Project no. **LSHB-CT-2006-019114**

Project acronym **PolExGene**

Project title **Biocompatible non-viral polymeric gene delivery systems for the *ex vivo* treatment of ocular and cardiovascular diseases with high unmet medical need**

Instrument **SPECIFIC TARGETED RESEARCH OR INNOVATION PROJECT**

Thematic Priority **SIXTH FRAMEWORK PROGRAMME PRIORITY 1**
Life Sciences, genomics and biotechnology for health

PUBLISHABLE FINAL ACIVITY REPORT

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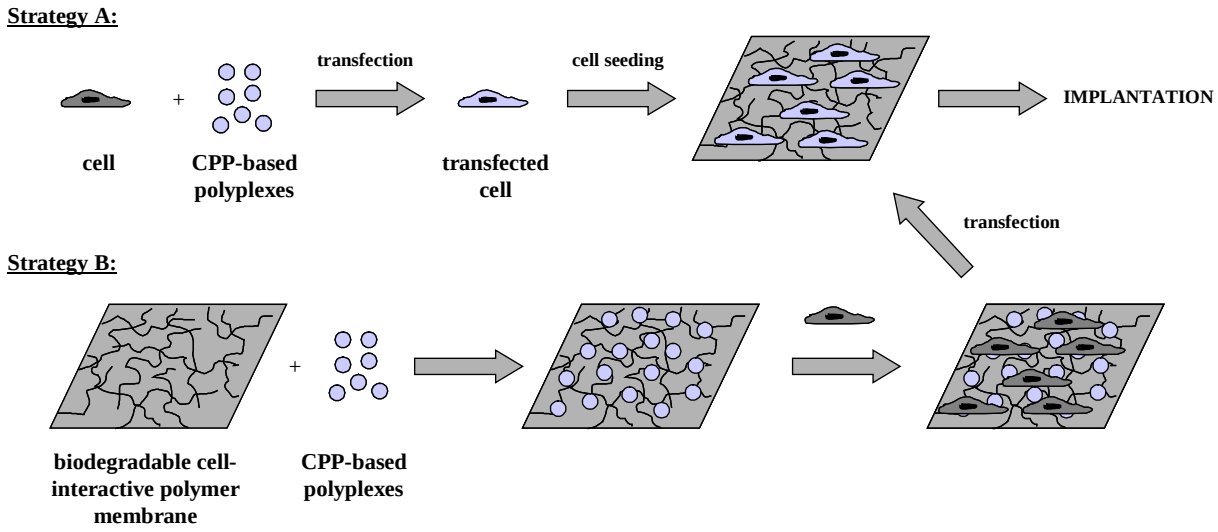
1. Project execution

Project objectives

The objective of the PolExGene project was to develop a non-viral *ex vivo* gene therapy that will be applied for both ocular and cardiovascular diseases with high unmet medical need. The research of the project focused on improving polymeric DNA-delivery by combining polyplexes with cell penetrating peptides (CPP). To circumvent the toxic and immunogenic problems related to viral vectors, the gene vectors developed in this project will be a non-toxic and non-immunogenic, biodegradable polymeric carrier based on multifunctional poly-α-aminoacids. The potential of the CPP-containing polyplexes regarding their transfection efficiency and the absence of any toxic or immunogenic side effects was evaluated using two gene transfer approaches. In a first approach, cells were transfected with polyplexes (i.e. polymer-DNA complexes) and then seeded on a polymer membrane prior to implantation. Alternatively, the polymer membrane were surface coated with polyplexes prior to cell seeding and implantation. In order to enhance the internalisation efficiency, the polyplexes were functionalised using CPP includig Penetratin and others. In order to improve the membrane-cell interaction and to enhance the cell proliferation and differentiation, the polymer membrane were functionalised with cell interacting peptides (CIP). Both approaches are schematically summarised in the figure below.



