Experimental Model 1	**Neuroretina organotypic culture**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Organotypic retina culture, from human and other mammals, has been demonstrably useful in improving our knowledge of retinal physiology and pathobiology. Furthermore, in organotypic cultures the morphology and functionality of the organ is temporarily retained, and experimental conditions are under control. In brief, eyes are dissected to remove the iris and the lens. The vitreous is then remove from the posterior eyecup by cotton swabs. The neuroretina is detached as a whole by paintbrushing and cutting the optic nerve. Finally, the neuroretina is unrolled in a Petri dish and cut into 5x5 mm explants. Samples are explanted on Transwell culture dishes with the photoreceptor layer facing the membrane, and cultured in Neurobasal A medium supplemented with B-27.
Scientific Publications	- Fernandez-Bueno I, Pastor JC, Gayoso MJ, et al. **Müller and macrophage-like cell interactions in an organotypic culture model of porcine neuroretina**. Mol Vis 2008; 14:2148-56. - Fernandez-Bueno I, Fernandez-Sanchez L, Gayoso MJ, et al. **Time course modifications in organotypic culture of human neuroretina**. Exp Eye Res 2012; 104:26-38. - Fernandez-Bueno I, Garcia-Gutierrez MT, Srivastava GK, et al. **Adalimumab (TNF-blocker) reduces the expression of GFAP immunoreactivity increased by exogenous TNFα in an organotypic culture of porcine neuroretina**. Mol Vis 2013. Epub ahead of print. - Rodriguez-Crespo D, Di Lauro S, Singh A, et al. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res 2014; 358:705-16. - Di Lauro S, Rodriguez-Crespo D, Gayoso MJ, et al. **A novel co-culture model of porcine central neuroretina explants and retinal pigment epithelium cells**. Mol Vis 2016;22:243-53 - Pastor JC, Coco R, Fernandez-Bueno I, et al. **Acute toxic damage after using a perfluoro-octane for retinal detachment surgery. Causes and clinical pictures**. Retina 2017;37:1140-1151 - Labrador-Velandia S, Alonso-Alonso ML, Di Lauro S, et al. **Mesenchymal stem cells provide paracrine neuroprotective resources that delay degeneration of co-cultured organotypic neuroretinal cultures**. Exp Eye Res 2019;185:107671
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 2	**Double and triple layered co-culture model**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retina is a complex structure consisting neuroretina and RPE layers. There are onsets of retinal diseases if these layers lose their structures and functions. There are many treatment strategies based on drugs as well as cells. Nevertheless it lacks appropriate in vitro models to test these treatment strategies before performing in vivo test. Experimental animal model development and testing a treatment strategy has many hurdles, hence, it could be interesting to develop co-culture models which can mimic, at least partially, in vivo retinal condition. Thus, it could solve many pre-clinical issues including minimizing number of experimental animals. Due to these reasons, it had been developed double and triple layered co-culture models. Double layered co-culture model consists two layers; a neuroretina organotypic culture and a monoculture of RPE cells or MSCs. Triple layered co-culture consists all these three layers. Important; These layers are physically separated. They are in comunications only through biomolecules which they secret. Cell secreted biomolecule profile, co-culture microenvironment condition could be modified using a drug property. RPE could be treated for degeneration. Neuroretina is under spontaneous degeneration.
Scientific Publications	- Rodriguez-Crespo D, Di Lauro S, Singh AK, Garcia-Gutierrez MT, Garrosa M, Pastor JC, Fernandez-Bueno I, Srivastava GK. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res. 2014;358(3):705-16. - Singh AK, Srivastava GK, García-Gutiérrez MT, Pastor JC. **Adipose derived mesenchymal stem cells partially rescue mitomycin C treated ARPE19 cells from death in co-culture condition**. Histol Histopathol. 2013;28(12):1577-83. - Di Lauro S, Rodriguez-Crespo D, Gayoso MJ, Garcia-Gutierrez MT, Pastor JC, Srivastava GK, Fernandez-Bueno I. **A novel co-culture model of porcine central neuroretina explants and retinal pigment epithelium cells**. Molecular Vision 2016;22:243-53
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 3	**Proliferative vitreoretinopathy (Intravitreal platelet-rich plasma)**
Target Disease	Proliferative vitreoretinopathy (PVR)
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	Intravitreal injection of 0.15ml of PRP, transconjuctival cryotherapy and vitrectomy are performed. PRP is prepared from fresh citrated rabbit blood and injected into the vitreous cavity through the equator, using a 25g needle after pupillary dilatation.
