The Potential Role of Primary Collagen Therapeutics in Treating Ocular Surface and Corneal Stromal Diseases

Jay S. Pepose, MD, PhD, ABO
Medical Director, Pepose Vision Institute
Professor of Clinical Ophthalmology and Visual Sciences
Washington University School of Medicine
St. Louis, Missouri

Co-Authors: **Robert O. Baratta, Eric Schlumpf, Brian J. Del Buono, Brian Ceresa, and David J. Calkins**

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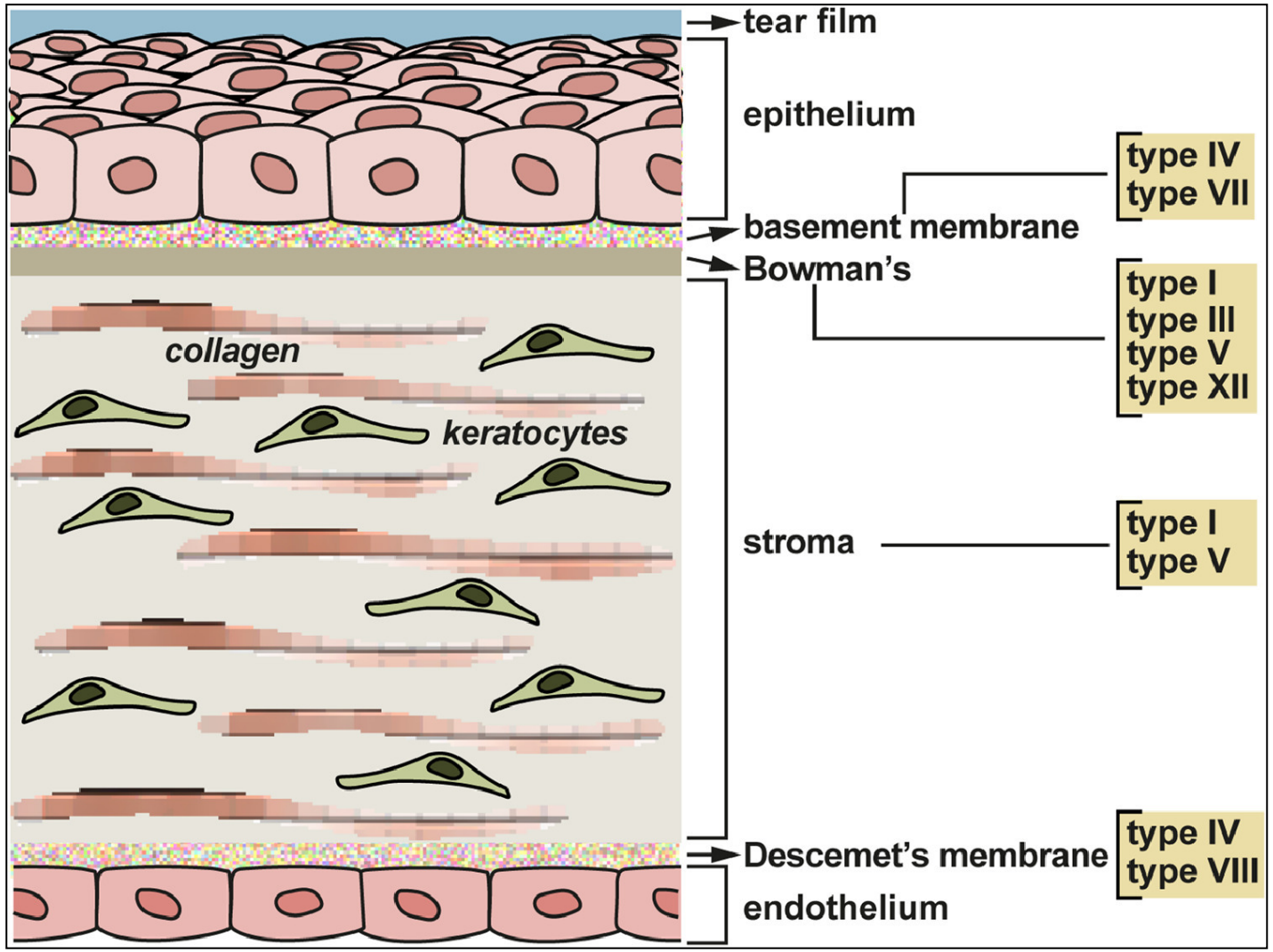
Corneal Collagens

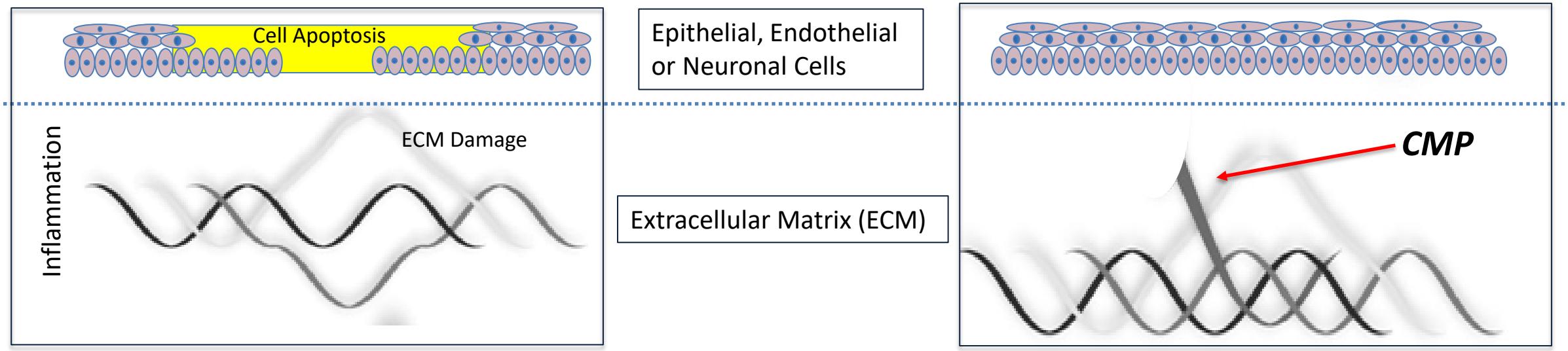


Baratta RO, Schlumpf E, Del Buono BJ, DeLorey SA, Calkins DJ. Surv Ophthalmol 2021 (In press); https://doi.org10.1016/j.surveyophthal.2021.04.006

