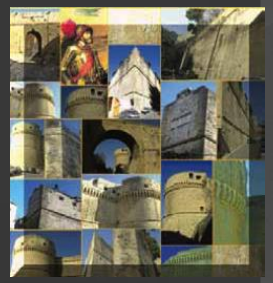
XV CONGRESSO SOCIETÀ OFTALMOLOGICA CALABRESE
"NEWS IN OFTALMOLOGIA"
2 - 3 Ottobre 2015
Grand Hotel Balestrieri Torre Melissa (KR)



Distacco di Retina e PVR

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Chairman: Claudio Azzolini MD

Presenter current disclosure information

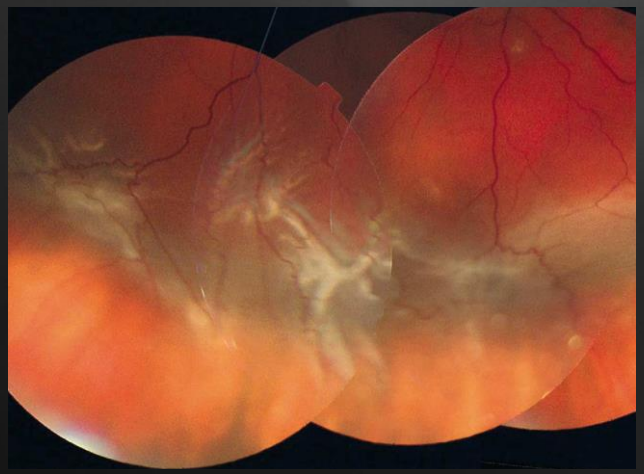
Receipt of grants/research support:

- **Novartis**
- **Bausch&Lomb**
- **Allergan**
- **Bayer**
- **Thrombogenics Diemme**
- **Medicalia**
- **B&D**

Azzolini MD has no affiliations with any product or pharmaceutical manufacturer

Definizione di PVR

- Processo cicatriziale
- Crescita e contrazione di membrane cellulari:
  - all'internodella cavità vitrea
  - su entrambe le superfici della retina
  - e processo fibrotico della retina stessa, in molti casi



Incidenza: Circa 5-10%

Pastor JC Prog Retin Eye Res. 2002

Etiologia della PVR

- Fattori Pre-operatori
  - persistenza del distacco retinico non trattato
  - dimensione della rottura
  - fattori infiammatori
- Fattori Intra-operatori
  - persistenza di pigmento
  - crio-laser pessia intensa
  - fattori infiammatori persistenti

Fattori coinvolti

Fattori cellulari

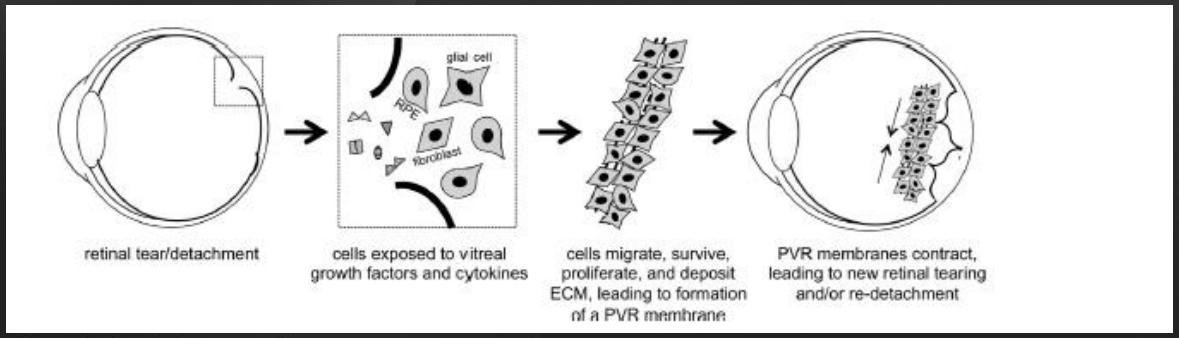
- Cellule EPR
- Cellule gliali
- Cellule di Muller
- Fibrociti
- Miofibroblasti

Fattori biochimici

- Citochine
- Fattori di crescita
- Molecole di adesione

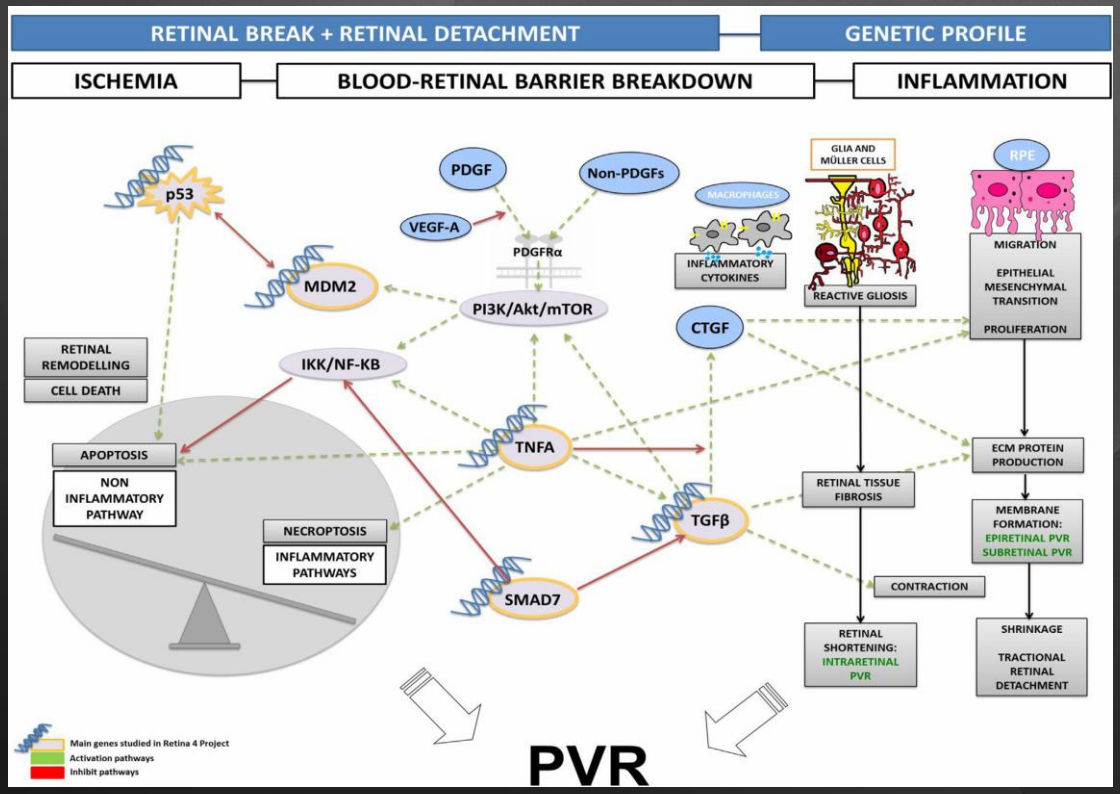
The Three (Overlapping) Biological Phases in the Development of Proliferative Vitreoretinopathy