Scientific Publications	- Piñon RM, Pastor JC, Saornil MA, Goldaracena MB, Layana AG, Gayoso MJ, Guisasola J. **Intravitreal and subretinal proliferation induced by platelet-rich plasma injection in rabbits**. Curr Eye Res 1992; 11:1047-55 - Goldaracena MB, Pastor JC, Saornil MA, Garcia-Layana A, De la Fuente LF. **[An effective model of PVR]**. Arch. Soc. Esp. Oftal. 1994; 67:127-34 - Goldaracena MB, Garcia-Layana A, Pastor JC, Saornil MA, de la Fuente F, Gayoso MJ. **The role of retinotomy in an experimental rabbit model of proliferative vitreoretinopathy**. Curr Eye Res 1997; 16:422-7
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 4	**Proliferative vitreoretinopathy (Intravitreal platelets)**
Target Disease	Proliferative vitreoretinopathy (PVR)
Species	Pig (*Sus scrofa domestica*)
Short Description	Animals receive four 3-mm-long retinectomies in the posterior retina, a partial mechanical vitectomy (about 1ml of central vitreous), and six cryoapplications at the retinal periphery. In addition, platelet concentrated plasma (10^6 platelets/mm^3) is injected intravitreally.
Scientific Publications	- Garcia-Layana A, Pastor JC, Saornil MA, Gonzalez G. **Porcine model of proliferative vitreoretinopathy with platelets**. Curr Eye Res 1997; 16:556-63
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 5	**Autologous translocation of retinal pigment epithelium-choroid graft**
Target Disease	Age-related macular degeneration (AMD)
Species	Pig (*Sus scrofa domestica*)
Short Description	Autologous translocation of the choroid and RPE is carried out in porcine eyes closely following a technique described by van Meurs & Van Den Biesen (2003). Briefly, a standard 20G pars plana lensectomy and a pars plana vitrectomy are performed. The central retina is detached by the subretinal injection of Ringer's lactate solution through a retinotomy performed with a 39G angled rigid microinjection cannula. Recipient RPE and Bruch's membrane are left intact. Afterwards, demarcation of a rectangular area of 2x2 mm, using an endolaser probe, at the donor nasal midperiphery is performed. Using a bimanual technique with vitreous forceps and scissors, a full-thickness graft of neuroretina, RPE and choroid is carefully separated from the sclera. The graft, then consisting of the RPE and choroid, is translocated to the recipient bed in the central and a bubble of perfluorooctane is injected. Finally, after fluid⁄air exchange, the eye is filled up with 1000 Cst silicone oil.
Scientific Publications	- Fernandez-Bueno I, Rodriguez de la Rua E, Hileeto D, Parrado ML, Regueiro-Purriños M, Sala-Puigdollers A, Srivastava GK, Gonzalo-Orden JM, Pastor JC. **Histology and immunochemistry evaluation of autologous translocation of RPE-choroid graft in porcine eyes**. Acta Ophthalmol Scan 2012. Epub ahead of print.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 6	**Endophthalmitis (Intravitreal S. aureus)**
Target Disease	Endophthalmitis
Species	Rabbit (*Oryctolagus cuniculus*)
Short Description	Rabbits were injected with approximately 200 CFU of a typed strain of S. aureus intravitreally.
