UNIVERSIDAD de VALLADOLID

COOPERATION BETWEEN LIVERPOOL AND VALLADOLID UNIVERSITIES

Academic year 2011-2012

VISITS AND EXCHANGE OF STUDENTS FOR THE PURPOSE OF STUDY AND RESEARCH

LIVERPOOL CO-ORDINATOR:

Dr. Luminita Paraoan
Department of Eye and Vision Sciences, Institute of Ageing and Chronic Disease
University of Liverpool
UCD Building, Daulby Street
Liverpool L69 3GA, United Kingdom

Tel: +44(0)151 706 4101
Fax: +44(0)151 706 5934
E-mail: luminita.paraoan@liverpool.ac.uk

VALLADOLID CO-ORDINATOR:

Dr. Yolanda Diebold
Institute for Applied Ophthalmobiology – IOBA
University of Valladolid
Edificio IOBA, Campus Miguel Delibes
Paseo de Belén, 17
47011 Valladolid, Spain

Tel: +34 983 184750
Fax: +34 983 184762
E-mail: yol@ioba.med.uva.es
Interest areas of staff in the Eye and Vision Science, University of Liverpool, UK

Potential project placements for postgraduate students from IOBA, Valladolid

Dr Luminita Paraoan  lparaoan@liverpool.ac.uk  Dr Lyndsay Davies, Dr Paul Kay

Ocular Molecular Biology Group  www.liv.ac.uk/paraoan

Projects investigating gene expression and regulation in RPE cells, uveal melanocytes and trabecular meshwork cells with particular interest in proteolysis, programmed cell death, intracellular trafficking and secretion. Our projects have applicability for AMD, uveal melanoma, PVR, glaucoma, diabetic retinopathy. Molecules and pathways of interest are cathepsins, cysteine proteinase inhibitors like cystatin C, p53 pathway, PERP, etc.

Examples of projects:

1. Regulation of proteolysis and its role in age-related macular degeneration

Cystatin C is a biochemically well-characterized, strong inhibitor of cysteine proteinases. In the eye, in non-pathological conditions, cystatin C is synthesized at fairly abundant levels by the retinal pigment epithelium (RPE), which in vivo forms a monolayer of cells supporting the function of the neuroretina. RPE secretion of cystatin C appears polarized basally in vitro, suggesting an extracellular function, at least in part, in or around the basement membrane of the epithelium (Paraoan et al, 2001).

A surprising association of a variant cystatin C with eye pathology has recently unveiled a possible novel mechanism of cystatin C involvement in pathogenesis. Specifically, variant B cystatin C, which has one amino acid change in the signal sequence responsible for directing the protein to the secretory pathway and is associated with increased risk of developing a type of age-related macular degeneration, is characterized by aberrant intracellular trafficking and impaired secretion by RPE cells (Paraoan et al, 2004; Paraoan et al, 2010). The current project aims to investigate the consequences in relation to cell death or apoptosis of the expression by RPE cells of this variant of cystatin C. The project will involve cell culture, transfection of GFP fusion constructs and general molecular biological techniques for which training will be provided, as needed.
Key References:


2. How is apoptosis triggered by the p53 effector PERP?

Apoptosis (programmed cell death) is a vital process for the death and safe removal of abnormal or damaged cells from the body. In cancer for example, this mechanism is often impaired, causing an accumulation of abnormal cells. We have shown that a gene called PERP, which is known to cause cell death, occurs at much lower levels in an aggressive form of the most common eye cancer (uveal melanoma, UM), compared with less aggressive UM tumours (Paraoan et al. 2006). However, when PERP levels are increased, UM cells readily undergo apoptosis (Davies et al. 2009). This project will make use of this experimental model to determine the effect that known inducers of apoptosis have on the levels of endogenous PERP mRNA and protein and will assess whether increased levels of PERP enhance the ability of these inducers to initiate apoptosis. The effect on other proteins involved in apoptosis will also be examined. Experimental procedures will involve general molecular biological techniques, cell culture, transfection, Western blotting, PCR and appropriate training will be provided.

Key References:


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**Professor Ian Grierson**  [eye123@liv.ac.uk](mailto:eye123@liv.ac.uk)

1) Dry eye – the effects of a range of drugs particularly glaucoma drops on the epithelial and stromal cells of the conjunctiva

2) Progenitor cell activity in the anterior chamber structures of the eye.

3) Progenitor activity in the Lamina Cribrosa. Does the optic nerve head have stem cells?

4) Trabecular meshwork cell dynamics – cell loss mechanisms and senescent changes with ageing and disease.

