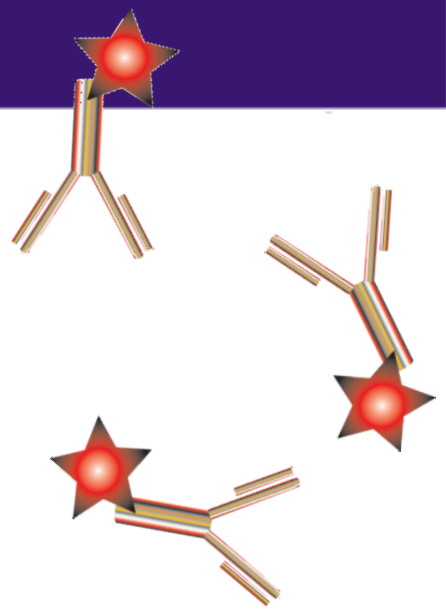
RayBiotech, Inc.
the protein array pioneer company

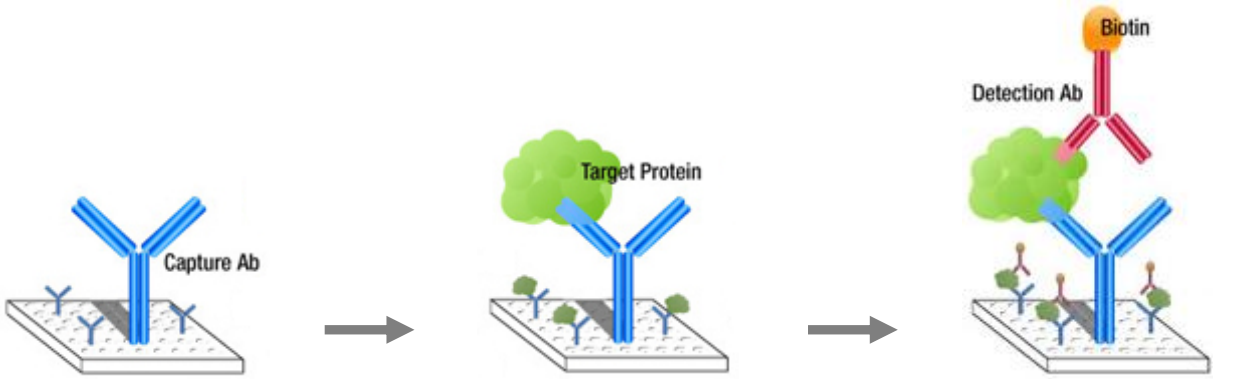
# The Application of Protein Array Technology to Investigations of Ocular Disease