Scientific Publications	- Del Nozal MJ, Bernal JL, Pampliega A, Marinero P, López MI, Coco R. **High-performance liquid chromatographic determination of vancomycin in rabbit serum, vitreous and aqueous humour after intravitreal injection of the drug**. J Chromatogr A 1996; 727:231-8 - Coco RM, López MI, Pastor JC, Nozal MJ. **Pharmacokinetics of intravitreal vancomycin in normal and infected rabbit eyes**. J Ocul Pharmacol Ther 1998; 14:555-63. - Coco RM, Lopez MI, Pastor JC. **Pharmacokinetics of 0.5 mg of a single and multiple dose of intravitreal vancomycin in infected rabbit eyes.** J Ocul Pharmacol Ther 2000;16(4):373-81.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 7	**Fresh retinal pigment epithelial (RPE) cells**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retinal diseases such as dry AMD, retinitis pigmentosa and many others are still non-curable or currently used therapeutic approaches are insufficiently effective. Their pathogenesis, likely multifactorial, involving a complex interaction of metabolic, functional, genetic and environmental factors, remain poorly understood. Although major abnormalities are seen in four functionally interrelated tissues, i.e., photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaries, the impairment of RPE cell functions is an early and crucial event in the cellular and molecular pathways leading to clinically relevant retinal changes. Detecting changes in RPE cells and repairing the pathways involved by using cellular and molecular based strategies are crucial for developing an effective therapy. For this reason, our laboratory is involved in investigation based on human RPE cells. We develop collaboration with the Universitary Hospital of Valladolid for obtaining human eye globes for isolating, cultivating pure fresh human RPE cells using our established protocols (Immunol Methods. 2013 Jan 11). Furthermore, porcine eye resembles with human eye in many properties such as similar size, anatomy and histology. Furthermore, retinal development in pig eye shows substantial similarity to human retinal development. These characteristics make pig eyes and their RPE cells an ideal model for performing pre-clinical tests. For this reason, our laboratory is involved in investigation based on pig RPE cells. We develop a collaboration with slaughter-house, Valladolid for obtaining pig eye globes for isolating, cultívating pure fresh pig RPE cells using our established protocols (Exp Eye Res. 2011;93:956-62.). In summary, we offer fresh pure human and porcine RPE cells for investigation purpose.
Scientific Publications	- Srivastava GK, Reinoso R, Singh AK, Fernandez-Bueno I, Martino M, Garcia-Gutierrez MT, Pastor JC, Corell A. **Flow cytometry assessment of the purity of human retinal pigment epithelial primary cell cultures**. J Immunol Methods. 2013 Jan 11. - Srivastava, G., Martin, L., Singh, A., Rodriguez-Cabello, J. and Pastor, J. **Evaluation of human retinal pigment epithelial cells growth on elastin-like recombinamers substrates**. Acta Ophthalmologica, 2011;89: 0. - Srivastava GK, Reinoso R, Singh AK, Fernandez-Bueno I, Hileeto D, Martino M, Garcia-Gutierrez MT, Merino JM, Alonso NF, Corell A, Pastor JC. **Trypan Blue staining method for quenching the autofluorescence of RPE cells for improving protein expression analysis**. Exp Eye Res. 2011;93:956-62.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 8	**Fresh retinal stem cells (RSCs) from ciliary margin**
Target Disease	Retinal degeneration
Species	Human, Pig (*Sus scrofa domestica*)
Short Description	Retinal diseases such as dry AMD, retinitis pigmentosa and many others are still non-curable or currently used therapeutic approaches are insufficiently effective. The pathogenesis remains poorly understood. Major abnormalities are seen in four functionally interrelated tissues, i.e., photoreceptors, retinal pigment epithelium (RPE), Bruch's membrane and choriocapillaries. Stem cell therapy showed favorable out comes. Retinal stem cells (RSCs) are present during embryonic development; they persist in quiescent forms in the adult mammalian eye in ciliary marginal zone. They have the ability to differentiate into different retinal cell type. Thus, they are promising for developing stem cell based treatments for retinal diseases. For this reason, our laboratory is involved in investigation based on human RSCs. We develop collaboration with Universitary Hospital of Valladolid for obtaining human eye globes for isolating, cultivating pure fresh human RSCs using our established protocols. Furthermore, porcine eye resembles with human eye in many properties such as similar size, anatomy and histology. Furthermore, retinal development in pig eye shows substantial similarity to human retinal development. These characteristics make pig eyes and their RSCs an ideal model for performing pre-clinical tests. For this reason, our laboratory is involved in investigation based on pig RSCs. We develop collaboration with slaughter-house, Valladolid for obtaining pig eye globes for isolating, cultivating pure fresh pig RSCs using our established protocols. In summary, we offer fresh pure human and porcine RSCs for investigation purpose.