Collagen, as a Part of the Corneal Epithelial and Stromal ECM, Plays a Key Role in Cell Regeneration and Inflammation

Auto-immune, degenerative, metabolic or infectious disease:

Restoration of tissue homeostasis:



The rapid repair of damaged ECM collagen restores cell signaling ligand binding sites, disrupted by disease, for important inflammation modulation

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Matrix Metalloproteinases

MMPs in the Anterior Segment	
Tear Film	MMP-1, -2, -8, -9
Cornea	
Epithelium	MMP-1, -9, -10, -12, -13, -14
Stroma	MMP-1, -2, -3, -14
Endothelium	MMP-2[a], -9[a]
Aqueous Humour	MMP-2, -3, -9
Lens	MMP-2, -9, -14
Trabecular Meshwork	MMP-2, -3, -9
Uveoscleral Outflow	MMP-1, -2, -3, -9
Conjunctiva	MMP-1, -2, -3

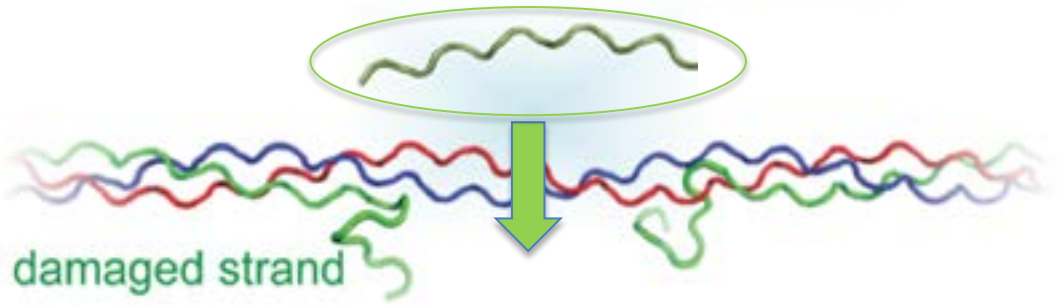
Upregulated MMP-9
Persistent epithelial defect
Dry eye disease
Sjögrens syndrome
Rosacea
Pterygium
Recurrent erosions
Alkali/thermal corneal burn
OCP/GVHD
Keratoconus
Microbial Keratitis
Mooren's ulcer
Peripheral ulcerative keratitis (RA, SLE)

Wong TTL, Sethi C, Daniels JT, et al. Surv Ophthalmol 47:239-256, 2002.

Collagen Mimetic Peptide's Mechanism of Action is the Direct Molecular Repair of Damaged Triple Helix Collagen

***Collagen Mimetic Peptide (CMP)* Repair – Molecular View in Inflammatory Disease**

CMP is specifically designed to intercalate into damaged triple helix collagen



CMP:

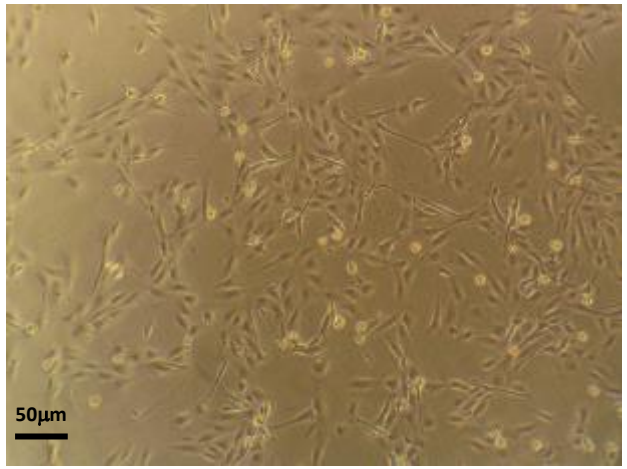
- ✓ ***Works rapidly***
- ✓ ***No off-target effects***
- ✓ ***Repairs collagen tissues***
- ✓ ***Restores tissue to homeostasis***

CMP *repairs Type I collagen triple helices, found in all collagen types; these Type I domains host a variety of important cell signaling proteins*