Valerie S. Jones, Ph.D.
RayBiotech, Inc.
Manager, Marketing & Tech Support

Confidential – All Rights Reserved

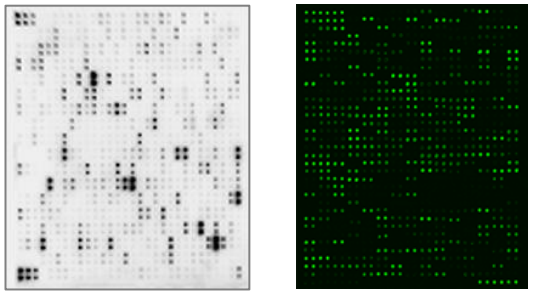
# Antibody array methodology



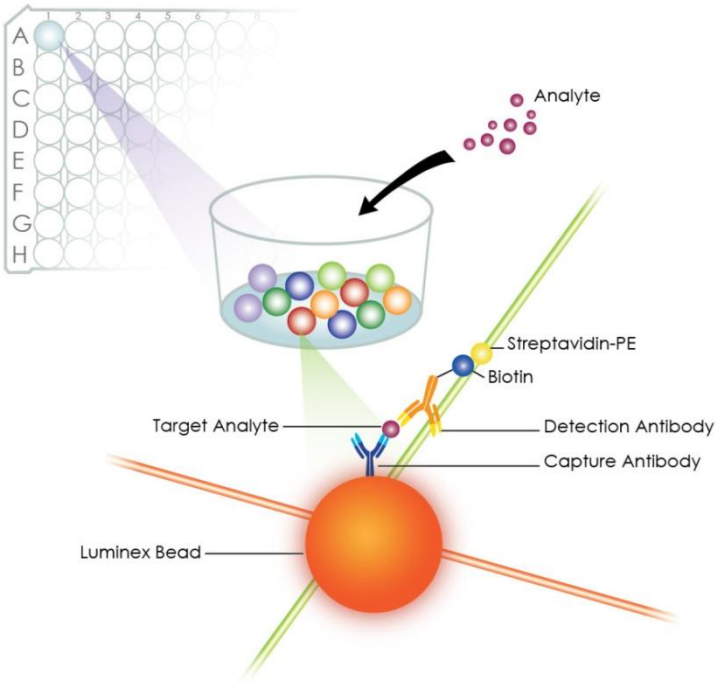

Array support spotted with multiple antibodies

Incubate sample with array

Detect bound proteins



# Cytometric bead assay methodology



# Cytokine Signatures of Vitreoretinal Disorders in Vitreous Humor

**Can disease-specific cytokine profiles be identified for various different vitreoretinal diseases?**

| | |
|---|---|
| Proliferative Diabetic Retinopathy | *n* = 10 |
| Proliferative Vitreoretinopathy | *n* = 8 |
| Chronic Uveitis | *n* = 8 |
| Lens-Induced Uveitis | *n* = 15 |
| Epiretinal Membrane (control) | *n* = 8 |

Collect vitreous; multiplex bead analysis (19 cytokines)

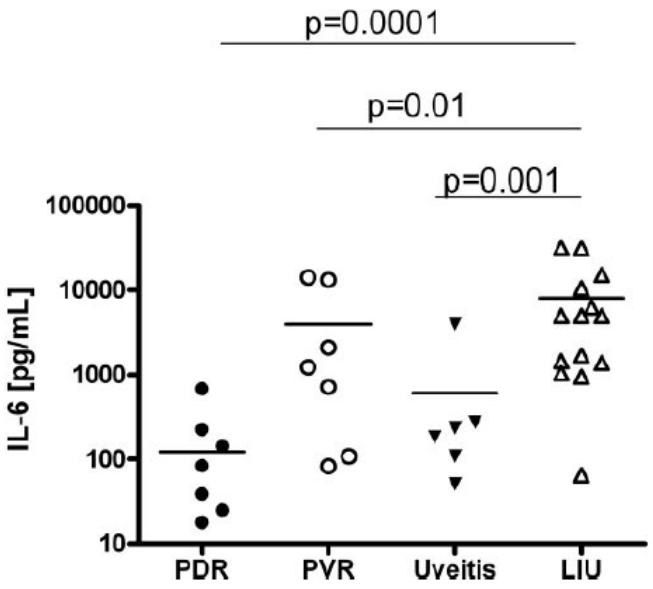
Vitreous samples were taken from patients undergoing vitrectomy for either inflammatory or proliferative vitreoretinal disease

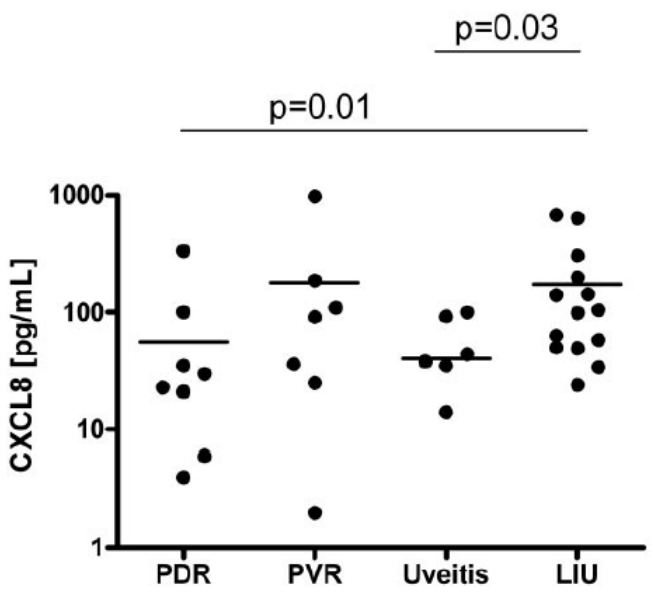
Banerjee, S., et al., *Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders.* Invest Ophthalmol Vis Sci, 2007. **48**(5): p. 2203-7.