Scientific Publications	- Srivastava GK, Rodríguez-Crespo D, Casado-Coterillo C, Singh AK, Garcia-Gutierrez MT., Coronas J, Pastor JC. **Evaluation of retinal stem cells adhesion, growth, viability and protein expression on chitosan surface**. ISER meeting, Berlin, July, 2012. - Srivastava GK, Rodríguez-Crespo D, Singh AK, Casado-Coterillo C, Fernandez-Bueno I, Garcia-Gutierrez MT, Coronas J, Pastor JC. **Chitosan feasibility to retain retinal stem cell phenotype and slow proliferation for retinal transplantation**. BioMed Research International 2014; Epub 2014 Feb 2.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 9	**Chronic-Inflammatory Ocular Surface (SV-40 immortalized human corneal epithelial (HCE) cell line)**
Target Disease	Chronic-inflammatory ocular surface diseases
Species	Human
Short Description (max. 250 words)	Although there are available therapies for acute-moderate ocular surface diseases, there is a lack of effective therapy for the treatment of more chronic-severe inflammatory diseases. These chronic-severe diseases are characterized for the possibility of corneal damage, that may lead to a sight-threatening outcome. It is clear that secreted molecules from inflammatory cells are responsible for many of the features observed in ocular inflammatory diseases. However, there is growing evidence showing that epithelial cells are active participants in inflammation, as they express and produce a large number of molecules implicated in the initiation and perpetuation of the inflammation process. For that reason, epithelial cells, and in our case corneal epithelial cells, are considered as a potential target for therapeutic interventions. This experimental model represents an in vitro model of ocular inflammation for the study of the implication of the corneal epithelium in the development of chronic-severe inflammatory diseases that may lead to visual loss. In this model, the SV-40 immortalized human corneal epithelial (HCE) cell line (Araki-Sasaki et al., IOVS 1995) has been exposed to different cytokines, including Th1 (INF-γ), Th2 (IL-4, IL-13) and Th17 (IL-17), and to TNF-α and TGF-β. HCE response in terms of cytokine/chemokine secretion and/or several receptor types' expression has been characterized.
Scientific Publications	- Enríquez de Salamanca A, Calder V, Gao J, Galatowicz B, Garcia-Vazquez C, Fernández I, Stern ME, Diebold Y, Calonge M. **Cytokine responses by conjunctival epithelial cells: an in vitro model of ocular inflammation**. Cytokine. 2008; 44:160-167 - Contreras-Ruiz L, Schulze U, García-Posadas L, Arranz-Valsero I, López-García A, Paulsen F, Diebold Y. **Structural and functional alteration of corneal epithelial barrier under inflammatory conditions**. Curr Eye Res. 2012;37(11):971-81. - Benito MJ, Calder V, Corrales RM, García Vázquez C, Narayanan S, Herreras JM, Stern ME, Calonge M, Enríquez de Salamanca A. **Effect of TGF-β on ocular surface epithelial cells**. Exp Eye Res. 2013; 107:88-100. - Arranz-Valsero I, Schulze U, Contreras-Ruiz L, García-Posadas L, López-García A, Paulsen F, Diebold Y. **Involvement of corneal epithelial cells in the Th17 response in an in vitro bacterial inflammation model**. Mol Vis. 2013; 19:85.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 10	**Limbal Stem Cell Deficiency**
Target Disease	Limbal Stem Cell Deficiency
Species	Pig (*Sus scrofa domestica*)
Short Description (max. 250 words)	An animal model of total LSCD was developed in pigs. 4-week-old male pigs were tranquilized with an intramuscular injection of Midazolam (0.35 mg/kg), Ketamine (5 mg/kg) and Atropine (0.02 mg/kg). Anesthesia induction was performed with an intravenous injection of Propofol (3-4mg/kg). Ocular surface was rinsed with sterile saline and 2.5% iodine solution. N-heptanol-based denudation of the corneal surface was done. Then, 360º cryogenic lesions were applied to the entire limbal area. A device was constructed to this end and the procedure was standardized. Intraorbital triamcinolone was immediately injected after surgery and systemic antibiotics were given for the 1st post-op week. After creation of the injury the eyelid was closed. Animals were weekly evaluated and scored by the same two independent researchers for: edema, opacification, neovascularisation, and re-epithelization. Photographs were taken at each evaluation point. Corneal impression cytologies were taken weekly to detect goblet cells. Histopathology analyses at the end of follow-up evaluated the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea. Additional evaluations were performed by laser scanning confocal microscopy (HRT-III Rostock Cornea Module) at the end of follow-up (12 weeks). Severe corneal scarring, neovascularization, opacification, and epithelial defects (fluorescein staining in green) were observed after 4 weeks and were maintained throughout the follow-up, resembling human LSCD. The presence of goblet cells in the central cornea was observed in corneal impression cytologies, resembling the conjunctival in-growth of human LSCD. Confocal microscopy demonstrated vascularization, inflammatory cells and goblet cells in the central cornea and complete (360º) limbal destruction, corroborated by pathology examination.