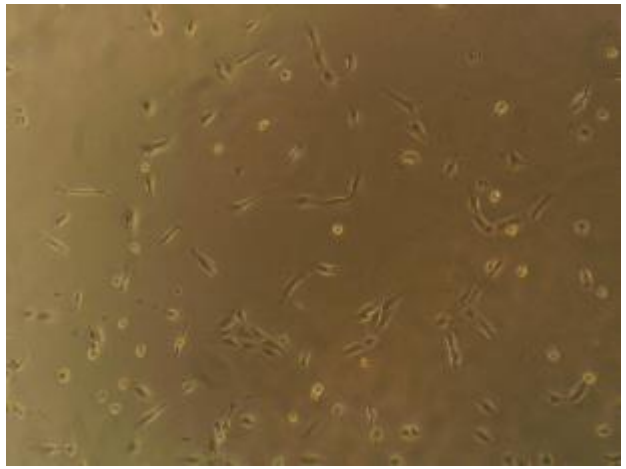
Epithelial Cell Regeneration

ARPE19 CELL Regeneration, 19 hours after plating

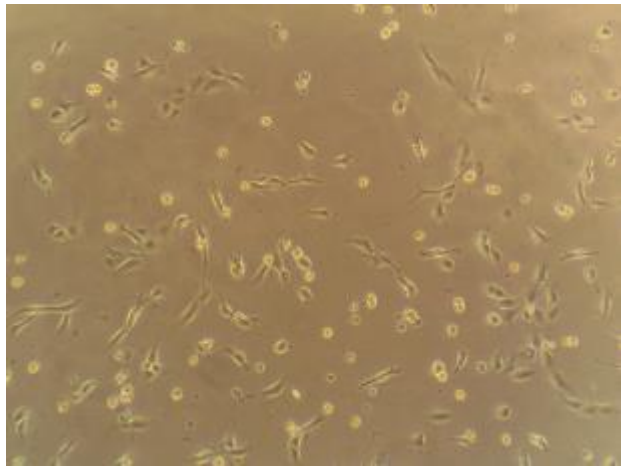
Collagen



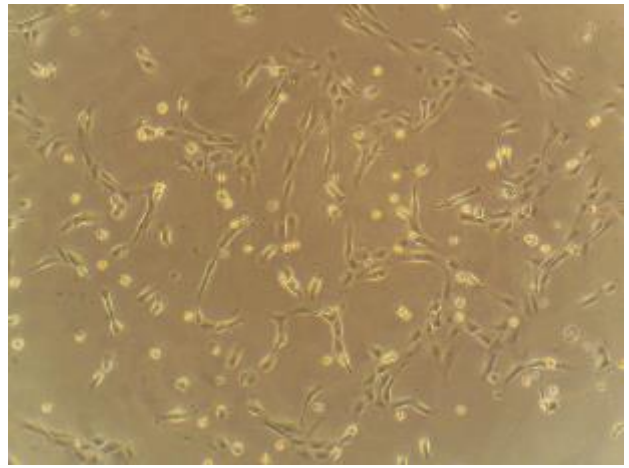
Cut collagen



Cut collagen + CMP A

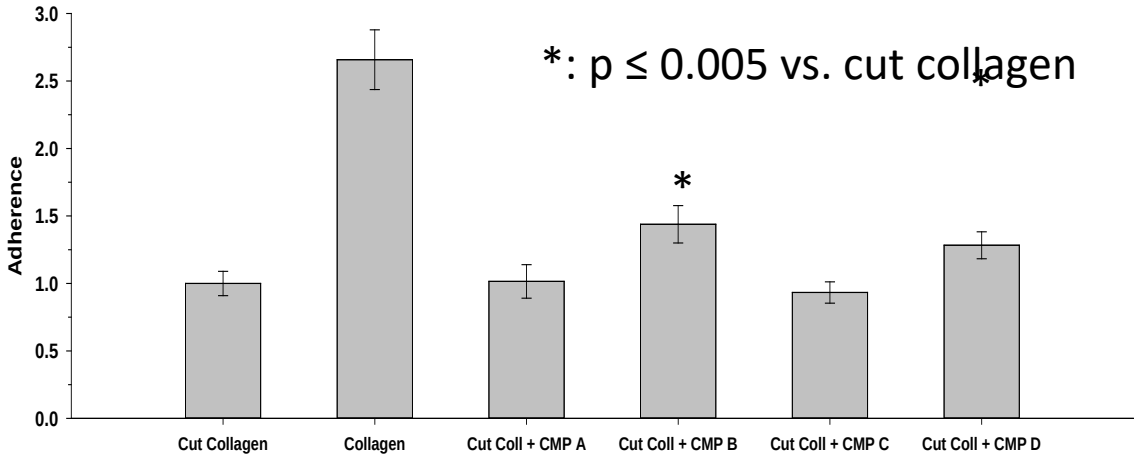


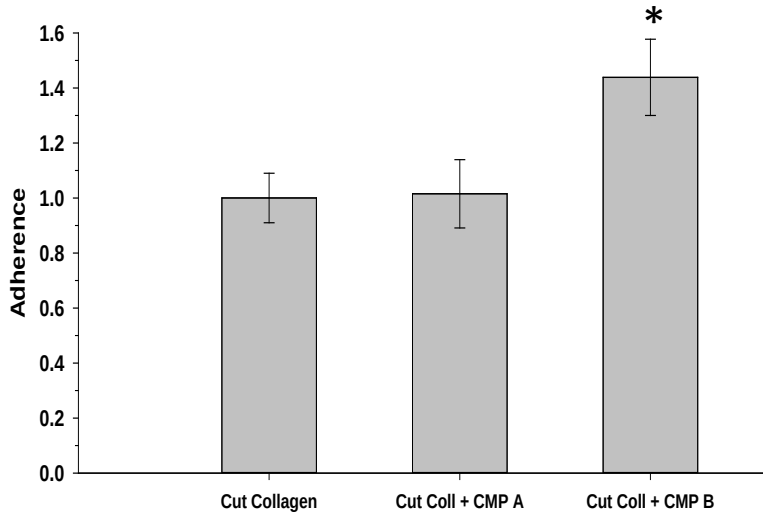
Cut collagen + CMP B



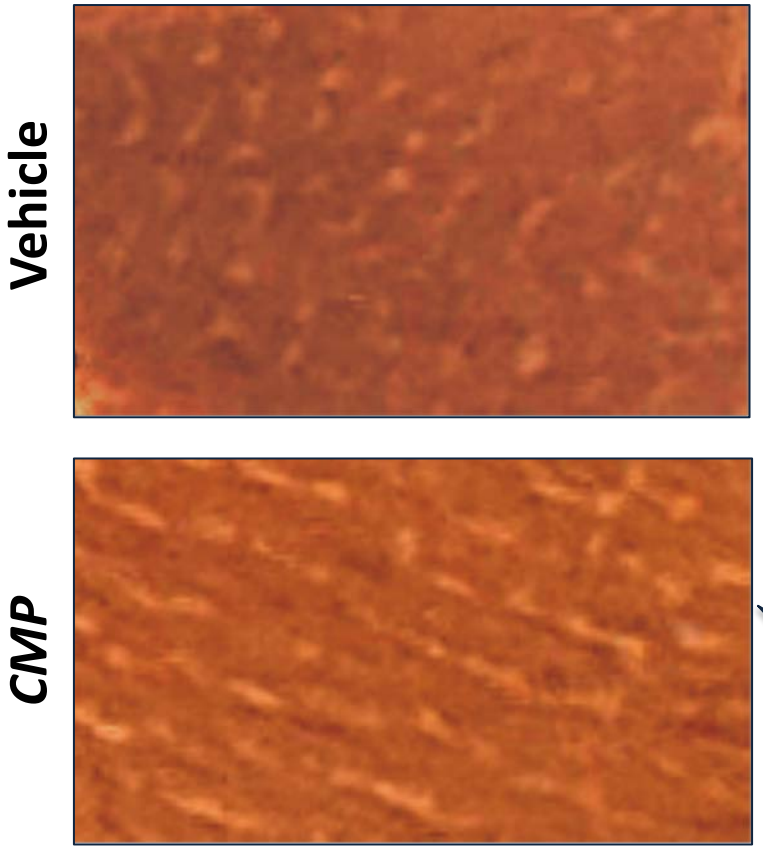
All data normalized with respect to cut collagen condition because of variability in its effectiveness to inhibit adherence