# Inflammatory profiles detected in proliferative retinopathies

| | ERM (control) | PDR | PVR | Chronic Uveitis | LIU |
|---|---|---|---|---|---|
| IL-6 | 6 (0–25) | 32 (0-686) | 975 (0–14182) | 145 (0–4019) | 5000 (65–31770) |
| IL-8 | 5 (0–27) | 22 (0-335) | 63.5 (0–977) | 36 (0–100) | 99 (0–682) |
| IL-12 | ND | 14 (0-48) | 15 (0–88) | ND | 3 (0–19) |
| MCP-1 | 449 (34–1228) | 1310 (0-7918) | 6101 (128–8777) | 635 (0–3102) | 5350 (1350–13688) |
| MIP-1β | ND | 4.5 (0-33) | 22 (0–133) | ND | 15 (0–41) |
| RANTES | ND | 3 (0-26) | ND | ND | ND |
| VEGF | ND | 246 (0-3193) | 975 (0–14182) | ND | 286 (0–3161) |
| FGF | ND | ND | 28 (0-255) | ND | ND |

# LIU vitreous contains significantly greater levels of IL-6 and IL-8 compared to CU

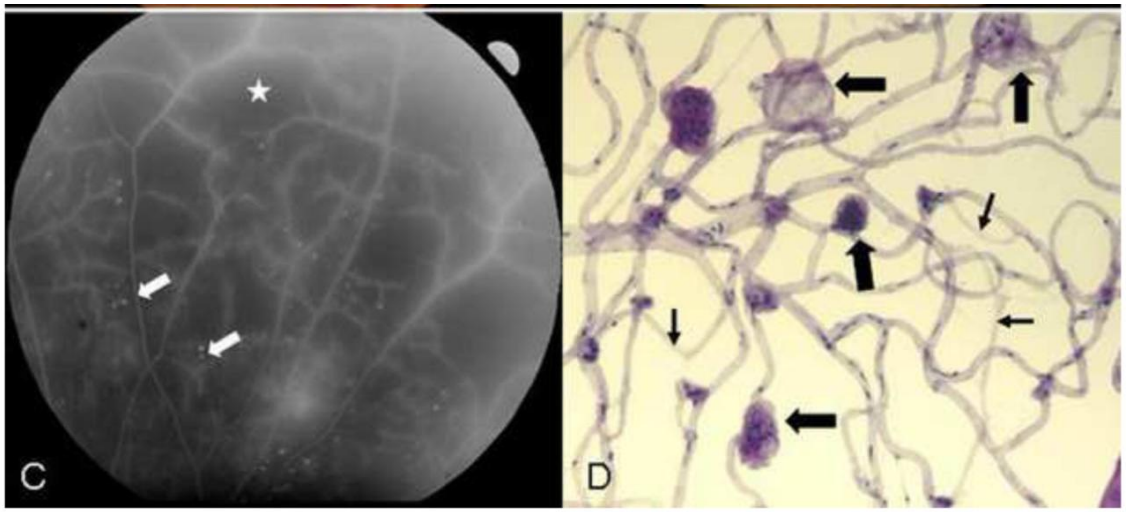



Banerjee, S., et al., *Multiplex bead analysis of vitreous humor of patients with vitreoretinal disorders.* Invest Ophthalmol Vis Sci, 2007. **48**(5): p. 2203-7.

