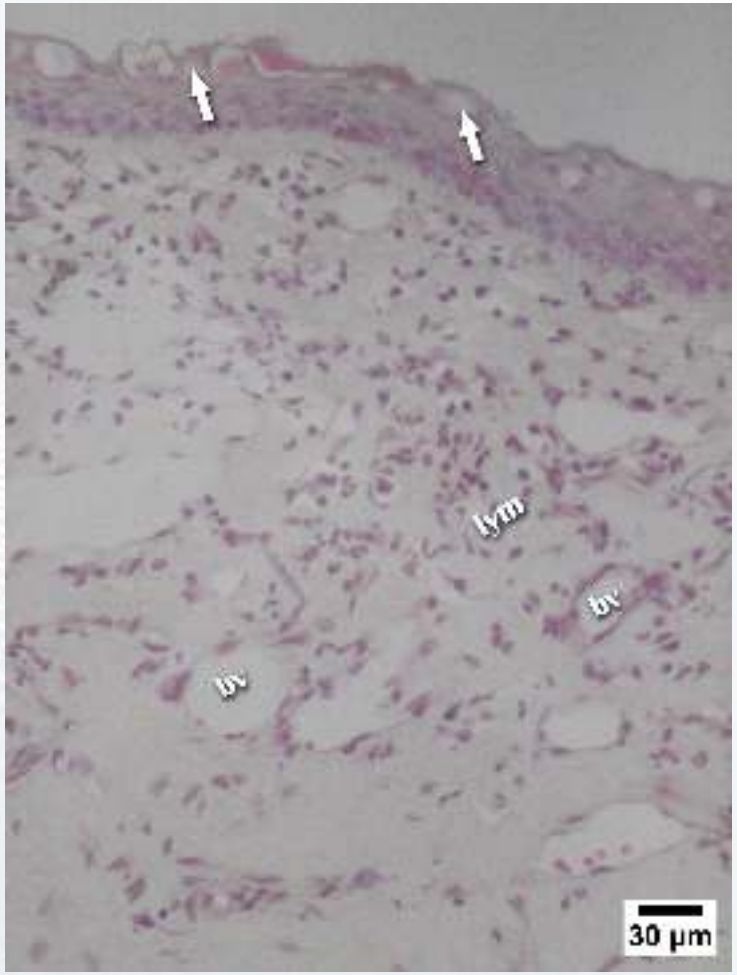
Alkali burn group (X 500)



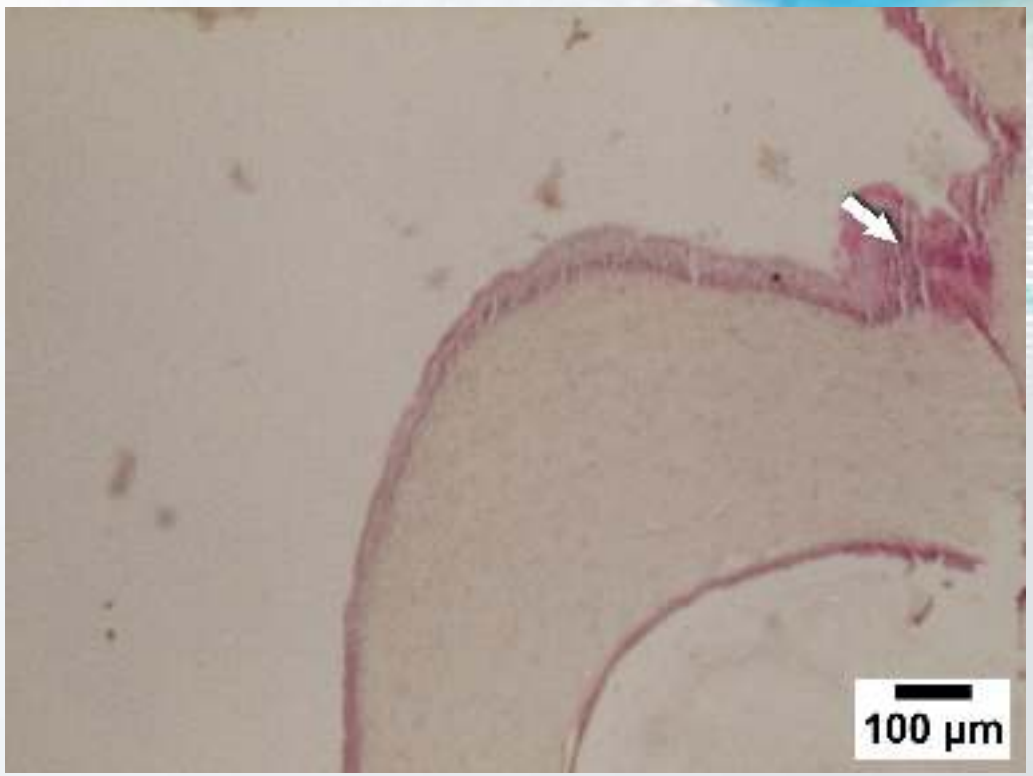
hyperkeratosis (K) dyskeratotic cells (arrows), dense lymphocytic infiltrate (Lym), blood vessels (bV), interstitial oedema (oed) fibroblasts (F)