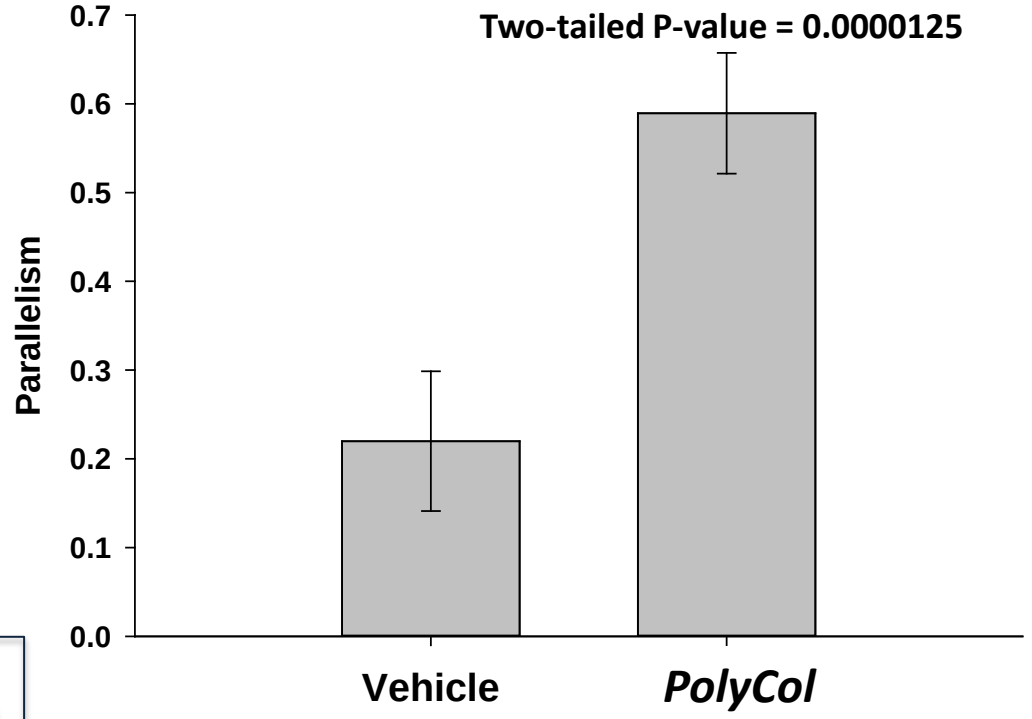
*: $p \leq 0.005$ vs. cut collagen





Threefold Improvement in Collagen Fiber Organization





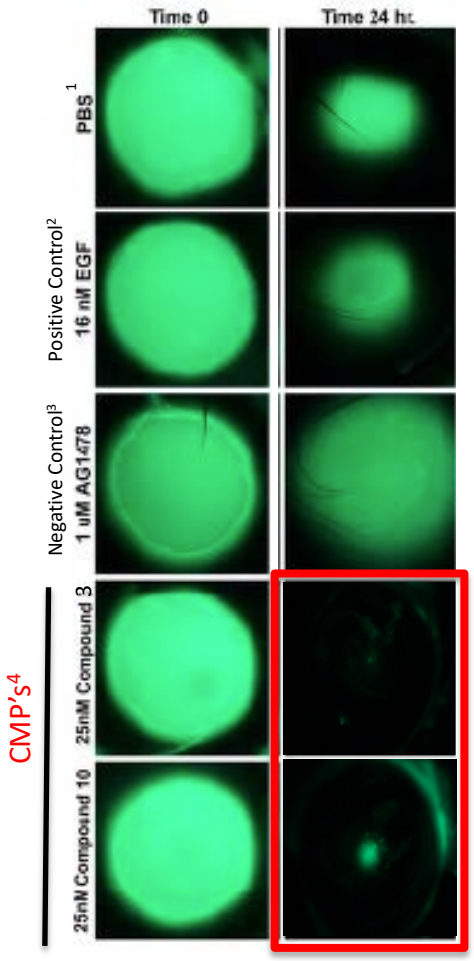
* *in vitro* test: collagen base damaged with collagenase, then treated with vehicle (control) or ***CMP***

** Testing conducted at Vanderbilt University, 2019

Damaged collagen in membranes such as Bowman's layer in the cornea inhibit the recovery of the cell layers they support (e.g. corneal epithelium). Differential Contrast photomicrography.