# Diabetic Retinopathy

- The leading cause of visual impairment in the U.S.
- The most common complication of diabetes, where chronic hyperglycemia culminates in vascular damage in the retina
- Oxidative stress, altered PKC signaling, increases in AGEs

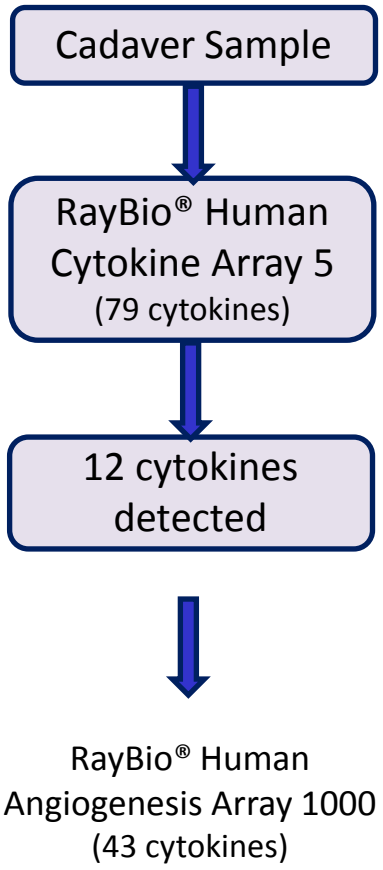



**PDR (fluorescein angiogram)** **Isolated retinal microvessels**

Tang, Johnny, and Timothy S. Kern. "Inflammation in diabetic retinopathy." *Progress in retinal and eye research* 30.5 (2011): 343-358.

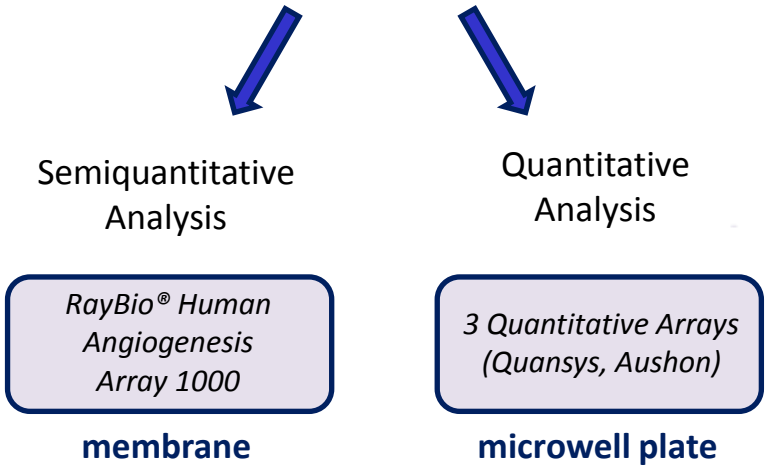
# Exploring Vitreal Cytokine Profiles in DR

## Preliminary Screen




## Follow up Study

| | | |
|---|---|---|
| **Non-diabetic controls** | Epiretinal Membrane | *n* = 9 |
| | Retinal Detachment | *n* = 6 |
| **Diabetic** | NPDR | *n* = 14 |
| | PDR | *n* = 13 |



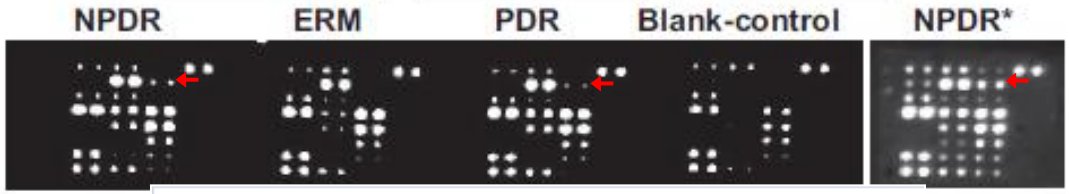

Schwartzman, ML, et al. "Profile of lipid and protein autacoids in diabetic vitreous correlates with the progression of diabetic retinopathy." *Diabetes* 59.7 (2010): 1780-1788.