Histological findings

Alkali burn group



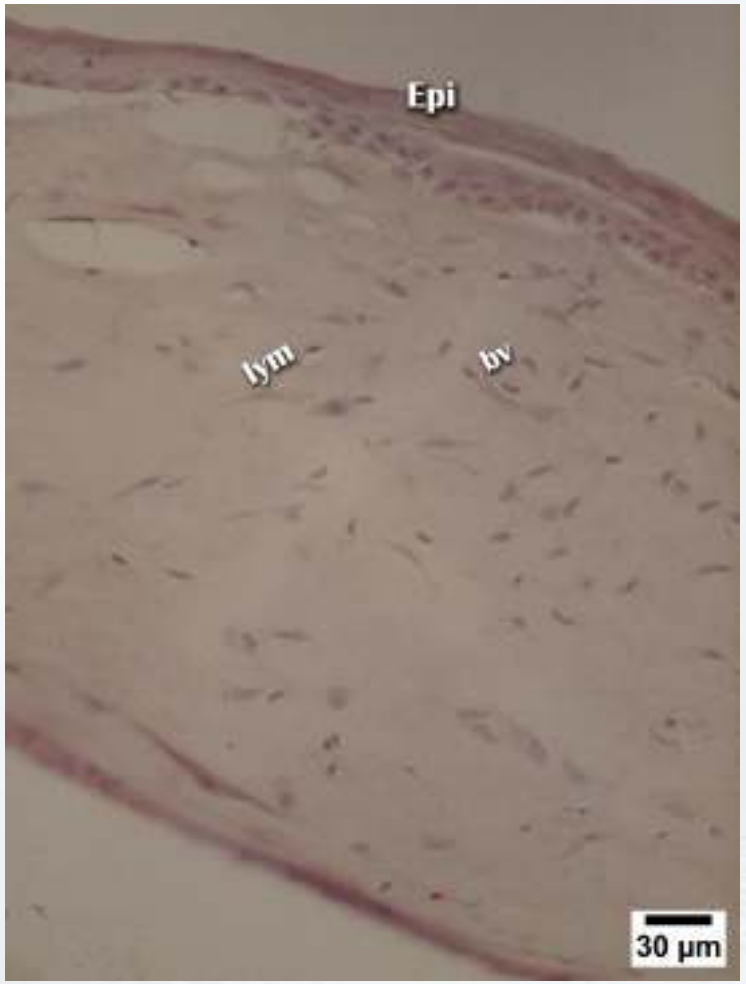
acantholysis (arrow) (X 500)



conjunctival epithelial cells invading the cornea surface (arrow) (X 125)

Histological findings

Stem cells group (X500)



Conclusion

Subconjunctival injection of BM-MSCs offers a promising treatment for LSCD by reconstruction of damaged corneal epithelium and acceleration of wound healing.

BM-MSCs exerts anti-inflammatory, anti-angiogenic, and anti-apoptotic effects to suppress corneal inflammation, neovascularization, and apoptosis.

MSCs clinical trials for ocular diseases

- Ongoing clinical trials are focusing on evaluating the safety, efficacy, and mechanisms of MSCs for various ocular diseases.
- Preliminary results are promising, demonstrating improvements in visual acuity and structural repair in patients with corneal and retinal conditions.

Liang et al., 2021	**severe ocular burns**
Agorogiannis et al., 2012	**facilitate the epithelial healing in corneal epithelial defect patients.**
Weng et al., 2012	**dry eye disorder associated with chronic graft-versus-host disease (GVHD) in 12 patients**
Møller-Hansen et al., 2024	**dry eye disease (DED) secondary to Sjogren's syndrome (SS)**

Future directions

- MSC-based therapies hold significant potential for treating a wide range of ocular diseases. However, further research is essential to address the current challenges and pave the way for widespread clinical adoption.

- **Development of engineered MSCs** with enhanced therapeutic properties.
- **Exploring exosome-based therapies**, where extracellular vesicles derived from MSCs could offer a safer and equally effective alternative.
- **Combining MSC therapy with gene editing** or biomaterials to improve integration and functionality in ocular tissues.

Thanks!

Do you have any questions?

ahmed.mostafa@rio.sci.eg
+2 010 9696 87 99
https://www.researchgate.net/profile/Ahmed-Ata