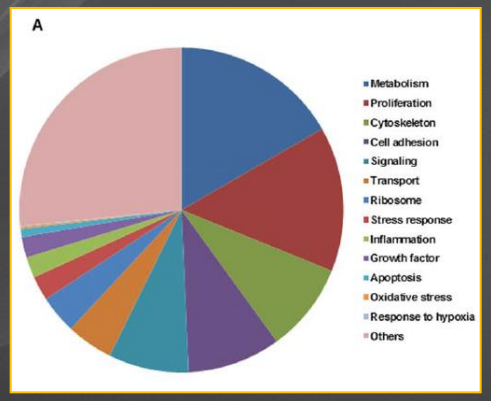
Cell migration (Grade A)	Retinal pigmented epithelial cells migrate through a retinal break into the vitreal cavity Glial cells migrate onto the retinal surface
Contraction (Grade B)	Blood–retinal barrier damage leads to progressive exudation of blood components, such as fibrin, elastin, fibronectin, growth factors, and cytokines
Cell proliferation (Grades C–D)	Collagen synthesis is evidenced by the presence of clearly demarcated membranes, which exert traction on the retina

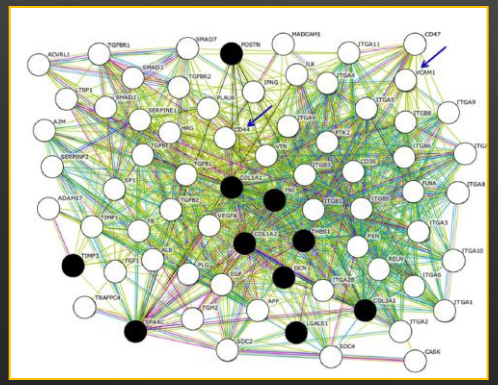


- J Garweg. Surv Ophthalmol 2013

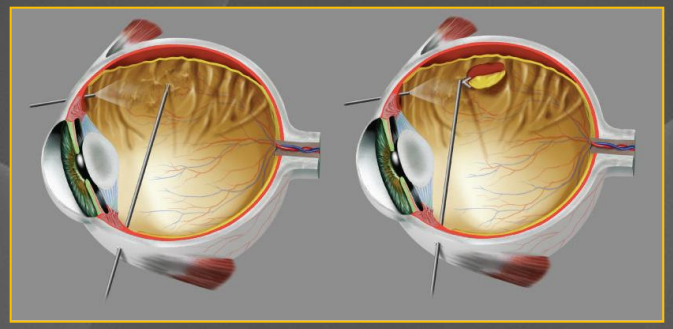
Patogenesi PVR

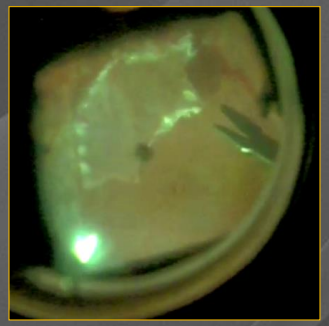




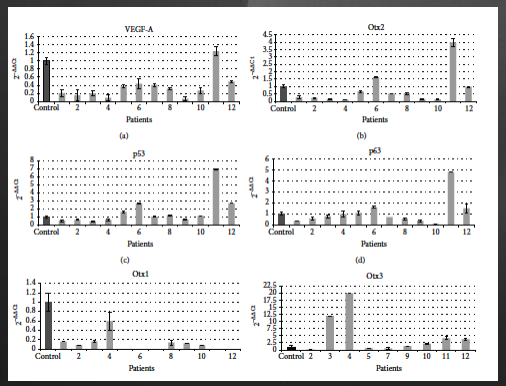


Pastor JC Prog Retin Eye Res. 2015



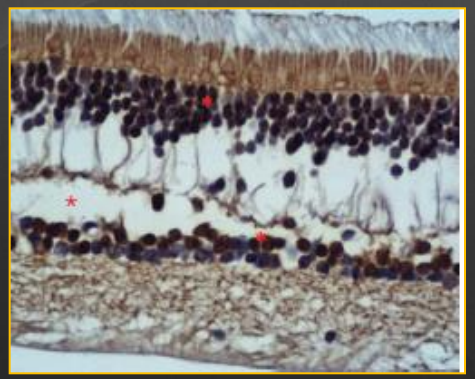


RNA isolation and reverse transcription
Quantitative real-time reverse transcriptase PCR



p53, VEGF-A
omeobox genes (OTX 1, OTX 2, OTX 3)

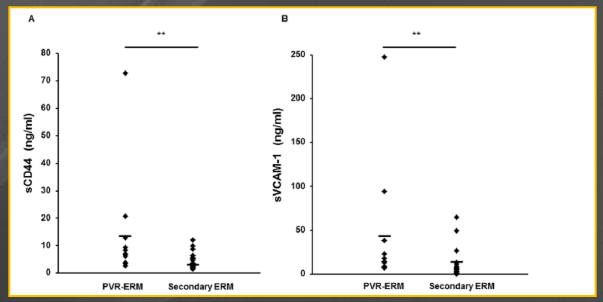
Immunoistochemical assay on human retinal autoptic tissue