# Patient vitreous exhibits varying levels of inflammatory and angiogenic factors

**individual samples** →




**pooled samples** →




← **rapid transition to proliferative diabetes**

| | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| A | bFGF | PDGF-BB | VEGF-D | POS |
| B | ENA-78 | MCP-1 | VEGF | NEG |
| C | EGF | Leptin | Thrombopoietin | blank |
| D | Angiogenin | IL-8 | TIMP-1 | blank |
| E | NEG | IL-6 | TIMP-2 | blank |
| F | NEG | IGF-1 | TGF-β1 | blank |
| G | POS | IFN-γ | RANTES | blank |
| H | POS | GRO | PlGF | blank |

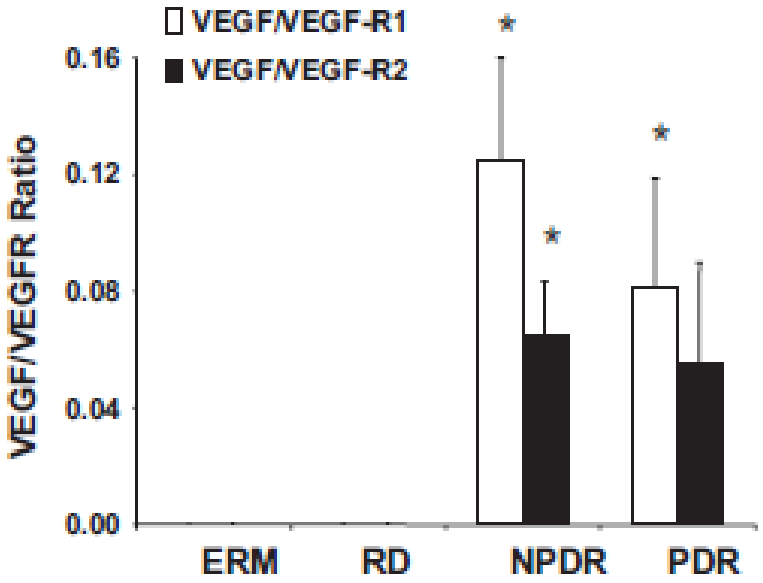
Schwartzman, ML, et al. "Profile of lipid and protein autacoids in diabetic vitreous correlates with the progression of diabetic retinopathy." *Diabetes* 59.7 (2010): 1780-1788.

# Diabetic vitreous displays increases in inflammatory and angiogenic factors

| | | ERM | RD | NPDR | PDR |
|---|---|---|---|---|---|
| chemokines | GROα | ND | 147 ± 83 | 104 ± 50 | ND |
| | IL-8 | 5 ± 3 | 214 ± 130 | 87 ± 42 | 52 ± 25 |
| | IP-10 | 18 ± 7 | 193 ± 86 | 48 ± 20 | 46 ± 19 |
| | MCP-1 | 856 ± 159 | 4,519 ± 1,136 | 1,610 ± 520 | 1,414 ± 210 |
| | RANTES | 14 ± 11 | 70 ± 22 | 199 ± 176 | 21 ± 10 |
| | TARC | 4 ± 2 | 57 ± 17 | 13 ± 7 | 6 ± 2 |
| angiogenic factors | ANG-2 | 1,326 ± 650 | 2,610 ± 740 | 3,641 ± 1,218 | 3,528 ± 1,753 |
| | bFGF | 756 ± 756 | ND | 636 ± 300 | 235 ± 235 |
| | HGF | 27,704 ± 7,912 | 10,846 ± 1,275 | 29,106 3,821 | 24,786 ± 3,009 |
| | PDGF-BB | 872 ± 469 | ND | 969 ± 360 | 366 ± 261 |
| | TIMP-1 | 367,841 ± 84,766 | 567,205 ± 84,921 | 352,477 ± 50,960 | 431,310 ± 54,912 |
| | TIMP-2 | 253,031 ± 44,718 | 126,403 ± 24,625 | 257,830 ± 21,453 | 286,248 ± 31,168 |
| | TNF-α | 31 ± 19 | ND | 153 ± 97 | 125 ± 118 |
| | VEGF | ND | ND | 647 ± 308 | 307 ± 159 |
| receptors | VEGF-R1 | 3,779 ± 360 | 2,735 ± 333 | 4,114 ± 830 | 3,606 ± 607 |
| | VEGF-R2 | 11,823 ± 1,460 | 8,676 ± 1,239 | 8,026 ± 1,809 | 6,430 ± 728 |

Schwartzman, ML, et al. "Profile of lipid and protein autacoids in diabetic vitreous correlates with the progression of diabetic retinopathy." *Diabetes* 59.7 (2010): 1780-1788.