5) Macular pigment in ageing – do supplements and nutritionals make a difference?

6) Cross linked actin networks and eye disease.

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**Dr Paul Knox**  [pcknox@liv.ac.uk](mailto:pcknox@liv.ac.uk)  [pcwww.liv.ac.uk/~pcknox](http://pcwww.liv.ac.uk/~pcknox)

**Visuomotor Control Projects.**

Eye movements are critical for clear and efficient vision. The control of eye movement is therefore a key function of the central nervous system, involving cortex, basal ganglia, midbrain, cerebellum and brainstem. Careful measurements of eye movements can therefore often both reveal the presence of problems, and also indicate which specific aspects of the control circuitry are affected. Current projects in the oculomotor laboratory in Eye and Vision Sciences include studies of inhibitory control of eye movement behaviour, cross population studies of saccadic eye movements and studies of dynamic alignment control. The main technique employed is quantitative parametric analysis of eye tracking recordings. We also have access to both fMRI (with in-scanner eye tracking) and TMS.

**Recent Relevant Publications:**


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**Dr Yalin Zheng**  
yzheng@liv.ac.uk

Projects on automated retinal image analysis and grading for visiting students.

These projects may suitable for electronic engineering/ computer science / physics / engineering students who want to do a project in image processing.

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**Dr Sharon Mason , Dr Carl Sheridan** (carlos@liv.ac.uk)

Comparing the growth and phenotype of Limbal stem cells on differently treated/stored decellularised amniotic membrane.

Cultured limbal stem cell therapy has been used successfully to improve the vision of patients with limbal stem cell deficiency for some time now. Cells are usually cultured on human amniotic membrane, which acts as a carrier and helps maintain the correct cell phenotype. It has been found that the way the membrane is treated prior to use as well as the storage conditions can substantially affect the growth of the cells and whether they have a corneal or stem cell identity. A major problem with the use of amnion is the large storage capacity required for the amnion, and storage of amnion at room temperature would make it a much easier material to work with.

During this project we will determine the effect of chemical composition of the storage medium, temperature of storage, and effect of irradiation on amniotic membranes ability to support the growth of limbal cells and the stem cell population in particular. Growth will be measured and phenotype assessed using immunofluorescent staining.

The project could be a 3 month or 6 month project. Please contact for more information.

References:


Dr Rachel Williams (rlw@liverpool.ac.uk), Dr Victoria Kearns, Dr Carl Sheridan and Mr Theodor Stappler

Development of the surgical procedure for cell transplantation treatment for age-related macular degeneration.

Age-Related Macular Degeneration (AMD) is the main cause of irreversible vision loss in people above 65 years in the West. A potential treatment is transplantation of a sheet of functioning retinal pigment epithelial (RPE) cells under the macula. Cells would be grown on an artificial substrate (which would act as a substitute for Bruch’s membrane) and the substrate and monolayer of cells implanted as a single structure.

Our group has previously demonstrated that primary RPE and IPE cells can be cultured and grown to a monolayer on porous polymer substrates. We now need to develop the surgical procedure for transplantation of the cell/substrate construct under the retina. This development process will use enucleated pig eyes and the ‘wet-lab’ surgical setup we have established in the laboratory. The student will learn the surgical techniques, work with the cell/substrate constructs, consider new instrument designs to facilitate the surgery and evaluate the integrity of the cell monolayer post transplantation using histology. This work will lead to subsequent in vivo studies in the pig model.

Dr Victoria Kearns (vkearns@liverpool.ac.uk), Dr Carl Sheridan (carlos@liv.ac.uk), Dr Rachel Williams

Comparison of IPE and RPE grown on artificial substrates for tissue engineering treatment for age-related macular degeneration.

Age-Related Macular Degeneration (AMD) is the main cause of irreversible vision loss in people above 65 years in the West. A potential treatment is transplantation of a sheet of functioning retinal pigment epithelial (RPE) cells under the macula. Cells would be grown on an artificial substrate (which would act as a substitute for Bruch’s membrane) and the substrate and monolayer of cells
implanted as a single structure. Iris pigment epithelium (IPE) is derived from the same embryonic origin as RPE and has been shown to have several of the same functions. IPE can be harvested more easily than RPE and would not be diseased like the RPE of an AMD patient.

Our group has previously demonstrated that primary bovine RPE and IPE cells cultured on porous polyurethane substrates (developed in partnership with a commercial partner) exhibit some characteristics of a differentiated monolayer: actin belts and tight and adherens junctions. We want to assess the ability of the IPE to perform RPE functions, such as phagocytosis of photoreceptor outer segments and transport of nutrients. Obtaining these data is vital for progressing to animal implantation studies, which would contribute towards moving this treatment towards clinical implementation. Students will isolate and culture bovine RPE and IPE cells on polyurethane substrates and characterise the cellular response.