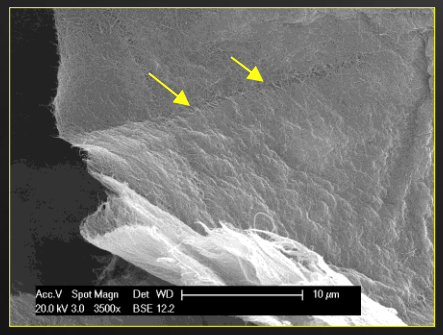
Otx proteins on photoreceptor, bipolar cells, ganglion cells in human retina (first time found in human health and PVR retina)

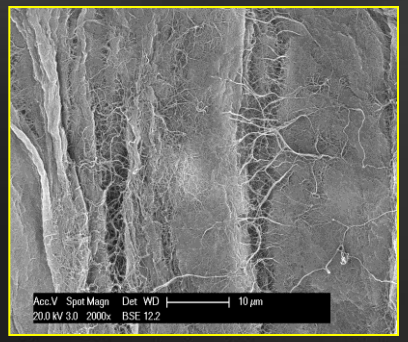
- Azzolini C et al, Expression of VEGF-A, Otx Homeobox and p53 Family Genes in Proliferative Vitreoretinopathy, Mediators of Inflammation 2013

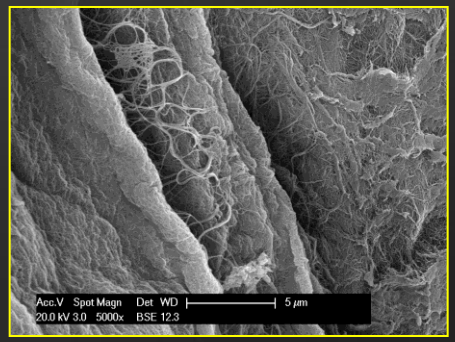
Confronto espressione genica tra PVR ERM e ERM: tendenza proliferativa diversa

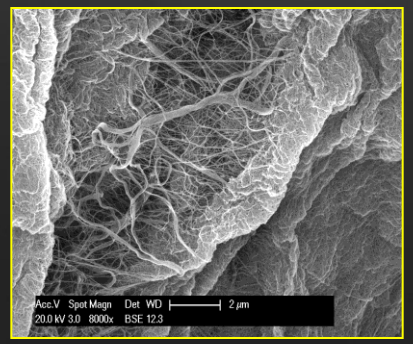


- Asato R. 2013 PLoS ONE 8(1): e54191







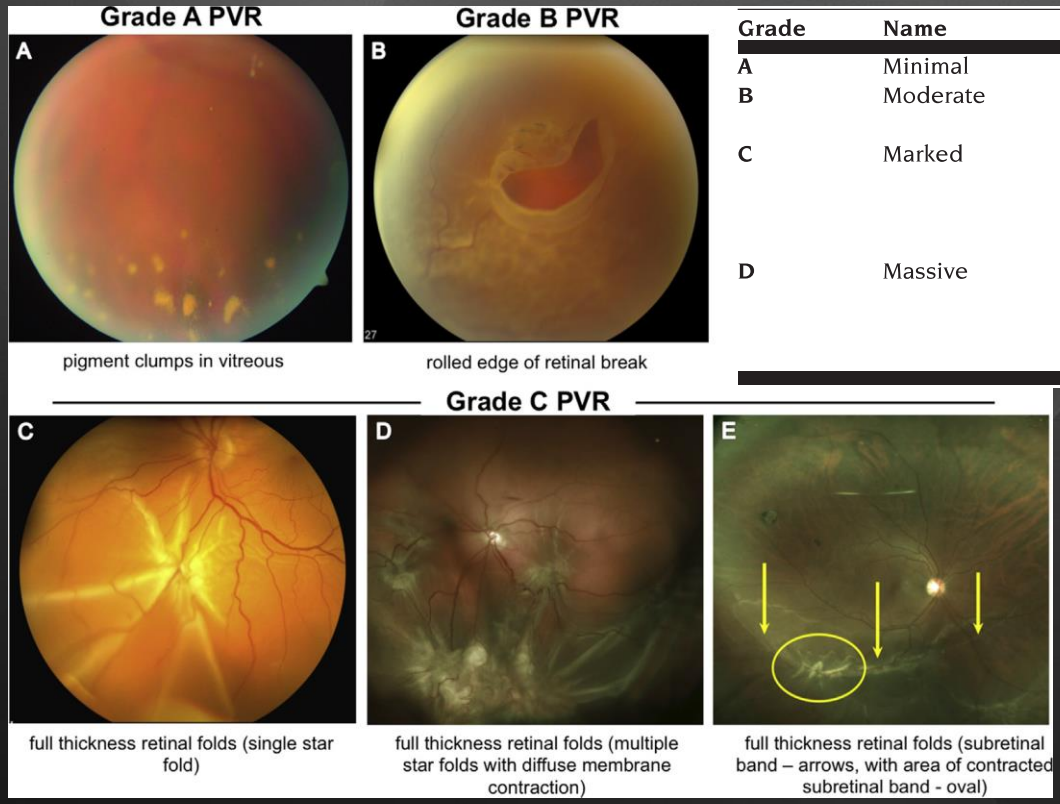


- Azzolini et al, Club Gonin 2014

Classificazione della PVR

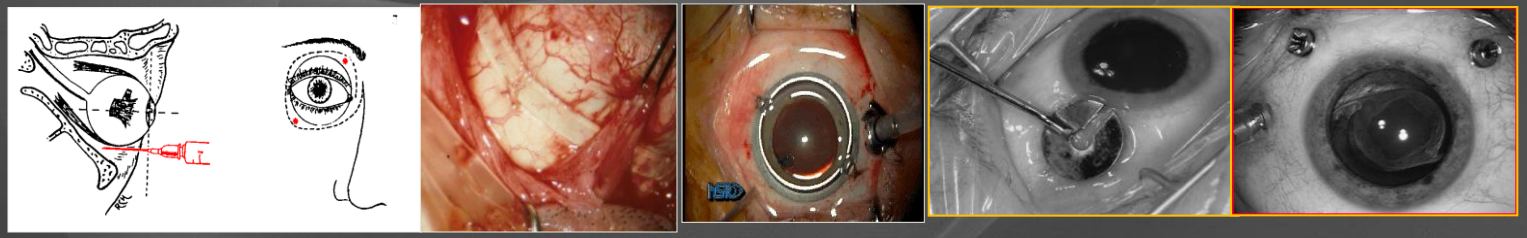
- Caratterizzazione topografica
  - rispetto al neuroepitelio
  - posteriore, anteriore, circonferenziale equatoriale
- Valutazione secondo la gravità
  - pigmento diffuso
  - contrazione retinica a stella
  - contrazione circonferenziale con distacco imbutiforme