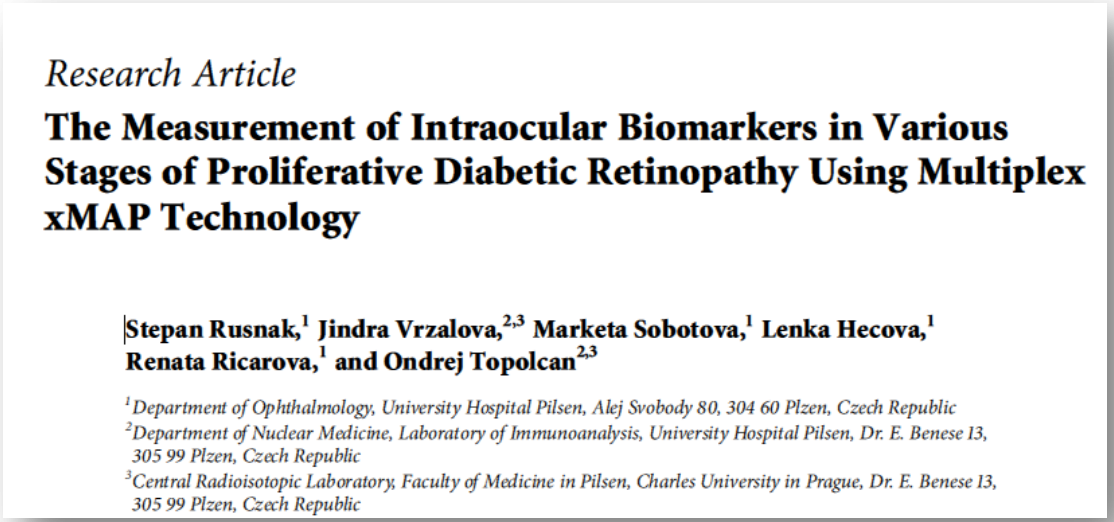
# Increased ratios of VEGF to VEGF receptors in the vitreous of DR patients




Schwartzman, ML, et al. "Profile of lipid and protein autacoids in diabetic vitreous correlates with the progression of diabetic retinopathy." *Diabetes* 59.7 (2010): 1780-1788.

# Recent insight

**"The concentrations of IL-6, TGF$\beta$-1, and VEGF correlate with the severity of PDR. In future, assessment of PDR biomarkers in intraocular fluid could be effective method for treatment monitoring and early detection of PDR progression."**



*Research Article*

**The Measurement of Intraocular Biomarkers in Various Stages of Proliferative Diabetic Retinopathy Using Multiplex xMAP Technology**

**Stepan Rusnak,[1] Jindra Vrzalova,[2,3] Marketa Sobotova,[1] Lenka Hecova,[1] Renata Ricarova,[1] and Ondrej Topolcan[2,3]**

[1] *Department of Ophthalmology, University Hospital Pilsen, Alej Svobody 80, 304 60 Plzen, Czech Republic*
[2] *Department of Nuclear Medicine, Laboratory of Immunoanalysis, University Hospital Pilsen, Dr. E. Benese 13, 305 99 Plzen, Czech Republic*
[3] *Central Radioisotopic Laboratory, Faculty of Medicine in Pilsen, Charles University in Prague, Dr. E. Benese 13, 305 99 Plzen, Czech Republic*

Rusnak, Stepan, et al. *Journal of Ophthalmology* 501 (2015): 424783.