Scientific Publications	- Galindo S, Plata-Cordero M, Vuelta E, Iglesias J, Regueiro M, Gonzalo-Orden M, Hileeto D**,** Nieto-Miguel T, Herreras JM, Calonge M. **Ocular Surface Failure Due to Limbal Stem Cell Deficiency (LSCD): Development of Two Efficient Animal Models.** Invest Ophthalmol Vis Sci 2012; 53: E-Abstract 3516
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 11	**Partial limbal stem cell deficiency (Rabbit)**
Target Disease	Limbal stem cell deficiency
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	A partial Limbal Stem Cell Deficiency (LSCD) model was created in rabbits. Rabbits (weighing 4-4.5 kg) were anesthetized with intramuscular injection of 50 mg/Kg ketamine (Imalgene 1000®, Merial, Lyon, France) and 7 mg/Kg xilasyn (Rompun®, Bayer AG, Leverkusen, Germany), complemented with topical ophthalmic anesthetic combined (Colircusí®, Alcon, Barcelona, Spain). Denudation of corneal surface was made by cotton swab soaked with n-hepanol for 1 minute, and then, cornea was stained with fluorescein sodium to ensure of the removal of corneal epithelium. Surgical 180º limbal peritomy (temporal limbus, from 7 to 1 o'clock) was performed by crescent knife. Topical anti-inflammatory and antibiotics (Maxitrol® and Tobrex®, Alcon), intramuscular analgesics (0.02 mg/Kg buprenorphine, Buprex®), and subcutaneous antibiotics (5 mg/Kg enrofloxacin, Alsir®, Esteve, Barcelona, Spain) were administered daily for 5 days. Corneal neovascularization, corneal opacity and epithelial defects were clinically scored weekly by two different researchers. At the end of follow-up (11 weeks), histopathology and immunofluorescence analyses evaluated the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea and limbus. Rabbit corneas developed neovascularization, opacification, and epithelial defects after 3 weeks, resembling mild human LSCD. Histopathology and immunoflourescence analyses showed complete destruction of the injured area (180º) and mild inflammation of the non-injured area. New ocular treatments could be applied in this model in order to evaluate their effect in a mild LSCD.
Scientific Publications	- Galindo S, Herreras JM, López-Paniagua M, Rey E, de la Mata A, Plata-Cordero M, Calonge M, Nieto-Miguel T. **Regenerative effect of human adipose tissue-derived mesenchymal stem cells in experimental corneal failure due to limbal stem cell niche damage**. Stem Cells 2017;35:2160-74
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 12	**Total limbal stem cell deficiency (Rabbit)**
Target Disease	Limbal stem cell deficiency
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	A total Limbal Stem Cell Deficiency (LSCD) model was created in rabbits. Rabbits (weighing 4-5 kg) were anesthetized with intramuscular injection of 50 mg/Kg ketamine (Imalgene 1000®, Merial, Lyon, France) and 7 mg/Kg xilazine (Rompun®, Bayer AG, Leverkusen, Germany), complemented with topical ophthalmic anesthetic combined (Colircusí®, Alcon, Barcelona, Spain). Denudation of corneal surface was made by cotton swab soaked with n-hepanol for 1 minute, and then, cornea was stained with fluorescein sodium to ensure of the removal of corneal epithelium. Surgical 360º limbal peritomy was performed by crescent knife. Topical anti-inflammatory and antibiotics (Maxitrol® and Tobrex®, Alcon), intramuscular analgesics (0.02 mg/Kg buprenorphine, Buprex®), and subcutaneous antibiotics (5 mg/Kg enrofloxacin, Alsir®, Esteve, Barcelona, Spain) were administered daily for 5 days. Corneal conjunctivalization, corneal neovascularization, corneal opacity and epithelial defects were clinically scored weekly by two different researchers. At the end of follow-up (11 weeks), histopathology and immunofluorescence analyses were performed to evaluate the degree of damage created in the limbal niche and the presence of inflammation and goblet cells (as a sign of conjuntival in-growth) in the central cornea and limbus. Rabbit corneas developed neovascularization, opacification, and epithelial defects, after 3 weeks, resembling moderate/severe human LSCD. Histopathology and immunofluorescence analyses showed complete destruction of the limbus and inflammation of corneal and limbal stroma. New ocular treatments could be applied in this model in order to evaluate their effect in a moderate/severe LSCD.