Baratta RO, Del Buono DJ, Schlumpf E, Ceresa BP, Calkins DJ. Frontiers in Pharmacology (submitted)

In vivo Healing in Mouse Eye Wounds



[1] PBS: Phosphate Buffered Saline

[2] EGF: 100ng/ml Epidermal Growth Factor; positive control

[3] AG1478: Tryphostin AG,epidermal growth factor receptor inhibitor; negative control

[4]Collagen Mimetic Peptides; Cmpd 3 ***sequence 3***, Cmpd 10 ***sequence 10*** with linked Substance P

Experiment Description

Mouse eye wounds (1.5mm diameter) created via trephine, followed by an Alger brush scouring technique. The wounds were designed to penetrate into the anterior stroma, damaging and exposing collagen.

Results

- Fluorescein marks the damage to the stroma, and treated showed a *marked reduction in the diameter of the fluorescein staining*
- This result indicates *significant stromal recovery as well as epithelial regeneration.*

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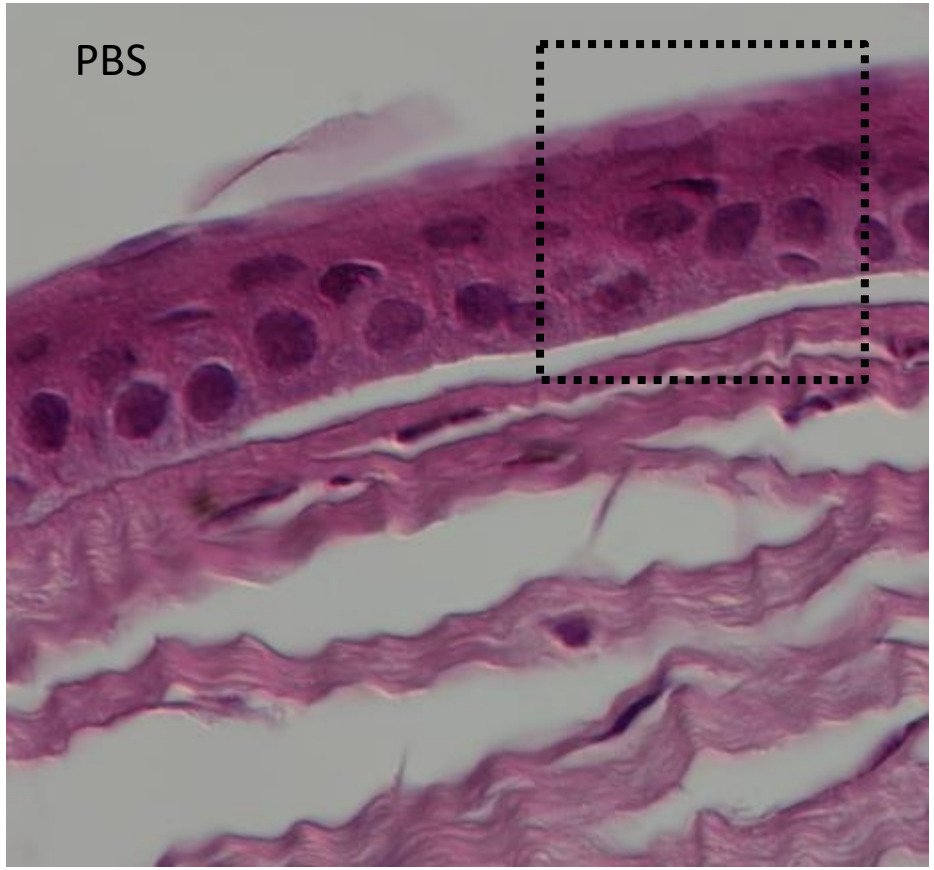
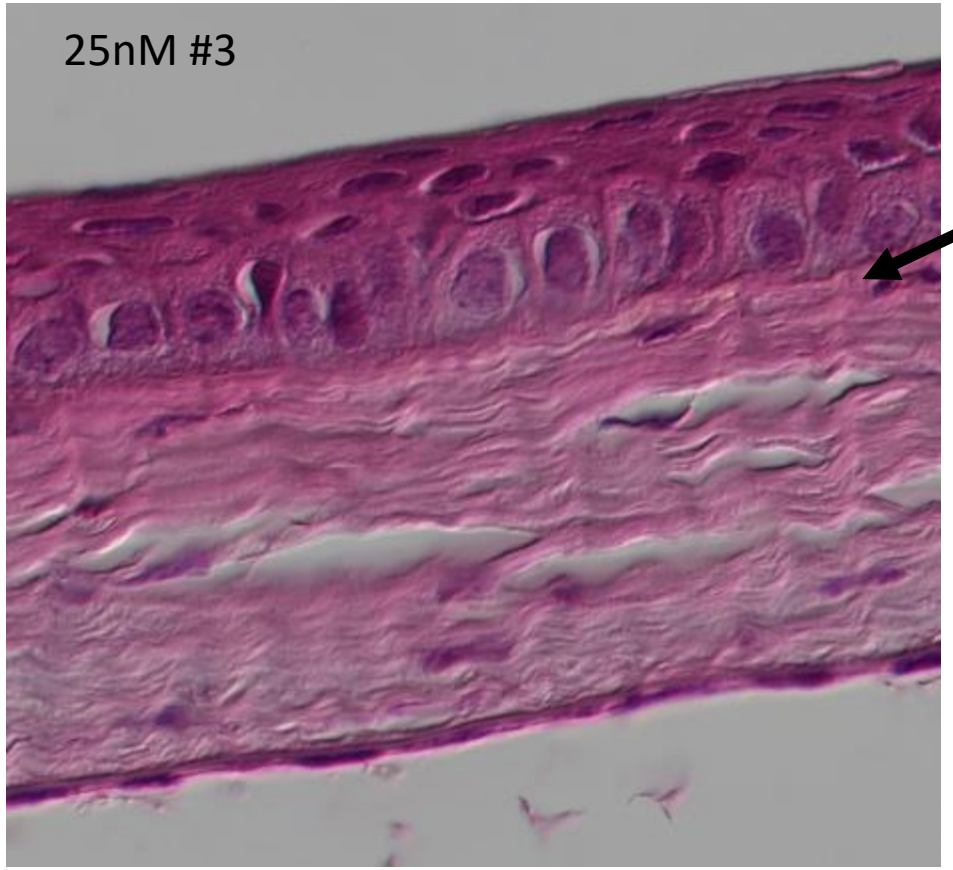
Epithelial Cell Adherence to Bowman's, Regularity and Stromal Recovery