The project will involve the following techniques: isolation of primary cells from animal tissue, tissue culture, immunocytochemistry, fluorescence microscopy, dextran transport assay, phagocytosis assay. The project could be a 3 month or 6 month project. Please contact for more information.
Interest areas of staff in the IOBA, University of Valladolid (Spain)

Potential project placements for postgraduate students from the Eye and Vision Science, University of Liverpool (UK)

Ocular Surface Group → Research Lines in Ocular Surface Physio-Pathology

RL1 - Advanced Therapies

P.I. Dr. Margarita Calonge (calonge@ioba.med.uva.es)

Target diseases: Corneal blindness caused by limbal stem cell deficiency.

Fields of research:

- Cell therapy (Dr. Teresa Nieto, tnietom@ioba.med.uva.es)
- Tissue engineering (Dr. Teresa Nieto, tnietom@ioba.med.uva.es)
- Gene therapy (Dr. Yolanda Diebold, yol@ioba.med.uva.es)

Visiting students may be enrolled in projects involving optimization of human limbal epithelial stem cells culture techniques, growth of limbal epithelial cells and mesenchymal stem cells on biopolymeric matrices and scaffolds, or in projects related to the testing of safety, biocompatibility and preliminary efficacy of novel advanced therapies to repair ocular surface failure in animal models of limbal stem cell deficiency.

Recent related publications:


**RL2 - Inflammation**

**P.I. Dr. Margarita Calonge (calonge@ioba.med.uva.es)**

**Target diseases:** Dry Eye Syndrome, allergy, other immune-based diseases.

**Fields of research:**

- In vitro models development (Dr. Yolanda Diebold, yol@ioba.med.uva.es)
- Biomarkers and new therapies (Dr. Amalia Enríquez-de-Salamanca, amalia@ioba.med.uva.es)
- Environmental stress (Dr. María J. González, aluche@ioba.med.uva.es)
- Contact lenses (Dr. María J. González, aluche@ioba.med.uva.es)

Visiting students may be enrolled in projects involving ocular inflammation in vitro models, the analysis of putative diagnostic, disease, or therapeutical biomarkers in ocular surface samples, such as tears or cell cytology samples, and inflammation-related events in primary cultures of different ocular surface cell types (corneal, limbus, and conjunctiva).

**Recent related publications:**

- Calonge M, Enriquez-de-Salamanca A, Diebold Y, González-García MJ, Reinoso R, Herreras JM, Corell A.

**RL3 – Nanomedicine**

P.I. Dr. Yolanda Diebold ([yol@ioba.med.uva.es](mailto:yol@ioba.med.uva.es))

**Target diseases:** Dry Eye Syndrome, allergy, other immune-based diseases.

**Fields of research:**

- Ocular application of drug delivery systems, gene therapy, and gene silencing.

Visiting students may be enrolled in projects involving the analysis of new drug delivery systems in terms of biocompatibility for ocular topical administration, as well as in vitro proof-of-concept efficacy of loaded drug (either model molecules or experimental bioactive compounds). The analysis may include cell proliferation and citotoxicity assays, intracellular trafficking of loaded bioactive compounds and/or the vehicle, and the study of the potential therapeutical effect of loaded drug in in vitro disease models.

**Recent related publications:**


**RL4 – Physiology and Immunology**

P.I. Dr. Alfredo Corell ([acorell@ped.uva.es](mailto:acorell@ped.uva.es))

**Field of research:**

- Characterization of eye-associated lymphoid tissue in healthy individuals (Dr. Roberto Reinoso, [reinosot@ioba.med.uva.es](mailto:reinosot@ioba.med.uva.es)).
- Diagnostic systemic Immunology (allergies, autoimmunity, immunodeficiencies, infections, transplantation, external quality control) (Dra. Carmen Martín Alonso,
Visiting students may be enrolled in projects involving the analysis and characterization of the EALT (eye-associated lymphoid tissue) both in healthy individuals as well as in target diseases (mainly inflammatory eye diseases: dry eye, conjunctivitis, etc.). The EALT analysis may include cell viability and proliferation as well as cell lineage identification by means of flow cytometry in epithelial cell suspensions obtained by brush cytology; alternatively the systemic immune system studies includes a wide spectrum of diagnostic and research methods.