Scientific Publications	- Galindo S, Herreras JM, López-Paniagua M, Rey E, de la Mata A, Plata-Cordero M, Calonge M, Nieto-Miguel T. **Regenerative effect of human adipose tissue-derived mesenchymal stem cells in experimental corneal failure due to limbal stem cell niche damage**. Stem Cells 2017;35:2160-74
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 13	**Wound healing in vivo model (Rabbit)**
Target Disease	Corneal wounds
Species	Rabbit (*Oryctolagus cuniculus*): New Zealand White
Short Description (max. 250 words)	New Zealand white rabbits (2.5-3 kg) were anesthetized with intramuscular injection of ketamine (50 mg/kg) and xylazine (7 mg/kg), complemented with two drops of topical ophthalmic anesthesia (Combined double anesthetic, Colircusí®). In addition, systemic analgesics (0.02 mg/kg buprenorphine) and antibiotics (5 mg/kg enrofloxacin) were administered before performing corneal damage. A filter paper disc (6 mm in diameter) soaked with 1-heptanol, was positioned in the center of each right corneal surface and left in place for 1 minute. The disc was then removed, and the damaged corneal epithelium was washed gently with sterile saline. The epithelial defects were revealed with 5 μl of sodium fluorescein (Colircusí Fluotest®), after 1 minute the excess of fluorescein was washed with sterile saline and the ocular surface was photographed using cobalt blue light and yellow barrier filter, immediately after injury (t=0) and at 8, 24, 32, 48, 56 and 72 hours after injury. The stained area was measured by computerized planimetry with NIH Image (Image J software). At the end of the follow-up (72 h) the animals were euthanized by intravenous injection of pentobarbital sodium (Dolethal®) under anesthesia. Fluorescein staining revealed that the epithelial defect decreased along the time until wounds were completely closed. Topical ocular administration of new treatments could be applied in this model in order to evaluate the wound healing rate.
Scientific Publications	
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 14	**Engineered human conjunctival-like tissue to study ocular surface inflammatory diseases (3D model of human conjunctiva)**
Target Disease	Inflammatory diseases of the ocular surface
Species	Human
Short Description (max. 250 words)	Inflammatory ocular surface diseases are very prevalent among the global population. Patients demand more efficacious, new treatments for their diseases and, at the same time, governments and pharmaceutical companies are concerned about the cost of the research needed to develop new drugs. The increasing use of three-dimensional models has shown their utility in decreasing research costs by providing more reliable results and reducing the use of animals in research. The conjunctiva is involved in different ocular surface diseases, playing an active role in the pathophysiology of common conditions such as dry eye disease, Sjögren's syndrome, and allergic conjunctivitis, among others. To date, the majority of in vitro investigations concerning the conjunctival tissue have been carried out using monolayer culture techniques that do not recapitulate the complexity of the whole tissue. For those reasons, we have developed a three-dimensional model of the human conjunctiva that can be used to perform pathophysiology experiments and test drug response. Briefly, fibrin-based matrices (derived from human plasma or plasma cryoprecipitate) were used as scaffolds, and primary cells obtained from cadaveric conjunctival tissue were seeded (fibroblast inside and epithelial cells on the surface). Characterization of conjunctival constructs showed epithelial cell stratification, cell polarization and functionality. Conditions such as desiccation and exposure to IL-13 were used to in vitro mimic dry eye disease or allergy. In response to those stimuli, conjunctival constructs increased IL-6 and MUC5AC production. Therefore, this three-dimensional model can be used to study ocular inflammatory diseases and test novel therapies.