Corneal epithelial cells form 5-7 layers, with older cells nearer the surface. We quantified number of nuclei in multiple samples of fixed area covering the wound zone.



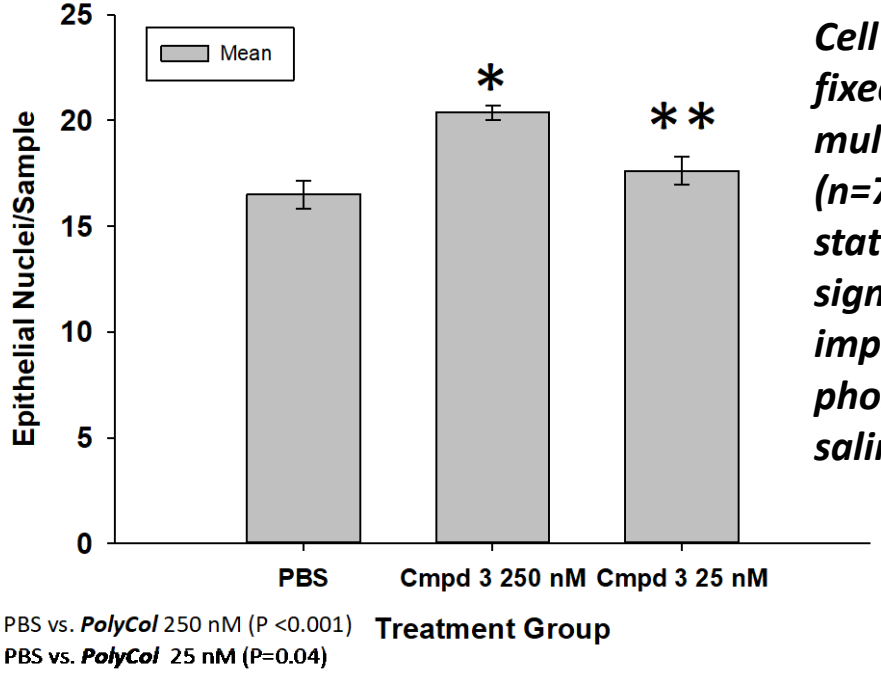


New epithelial cells adhere to Bowman's layer and migrate towards the surface to form flat cells as they age. Adherence is a property necessary for corneal healing.

We quantified the total length of adherence for a segment of fixed length (wound size).

24 hrs
100x Differential Interference Contrast Optics
haematoxylin and eosin stain (**H&E stain**)

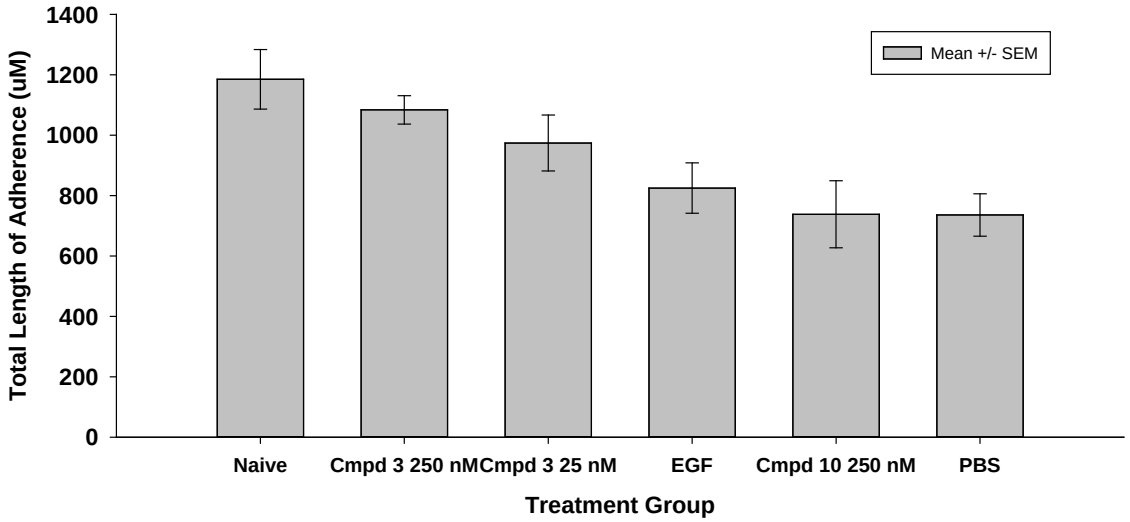
Formation of New Epithelial Cells at the Corneal Surface at 24 Hrs vs. PBS



* PBS vs. ***PolyCol*** 250 nM (P <0.001)
** PBS vs. ***PolyCol*** 25 nM (P=0.04)

Cell nuclei counts in a fixed area across multiple samples (n=7) demonstrated statistically significant improvement over phosphate buffered saline controls

Adherence of Epithelium to Bowman's Layer at 24 Hrs vs. PBS.