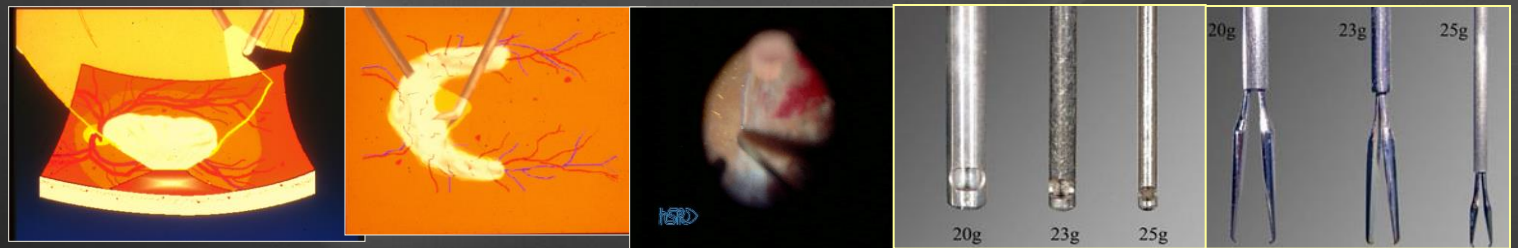
Classificazione della PVR

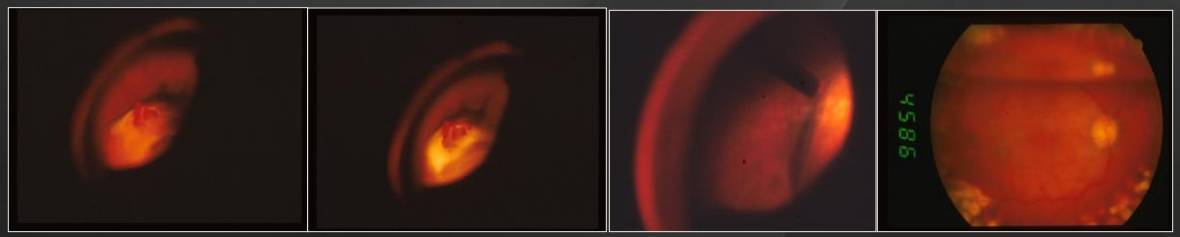


Grade	Name	Clinical Signs
A	Minimal	Vitreous haze, vitreous pigment clumps
B	Moderate	Wrinkling of inner retinal surface, rolled edge of retinal break, retinal stiffness, vessel tortuosity
C	Marked	Full-thickness fixed retinal folds
		C-1 one quadrant
		C-2 two quadrants
		C-3 three quadrants
D	Massive	Fixed retinal folds in four quadrants
		D-1 wide funnel shape
		D-2 narrow funnel shape*
		D-2 closed funnel (optic nerve head not visible)

Tecnica chirurgica

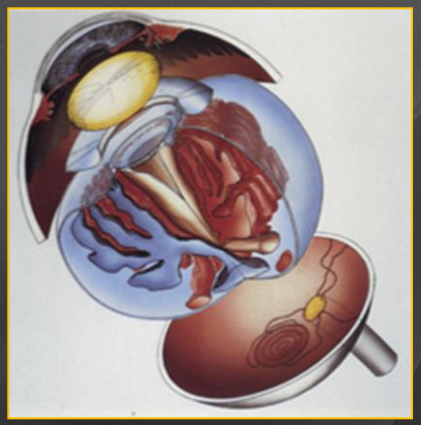






Vitreous body

- gelatinous structure (98-99% water)
- mainly hyaluronic acid and different types of collagen
- water on a bounded form to the glycosaminoglycans (15-20%)



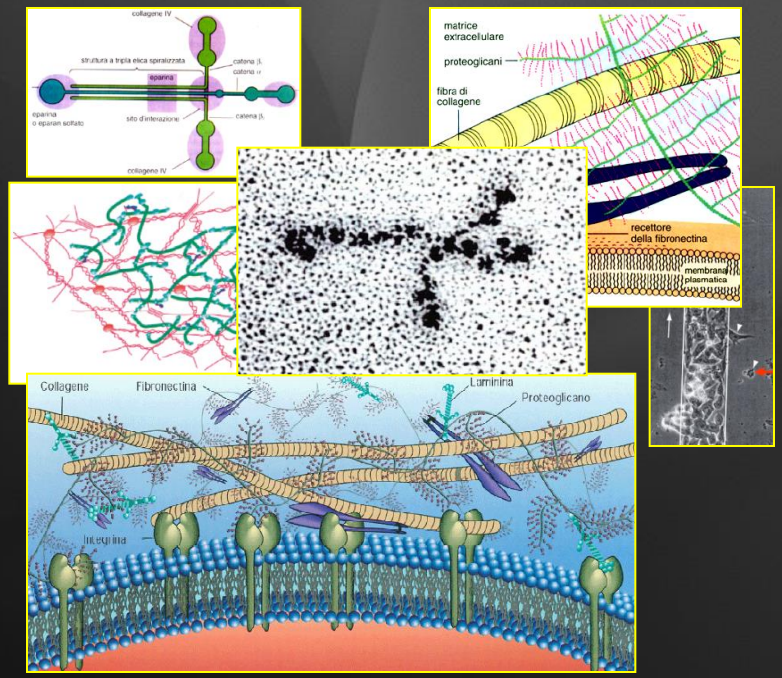
Physical characteristics of the vitreous	
Weight	4 g
Density	1.0053–1.008 g/cm^3
Refractive index	1.3345–1.3348
Viscosity	300–2000 cP
pH	7.0–7.4

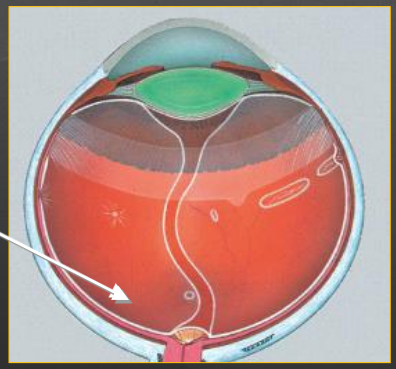
Biochemical composition of the vitreous.