Scientific Publications	García-Posadas L, Soriano-Romaní L, López-García A, Diebold Y. **An Engineered Human Conjunctival-Like Tissue to Study Ocular Surface Inflammatory Diseases**. PLoS ONE 12(3): e0171099. doi:10.1371/journal.pone.0171099
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 15	**In vitro human corneal wound healing model**
Target Disease	Corneal wound healing under inflammatory conditions
Species	Human
Short Description (max. 250 words)	The ability of the cornea to heal and maintain its clarity has paramount importance in preserving the eyesight. Corneal opacity insults, such as trauma, eye surgery, or inflammatory diseases of the anterior part of the eye. In the ocular surface, a pathologic wound healing process, along with local inflammation and neovascularization can induce failure in the functional recovery of the ocular surface tissues, which can lead to corneal blindness. In particular, corneal healing process under inflammatory conditions is not fully understood. In this model, confluent monolayers of the SV-40 immortalized human corneal epithelial (HCE) cell line (Araki-Sasaki et al., IOVS 1995) are consistently wounded, creating a cell-free area, based on the technique described by Liang et al. (Liang et al., 2007). Cultures are gently washed with DMEM/F12 to remove loose cells. Cells are then exposed to different cytokines in culture medium. Cells for control conditions are also scratched, washed, and maintained in culture medium after the scratch. Immediately after the scratch and/or at different time points, at least four images of the scraped area are captured using phase contrast microscopy. The remaining wounded area and the scratch width at six different points per image are measured. The same scratched area is selected for the measurements at each time of study. Using this model, influence of inflammatory molecules or treatments in corneal wound closure can be studied, and relative contribution of cell proliferation and cell migration processes analyzed.
Scientific Publications	Arranz-Valsero I, Soriano-Romaní L, García-Posadas L, López-García A, Diebold Y. I**L-6 as a corneal wound healing mediator in an in vitro scratch assay**. Exp Eye Res 2014; 125: 183-192. DOI 10.1016/j.exer.2014.06.012
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 16	**Oxidative stress based cell culture model (line ARPE19)**
Target Disease	Retinal degeneration
Species	Human
Short Description (max. 250 words)	Retina is a principal anatomical structure for vision. The retina is exposed to oxidative stress along life span, which produce damage in retinal structure obstructing its proper function. The retinal pigment epithelial cells layer is a crucial part of retina. Our laboratory has established in vitro cell culture models applying oxidative stress. The Glucose oxidase (GOx) enzyme is applied for generation of H2O2 which produces continues oxidative stress in cell culture. The H2O2 is applied for acute oxidative stress. Depending on experimental requirements it can be selected an oxidative stress model. The developed oxidative stress based cell culture models can be used for testing anti-oxidant effects.
Scientific Publications	Rodriguez-Crespo D, Di Lauro S, Singh AK, Garcia-Gutierrez MT, Garrosa M, Pastor JC, Fernandez-Bueno I, Srivastava GK. **Triple-layered mixed co-culture model of RPE cells with neuroretina for evaluating the neuroprotective effects of adipose-MSCs**. Cell Tissue Res. 2014 Dec;358(3):705-16. Rodriguez-Crespo D. **Evaluación del cocultivo de células madre mesenquimales y células del epitelio pigmentario de la retina en un modelo de cultivo organotípico de neurorretina de cerdo**. Master thesis. 2013. IOBA, Universidad de Valladolid.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 17	***In vitro* culture of limbal epithelial stem cells**
Target Disease	Limbal stem cell deficiency
Species	Human
Short Description (max. 250 words)	Transplantation of in vitro cultured limbal epithelial stem cells (LESCs) is a treatment widely used for LESC deficiency. However, the number of limbal tissue donors is limited, and protocols for LESC cultivation often include compounds and/or feeder layers that can induce side effects and/or increase the cost of the culture procedure. We investigated the feasibility of obtaining more than one limbal primary culture (LPC) from the same biopsy using a culture medium in which several potentially harmful compounds were replaced at the same time by biosafe supplements, allowing the LESC cultivation without feeder layers. Our group established feeder layer-free LPCs with three culture media: (1) a modified supplemental hormonal epithelial medium, containing potential harmful components (cholera toxin, dimethylsulfoxide, and fetal bovine serum [FBS]), (2) IOBA-FBS, a medium with FBS but with no other harmful supplements, and (3) IOBA-HS, similar to IOBA-FBS but with human serum instead of FBS. Additionally, the same limbal explant was consecutively cultured with IOBA-HS producing three cultures. LPCs were characterized by real-time reverse transcription polymerase chain reaction and/or immunofluorescence. IOBA-HS enables the culturing of up to two biosafe homologous LPCs from one limbal tissue under feeder layer-free conditions. The routine use of this culture medium could improve both the biosafety and the number of available LPCs for potential clinical transplantation, as well as decrease the expense of the culture procedure.These systems could be used as in vitro model of limbal stem cell culture.