Recent related publications:


RL5 – Clinical trials

P.I. Dr. José M. Herreras (herrer@ioba.med.uva.es)

Target diseases: Ocular surface inflammation, limbal stem cell deficiency, intraocular inflammation (Uveitis), contact lens-associated pathologies.

Field of research:

- Cell therapy (Dr. Margarita Calonge, calonge@ioba.med.uva.es)
- Drug therapy (Dr. José M. Herreras, herrer@ioba.med.uva.es)
- Contact lenses (Dr. María J. González, aluche@ioba.med.uva.es)
Visiting students may be enrolled in ongoing clinical trials participating in data gathering, clinical examination, laboratory analyses of collected samples, and result interpretation.

Recent related publications:

- Pavesio C, Zierhut M, Bairi K, Comstock TL, Usner DW; Fluocinolone Acetonide Study Group (*Margarita Calonge is member of this study group*). Evaluation of an intravitreal fluocinolone acetonide implant versus standard systemic therapy in noninfectious posterior uveitis. Ophthalmology. 2010; 117:567-575.

Retina Group → Research Lines in Retina Physio-Pathology

**RL1 – Biomaterials for RPE transplantation; cell therapy for AMD**

P.I. Dr. Girish Kumar Srivastava ([girish@ioba.med.uva.es](mailto:girish@ioba.med.uva.es))

**Target disease:** Dry forms of AMD

**Fields of research:**

- Cell therapy
- Tissue engineering

Visiting student may be enrolled in the projects dealing with search of an appropriate biomaterial for RPE cell transplantation in submacular space in patients suffering with AMD. New biomaterials with ARPE19, hRPE, pRPE, CBSCs and hAd-MSCs have been tested in vitro to evaluate that these cells maintain their viability, adhesion and growth, phenotype and functional characteristics over surfaces of these biomaterials. The involvement of the visiting student in these projects will support to understand Cell-Biomaterial interactions.

Recent related publications:

UNIVERSIDAD de VALLADOLID


**RL2 – Retinal organotypic cultures and animal models**

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

**Target disease:** Dry forms of AMD; Proliferative vitreoretinopathy; retinal detachment

**Fields of research:**

- Animal model development (Dr. Iván Fernández Bueno, [ifernandezb@ioba.med.uva.es](mailto:ifernandezb@ioba.med.uva.es))
- Surgical procedures development (Dr. Iván Fernández Bueno, [ifernandezb@ioba.med.uva.es](mailto:ifernandezb@ioba.med.uva.es))
- Cell transplantation (Dr. Girish Kumar Srivastava, [girish@ioba.med.uva.es](mailto:girish@ioba.med.uva.es), Dr. Iván Fernández Bueno, [ifernandezb@ioba.med.uva.es](mailto:ifernandezb@ioba.med.uva.es))

Visiting students may be enrolled in the projects dealing with organotypic culture of neuroretina and in vivo experiments in animal model. Neuroretina cultures are used to analyze cellular modifications after retinal detachment and to test the effects of different drugs on neuroprotection and retinal gliosis. Also, visiting students could learn to prepare neuroretinal samples for culturing, to process them for histological and immunochemical analysis, and how to evaluate retinal modifications during culture.

Furthermore, some polymers with hRPE cells have been tested in vitro, and in vivo evaluation of these polymers in pig model are under process. We are also involved in study of hAd-MSCs differentiation using different techniques. Some results are very favourable to evaluate those results in vivo in porcine model. A porcine model with macular atrophy is under development with new approaches, sidewise developing new surgical techniques for transplantation. Visiting student could be involved in projects related with testing of safety, biocompatibility and preliminary efficacy of transplantation of polymers with hRPE cells and/or hAd-MSCs in a porcine model of macular atrophy.

**Recent related publications:**

- Fernandez-Bueno I, Pastor JC, Gayoso MJ, Alcalde I, Garcia MT. Müller and macrophage-like cell

**RL3 –Genetics of Inflammation and Repairing Process in the Retina. Retina 4 Project**

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

**Target disease:** Proliferative Vitreoretinopathy; Retinal detachment

**Fields of research:**
- Search for PVR biomarkers (Dr. Jimena Rojas, *jimena@ioba.med.uva.es*)
- Identification of potential therapeutic targets in PVR (M.Sc. Salvador Pastor, *salva_pastor@hotmail.com*)
- Development of tools for predicting the risk of PVR (M.Sc. Itziar Fernandez, *Itziar.fernandez@ioba.med.uva.es*)

Visiting students may be enrolled in projects related to genetics contribution of inflammation in the multifactorial retinal diseases, such as PVR through the identification of new PVR biomarkers (*LTA, Tp53, Mdm2*) in DNA samples, testing their functional role and searching new potential therapeutic targets in primary cultures of different cell types (Cos-7, ARPE19) and analyze the role of other cell death pathways after RD or in projects related to develop kits for predicting the risk of PVR after RD analyzing clinical and genetic factor and developing new diagnostic formulas.