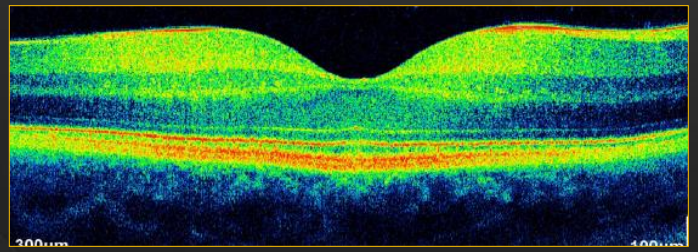
Subgroups	Molecule	Action
Protein	Albumin (40%)	
	Iron binding protein (30%) like transferrin	Protective effect to reduce iron toxicity
	Collagens	Structure of the vitreous
	Type II (60–70%)	
	Type IX (25%)	
	Type V/IX (10–25%)	
	Type IV (<10%)	
Glycosaminoglycan	Hyaluronic acid (66–115 microgram/mL concentration)	Determine the vitreous body viscosity
	Chondroitin sulfate	Major component of extracellular matrix
	Versican	
	Type IX collagen	
	Heparan sulfate	It maintains adequate spacing between the collagen fibrils
Metabolites	Glucose	To support the enzymatic activity
	Lactic acid	
	Ascorbic acid	Neovascularization inhibitor Increase proliferation of hyalocytes Potent antioxidant
	Amino acids	Metabolic cells maintenance
	Fatty acids unsaturated (50–55%)	Metabolic cells maintenance
	Prostaglandins (100 picogram/mL)	Cells regulation
	PGE2	Cells regulation
	PGF2alpha	Cells regulation
	Prostacyclin	Cells regulation
	Thromboxane	Cells regulation
Cells	Hyalocytes	Vitreous matrix creation and maintenance
	Fibrocytes/fibroblasts	Vitreous matrix creation and maintenance
	Macrophages	Cells and matrix regulation and degradation
	Enzymes and metabolic activity: ACE	Cells regulation

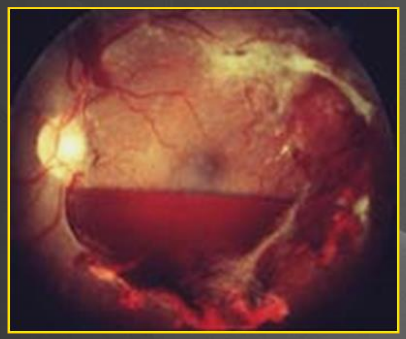
Vitreoretinal junction

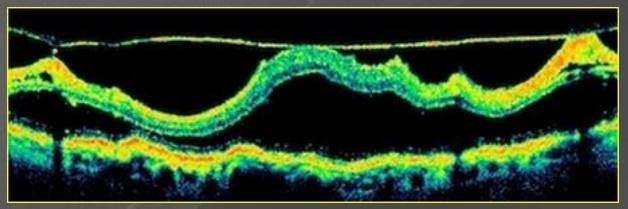
- stabilization of vitreoretinal adhesions
- glycoproteins in vitreomacular interface