To reach the final project goal, different interrelated work packages were implemented: (1) selection of CIP and CPP, (2) development of CPP-containing polymers, (3) development of CIP-containing polymer membranes, (4) preparation of plasmids and CPP-containing polyplexes, (5) characterisation of polyplex-cell and polymer membrane-cell interactions, (6) study on immunological properties of polyplexes and polymer membranes and (7) polymer membrane implantation in test animals. Work package 8 was devoted to the project management.

Contractors involved

The PolExGene consortium was comprised of 9 partners from 6 different countries (Belgium, Finland, Germany, France, Czech Republic, UK). The different partners and the responsible persons per partner are summarised below:

- Ghent University, Belgium (Ghent), Prof. E. Schacht and Prof. P. Dubruel
- Helsigin Yliopisto, Finland (Helsinki), Prof. A. Urtti
- Fraunhofer Institute for Biomedical Engineering, Germany (St. Ingbert), Dr. H. Thielecke
- Institute of Microbiology, Czech Republic (Prague), Prof. B. Rihova
- Ecole Normale Supérieure, France (Paris), Dr. A. Joliot
- Eberhard-Karls-University Tübingen, Germany (Tübingen), Prof. E. Zrenner
- Ark Therapeutics Group Ltd, United Kingdom (London), Dr. D. Ellam
- University of Kuopio, Finland (Kuopio), Dr. T. Wirth
- Centre National de la Recherche Scientifique, France (Montpellier), Prof. J. Martinez

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Work performed and obtained results

Development and characterisation of CPP-containing polyplexes

In order to prepare polymer-DNA complexes (i.e. polyplexes) which contain cell penetrating peptides (CPP), three different components were required: cationic polymers, plasmid DNA and CPP. A large part of the work performed covered the development of these three polyplex constituents.

A series of water soluble cationic (co)polymers based on poly-α-amino acids were developed and characterised for their chemical structure and molecular weight. The polymers developed contained various side groups including tertiary amines, primary amines, hydroxyl groups, imidazole groups, guanidine functions or combinations thereof. All the polymers developed had in common that they are positively charged and can thus interact with DNA through electrostatic binding, forming polyelectrolyte complexes (PEC).

As second polyplex component, a series of therapeutic plasmids were also developed. The plasmids developed included among other an EBNA1-ORIP plasmid with the SEAP marker under the viral CMV promoter or a human tyrosinase promoter. For therapeutic use, VEGF-R1, VEGF-R2 and VEGF-R3 were cloned into these plasmids. Additionally, these genes have been cloned in replicating Ebstain-Barr virus nuclear antigen (EBNA) plasmids. Finally, cloning of IK-17 or Mertk into the pCDNA3 Plasmid was realised.

The last polyplex constituent which was developed included different types of CPP. As an example, the following CPP were developed and characterised: Penetratin, poly-arginine, TAT and KALA. To test the biological behaviour of the CPP developed, an impedance chip with fluidic system was developed. The chip can be used to study the effects of CPP on single cell level. A similar chip can also be applied to measure the polyplex-cell interactions.

Using the materials developed, a variety of techniques were used to study the interaction between DNA and the (co)polymers developed. The DNA condensation capacity was studied using ethidium bromide exclusion tests and agarose gel electrophoresis. The studies indicated that all polymers studied are able to condense DNA. Dynamic light scattering and zeta potential measurements further indicated that the polyplexes formed possess a net positive charge at charge ratios where DNA condensation occurs. In addition, the polyplexes formed are small enough to be taken up through endocytosis. Addition of CPP to pre-formed polyplexes does not alter the stability of the polyplexes.

After the polymer development and the physico-chemical evaluation of the polyplexes, a biological evaluation of the materials was performed. This covered a series of transfection studies using a variety of cells: mammalian epithelial cell line (CV1), a human retinal pigment epithelium (RPE) cell line (ARPE19) and primary RPE cells. The final results show that some polymer derivatives developed within the framework of PolExGene possess a higher transfection efficiency compared to PEI while the toxicity is lower or similar compared to PEI.