# Keratoconus

- Keratoconus is idiopathic cornea degeneration that leads to progressive corneal thinning and distortion of its normal curvature.
- Characterized by corneal stromal degeneration, reduction in resident keratocytes and collagen lamellae
- Leads to significant visual impairment with high amounts of irregular astigmatism and myopia. One of the leading indications for corneal transplant. Affects 1 out of 2000



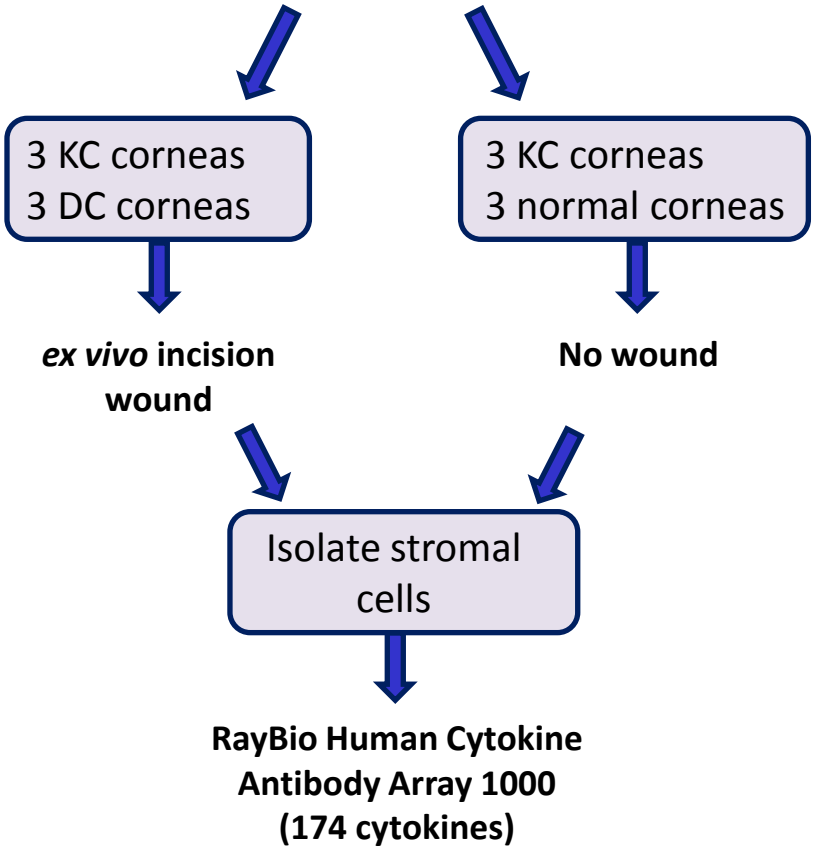
Courtesy of Wikimedia Commons

**Although it has been historically defined as a non-inflammatory condition, recent literature supports a possible role of inflammatory agents in the course of the disease.**

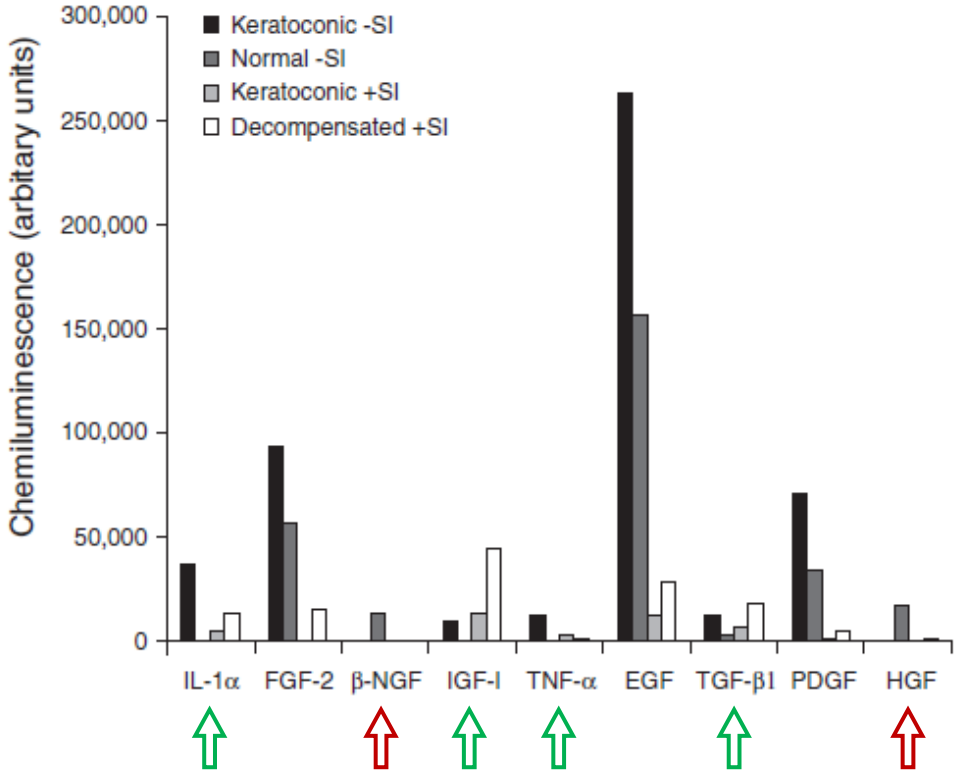
# Is the wound reparative response altered in corneas of keratoconic patients?