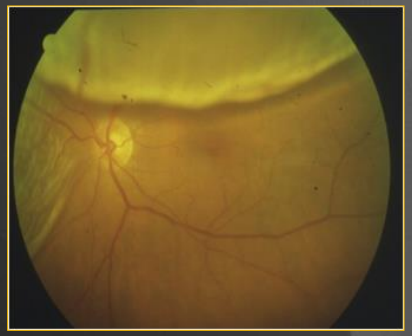


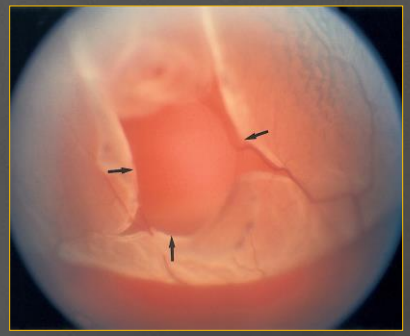


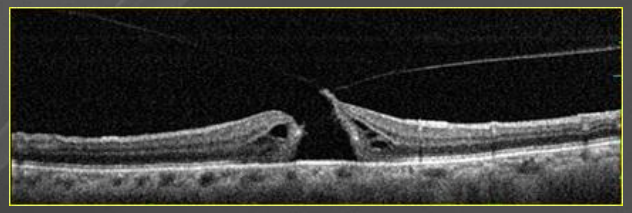


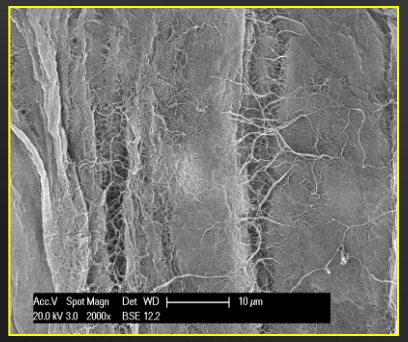


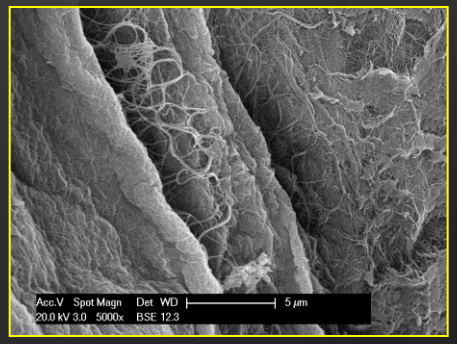


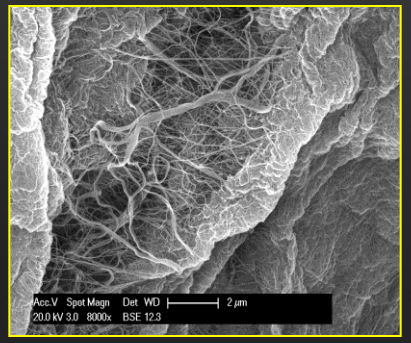






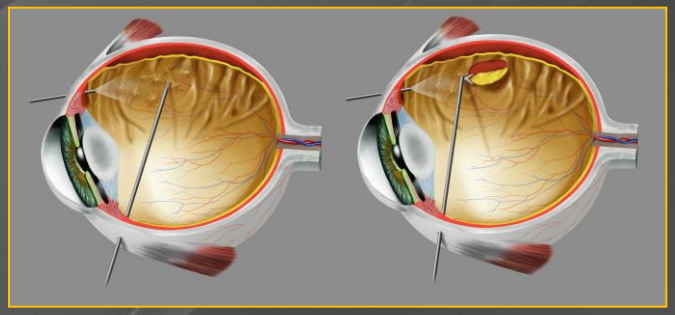




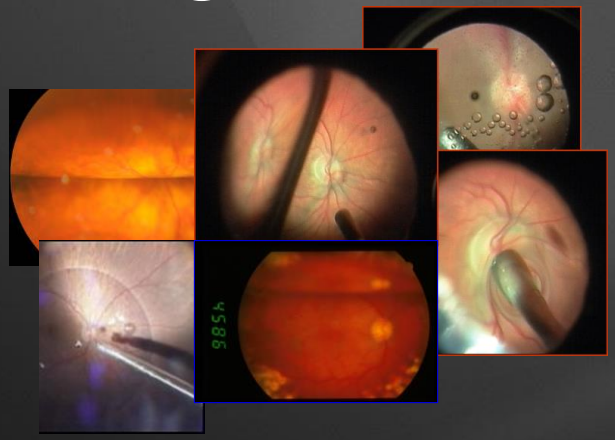


- Azzolini et al, Club Gonin 2014

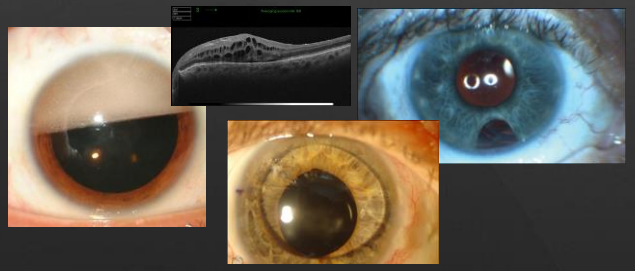
Current vitreous substitutes



- short-term
- temporary
- long-term



- complications

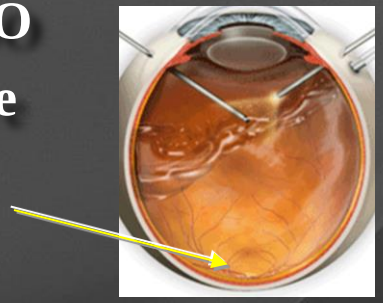


- Kleinberg TT, Vitreous substitutes: a comprehensive review, Surv Ophthalmol 2011
- Donati S, Azzolini et t al, Vitreous substitutes: the present and the future Biomed Res Int 2014
- Azzolini C et al, Arch Ophthalmol 1992

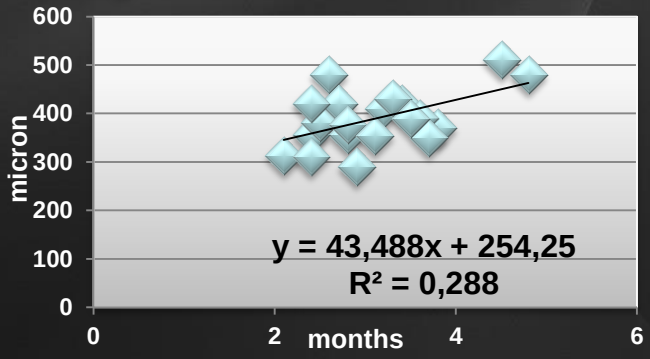
Many complications from vitreous substitutes

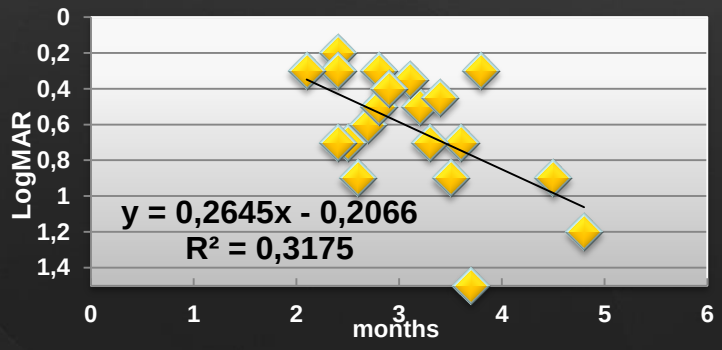
Example of silicone oil

- decrease molecular transport in vitreous space
- permanence of inflammatory substances between SO and macula
- mechanical floating of SO
- dangerous light exposure



Significant correlation between time of SO permanence, ME and VA





- Azzolini C, Macula Society, Scottsdale 2015

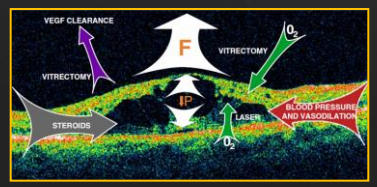
Vitreous substitutes properties

Passive properties (filling action):

- **structural function, control intraocular hemorrhage, maintain IOP**

Active properties:

- **to interact with eye biology and metabolism**
- **transportation of substances, ions, oxygen**
- **to mantain integrity and transparency over time**
- **correct refractive index..**



Ideal testing protocol

- light trasmittance
- kinetics of hydratation and water swelling
- oscillatory and shear-stress analysis
- shear-creep analysis
- evaluation of solute diffusion
- in vitro and in vivo biocompatibility
- optical properties
- degradation during injection