Total length of epithelial adherence with 250 nM Cmpd 3 is similar to unwounded cornea (p=0.24). Cmpd 3 is also more effective than EGF (p=0.04).

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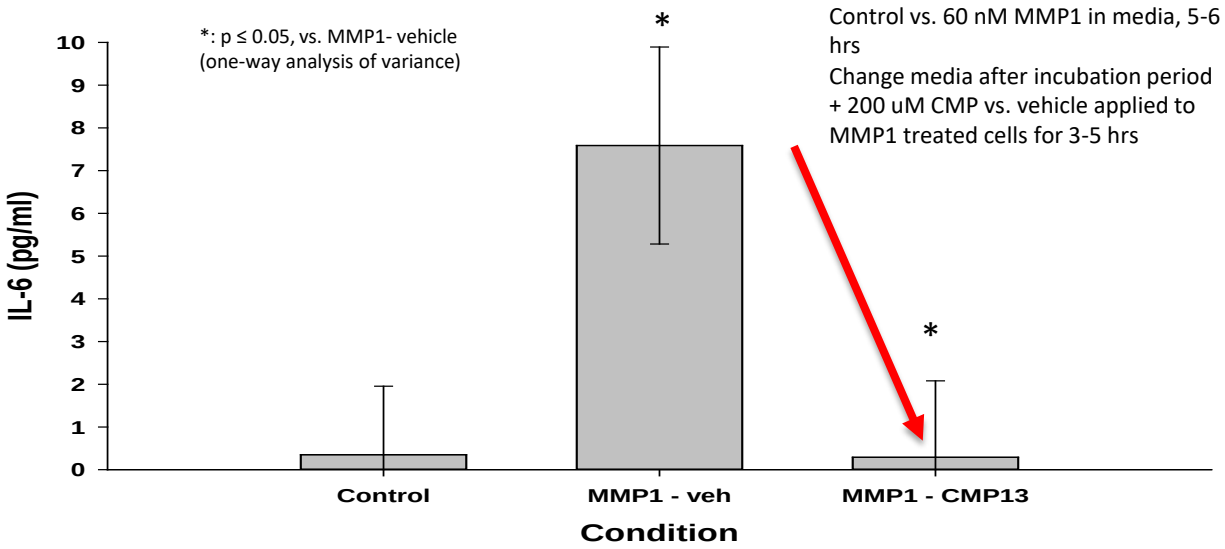
Restoration of Cell Signaling Binding Sites, Reducing Inflammatory Cytokines

CMP reduced IL-6 secretion by 96%



ARPE-19 Cells, application of MMP-1 and CMP

IL-6 secretion caused by MMP-1 application, is modulated, suggesting collagen repair reduces inflammation and can break chronic inflammatory cycles