Cornea biopsies from penetrating keratoplasty

| | |
|---|---|
| **Keratoconus** | **6** |
| **Decompensated (non-KC) donor** | **3** |
| **Normal donor** | **3** |

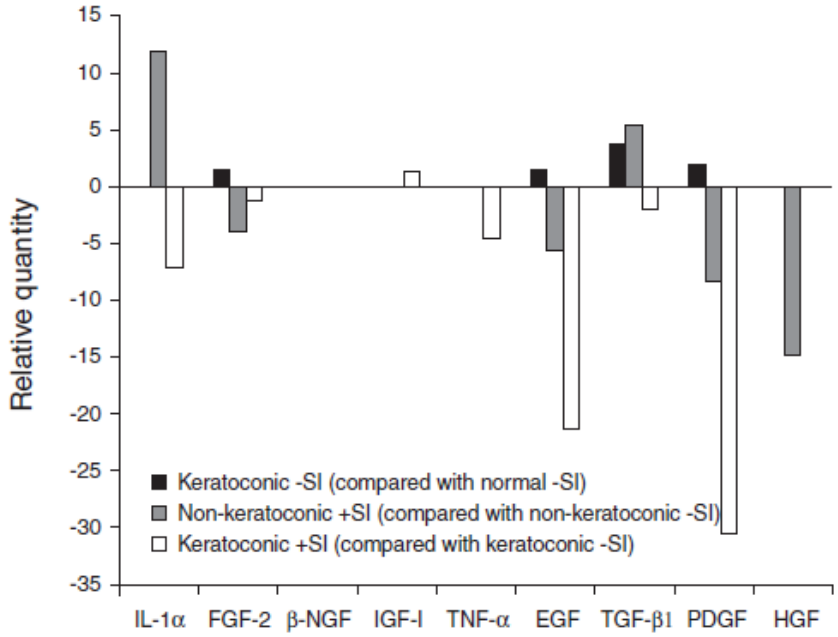


# Keratoconic corneas exhibit a similar cytokine profile to wounded corneas




Cheung, Isabella MY, Charles NJ McGhee, and Trevor Sherwin. "Deficient repair regulatory response to injury in keratoconic stromal cells." *Clinical and Experimental Optometry* 97.3 (2014): 234-239.

# Keratoconic corneas exhibit a blunted wound repair response




Cheung, Isabella MY, Charles NJ McGhee, and Trevor Sherwin. "Deficient repair regulatory response to injury in keratoconic stromal cells." *Clinical and Experimental Optometry* 97.3 (2014): 234-239.

# Conclusions

- Corneas affected by keratoconus appear perpetually in an injured state.
- Furthermore, keratoconic corneas may be incapable of orchestrating a normal reparative response to an insult. Over time, this may lead to insufficient restoration of the diseased tissue following damage.




# THANK YOU