1. **Cell therapy for Age-Related Macular Degeneration (AMD); Biomaterials for Retinal Pigment Epithelium (RPE) transplantation.**

P.I. Dr. Girish Kumar Srivastava (girish@ioba.med.uva.es).

**Target disease:** Dry forms of AMD.

**Fields of research:**

- Cell therapy.
- Tissue engineering.

The aim of this research line is to develop cell therapy as the future treatment of AMD by RPE transplantation in the submacular space of the retina. For this, we try to differentiate stem cells from adipose tissue and ciliary body into RPE cells, using different techniques as EPR and neuroretinal co-cultures, adding growth factors and others. We also search for new biomaterials as a substitute of Bruch membrane, which can maintain the characteristics of these cells. We evaluate viability, adhesion and growth, phenotype and functional characteristics of different human and porcine cell types (ARPE19, adult RPE, fibroblasts, AT-SC and CB-SC) over surfaces of different biomaterials, tested in vitro, and finally we will test them in an animal model in vivo conditions.

**Recent related publications:**


Retinal pigment epithelial (RPE) cells are currently in the "spotlight" of cell therapy approaches to
some retinal diseases. The analysis of the expressed proteins of RPE primary cells is an essential step for many of these approaches. But the emission of autofluorescence by RPE cells produces higher background noise interference thereby creating an impediment to this analysis. Trypan Blue (TB), a routinely used counterstain, has the capacity to quench this autofluorescence, not only in cell cultures but also in tissue samples. This method should significantly increase the quality and value of RPE cell protein analysis, as well as other cell protein analysis performed by Flow cytometry (FC) and Immunohistochemistry (IHC) techniques.


The aim of this study is to investigate the use of elastin-like recombinamers (ELRs) as a substrate that can maintain the growth, phenotype, and functional characteristics of retinal pigment epithelial (RPE) cells efficiently and as a suitable carrier for the transplantation of autologous RPE cells for treatment of age-related macular degeneration (AMD). These results should be extended to further studies with fresh human RPE cells and in vivo studies to determine whether this ELR-RGD matrix could be used as a Bruch's membrane prosthesis and carrier for transplantation of RPE cells in patients suffering with AMD.

2. Retinal healing and repairment

P.I. Dr. Iván Fernández Bueno (ifeñandezb@joba.med.uva.es),

Target disease: Retinal detachment; retinal degenerations.

Fields of research:

- Neuroprotection.
- Retinal healing, inflammation and repairment.
We work with human and porcine neuroretina co-cultures to evaluate cell modifications that occur after the separation of RPE from neuroretina and the loss of vascularisation. We also test the effects of different drugs involved in neuroprotection and retinal gliosis. Professor Nicolás Cuenca, from Alicante University, collaborates in this study.

Recent related publications:


We developed an organotypic culture of porcine neuroretina as a model to analyze glial cell modifications, mainly Müller cells, that occur after a retinal detachment. We observed that the addition of a blood-derived mononuclear fraction (MNF; monocytes and lymphocytes) as a source of macrophages, stimulates modifications of Müller cells, producing a wider intraretinal reactive gliosis and tissue proliferation at the subretinal space (outer layers of the retina). These findings emphasize the role of macrophage-like cells in the production of changes in retinal structure observed after retinal detachment in humans.

3. Genetics of inflammation in the retina

P.I. Dr. Rogelio González-Sarmiento (gonzalez@usal.es), Dr. J. Carlos Pastor (pastor@ioba.med.uva.es).

Target disease: Proliferative Vitreoretinopathy (PVR); Retinal detachment (RD).

Fields of research:

- Find PVR biomarkers (Dr. Jimena Rojas, jimena@ioba.med.uva.es).
Identify potential therapeutic targets for the treatment of PVR (Salvador Pastor, salva_pastor@hotmail.com).

Develop formulas for predicting the risk of PVR (Itziar Fernandez (itziar.fernandez@ioba.med.uva.es).

This research line studies genetic contribution to retinal inflammation in multifactorial inherited retinal diseases, as PVR, through new biomarkers identification (LTA, Tp53, Mdm2) in DNA samples.