Development and characterisation of polymer membranes

In addition to the CPP-functionalised polyplexes, the development and characterisation of polymer membranes was a second important part of the PolExGene project. Various generations implantable polymer membranes were developed including a polyimide and a biodegradable polymer sheet as base material. In order to enhance the cell-interactive properties of both polymer sheets, a variety of constituents of the extra-cellular matrix were immobilised on the polymer sheets. Gelatin was the first candidate ECM-like material. Gelatin was therefore completely screened for its biocompatibility. This is of the utmost importance since chemical modification of gelatin was required to enable immobilisations on the implantable polymer sheets.

Finally, the polymer sheets developed were applied for the originally envisaged cardiovascular and ocular applications. Therefore, a large number of *in vivo* studies were performed. The tests have clearly revealed the potential of the selected PolExGene approach. The consortium is now ready to bring the technology one step further in the coming years. The consortium hopes to acquire soon the required funds to enable the various pre-clinical and clinical tests.

Publishable results of dissemination of knowledge plan

A Knowledge an Innovation Board has been implemented to take care about dissemination of knowledge created as a result of the project. This Board implemented this Plan for using and disseminating the knowledge. The Plan was discussed and updated each 6 months.

Publications

Peer reviewed publications are one of the most direct ways to reach the scientific community. Since the project is multidisciplinary, there is a unique occasion to reach different scientific communities through the most relevant journals of the different fields. So far, two papers have been published as a result of the PolExGene project:

http://www.natureprotocols.com/2009/04/23/optimized_transfection_protoco.php

Van Vlierberghe, S., Vanderleyden, E. Dubruel, P., Devos, F., Schacht, E. Affinity Study of Novel Gelatin Cell Carriers for Fibronectin. *Macromolecular Bioscience* 2009, 9, 11, 1105-1115

At present, 15 other papers are in preparation to be submitted Q2 of 2010 at latest. An additional five reviews on PolExGene related research are anticipated as the minimum number.

Project web-site

Website can be found at http://www.polexgene.eu

During the first term of the project, the website was developed and set up. The web site had two objectives: on the one hand it was intended to disseminate the project among the scientific community as well as the general public. On the other hand, this web served to exchange information among the partners of the project. After the end of the PolExGene project, the website will be kept active for at least one year.

Conferences

The partners have spread the results of the projects on conferences held in Europe and outside (see further for detailed description). The coordinators (Schacht and Dubruel) are respectively member of the council of the European Society for Biomaterials (Vice-President) and spokesperson of the ESB-Young Scientist Forum. This was one way to enhance the visibility of the PolExGene project.

Networking with other projects, institutions

Another important measure to spread the project activities, was to organize an internal lobbying group aimed at keeping the national authorities and funding agencies informed and interested on the work and progress of the project. The direct or indirect participation of existing networks and institutions to which the partners of the project are associated (e.g. the European Society for Biomaterials, the European Society of Gene Therapy, the European Platform for Patients Organisations, Science and Industry, …) was being used to facilitate a wide coverage of the goals and results of the project as appropriate.

2. Dissemination and use

Section 1 - Exploitable knowledge and its use

THIS PART SHOULD BE DISCUSSED WITH THE EU PROJECT OFFICER BEFORE ONLINE PUBLICATION

Exploitable Knowledge (description)	**Exploitable product(s) or measure(s)**	**Sector(s)** of application	Timetable for commercial use	Patents or other IPR protection	**Owner** & Other Partner(s) involved
1) Hydrogel coated polyester membrane	*Implant material*	*Medical*	*2015*	*An application patent will be aimed at*	To be defined February 2010
2) Cationic polymers as protein delivery system	*Protein delivery system*	*Medical*	*2018*	*To be investigated*	To be determined Q2 2010
3) polymetha-crylates as ocular implant material	*Implant for ocular applications*	*Medical Optical*	*2015*	*Under investigation with Tech Transfer department of the coordinator*	UG

1) In terms of exploitable results, the biodegradable polymer sheets (VIVOSORB) coated with various biopolymers have a clear potential to be exploited. The VIVOSORB materials as such are biocompatible but not cell interactive. By applying the PolExGene hydrogel coating mimicking the extra-cellular matrix, the materials become cell-interactive while maintaining their bulk properties. At present, the coordinator has signed a NDA with POLYGANICS to screen the possibilities of bringing these modified biodegradable polyesters to the market for a variety of applications including ocular and cardiovascular. The data obtained so far using those materials will of course also be the basis for future research activities within the consortium. The surface modification was proposed by the research group of the coordinator.