**Recent related publications:**
RL4 –Degenerative diseases of the Retina

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

Target disease: ARMD, Inherited Retinal Diseases.

Fields of research:

- Identification of potential gene markers of ARMD and new mutations in inherited eye diseases
- Definition of the natural history of Dry ARMD
- Development of new low vision aids for them

Visiting students may be enrolled in projects related to the testing of new low vision aids in order to familiarize to new ITC applied to visual impairment. They can also learn to collect data for our clinical studies. That may help students to better perform phenotyping in clinical research studies.

Recent related publications:

• RM Coco, RM Sanabria, I Fernandez. “General Practitioners’ knowledge gaps on ARMD and effectiveness of an e-learning training” 2012 Medical Education (accepted).

Refractive Surgery and Vision Rehabilitation Group ➔ Research Lines

RL1 – Refractive Surgery

P.I. Dr. Miguel Maldonado (maldonado@ioba.med.uva.es)

Target disease: Refractive surgery-associated problems.

Fields of research:

- New diagnostic technology for corneal physiology
- Modulation of corneal wound healing
- Refractive surgery and quality of vision
- Evaluation of diagnostic and therapeutic technology in vision science

Visiting students may be enrolled in projects involving novel ways of assessing non-invasively corneal function in the in-vivo eye by means of impedance measurements, the inhibition of corneal fibrosis by means of anti-TGF-beta peptides, advanced surface ablation procedures with the excimer laser for the correction of myopia, astigmatism and myopic astigmatism focused on the visual function under mesopic and scotopic lighting conditions, and the assessment of the reliability of diagnostic devices by analysing their reproducibility, repeatability and inter-changeability with similar devices.

Recent related publications:

- López-Miguel A, Correa-Pérez, ME, Miranda-Anta S, Iglesias-Cortiñas Dario, Coco-Martín MB,


**RL2 – Vision Rehabilitation**

**P.I. Dr. Begoña Coco (bego@ioba.med.uva.es)**

**Target disease:** Low vision patients affected by any of the following diseases: AMD, hereditary retina diseases, and diabetic retinopathy.

**Fields of research:**

- Quality of life and vision rehabilitation
- Reading performance in low vision patients

Visiting students may be enrolled in projects involving the evaluation of the impact of vision rehabilitation programs upon patient’s perceived quality of life and search for associations between improvements in visual function and the fields of the quality of live that are enhanced by such an improvement. Additionally, we are developing new ad hoc vision rehabilitation programs that are meant specifically for improving reading performance in those patients who suffer from retinal disease that leads to central vision loss. Visiting students may be enrolled in projects aimed at evaluating the effectiveness of those programs in such patients.

**Recent related publications:**

Tele-Ophthalmology Research Group → Research Lines

P.I. Dr. María Isabel López (maribel@ioba.med.uva.es)

Dr. Roberto Hornero (robhor@tel.uva.es)

Target diseases: Diabetic retinopathy, diabetic macular edema, age-related macular degeneration

Fields of research:

- Research and development of eye fundus image analysis techniques to automatically detect characteristic clinical signs of diabetic retinopathy: hard exudates, cotton wool spots, hemorrhages and microaneurysms.
- Research, development and establishment of Telemedicine in Ophthalmology services: Tele-Ophthalmology.

Visiting students may be enrolled in projects involving optimization in the detection techniques of the fovea, automatic grading of DR, or in projects related to the automatic detection of age related macular degeneration.

Recent related publications:

- Isabel de la Torre, Francisco Javier Díaz, Miriam Antón, José Fernando Díez, Beatriz Sainz, Miguel López, Roberto Hornero, María Isabel López, "Choosing the most efficient database for a Web-based system to store and exchange Ophthalmologic Health Records", Journal of Medical Systems, 35, pp. 1455-1464, November, 2011.
- Isabel de la Torre, Francisco Javier Díaz, Míriam Antón, Mario Martínez, José Fernando Díez, Daniel Boto, Miguel López, Roberto Hornero, María Isabel López, "Performance Evaluation of a Web-Based System to Exchange Electronic Health Records Using Queueing Model (M/M/1)", Journal of Medical Systems, July, 2010.