The ideal vitreous substitute
Mimic the native vitreous
Be easily manipulable during surgery
Have similar viscoelastic proprieties
Be clear and transparent
Have refractive index and density similar to native vitreous
Be biologically and chemically inert
Be hydrophilic and insoluble in water
Be able to maintain the IOP within a physiologic range and support the intraocular tissues in proper position
Allow movement of ions and electrolytes and maintain the concentration of certain substances (oxygen, lactic acid, and ascorbic acid)
Be clear
Not induce toxic reactions
Be biocompatible
Be easily available, stable, and injectable through a small syringe
Be able to maintain its light transparency post-op without undergoing opacification

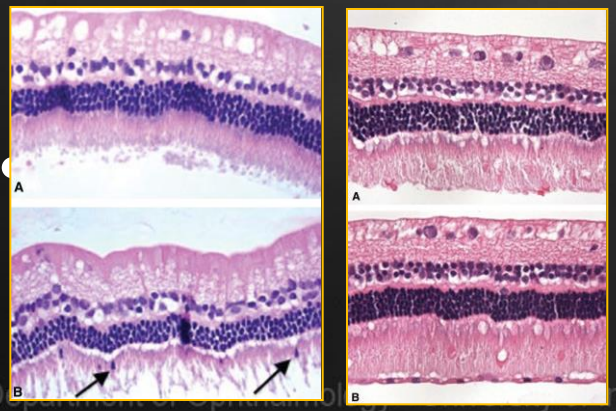
Experimental substitutes

- new substances
- natural polimers
- hydrogels
- smart hydrogels
- transplant and implants
- vitreous regeneration

- Cutler NL et al, Transplantation of human vitreous: a preliminary report, Arch Ophthalmol 1946
- Sebag J e al, Morphology and ultrastructure of human vitreous fibers, Inv Ophth & Vis Sci 1989
- Liu Y et al, Technical standards of a foldable capsular vitreous body in term of mechanical, optical, and biocompatible properties, Artificial Organs 2010
- Rizzo S et al, Heavy silicone oil (Densiron-68) for the treatment of persistent macular hole, Graefe's Archives for Clinical and Experimental Ophthalmology 2009
- Baino F et al, Towards an ideal biomaterial for vitreous replacement: historical overview and future trends, Graefe's Archives for Clinical and Experimental Ophthalmology 2011
- Vijayasekaran S et al, Poly (1-vinyl-2-pyrrolidinone) hydrogels as vitreous substitutes: histopathological evaluation in the animal eye, Journal of Biomaterials Science 1996
- Tao Y et al, Evaluation of an in situ chemically crosslinked hydrogel as a long-term vitreous substitute material, Acta Biomaterialia 2013
- ……

Decafluoro-di-n-pentyl ether (DFPE)

- **miscible in perfluoro-n-octane**
- **miscible in silicone oil to about 20%**
- **properties: optical clarity, inertness, hydrophobicity, low vapour pressure**
- **minimal adverse effects in retina rabbits**
- **act on both superior and inferior retina**



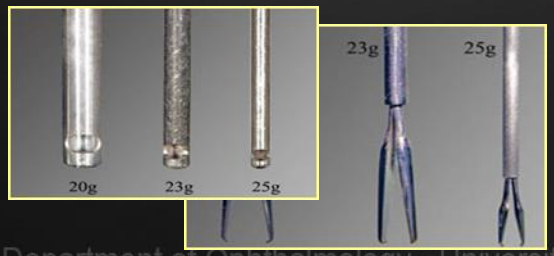
superior retina

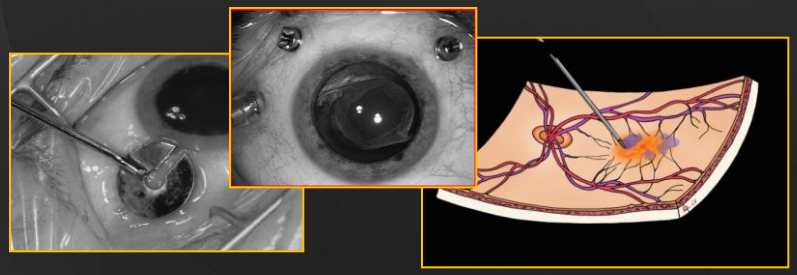
inferior retina

- Santos RAV et al, DFPE- partially fluorinated ether, Retina 2013

Natural polymers

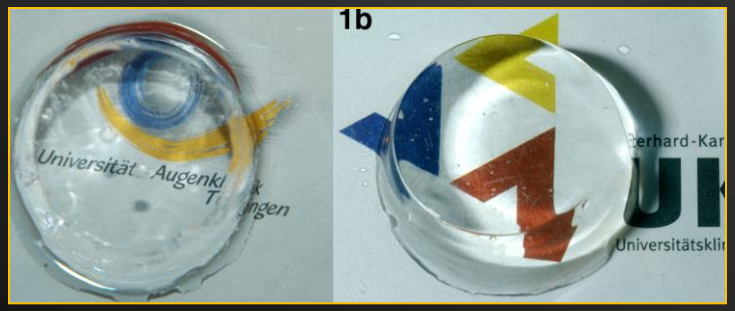
- HA and collagen: great biocompatibility, short degradation time
- gelatine, polygeline methiylated collagen: poor results
- gel hyalan (**cross-linked reinforced** molecules of HA formaldehyde, divynil sulfone and gellan molecules): excessive water solubility
- dihydrazide photo-cross-linking reaction: limited inflammation and toxic reaction, but short degradation time. **The injection procedure alters the gel reducing integrity and stability**
- the cross-linking processes by in situ gelification and the intraocular injection of cellular components to actively produce polymer matrix **represent a possible solution**