2) Most of the cationic polymers developed within the framework of the PolExGene project possess a lower transfection efficiency but also a lower toxicity. One of the factors responsible for the lower transfection might be the low polyplex uptake in the cell nucleus. Therefore, it if of large importance to screen if the materials can not be applied for the delivery of proteins since entry in the cytoplasm is the endpoint of most protein delivery systems.

3) In the framework of a FW7 project in which the coordinator is involved (PHOSFOS), a series of polymethacrylates have been developed to be applied in the optical field (waveguides, optical fibre coatings, …). Recently, the biocompatibility of those materials has been studied and proven. Due to the large variety of material properties that can be developed as well as the surface functionalisation which is feasible, the potential of the materials for PolExGene related research will be investigated.

Section 2 – Dissemination of knowledge

An overview table including the dissemination of knowledge during the PolExGene project is given below.

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2006-2009	**Bachelor, Master and PhD students (20 in total during the project)**	Research	**ALL**	-	**ALL**
	Courses				
2010 onwards	Biomaterial-cell interaction core laboratory as part of Biocenter Finland network "Stem cells and biomaterials"	Teaching	**FI**	-	**UH.FP**
2009 onwards	Biomaterials master's course	Teaching	**FI**	-	**UH.FP**
2007 onwards	Biopolymers master's course	Teaching	**BE**	-	**UG**
2007 onwards	Biomaterials master's course	Teaching	**BE**	-	**UG**
2009	**Publications: 2**	Research	**WORLD**	-	**UG, UH.FP and ENS**
2010 - …	**Publications: 15**	Research	**WORLD**	-	**ALL 1**
2006	**Project web-site**		**WORLD**	-	
	Conferences				
2010	11th Japan Belgium Symposium on Polymer Science	Research/Industry	**WORLD**		**UG 1**
2010	I-Sup 2010	Research/Industry	**WORLD**		**UG 2**
2009	EU coordinator days	Research/EU	**WORLD**		**UG 3**
2009	ENFI 2009 (Hasselt)	Research	**WORLD**		**UG 4**
2009	Amsterdam	Research	**WORLD**		**UG 5**
2009	European Vision and Eye Research (EVER) Conference, Portoroz, Slovenia,	Research	**WORLD**		**UH.FP 1**
2008	Retina International, Helsinki, Finland	Research	**WORLD**		**UH.FP 2**
2007	Annual Controlled Release Society meeting, San Diego,	Research	**WORLD**		**UH.FP 3**

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
	US				
2010	International conference of electrical bioimpedance 2010, Gainesville, Fl	Research	**WORLD**		**IBMT 1**
2009	35rd International Conference on Micro- and Nano Engineering, Ghent, Belgium	Research	**WORLD**		**IBMT 2**
2009	DECHEMA - Conference Organotypic Tissue Culture for Substance Evaluation, Potsdam, Germany	Research	**WORLD**		**IBMT 3**
2009	Summer School BioMEMS, Saarbrücken, Germany,	Research	**WORLD**		**IBMT 4**
2009	BioKorea 2009 Conference, Seoul, Korea	Research	**WORLD**		**IBMT 5**
2009	Materials Valley Workshop, Hanau, Germany	Research	**WORLD**		**IBMT 6**
2009	Deutsche Keramische Gesellschaft Workshop, Hardheim, Germany	Research	**WORLD**		**IBMT 7**
2008	MSE 2008 Conference, Nuremberg, Germany	Research	**WORLD**		**IBMT 8**
2008	Nano2Life Research School, Saarbruecken, Germany	Research	**WORLD**		**IBMT 9**
2007	Trade fair Biotechnica, Hannover, Germany,	Research	**WORLD**		**IBMT 10**
2007	33rd International Conference on Micro- and Nano Engineering 2007, Copenhagen	Research	**WORLD**		**IBMT 11**
2006	Korea-EU Workshop, Saarbruecken, Germany	Research	**WORLD**		**IBMT 12**
2009	2nd European Congress of Immunology. Berlin, Germany	Research	**WORLD**		**IMIC 1**
2009	4th ENII – MUGEN Summer School in Advanced Immunology. Capo Caccia, Sardinia, Italy	Research	**WORLD**		**IMIC 2**