We look for therapeutic targets in primary cultures of different cell types (Cos-7, ARPE19) for the treatment and prevention of PVR, and we aim to develop new formulas for predicting the risk of PVR after a retinal detachment, analyzing clinical and genetic factors. We also study the role of other cell death pathways after RD.

**Recent related publications:**


This study analyzed the importance of the variability of the p53 tumor suppressor protein in the functional prognosis after a stroke. As a result, we showed that poor functional outcome after either ischemic or hemorrhagic stroke was linked to the Arg/Arg genotype at residue 72 of p53. These results suggest that the Tp53 Arg/Arg genotype governs neuronal vulnerability to apoptosis and can be considered as a genetic marker predicting poor functional outcome after a stroke.

We studied genetic vulnerability to suffer a PVR and reported the association observed to the tumor necrosis factor (TNF) locus. The strong association found in the rs2229094(T→C) of the LTA gene may indicate an important role of this polymorphism in the development of PVR. If supported in extended studies, the rs2229094(T→C) may have significant implications regarding the genetic risk of the retinal repairing process.


We tried to identify which of 14 algorithms best predicts the genetic risk for development of proliferative vitreoretinopathy (PVR) in patients who are experiencing primary rhegmatogenous retinal detachment. Genetic variables may be useful to predict the likelihood of the development of PVR. The predictive capabilities of these models are as good as those observed with clinical approaches. These results need external validation to estimate the true predictive capability and select the most appropriate ones for clinical use.

CLINICAL RESEARCH LINES

4. Degenerative diseases of the retina

P.I.: Dr. Rosa M. Coco (rosa@ioba.med.uva.es), Dr. M. Rosa Sanabria (rsanabria@ioba.med.uva.es).

Target diseases: Age-related macular degeneration (AMD); Inherited Retinal Diseases.

Fields of research:

- Identify potential gene markers of AMD and new mutations in inherited eye diseases.
- Define natural history of Dry AMD.
Development of new low vision aids for these patients.

This research line is focused on visual disability, low vision tests and development of new aids designed to improve the functional capability of these patients.

**Recent related publications:**


Two cases of patients suffering Sjögren-Larsson syndrome were described in this study. Much of the clinical variation in SLS cannot be explained by the genotype alone, age of patients and the effect of other modifier genes also play a role. We described a new mutation.


We studied the occurrence of PRPH2 mutations in patients presenting macular dystrophies and described their phenotype-genotype correlation. New phenotypes were found for known mutations. No phenotype variation was observed in the members of the 3 families. A new mutation in PRPH2 gene was found.


We described a case of a 79 year old man, with a diagnosis of retinitis pigmentosa, who progressively lost visual acuity and visual field, and presented bilateral iris stromal atrophy, glaucoma and retinochoroidal degeneration. We asked several experts for their opinion about this therapeutic challenge.

In this study, we described a case in which we confirmed pathological findings observed on posterior pole biomicroscopy by full-field electroretinogram, microperimetry and optical coherence tomography (OCT). We found functional defects in a pathology that supposedly did not involve functional retinal loss in the past.


Aggressive treatment in Punctate Inner Choroidopathy was proposed to avoid the appearance of choroidal neovascularisation that can threaten visual acuity. We can improve functional prognosis by reducing choroidal inflammation.

**Development of animal models for biomedical investigation in ophthalmology and vision sciences.**

P.I.: Dr. Iván Fernández Bueno (ifernandezb@ioba.med.uva.es).

With a high degree of experience in the development of animal models for investigation in the field of ophthalmology, we could develop in our installations any animal model described in literature before. We comply with the instructions and requirements proposed by the Ethical Committee for Animal Experimentation and Wellbeing, from the University of Valladolid and the Ethical Committee from the University of León, which comply with the current European Union and Association for Research in Vision and Ophthalmology (ARVO) law.

**Animal models published by members from IOBA:**


Experimental model of allergic conjunctivitis to ragweed in guinea pig (Curr Eye Res 1995).


Organotypic culture model of porcine neuroretina (Mol Vis 2008).


Limbal deficiency porcine model (developing since 2009).

EPR-choroid autotransplant porcine model (developing since 2010).

Limbal deficiency rabbit model (developing since 2010).

Dry forms of age-related macular degeneration porcine model (developing since 2010).