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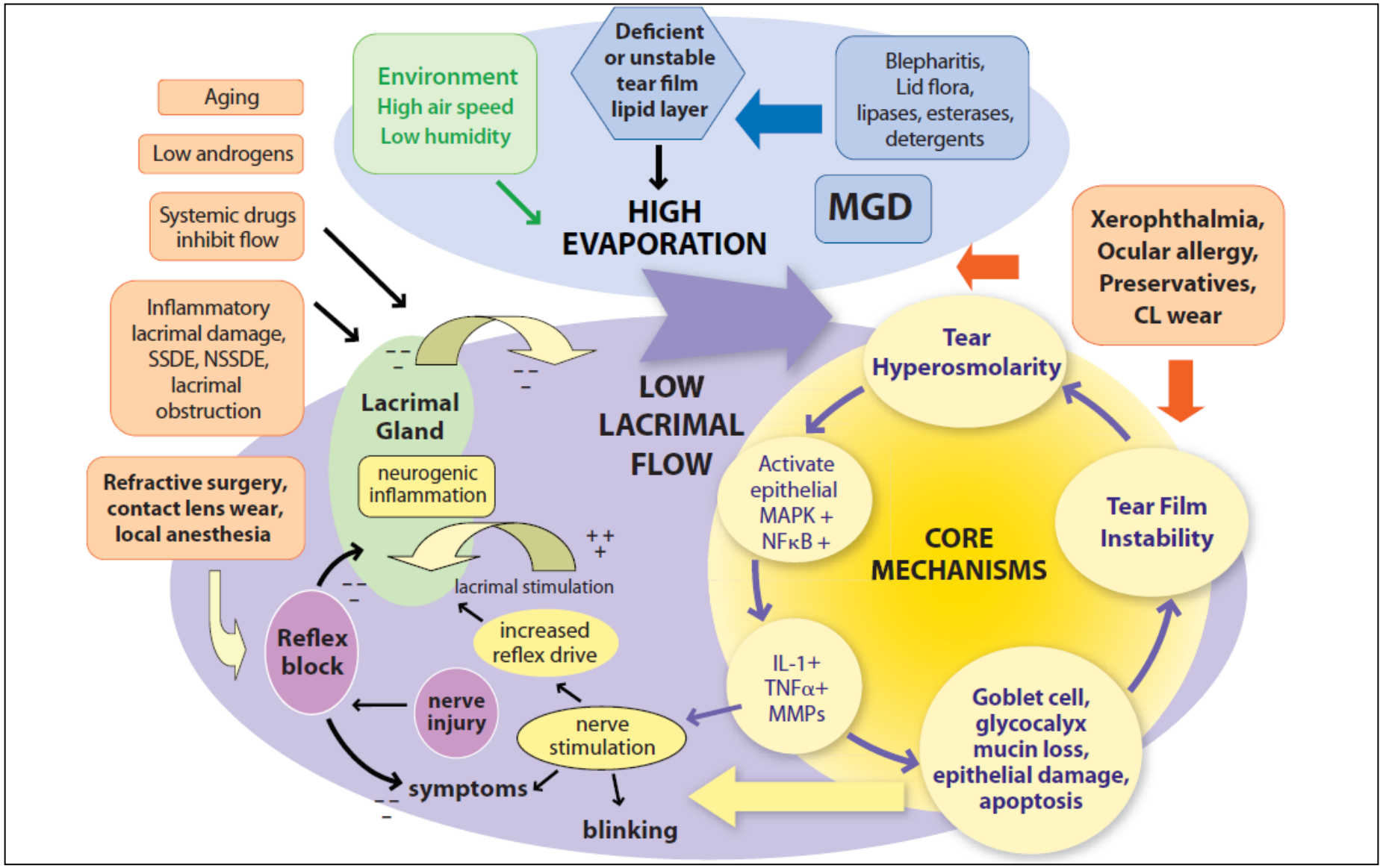
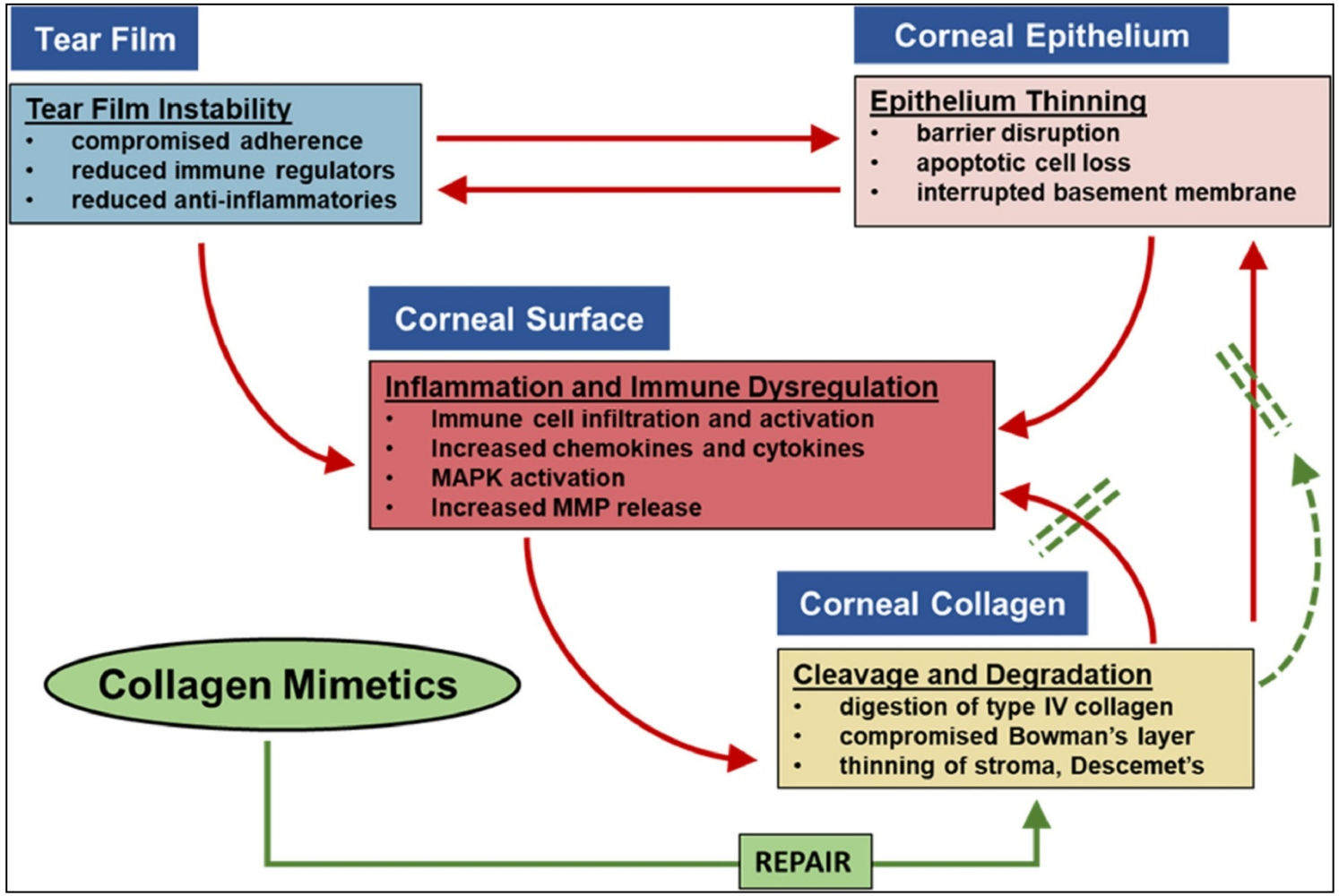
Vicious Cycle of Increasing Tear Hyperosmolarity, Inflammation and Ocular Surface Damage Accompanies Breakdown of Homeostatic Mechanisms



Bron AJ, Yokoi N, Gaffney E, Tiffany JM. Predicted phenotypes of dry eye: proposed consequences of its natural history. Ocular Surf 2009; 7:78-92

Potential Role of CMP in Dry Eye Disease



- Many common diseases affecting the ocular surface and corneal stroma are associated with elevated levels of MMPs. damaged collagen, and epithelial cell apoptosis, including DED.

- Collagen mimetic peptides have been shown both in vitro and in animal models to promote epithelial cell wound healing and adherence, restore immunomodulatory binding sites associated with lower levels of inflammatory cytokines, such as IL-6, which play a central role in the MAPK driven inflammatory cascade in patients with chronic DED.

- CMP is now being studied in a FDA registered phase 2 masked, randomized, placebo controlled clinical trial of patients with dry eye disease.

Baratta RO, Schlumpf E, Del Buono BJ, DeLorey SA, Calkins DJ. Surv Ophthalmol 2021 (In press)