Planned /actual Dates	Type	Type of audience	Countries addressed	Size of audience	Partner responsible /involved
2008	25th Congress of Czech and Slovak Allergologists and Clinical Immunologists AND 12th Congress of Czech and Slovak Immunologists. Prague, Czech Rep.	Research	**WORLD**		**IMIC 3**
2008	25th Congress of Czech and Slovak Allergologists and Clinical Immunologists AND 12th Congress of Czech and Slovak Immunologists. Prague, Czech Rep.	Research	**WORLD**		**IMIC 4**
2008	6th International Workshop on Drug Delivery systems for Nanomedicine. Liblice Castle, Czech Rep.	Research	**WORLD**		**IMIC 5**
2008	6th International Workshop on Drug Delivery systems for Nanomedicine. Liblice Castle, Czech Rep.	Research	**WORLD**		**IMIC 6**
2009	Journées de l'Ecole Doctorale "Complexité du Vivant"	Research	**FR**		**ENS 1**
2009	Séminaires du Collège de France	Research	**FR**		**ENS 2**

In what follows, a detailed description of all items listed in the above table is given.

UG, UH.FP and ENS: two papers are already published at present

1) http://www.natureprotocols.com/2009/04/23/optimized_transfection_protoco.php

2) Van Vlierberghe, S., Vanderleyden, E. Dubruel, P., Devos, F., Schacht, E. Affinity Study of Novel Gelatin Cell Carriers for Fibronectin. *Macromolecular Bioscience* 2009, 9, 11, 1105-1115

ALL: At present, 15 publications are in preparation covering various subtopics of the PolExGene project. The consortium also plans to submit at least 5 review articles on key topics of the project.

UG 1: P. Dubruel, V. Toncheva, V. Vermeersch, S. Van Vlierberghe, E. Schacht, Polymers as versatile materials for biomedical applications
INVITED ORAL PRESENTATION

UG 2: S. Van Vlierberghe, V. Vermeersch, V. Toncheva, H. Thielecke, H. Bueth, E. Schacht, P. Dubruel, Polymer membranes – A versatile tool in regenerative medicine
ORAL PRESENTATION

UG 3: PolExGene Consortium, Biocompatible non-viral gene delivery systems for the ex vivo treatment of ocular and cardiovascular diseases with high unmet medical need
INVITED ORAL PRESENTATION

UG 4: S. Van Vlierberghe, V. Vermeersch, V. Toncheva, H. Thielecke, H. Bueth, P. Dubruel, E. Schacht, Biopolymer Functionalized Polyimide Membranes for Ocular Gene Therapy Applications
POSTER AND BRIEF ORAL PRESENTATION

UG 5: S. Van Vlierberghe, P. Dubruel, E. Schacht, Cell-Interactive Biopolymer-based Hydrogels designed for Tissue Engineering
INVITED ORAL PRESENTATION

UH.FP 1: A. Urtti, Retinal Pigment Epithelium Cell Models
INVITED ORAL PRESENTATION

UH.FP 2: A. Urtti, Pharmacological treatment of retinal diseases
INVITED ORAL PRESENTATION

UH.FP 3: A. Urtti, Retinal Pigment Epithelium: Key Player in Posterior Segment Ocular Drug Delivery
INVITED ORAL PRESENTATION