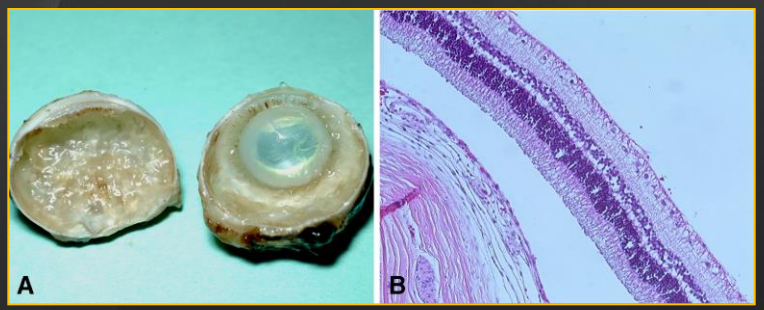


Synthetic polymers: hydrogels

- hydrofilic polymers that form a gel network when cross-linked, capable of swelling by absorbing several times their own weight in water
- polyacrylamide, copoty(acrylamide), **ADCON hydrogel**, poly(vinyl alcohol, and other 15 principal molecules
- photoinitiator (UVA wavelength, disulfide, air oxidation, temperature..)
- advantages: handiness, optical properties, viscosity, elasticity
- disadvantages: inflammatory reaction, degradation, toxicity
- several discarded for toxicity or unable characteristics



Cross-linked biopolimer



6 weeks after UV-CHA implantation (rabbit model)

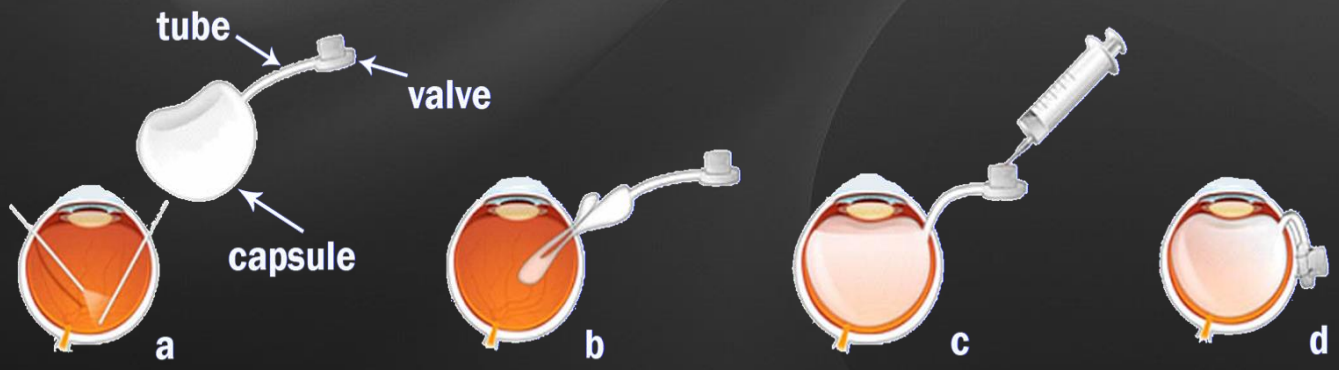
Smart hydrogels

- similar characteristics
- more interactive properties with the environment: glucose-, glutathione-, pH-dependent activity..
- reactivity to light, pressure, electric fields

- advantages: checked gelification expansion, reservoirs, interaction with retinal tissue, injected drugs, laser light, physical stimuli
- disadvantages: little information on possible phagocytosis, vacuolization, immune reaction

Transplant and implants

- transplant vitreal tissue: cataract, glaucoma, ocular atrophy
- artificial capsular bodies: silicone rubber elastomer filled with a saline solution
- several nanometer wide aperure to add drugs

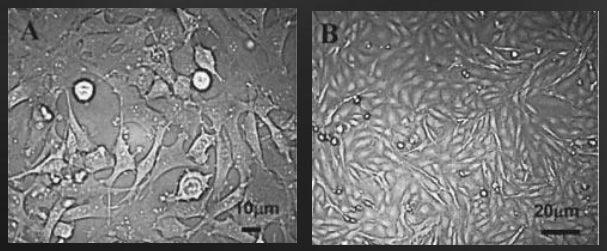


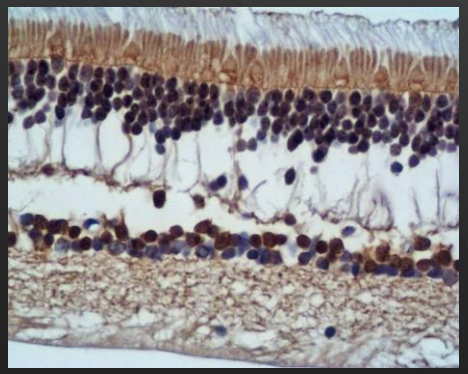
- Gao Q et al, A new strategy to replace the natural vitreous by a novel capsular artificial vitreous body with pressure control valve, Eye 2008

Vitreous regeneration

- **controlled hyalocytes proliferation** with specific growth factors (Bfgf stimulates, TGF-B1 inhibits) and production of HA
- reverse transcriptase-PCR analyzed expression profiles for several genes
- **individuating specific genetic pathways a novel approach to vitreous regeneration.** Otx homeobox, p53 family, and VEGF-A genes are expressed in PVR human retina.

Muller cells





- Azzolini C et al, Expression of VEGF-A, Otx Homeobox and p53 Family Genes in Proliferative Vitreoretinopathy, Mediators of Inflammation 2013

- **vitreous body has filling and active properties**
- **currently used vitreous substitutes for PVR have many disadantages**
- **ideal vitreous substitutes should have both passive and active properties**
- **polymeric hydrogels: suitable characteristics**

The ideal vitreous substitute
Mimic the native vitreous
Be easily manipulable during surgery
Have similar viscoelastic proprieties
Be clear and transparent
Have refractive index and density similar to native vitreous
Be biologically and chemically inert
Be hydrophilic and insoluble in water
Be able to maintain the IOP within a physiologic range and support the intraocular tissues in proper position
Allow movement of ions and electrolytes and maintain the concentration of certain substances (oxygen, lactic acid, and ascorbic acid)
Be clear
Not induce toxic reactions
Be biocompatible
Be easily available, stable, and injectable through a small syringe
Be able to maintain its light transparency post-op without undergoing opacification

Conclusion

Which are the advancements in biocompatibility and rheological properties of vitreous substitutes for PVR?

Answer:
We know better what we need,
but we have not yet