Scientific Publications	López-Paniagua M, Nieto-Miguel T, Galindo S, García-Posadas L, de la Mata A, Corrales RM, Calonge M, Diebold Y. **Optimization of Human Limbal Stem Cell Culture by Replating a Single Limbal Explant**. Methods Mol Biol. 2020;2145:39-49.. López-Paniagua M, Nieto-Miguel T, de la Mata A, Galindo S, Herreras JM, Corrales RM, Calonge M. **Successful Consecutive Expansion of Limbal Explants Using a Biosafe Culture Medium under Feeder Layer-Free Conditions**. Curr Eye Res. 2017 May;42(5):685-695. López-Paniagua M, Nieto-Miguel T, de la Mata A, Dziasko M, Galindo S, Rey E, Herreras JM, Corrales RM, Daniels JT, Calonge M. **Comparison of functional limbal epithelial stem cell isolation methods.** Exp Eye Res. 2016 May;146:83-94. López-Paniagua M, Nieto-Miguel T, de la Mata A, Galindo S, Herreras JM, Corrales RM, Calonge M. **Consecutive expansion of limbal epithelial stem cells from a single limbal biopsy.** Curr Eye Res. 2013 May;38(5):537-49.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid

Experimental Model 18	**Limbal epithelial stem cells-biopolymer complex biocompatibility (*in vitro*)**
Target Disease	Limbal stem cell deficiency
Species	Human
Short Description (max. 250 words)	Limbal epithelial stem cells (LESCs) are responsible for the renewal of corneal epithelium. Cultivated limbal epithelial transplantation is the current treatment of choice for restoring the loss or dysfunction of LESCs. To perform this procedure, a substratum is necessary for *in vitro* culturing of limbal epithelial cells and their subsequent transplantation onto the ocular surface. Our group has evaluated the *in vitro* biocompatibility of different biopolymers as cell carriers for LESCs: chitosan-gelatin and poly-L/DL-lactic acid 70:30 (PLA) films functionalized with type IV collagen (col IV). Cell viability and proliferation was studied in all the substrates. In addition, our group analyzed the expression levels for the LESC specific markers, K15, P63α and ABCG, and the expression levels of the corneal epithelial markers K3 and K12. Our results demonstrate that CH-G 20:80 membranes are suitable for the expansion and maintenance of stem cells derived from the limbal niche. These results strongly support the use of polymers as alternative substrata for the transplantation of cultivated limbal cells onto the ocular surface. In case of the PLA-col IV films, it was demonstrated that they promoted LESC attachment and helped to maintain their undifferentiated stem cell phenotype. Consequently, these substrata offer an alternative for the transplantation of limbal cells onto the ocular surface. This procedure could be used as a protocol model to test the biocompatibility of cell substrates.
Scientific Publications	de la Mata A, Mateos-Timoneda MA, Nieto-Miguel T, Galindo S, López-Paniagua M, Planell JA, Engel E, Calonge M. **Poly-l/dl-lactic acid films functionalized with collagen IV as carrier substrata for corneal epithelial stem cells**. Colloids Surf B Biointerfaces. 2019 May 1;177:121-129. de la Mata A, Nieto-Miguel T, López-Paniagua M, Galindo S, Aguilar MR, García-Fernández L, Gonzalo S, Vázquez B, Román JS, Corrales RM, Calonge M. **Chitosan-gelatin biopolymers as carrier substrata for limbal epithelial stem cells.** J Mater Sci Mater Med. 2013 Dec;24(12):2819-29.
Institution	IOBA-Eye Institute, University of Valladolid, Valladolid