IBMT 1: C. Kurz, H. Büth, H. Thielecke, Influence of transfection process on single cell impedance monitoring
ORAL PRESENTATION

IBMT 2: C. Kurz, S. Cho, H. Büth, H. Thielecke, Influence of the Electrode Radius on the Impedance Spectra of Cell-covered Disc-Electrode
CONFERENCE PROCEEDING

IBMT 3: H. Thielecke, C. Hildebrandt, H. Büth, Approaches for tissue culture-based tests with higher throughput
ORAL PRESENTATION

IBMT 4: H. Büth, Biohybrid Systems
ORAL PRESENTATION

IBMT 5: H. Thielecke, Cell-based biosensors and test systems – Approaches and applications
ORAL PRESENTATION

IBMT 6: H. Büth, Neue Ansätze für die zerstörungsfreie Charakterisierung von Einzelzellen und Zellverbänden
ORAL PRESENTATION

IBMT 7: H. Thielecke, In vitro Prüfungen zur biologischen Verträglichkeit und Funktion von Materialien für den Einsatz in Medizinprodukten
ORAL PRESENTATION

IBMT 8: H. Thielecke, Non-destructive cell-based tests to support the engineering of nanomaterials with a desired biological function
ORAL PRESENTATION

IBMT 9: H. Thielecke, Biological evaluation of nanomaterials and nanoparticles
ORAL PRESENTATION

IBMT 10: H. Büth, Product sheet presented
ORAL PRESENTATION

IBMT 11: S. Cho, H. Thielecke, Electrical Characterization of Cell Behaviour on Microelectrode
CONFERENCE PROCEEDING

IBMT 12: H. Thielecke, Cell-based test and manipulation systems for the evaluation and application of engineered nanomaterials/nanoparticles
ORAL PRESENTATION

IMIC 1: M. Sirova, V. Pakanova, P. Rossmann, L. Kovar, S. Van Vlierberghe, P. Dubruel, E. Schacht, B. Rihova, Immunogenicity of gelatin-based hydrogels supporting *ex vivo* gene therapy
POSTER

IMIC 2: V. Pakanova, M. Sirova, P. Rossmann, S. Van Vlierberghe, V. Vermeersch, V. Toncheva, P. Dubruel, E. Schacht, B. Rihova, Biocompatibility of polymers for gene delivery
POSTER

IMIC 3: V. Pakanova, M. Sirova, P. Rossmann, P. Dubruel, S. Van Vlierberghe, E. Schacht, B. Rihova, Immunogenicity of polymers for gene delivery: I. Biocompatibility and immunocompatibility of hydrogels
POSTER

IMIC 4: M. Sirova, L. Kovar, V. Pakanova, P. Dubruel, S. Van Vlierberghe, E. Schacht, B. Rihova, Immunogenicity of polymers for gene delivery: II. Immunocompatibility of hydrogels and polyplexes evaluated using multiplex cytokine detection
POSTER

IMIC 5: V. Pakanova, M. Sirova, P. Rossmann, P. Dubruel, S. Van Vlierberghe, E. Schacht, B. Rihova, Immunogenicity of polymers for gene delivery: Biocompatibility and immunocompatibility of hydrogels
POSTER

IMIC 6: M. Sirova, L. Kovar, V. Pakanova, P. Rossmann, P. Dubruel, S. Van Vlierberghe, V. vermeersch, E. Schacht, B. Rihova, Immunogenicity of polymers for gene delivery
PRESENTATION

ENS 1: L. Tibaldi, E. Dupont, V. Lebled A. Joliot, Cell Penetrating peptides
POSTER

ENS 2: L. Tibaldi, E. Dupont, V. Lebled A. Joliot, Protein and DNA Vectorisartion
ORAL PRESENTATION

Section 3 - Publishable results

As indicated in section 1 of this report, the coordinator is currently looking with a Dutch SME if some of the surface chemistry developed is prone to valorization. The European Officer will be informed on a regular basis in the coming months on the status of this as well as the two other topics discussed in section 1.

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